Review article

Global scenario and recent advances in molecular diagnosis and management of drug-resistant tuberculosis**Mohan B. Sannathimmappa¹, Prashanth Gowda Parameshwara², Rajeev Aravindakshan³, Vinod Nambiar¹**Department of ¹Microbiology, ²Pediatrics, College of Medicine and Health Sciences, National University of Science and Technology, Sohar Campus, Sultanate of Oman³All India Institute of Medical Sciences, Mangalagiri, Andhra Pradesh, India

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Corresponding author: **Mohan B. Sannathimmappa**. Email: drmohan123@gmail.com**ABSTRACT**

Tuberculosis (TB) is one of the oldest communicable bacterial diseases, spreads predominantly by inhalation of infected respiratory droplets. It is a curable and preventable disease yet remains the leading infectious cause of human death worldwide. The TB burden is high among developing nations of Asia and Africa. Major obstacles in controlling TB are patient's non-compliance to the anti-tubercular therapy, co-infection with Human immunodeficiency virus (HIV), low socioeconomic status, crowded living condition, inadequate rapid diagnostic testing facilities especially in resource-poor developing countries, delay in diagnosis and initiation of therapy, and the emergence of drug-resistant strains of *Mycobacterium tuberculosis* (MTB). Multidrug-resistant (MDR-TB), extensively drug-resistant (XDR-TB), and total drug-resistant (TDR-TB) MTB strains are difficult to treat and are associated with frequent treatment failures and high mortality. The recent advent of molecular techniques including nucleic acid amplification tests (NAATs) and whole-genome sequencing (WGS) have significantly ameliorated the rapid detection of TB cases and drug-resistant MTB. This, in turn, enabled the early initiation of therapy and development of novel treatment plans which is crucial for the global TB elimination target. The World Health Organization (WHO) 2020 guideline prioritizes the use of newer drugs as part of all-oral regimens for the treatment of MDR-TB. Apart from the use of newer drug delivery methods, host factors including immune functions and cytokine responses as well as mycobacterial enzymatic pathways are targeted in TB drug development. Adjuvant therapy employing host-directed approaches is increasingly studied through the time-tested pathogen-targeted approach remains the mainstay in the current treatment of MDR-TB.

Keywords: Drug-resistant tuberculosis; molecular methods; mutation; whole-genome sequencing; Xpert-MTB/RIF.

INTRODUCTION

Tuberculosis (TB) is one of the oldest communicable infectious diseases, spreads predominantly from person-person through inhalation of infected respiratory droplets. It is caused by an acid-fast, aerobic, and facultative intracellular bacteria called *Mycobacterium tuberculosis* (1). Tuberculosis predominantly affects lungs (pulmonary TB) but can affect any other parts of the body (extra-pulmonary TB). Human serves as a natural reservoir for *M. tuberculosis* (MTB) and its ability to establish latent infection has enabled the spread of infection to large number of people around the globe (1). TB is a curable and preventable disease, yet it remains the leading infectious cause of death especially in adults globally. About one-fourth of the world's population is infected with *M. tuberculosis*. Amongst, risk of developing TB disease, especially in individuals with immunosuppressed conditions is high (1). The emergence of drug-resistance is a major obstacle in the treatment and eradication of tuberculosis and the number of cases of drug-resistant TB is rising globally (1). The number of MDR-TB cases was estimated to be increased from 480 thousand in 2014

to 600 thousand in 2016 (1). Non-compliance/inadequate therapy with anti-tubercular drugs, co-infection with HIV, low socioeconomic status, crowded living conditions, and the presence of immunocompromised status are key factors associated with an increase in number of drug-resistant MTB cases (1).

Drug-resistance is a natural phenomenon and in MTB, it is predominantly acquired through spontaneous mutation, principally due to single nucleotide polymorphism in the chromosome. Furthermore, different mutations in target genes are linked to different levels of phenotypic resistance to anti-tubercular drugs (2). The emergence of multidrug-resistant (MDR-TB) and more recently, extensively drug-resistant (XDR-TB), and total drug-resistant strains (TDR-TB) of MTB have posed a serious challenge to global TB control strategies (2). Treatment of multidrug-resistant strains is complex and requires the use of more expensive and toxic drugs for longer duration (3). Also, it is associated with more frequent treatment failures compared to non-MDR strains (3). Understanding of the molecular basis of drug resistance in TB bacilli has improved in recent years. The knowledge of

molecular mechanisms of TB drug resistance helps immensely in designing a new drug, developing a highly sensitive rapid diagnostic test, targeting specific drug-resistant strain, and developing novel therapeutic strategies to combat multidrug-resistant strains. This review article focuses on epidemiology, recent advances in molecular diagnosis, and the management of drug-resistant *M. tuberculosis*.

Global scenario of TB

Tuberculosis (TB) has been the focus of medical publications from the earliest record in 1925. Though the rate of publications was slow for the first 20 years, the pace picked up from 1990 when the interest grew by leaps and bounds (4). Tuberculosis was and is a public health threat all over the world with markedly varying patterns from continent to continent. It is not only a biological threat but also a sociological one. The problem has assumed bigger proportions with the advent and addition of HIV and MDR-TB to the risk profile. The incidence of TB varies widely from 10 per 100,000 people to more than 500 per 100,000 people in Lesotho, Mozambique, Philippines, South Africa etcetera (5). The spectrum spans from most developed to least developed countries in terms of the burden of tuberculosis.

Eighteen countries had more than 100,000 cases of TB in 2016. The estimated number of new TB cases in the world is close to 10 million per year. India accounts for 27% of this estimated number, followed by Indonesia (10%), China (9%), and the Philippines (5%) (5). Most of these cases are in men (65%) and mainly among adults (90%). It can be safely assumed that only about 61% of these projected numbers are being reported to public health programs uncovering the task ahead for control activities (4). If one takes infections into consideration, a shocking 1.7 billion (23%) of the world's population will be showing signs of *M. tuberculosis* in their persons (4). If the severity of burden (TB incidence per million) is considered as a criterion for high burden, then a tally of 30 countries would be tagged, whereas by absolute numbers of incidence cases it would be about 20 as per WHO (4).

Another way to look at the burden would be by the mortality rate. One and a half million deaths are happening due to TB every year. This too varies widely like the morbidity statistics. High-income countries would show about a single death per 100,000 whereas the low-income countries would be showing as much as 40 deaths due to TB per 100,000 per year (4). There is always a variation in reporting as per the multiple causes of death especially in poor countries as well as under-reported cases (6). HIV has added a new dimension to TB which by itself causes more deaths than the former as a specific single entity. About 23.5% of deaths in patients with TB are precipitated by HIV co-infection while only

10% of the incident cases of TB are HIV positive. The majority (74%) of incident cases of HIV-TB were in the African region and 90% of these were men. Many of these countries are plagued by poverty, undernutrition, and smoking (5). Fatality in tuberculosis is also associated with diabetes mellitus and alcoholism too (6). Globally the gap between the incidence and number of newly diagnosed cases remains large (64%). As per 2018 report of WHO, out of 6.6 million notified cases of TB, only 24% of cases were tested for new TB, 30% for rifampicin resistance, and 70% for previously treated TB illness (1).

Drug resistance among TB cases became an added bane to the control of TB worldwide. Over 600,000 cases of drug-resistant TB were reported in 2016, majority of which (490,000) were multi-drug resistant. While 4.1% of newly detected cases were multi-drug resistant, 19% of previously treated cases had similar features. A significant proportion (47%) of the cases was reported from India, China, and Russia among other countries. Extremely resistant TB cases were also reported from 123 countries with features of resistance to fluoroquinolones in addition to at least one second-line injectable drug. About a quarter of a million deaths in 2016 were due to this group of cases alone (4). All said and done, the trends of tuberculosis, however, was that of a faint optimism. With increasing attention paid on TB and the emphasis on bacteriological fight against the disease, indices were showing a small yet significant decline. The death due to TB was on the decline even among those with HIV co-infection (5). Data tracking of TB also improved, and many countries linked the HIV and TB databases to reduce under-reporting of cases (6). The European region of WHO accounted for fastest reduction in the incidence of TB. TB is invariably linked with economic growth and development of individual countries and financial resources are determining factors for effective control of this disease (7). This has especially become relevant with the advent of COVID-19 to the detriment of TB control activities in resource-scarce countries (8). These two pandemics (if TB can also be called one) share similar risk groups such as the malnourished, smokers, diabetics, alcoholics, and people living with HIV (PLHIV).

Molecular diagnosis of drug-resistant *M. tuberculosis*

The conventional sputum smear microscopy and culture methods for TB diagnosis and detection of drug resistance in MTB require several weeks (3). This would lead to too much delay in the initiation of appropriate therapy. Hence, rapid and accurate detection of drug-resistant MTB is critical for the effective early treatment of TB patients and to achieve the goals of WHO "End TB Strategy" which

targets for 90% reduction in TB related deaths and 80% reduction in TB incidence by 2030 (1). Currently, several molecular diagnostic tests, based on nucleic acid amplification tests (NAATs) and sequencing, endorsed by WHO are available, and few more are under the stages of development and implementation. These molecular diagnostic tests have significantly improved the rapid detection of TB cases and drug-resistant strains of MTB (2, 9). However, each technique has its own advantages and limitations as summarized below.

DNA line probe assays

Line probe assays (LPAs) are primarily DNA–DNA hybridization assays by which different mutations can be detected simultaneously by using multiple probes (10). The complete assay turnaround time is approximately 5-7 hours. Several LPAs are available; however, most of them target the hot spot regions of drug resistance. These assays are found to have good sensitivity (94%) in smear-positive sputum, but sensitivity is low (44%) in smear-negative sputum samples (1, 2). At present, WHO recommends the use of DNA probe assays such as GenoType MTBDR*plus*, GenoType MTBDR*sl*, and Nipro NTM+MDR-TB for initial screening of drug resistance in sputum smear-positive samples (10). GenoType MTBDR*plus* is developed to detect isoniazid (INH) and rifampicin (RIF) resistance by detecting mutation in *rpoB*, *katG*, and the *inhA* promoter. GenoType MTBDR*sl* analyzes *gyrA*, *gyrB*, *rrs*, *embB*, and *eis* genes and thereby used to detect *Mycobacterium tuberculosis* complex and resistance to fluoroquinolones, ethambutol, and aminoglycosides. It is basically used for the initial diagnosis of Rifampicin-resistant TB (RR-TB) or multidrug-resistant TB (MDR-TB). Nipro NTM+MDR-TB can differentiate 4 important *Mycobacterium* species (*M. tuberculosis*, *M. avium*, *M. intracellulare*, and *M. kansasii*) that cause human disease and also helpful in detecting MDR-TB as it analyses mutation in *rpoB*, *katG*, and *inhA* genes (2, 10). To conclude, LPAs are rapid, simple, and easy to perform. However, these tests have certain limitations. The major drawback is LPAs require expensive laboratory equipment and complex laboratory infrastructure and hence are available generally in reference laboratories (2, 10).

Xpert MTB/RIF and Xpert MTB/RIF Ultra

Recent advent of rapid molecular tests such as Xpert-MTB/RIF has significantly improved the detection of MTB and RR-TB in sputum specimens (11). Basically, Xpert MTB/RIF assay is a semi-quantitative nested real-time PCR technique, requires minimum expertise, minimizes cross-contamination between samples, limits the misdiagnosis between MTB and non-tuberculous mycobacteria (NTM), and the results can be obtained rapidly within 2 hours. Additionally, sensitivity and specificity for smear-

positive samples is almost 99-100% compared to standard culture-based drug susceptibility tests (11). This would have a significant impact on treatment as it allows rapid initiation of therapy in MDR-TB cases. However, some studies have reported low sensitivity of Xpert-MTB/RIF in detecting paucibacillary TB cases and therefore its use in the diagnosis of smear-negative and extra-pulmonary cases is limited. This is particularly important in the diagnosis of TB in young children and HIV positive patients (11). Furthermore, it does not detect genes associated with isoniazid (INH) resistance, may give rise to false positive and false negative results in rifampicin resistance due to silent mutation and rifampicin resistance in mutations outside the hotspot region respectively (11).

Subsequent development of Xpert-MTB/RIF ULTRA that detects two additional targets (IS6110 and IS1081) has some advantages over Xpert-MTB/RIF assay. The new RIF/ULTRA assay has significantly higher sensitivity (10 times higher) in MTB detection in clinical samples as compared to Xpert-MTB/RIF. Therefore, it facilitates screening of clinical specimens with a lower number of bacilli and it would improve MTB detection particularly in sputum samples of children, patients co-infected with HIV, smear-negative pulmonary TB, and also extrapulmonary TB cases (2). In addition, it also overcomes false-negative results seen in Xpert-MTB/RIF assay due to certain silent mutations in RIF genes. However, the Xpert-MTB/RIF ULTRA assay has low specificity compared to Xpert-MTB/RIF assay, but there was no difference between the two tests in the detection of RIF resistance (12). Currently, WHO recommends Xpert-MTB/RIF ULTRA assay for initial screening of smear-negative sputum samples and for screening extra-pulmonary TB specimens such as cerebrospinal fluid, lymph node biopsy, tissue samples, gastric fluid, and others (12).

Loop-mediated isothermal amplification (TB-LAMP)

It is another rapid test recommended by WHO as an alternative test to sputum microscopy for the diagnosis of TB in patients with signs and symptoms of pulmonary TB, particularly in resource-poor developing countries since it has many advantages (13). It is a less expensive, simple manual assay which requires less than an hour to perform and the results can be read through the naked eye under ultraviolet light. However, it has many limitations. Firstly, it does not detect rifampicin resistance. Secondly, it should not be used for patients who are at high risk for MDR-TB and for people living with HIV. Lastly, it is less sensitive compared to Xpert-TB to detect smear-negative and culture-positive cases. Also, not recommended for the diagnosis of extra pulmonary TB (13). Hence, Xpert TB/ RIF

should be still preferred in countries with adequate infrastructure and facilities (13).

Gene drive MTB/RIF ID kit, Anyplex kits, and Digital PCR

The Genedrive MTB/RIF ID Kit is an innovative technique developed for the rapid detection of MTB and RR-TB from raw sputum samples (2). The greatest advantage is it is a portable low-power thermal cycling apparatus and used at TB point-of-care sites. It can detect as low as 5 genome copies and the overall sensitivity of Gene drive MTB/RIF ID test is better compared to Xpert-MTB/RIF assays (100% vs. 93%). Additionally, it is user friendly, stable, and has the capacity to work without air conditioning, affordable, and provides rapid results. Hence it can be implemented in many peripheral laboratories especially in low-income/developing nations (2).

The Anyplex kits were based on a semi-automated multiplex real-time PCR principle and are designed to detect drug-resistant MTB in clinical samples such as sputum, bronchial wash, fresh tissues, and culture isolates (2). Two widely used kits are Anyplex II MTB/MDR and MTB/XDR kits. Anyplex MTB/MDR detects most frequent mutations in *rpoB*, *katG*, and *inhA* associated with MDR-TB, while MTB/XDR kit detects around 13 main mutations in the *gyrA* and *rrs* genes and *eis* promoter associated with XDR-TB (14). The total turnaround time of these tests is about 3-4 hours. Evaluation studies found that these tests have specificity ranging 94-100% and sensitivity ranging 50-100%. The lowest sensitivity was observed for the detection of fluoroquinolone-resistant strains (15). Overall, these real-time PCR tests show low false-positive results compared to Xpert technology. Additionally, they are potential tools for the rapid detection of MDR and XDR strains in clinical samples especially in extra-pulmonary tuberculosis. However, the drawback is their limited number of targets (14, 15).

Another PCR based test is Digital PCR assay. It is basically a quantitative test that can detect and quantify different resistant subpopulations (hetero-resistance) of MTB that may result either from superinfection by two or more strains or evolve from a single isolate during treatment due to antibiotic pressure (2, 16). These assays can detect bacilli at concentrations as low as 1000 CFU/ml, and as little as one XDR-MTB among thousand susceptible *M. tuberculosis*. Hence, the digital PCR assay has the advantage of early detection of new mutations emerging during treatment which could require modification in the therapy (2, 16). Furthermore, newly developed digital PCR assays based on micro and nano-fluidic technologies are found to be simpler and more practical (17).

Whole-genome sequencing (WGS)

The development of WGS has been a major step forward in molecular diagnostics because whole-genome data can be analyzed rapidly in a single run. Unlike other molecular tests that are designed specifically to target a limited number of target regions, WGS provides whole genome sequence of *M. tuberculosis* and single nucleotide polymorphisms (SNPs) responsible for specific resistance (18). Therefore, this has certain additional advantages; allows species identification, screening of a sample for all types of mutations, detection of drug resistance, and predicting the organism evolution (2). These advances in molecular diagnostic techniques help immensely in early detection of TB patients and drug resistance and thereby initiating appropriate treatment regimen at the earliest for better outcomes. Despite of many invaluable advantages, implementation of this technology by WHO was hampered because of the high cost of equipment, need for a strong and advanced software tool, difficulty in the standardization of sample preparation and DNA extraction procedures, and the need of skilled personals for experiments, data acquisition, and interpretation of generated data (1, 19). However, these gene sequencing techniques are progressively becoming cheaper and simpler. Currently, they are utilized in research and drug resistance surveillance (1).

DNA microarrays

DNA microarray technology has the highest capacity to detect multiple targets in thousands of sequences in one reaction (20). This technology has been primarily employed in many studies for detecting drug-resistance associated mutations in *M. tuberculosis*. Even these assays have their own advantages and limitations (2). The turnaround time is approximately 20 hours, which is quite long compared to other molecular diagnostic methods. However, they show high sensitivity and specificity for the detection of mutations associated with drug resistance in MDR-TB and XDR-TB (2). Currently, most microarray tests are in the developmental stage, and in the future, they could become important diagnostic tools for the rapid detection of drug-resistant strains of *M. tuberculosis* in clinical samples (2).

MALDI-TOF MS assay

Matrix-assisted Laser Desorption Ionization Time-of-flight Mass Spectrometry assay, a novel and unique technique that can detect rapidly and accurately drug-resistant MTB is gaining importance in recent years. It involves the detection of microbes through the measurement of ionized analytes in the clinical sample. Drug-resistant MTB is identified by detecting the probable proteins or oligonucleotides connected to drug resistance. It has several

advantages compared to NAATs which are gold standard techniques for species identification and detection of drug-resistant MTB. MALDI-TOF MS assay does not require costly equipment, involves the simple procedure, less expensive, requires less expertise, and can be made available in peripheral laboratories, unlike NAATs which are presently restricted to reference laboratories (21).

Management of drug-resistant tuberculosis:

Novel treatment strategies:

Drug-resistant TB refers to tuberculosis caused by an isolate of *M. tuberculosis* that is resistant to one or more anti-tuberculous drugs. Drug resistance in mycobacterium tuberculosis is now widespread ranging from a global average of 4-6% to as high as 25% in countries like Kazakhstan and Ukraine (22). About 4% of new tuberculosis patients and about 20% of patients receiving retreatment have multidrug resistance (23).

Various degrees of drug resistance that have been defined are as follows (24):

- Monoresistance refers to resistance to a single first-line anti-TB drug only.
- Poly-resistance refers to resistance to more than one first-line anti-TB drug; the isolate may be resistant to either isoniazid (INH) or rifampin but not both.
- Multidrug resistance (MDR) refers to the resistance to at least both isoniazid and rifampicin and possibly additional agents.
- Pre-extensively drug-resistance (pre-XDR) refers to resistance to INH and rifampin and either quinolones or all injectable agents (streptomycin, amikacin, kanamycin, or capreomycin).
- Extensively drug-resistance (XDR) refers to resistance to at least INH, rifampin, and fluoroquinolones as well as either aminoglycosides (amikacin, kanamycin) or capreomycin or both.

- Totally drug-resistance TB (TDR) used inconsistently refers to resistance to all locally tested medications.

The 2020 WHO consolidated guidelines on drug-resistant TB treatment is based on the most recent and comprehensive evidence available (24). The guidelines, which replace all the previous recommendations, were based on a modified set of data that expanded on the initial individual patient dataset (over 13,000 patient records from more than 50 different studies done in 40 countries) used for 2019 American Thoracic Society (ATS) guidelines (25). However, the key difference between the two is that WHO guidelines allow for use of globally empirical regimens whereas the ATS recommends the use of microbiological data to develop a tailor-made regimen for the given strain of *M. tuberculosis*. Other differences and similarities between ATS and WHO guidelines are elaborated by the Expert Committee in the 2019 ATS guidelines.

Regimens for isoniazid-resistant tuberculosis

The WHO recommended treatment in cases with confirmed rifampicin-susceptible and isoniazid-resistant TB includes use of rifampicin, ethambutol, pyrazinamide and levofloxacin for 6 months. It is not recommended in the guideline to add streptomycin or any other injectable agents to the treatment regimen.

Multidrug-resistant tuberculosis (MDR-TB)

Short regimen

A Phase III RCT conducted in Bangladesh recently showed that a short regimen was non-inferior to a long duration regimen with respect to the primary efficacy outcome and was similar to the long duration regimen in terms of safety (26). The total duration of therapy was less than 12 months, compared with an individualized regimen of 18 to 24 months. The cost of treatment in such regimen is less than that of conventional regimens (27).

Table 1: Grouping of medicines recommended for use in longer MDR-TB regimens

Group	Recommendation	Drugs included
A	Include all three medicines	<ul style="list-style-type: none"> • Levofloxacin/Moxifloxacin • Bedaquiline • Linezolid
B	Add one or both medicines	<ul style="list-style-type: none"> • Clofazimine • Cycloserine/Terizidone
C*	Add to complete the regimen and when medicines from Groups A and B cannot be used	<ul style="list-style-type: none"> • Ethambutol • Delamanid • Pyrazinamide • Imipenem-cilastatin/Meropenem • Amikacin • Streptomycin • Ethionamide/Prothionamide • p-aminosalicylic acid

*The drugs are ranked by the relative balance of benefit to harm usually expected of each.

According to WHO, in patients with MDR-TB who have not been previously treated for more than 1 month with 2nd line drugs or in patients without known resistance to FQs and second-line injectable agents has been excluded, a shorter regimen (9 to 12 months) may be used. In case of intolerance to medicines in the shorter MDR-TB regimen or risk of toxicity, cases of disseminated TB, CNS TB, or extrapulmonary disease in HIV positive individuals and in pregnant women, a longer MDR-TB regimen is to be used. A shorter all-oral bedaquiline-containing regimen of 9 to 12 months' duration is recommended in this category. The regimen can be summarized as Initial phase of 4–6 months: Bdq (6 months)-Lfx-Cfz-Z-E-Hh-Eto followed by the Continuation phase of 5 months: Lfx-Cfz-Z-E. WHO recommends against changes in the duration or composition of this regime in programmatic usage.

Long regimen

Medicines used in longer MDR-TB regimens are grouped based on the strength of available evidence (Table 1). Medicines listed under Group A are considered highly effective and strongly recommended for inclusion in all regimens unless contraindicated. The medicines in Group C are the least effective drugs and should not be considered an automatic replacement of a group A or B drug. Longer regimens should include at least 4 drugs (all three Group A drugs and at least one Group B drug OR two drugs each from Group A and B) and at least 3 drugs are included for the rest of the treatment once bedaquiline is stopped. If the regimen cannot be composed with drugs from Groups A and B alone, Group C agents are added to complete it. Bedaquiline and delamanid may be included in patients aged more than 6 and 3 years, respectively.

The regimen can be summarized as 18 months: Bdq (at least 6 months) -(Lfx or Mfx)-Lzd-(Cfz or Cs) wherein the first 6 months of treatment comprises

four second-line agents and the remaining 12 months comprise the same agents except for bedaquiline.

Host directed therapy

Novel concepts in TB pathology have emerged due to a better understanding of various immune mediators involved in the disease pathogenesis (28). Host-directed therapy (HDT) is emerging as a promising area of research in the treatment of MDR-TB with a potential to reduce tissue damage through enhanced bacterial clearance (29).

Based on recently published reviews on the treatment of drug-resistant TB, a condensed list of selected drugs with their proposed mechanism of actions is presented in Table 2 (30-33). There is increasing availability of evidence to show that certain forms of HDT when used with the current treatment regimens could accelerate the response to treatment and improve the disease outcomes (32). A recent meta-analysis concluded that Vitamin D and other anti-inflammatory medications used as adjuncts may produce a favorable anti-inflammatory effect thereby leading to improved sputum smear conversion rate and radiological findings (34). Statins, which inhibit cholesterol biosynthesis and protein prenylation branches of the mevalonate pathway, are known to have broad anti-inflammatory and immunomodulatory activities. Recently, pravastatin belonging to the statin family is shown to increase anti-tubercular antibiotic efficacy in a mouse model (35). Tumour necrosis factor- α (TNF- α) is essential to the expression of acquired specific resistance following TB infection. However, trials evaluating anti-TNF- α which inhibits necrosis of infected cells in the granuloma have shown mixed results so far (36). Similarly, in a randomized trial of adjunctive interleukin (IL-2) in adults with pulmonary tuberculosis, the HDT did not enhance the bacillary clearance (37).

Table 2: List of selected adjuvants used as part of host directed therapy against drug-resistant tuberculosis

Agent	Justification for use	Status of evaluation
Cyclophosphamide	Enhanced anti-tumour immunity through selective depletion of Treg cells.	Undergoing phase II trials
Evofosfamide	Prodrug of the DNA alkylating agent bromo-isophosphoramide. Selective eradication of hypoxic cells.	Undergoing phase III trials
Banoxantrone	Selective activation to form AQ4. Topoisomerase II inhibition.	Undergoing phase I trials
Myo-inositol trispyrophosphate (ITPP)	Allosteric effect on haemoglobin. Enhanced tissue oxygenation and inhibition of hypoxia-induced angiogenesis.	Undergoing phase I trials
N-acetylcysteine	COX1 inhibition and mitigation of TNF- α -induced inflammatory response.	Randomized, phase II clinical trial
Ibuprofen	COX1/2 inhibition and suppression of prostaglandin H2 and thromboxane production.	Undergoing phase I trials
Vitamin D	Antimicrobial peptide (AMP)-expression. Augmentation of IL-32 and IL-15-mediated immune response*	Double-blind, randomized phase III clinical trials in various countries

Metformin	AMPK activation. Increased mitochondrial turnover and augmentation of CD8 T-cells.	Retrospective cohort or case control studies.
Zileuton	Increases in PGE2. Modulates IL-1 β -mediated immune pathways. Induction of apoptosis.	Undergoing phase I trials
Valproic acid/ Vorinostat	Histone deacetylase inhibition. Enhanced CD8 T-cell activity and autophagy.	Undergoing phase I trials
Imatinib mesylate	Tyrosine kinase inhibition. Reduction of colony forming units of the bacilli.	Undergoing phase I trials
Rapamycin	Inhibition of target of rapamycin (mTOR) and activation of autophagy	Undergoing phase I trials
Etanercept	TNF- α neutralization and disruption of granuloma formation.	Undergoing phase I trials
Bevacizumab	Anti-VEGF antibody. Vascular normalization and reduced hypoxic fractions**	Undergoing phase I trials

*IL- Interleukin; **VEGF- vascular endothelial growth factor

Novel drug delivery strategies

Since the conventional drug delivery methods tend to contribute to increased instances of drug toxicity, emergence of drug resistance, and extended duration of treatment, novel drug delivery systems are being evaluated. Such drug delivery technology includes but not limited to multiple variants of nanotechnology, microparticles, liposomes, niosomes, liquid crystals, polymeric micelles, and dendrimer (38).

Drugs recently approved by regulatory bodies

The US FDA approved pretomanid, a nitroimidazooxazine, in 2019 as a treatment option for XDR-TB or nonresponsive MDR-TB and treatment-intolerant TB (39). This bactericidal drug is known to inhibit mycolic acid synthesis leading to blocking of cell wall production in both actively replicating and non-replicating mycobacteria. In a set of patients administered a combination of pretomanid with bedaquiline and linezolid, the treatment was successful in nearly 90% of patients, far exceeding the previous success rates for XDR-TB treatment (40).

Bedaquiline, a diarylquinoline antimycobacterial, acts on both actively replicating and dormant mycobacteria by inhibiting mycobacterial ATP synthase. Bedaquiline which got FDA approval in 2012 as part of a 22-week multidrug regimen for pulmonary MDR-TB, was approved for patients aged 12 years or older in 2019 and for children as young as 5 years or older in 2020 (41). This recent approval was based on a trial in which bedaquiline was used for 6 months along with pre-existing combination therapy in children with MDR-TB (42). Currently, bedaquiline used on a case-by-case basis in HIV-positive cases, pregnant women, pediatric, and extra-pulmonary MDR-TB cases.

Delamanid, a bactericidal drug that inhibits mycolic acid biosynthesis and interferes with cell wall metabolism of mycobacteria was approved by European Medicine Agency (EMA) and Pharmaceuticals and Medical Devices Agency of Japan (PMDA) in 2014 (43). At the same time, in an

effort to tackle the growing public-health challenge of drug resistance, EMA provided a marketing authorization for Para-aminosalicylic acid, which was earlier used as the first-line treatment of TB before the introduction of more potent drugs like rifampicin. Further studies evaluating the long-term effectiveness of these drugs are recommended by the regulatory bodies.

As part of the pathogen-centric strategy, a variety of candidates including some of the agents approved for other clinical conditions are being evaluated in several Phase I to III clinical trials. Repurposed drugs such as antibiotics linezolid and doxycycline, pranlukast (FDA-approved for asthma treatment), clofazimine, sildenafil, and novel analogues of existing anti-TB drugs are some of the potential candidates. Novel anti-mycobacterial agents are being developed with the identification of enzymatic targets such as enoyl-acyl carrier protein reductase, transmembrane transport protein large, decaprenylphospho-beta-d-ribofuranose 2-oxidase, and the ubiquinol-cytochrome C reductase (44).

Update on TB prevention and control strategy

TB preventive treatment, prevention of transmission through infection prevention and control and BCG vaccination of children continue to remain the three major health care interventions for TB prevention. Considering that the efficacy of current preventive treatment ranges from 60% to 90%, prevention of active disease by *TB preventive treatment* is a critical component of the TB prevention and control strategy. The 2020 WHO consolidated guidelines on tuberculosis provides the details of this component as a separate module.

Following the implementation of *Stop TB Strategy* that underpins the Global Plan to Stop TB 2006–2015, the WHO reiterated its new policy as *End TB strategy* in May 2014 (23). The process involved consultations with representatives from countries with a high disease burden. The key principles of this strategy include government stewardship and accountability, strong coalition with civil society organizations/communities, an adaptation of the

strategy/targets at the country level with global collaboration. The following are the highlights of the structure of the expert recommendation, further details of which are beyond the scope of this review.

- *Integrated, patient-centered care and prevention:* Use of new molecular diagnostic testing platforms for early and accurate diagnosis and drug resistance, Mapping of high-risk groups for systematic screening. Continue BCG vaccination in high-prevalence countries.
- *Bold policies and supportive systems:* Multidisciplinary and multi-sectoral approach. Development of ambitious strategic plans. Scaling up the public-private mix approaches targeted to promote international standards for tuberculosis care. Health-in-all-policies approach.
- *Intensified research and innovation:* Point-of-care rapid diagnostic tests, new drugs and regimens. Development of new vaccines apart from the 12 vaccine candidates in clinical trials currently.
- The target set for 2035 are 90% reduction in deaths due to TB (in comparison to 2015) and a 90% reduction in TB incidence rate which amounts to less than 10 tuberculosis cases per 100,000 populations. The WHO document also provides detailed key global indicators and milestones for the post-2015 tuberculosis strategy.

CONCLUSION

The progressive global increase in drug-resistant tuberculosis highlights the importance of rapid TB diagnosis, early accurate detection of drug-resistant MTB, and early initiation of appropriate therapy for reduction of TB burden and its complications. Also, avoiding unnecessary isolation and treatment of false positive TB cases. Several molecular techniques that are developed or in verge of development such as LPAs, PCR based assays, DNA microarrays, and sequencing techniques are promising tools for achieving the goals of World Health Organization's End TB strategy. However, each molecular test has its advantages and limitations and most of the tests endorsed by WHO do not meet essential performance standards. Accuracy, affordability, ability to detect multiple mutations simultaneously in single reaction, minimum turnaround time (rapidity), and easy to perform are among the key factors that needs to be considered for the development and application of an ideal diagnostic test. A rapid molecular test involving simple testing procedures that can provide accurate results at an affordable cost facilitates its accessibility at different levels of microbiology laboratories especially in resource poor countries.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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