Original Research Paper

Volume - 15 | Issue - 02 | February - 2025 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

MULTI - DRUG RESISTANCE TUBERCULOSIS (MDR – TB) TREATMENT AND NEWER TECHNIQUES FOR DIAGNOSIS: A REVIEW

Asma Ansari — Department of Microbiology, Bhopal Memorial Hospital and Research Centre, Bhopal

Mohammad Nadeem Khan — Department of Microbiology, Bhopal Memorial Hospital and Research Centre, Bhopal

Shazia Mansoor* — Paramedical Institute, Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal
*Corresponding Author

ABSTRACT Tuberculosis is one of the leading cause of mortality in India as well as worldwide. The causative organism is Mycobacterium tuberculosis which is a global concern due to drug resistance. Recently there is an increase of 20% drug resistance strain of tuberculosis and some are resistant to more than one drug called multi – drug resistance tuberculosis (MDR – TB). To overcome this problem there is a need to early diagnosis of disease and proper treatment. This review discusses about diagnostic tools of tuberculosis and MDR – TB as well as treatment regime. The use of molecular techniques are helpful for early diagnosis and detection of mutations and drug resistance which provide guidance of treatment decisions.

KEYWORDS : Tuberculosis, MDR – TB, molecular techniques and mutations

INTRODUCTION

One of the leading cause of death worldwide and especially India is Tuberculosis caused by Mycobacterium tuberculosis and the concern of the World Health Organization (WHO) in this disease is drug resistant. The antimicrobial resistance of tuberculosis is one of the major public health threat as well as foster research. It had been reported that multidrug resistance tuberculosis (MDR – TB) had increased from 2009 and 20% for rifampicin and isoniazid annually. The rapid diagnosis of MDR – TB and molecular or phenotypic drug susceptibility testing (DST) had also been improved. Almost 8014 patients in 72 countries in 2016 were identified drug resistant XDR – TB. The drug resistance was also reported in second line injectable agent, amikacin, capreomycin or kanamycin. The death rate was found around 16 % to 28 % in MDR – TB and XDR – TB, respectively [1]. There are some novel treatment and diagnosis that offer a promising hope that M/XDR – TB is curable.

Drug Resistance Tuberculosis

The TB bacteria resistance may be on first line drug or multi drug. Types of drug resistant experienced in TB patients include Mono resistant TB caused by isolate that shows resistance to single first line drug (isoniazid, rifampicin, ethambutol or pyrazinamide). Poly resistant TB caused by resistance from more than one drug, but not resistant to both rifampicin and isoniazid simultaneously. MDR – TB isolate shows resistance to at least isoniazid and rifampicin. Pre – extensively drug resistant TB shows resistance to isoniazid, rifampicin and either fluoroquinolones or injectable agents (amikacin, kanamycin or capreomycin) but not both. Extensively drug resistance TB show resistance isoniazid and rifampicin as well as to any fluoroquinolone and at least one out of the three injectable agents (amikacin, kanamycin or capreomycin) and is rare form. The extensive MDR – TB resistant patients are comparatively less approximately 9% [2].

Drug Resistance Mechanism

The tuberculosis patients develop drug resistance by two ways primary and secondary. The primary resistance develops in patients exposed to drug resistance strain and secondary, also called acquired due to medication poor adherence, inadequate regimen in patients taking TB medication and malabsorption of drug. Most of the cases of MDR – TB reported from transmission rather than acquired due to exposure in high burden environment [3]. The mechanism of drug resistance in Mycobacterium tuberculosis bacteria (MTB) takes place due to mutations in bacterial chromosomes that may be random or spontaneous which leads specificagents susceptibility reduction [4]. This leads production of enzymes by bacteria which inactivates or disrupts drug activation or alters drug target or activates efflux pump at bacterial surface [5].

Treatment of Multidrug-resistant Tuberculosis

The MDR – TB drugs were classified in three groups by WHO in 2018 [6]. Group A includes highly effective drugs of MDR – TB which are fluoroquinolones, bedaquiline, and linezolid. Group B are second choice drugs which includes Clofazimine and either cycloserine or terizidone. Group C are given when A and B cannot be given due to toxicity and include all other drugs except isoniazid high dose, amoxicillin-clavulanate, kanamycin and capreomycin.

The mechanism of action Fluoroquinolones is DNA transcription inhibition of MTB and replication of bacteria by interfering in DNA gyrase, a tetramer composed of two α and two β subunits encoded by gyrA and gyrB genes [7]. The mutations in gyrA gene leads to MTB resistance and this drug mechanism is distant from rifampicin and isoniazid [8]. Fluoroquinolones have side effects and the adverse effects include gastrointestinal trouble, central nervous system related problems and QT interval prolongation [9]. The most common drugs recommended by WHO for MDR are Levofloxacin (750 mg daily dosage) and moxifloxacin (400 mg daily dosage) [10].

Bedaquiline has concentration dependant bacteriocidal effect concentration dependant causing cell death in both replicating and non – replicating MTB is a diarylquinoline compound that blocks proton pump flow of mycobacteria by inhibiting adenosine triphosphate synthetase [11]. The dosage approved by U.S. Food and drug administration is 400 mg daily for 14 days, followed by 200 my three times weekly for 22 weeks and it is also recommended that drug absorption increases with food [12]. The adverse effects of drug reported include nausea or vomiting, arthralgia or myalgia and QT prolongation. Nearly 2.8% patients were reported to have severe adverse events (SAE) [13].

Linezolid is oxazolidinone antibiotic prevent fusion of 30 S and 50 S ribosomal subunits and inhibits protein synthesis of MTB [14]. The dosage of drug varies 300 to 1200 mg daily, once or twice a day and as per WHO 600 mg is recommended daily dosage which is considered safer with lesser side effects for 6 months, whereas, some studies reported dosage of 300 mg daily dosage [15]. The adverse effect of drug includes peripheral neuropathy, optic neuropathy leading to blindness, anemia and thrombocytopenia [13].

A new MTB drug from nitro-dihydro-imidazooxazole class of compound included in group C, Delamanid inhibits mycoloc acid synthesis of bacterial cell wall can be used for MDR – TB aged 3 years or more. The drug is effective and safe similar like bedaquiline and its substitute [16].

Duration of Treatment

The standard treatment regimen for MDR – TB as per WHO is of two types shorter and longer and depends on drug combination and duration varies. The longer regimen is for 18 to 20 months and oral drugs preferred, in severe cases lasts for 6 to 7 months atleast four drugs until bequiline stopped [17]. The duration of shorter regimen is 9 to 11 months and this includes intensive phase lasting 4 to 6 months of seven drugs kanamycin, moxifloxacin, prothionamide, clofazimine, pyrazinamide, high-dose isoniazid, and ethambutol. It is followed by a

5-month course with moxifloxacin, clofazimine, pyrazinamide, and ethambutol [17]. Exclusion criteria includes resistance except isoniazid, medicine intolerance, toxicity [17], pregnancy, disseminated TB meningitis [4] or HIV patients [5].

Diagnosis of Tuberculosis

The common and definitive test for diagnosis of tuberculosis is done by detection of Mycobacterium tuberculosis bacilli in clinical specimen of patient with acute disease and symptoms. The clinical specimen taken for diagnosis is sputum for diagnosis of pulmonary disease, but due to mucoid and viscous nature sometimes difficult to diagnose by acid fast staining and culture takes time for growth [18]. Extra pulmonary tuberculosis require different procedures and sample will vary depend on site of infection. Several new techniques are adopted for rapid diagnosis of tuberculosis which include molecular tests which takes less time than culture.

Molecular tests can be used for number of biological samples include sputum, pleural fluid, CSF, urine, stool and blood [19]. The first line and second line of drug resistance can be determined by nucleic acid amplification (NAATs) technique. NAATs technique involve detection and amplification of nucleic acids and is one of the advance technique require laboratory facilities and skilled technical staff [20]. The technique also determines mutations related to first and second drug resistance which helps in treatment plan of patient. A number of commercial tests are available and recent one, a new version test, Xpert MTB/RIF Ultra introduced. The Xpert MTB/RIF is an automated PCR (polymerase chain reaction) based test to identify DNA of bacteria from clinical specimens and detect drug resistance [21]. The drug resistance mainly found due to rifampicin and assay determines drug resistance 15% - 30% of rifampicin rpoB gene mutations. It is a sensitive technique and gives better result than Xpert assay [22]. A new version of test introduced Xpert MTB/RIF Ultra which has higher sensitivity than Xpert MTB/RIF in patients with suspected TB meningitis and patients with HIV [23]. The assay can also determine mutations not only rpoB mutation for rifampicin, but improved one can determine additional resistance to isoniazid, fluoroquinones and second line injectables (SLID) with accuracy. But unlike other assay it all require skilled expertise [24]. The availability of new software used in instruments for example Fluorotype MTB one of them helps to recognize mutations whether known or unknown with resistance as well as susceptibility [25].

The GeneXpert technology is another method and some of the common techniques similar like Xpert MTB/RIF assay are Truenat MTB test (Molbio Diagnostics, India) used in India, which is sensitive and specific one [26].

The best method is whole genome sequencing (WGS) of entire genome of Mycobacterium gives a complete information about mutation and drug resistance. To perform WGS large amount of bacterial DNA required, therefore, culture of bacteria is required which is a drawback of this technique [27]. A recent technique known as deep sequencing detects microhetero resistance by conventional culture techniques but clinically not used commonly[28].

Diagnosis of Multidrug – Resistance Tuberculosis

The treatment of MDR – TB depends on diagnosis which should be rapid and precise drug sensitivity test (DST) for selecting effective drug [29]. DST involve phenotypic tests in which growth and metabolic inhibitors in media containing anti-TB drug and without drug and molecular test to detect genes responsible for drug resistance [5]. The conventional phenotypic DST include culture methods on agar and egg based media and drug sensitivity of bacteria determined by three different types proportion methods, one which provide measure of drug susceptibility of bacteria, second one is resistance ratio method and third is absolute concentration method to determine minimal inhibitory concentration [30]. The procedure of bacterial growth and sensitivity takes 2 to 3 months and liquid culture DST takes less time as compared to solid media, but contamination and cross infection is a drawback. To overcome these problems rapid culture technique has been developed and adopted which gives result within a month, examples are BACTEC 460 (detects Carbon dioxide production and used commonly), Mycobacteria Growth Indicator Tube (MGIT) (detects oxygen consumption and used commonly), Septi – Check, Myco – ESP Culture System II, BacT/Alert MB susceptibility $_{kit}$(31)

The molecular DST method is comparatively to conventional phenotype is rapid and accurate to diagnose MDR – TB through amplification of nucleic acids and detects mutations in specific genes for drug resistance [32]. They are of two types probe based assays and sequence based assays. The probe based include line probe assays (LPA) and GeneXpert, both are commercially available. The LPAs are used on MDR – TB patients by direct testing with culture positive or indirect testing with smear positive. The GeneType MTBDRplus version 1 consists of three steps DNA extraction, amplification by multiplex polymerase chain reaction (PCR) and reverse hybridization. The method detects rifampicin resistance by mutations in rpo gene with 98.7% accuracy for isoniazid resistance mutations detected in katG gene and inhA promoter region with 84.3% accuracy [33]. The WHO recommended GeneType MTBDRsl to detect ethambutanol resistance in embB gene, fluoroquinolones resistance in gyrA and gyrB genes and injectable agents mutation in rrs leading to resistance in kanamycin, amikacin and capreomycin [34].

The diagnosis of pulmonary and extrapulmonary TB and rifampicin resistance in adults and children as recommended by WHO by molecular assays, Xpert MTB/RIF and Xpert MTB/RIF (Ultra) [35]. The Xpert MTB/RIF is fully automated real time PCR based assay detects MTB and rifampicin resistance within 2 hours and its enhanced version is Xpert Ultra which is more sensitive especially in extrapulmonary cases and HIV infected patients, as well as rifampicin resistance. But still probe based assays cannot determine resistance for mutations which is outside target genetic region and second line resistance [35]. To overcome these problems, next generation sequencing (NGS) technique can be used which gives detailed sequence information of the targeted genome or whole genome sequencing. NGS detects first line and second line drug resistance phenotypes, also provide information to detect transmission in outbreak situation [37].

CONCLUSION

Multi – drug resistance tuberculosis MDR-TB remains a major concern in India and to control tuberculosis. A rapid diagnostic tool and proper effective treatment is required. The present review focusses on drug resistance, treatment and diagnostic tools. The advanced molecular tools are helpful and found effective to diagnose MDR – TB. There is still a need to further research to eradicate tuberculosis by adopting a shorter regimens and new agents assessment.

REFERENCES

1. Lange C., Chesov D., Heyckendorf J., Leung C. C., Udwadia Z. & Dheda K. (2018). Drug-resistant tuberculosis: An update on disease burden, diagnosis and treatment. Respirology, 23(7), 656 - 673.
2. Jang J. G. & Chung J. H. (2020). Diagnosis and treatment of multidrug-resistant tuberculosis. Yeungnam Univ. J. Med., 37(4), 277 - 285.
3. Kendall E. A., Fofana M. O. & Dowdy D. W. (2015). Burden of transmitted multidrug resistance in epidemics of tuberculosis: a transmission modelling analysis. Lancet Respir. Med., 3, 963 - 972.
4. David H. L. (1970). Probability distribution of drug-resistant mutants in unselected populations of Mycobacterium tuberculosis. Appl. Microbiol., 20, 810 - 814.
5. Kim S. J. (2005). Drug-susceptibility testing in tuberculosis: methods and reliability of results. Eur. Respir. J., 25, 564 - 569.
6. Zhang Y. & Yew W. W. (2015). Mechanisms of drug resistance in Mycobacterium tuberculosis: update 2015. Int. J. Tuberc. Lung Dis., 19, 1276 - 1289.
7. Moadebi S., Harder C. K., Fitzgerald M. J., Elwood K. R. & Marra F (2007). Fluoroquinolones for the treatment of pulmonary tuberculosis. Drugs, 67: 2077 – 2099.
8. Sirgel F. A., Warren R. M., Streicher E. M., Victor T. C., Helden P. D. & Böttger E. C. (2012). gyrA mutations and phenotypic susceptibility levels to ofloxacin and moxifloxacin in clinical isolates of Mycobacterium tuberculosis. J. Antimicrob. Chemother., 67, 1088 - 1093.
9. Nahid P., Mase S. R., Migliori G. B., Sotgiu G., Bothamley G. H., Brozek J. L., et al. (2019). Treatment of drug-resistant tuberculosis. An official ATS/CDC/ERS/IDSA clinical practice guideline. Am. J. Respir. Crit. Care Med., 200, e93 - 142.
10. Kang Y. A., Shim T. S., Koh W. J., Lee S. H., Lee C. H., Choi J. C., et al. (2016). Choice between levofloxacin and moxifloxacin and multidrug-resistant tuberculosis treatment outcomes. Ann. Am. Thorac. Soc., 13, 364 - 70.
11. Koul A., Dendouga N., Vergauwen K., Molenberghs B., Vranckx L., Willebrords R., et al. (2007). Diarylquinolines target subunit c of mycobacterial ATP synthase. Nat. Chem. Biol., 3, 323 - 324.
12. Centers for Disease Control and Prevention (2013). Provisional CDC guidelines for the use and safety monitoring of bedaquiline fumarate (Sirturo) for the treatment of multidrug-resistant tuberculosis. MMWR Recomm. Rep., 62(RR-09), 1–12.
13. Lan Z., Ahmad N., Baghaei P., Barkane L., Benedetti A., Brode S. K., et al. (2020). Drug-associated adverse events in the treatment of multidrug-resistant tuberculosis: an individual patient data meta-analysis. Lancet Respir. Med., 2020, 8, 383 - 94.
14. Livermore D.M. (2013). Linezolid in vitro: mechanism and antibacterial spectrum. J. Antimicrob. Chemother. 51(Suppl 2), 9 - 16.
15. Koh W. J., Kang Y. R., Jeon K., Kwon O. J., Lyu J., Kim W. S., et al. (2012). Daily 300 mg dose of linezolid for multidrug-resistant and extensively drug-resistant tuberculosis: updated analysis of 51 patients. J. Antimicrob. Chemother., 67, 1503 - 1507.
16. Ferlazzo G., Mohr E., Laxmeshwar C., Hewison C., Hughes J., Jonckheere S., et al. (2018). Early safety and efficacy of the combination of bedaquiline and delamanid for the treatment of patients with drug-resistant tuberculosis in Armenia, India, and South Africa: a retrospective cohort study. Lancet Infect. Dis., 18, 536 - 544.
17. World Health Organization (2019). WHO consolidated guidelines on drug-resistant tuberculosis treatment [Internet]. Geneva: World Health Organization.
18. Zumla A., Raviglione M., Hafner R. & Reyn C. F. (2013). Tuberculosis. N. Engl. J. Med. 368, 745 - 755.
19. Boehme C. C., Nabeta P., Hillemann D., Nicol M. P., Shenai S., Krapp F., Allen J., Tahirli R., Blakemore R., Rustomjee R. et al. (2010). Rapid molecular detection of tuberculosis

and rifampin resistance. New Engl. J. Med., 363, 1005 - 1015.
20. Boehme C. C., Nicol M. P., Nabeta P., Michael J. S., Gotuzzo E., Tahirli R., Gler M. T., Blakemore R., Worodria W. & Gray C. et al. (2011). Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet, 377, 1495 – 1505.
21. Alland D., Rowneki M. & Smith L., et al. (2015). Xpert MTB/RIF Ultra: a new near-patient TB test with sensitivity equal to culture. In: Conference on Retroviral and Opportunistic Infections, Seattle, WA.
22. Bahr N. C., Nuwagira E., Evans E. E., Cresswell F. V., Bystrom P. V., Byamukama A., Bridge S. C., Bangdiwala A. S., Meya D. B. & Denkinger C. M. et al. (2018).Diagnostic accuracy of Xpert MTB/RIF ultra for tuberculous meningitis in HIV- infected adults: a prospective cohort study. Lancet Infect. Dis., 18, 68 - 75.
23. Nathavitharana R. R., Cudahy P. G., Schumacher S. G., Steingart K. R., Pai M. & Denkinger C. M. (2017). Accuracy of line probe assays for the diagnosis of pulmonary and multidrug-resistant tuberculosis: a systematic review and meta-analysis. Eur. Respir. J., 49, 1601075.
24. Hofmann T. S., Ingen J., Feldmann K., Turaev L., Uzakova G. T., Murmusaeva G., Soolingen D. & Hoffmann H. (2009). Mechanisms of heteroresistance to isoniazid and rifampin of Mycobacterium tuberculosis in Tashkent, Uzbekistan. Eur. Respir. J., 33, 368 - 374.
25. Nikam C., Kazi M., Nair C., Jaggannath M., M. M. R. V., Shetty A. & Rodrigues C. (2014). Evaluation of the Indian TrueNAT micro RT-PCR device with GeneXpert for case detection of pulmonary tuberculosis. Int. J. Mycobacteriol., 3(3), 205 – 210.
26. Pankhurst L. J., Del O. E. C., Votintseva A. A., Walker T. M., Cole K., Davies J., Fermont J. M., Gascoyne-B. D. M., Kohl T. A. & Kong C. et al. (2016). Rapid, comprehensive, and affordable mycobacterial diagnosis with whole-genome sequencing: a prospective study. Lancet Respir. Med., 4, 49 – 58.
27. Schleusener V., Koser C. U., Beckert P., Niemann S. & Feuerriegel S. (2017). Mycobacterium tuberculosis resistance prediction and lineage classification from genome sequencing: comparison of automated analysis tools. Sci. Rep., 7.
28. World Health Organization (2019). WHO consolidated guidelines on drug-resistant tuberculosis treatment [Internet]. Geneva: World Health Organization.
29. Canetti G., Rist N. & Grosset J. (1963). Measurement of sensitivity of the tuberculous bacillus to antibacillary drugs by the method of proportions. Methodology, resistance criteria, results and interpretation. Rev Tuberc Pneumol (Paris), 27, 217 - 72.
30. Koh W.J., Ko Y., Kim C. K., Park K. S. & Lee N. Y. (2012). Rapid diagnosis of tuberculosis and multidrug resistance using a MGIT 960 system. Ann. Lab. Med., 32, 264 – 269.
31. Coll F., McNerney R., Preston M. D., Guerra A. J. A. & Warry A. & Hill-Cawthorne G, et al. (2015). Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences. Genome Med., 7(51).
32. Hillemann D., Rüsch G. S. & Richter E. (2007). Evaluation of the GenoType MTBDRplus assay for rifampin and isoniazid susceptibility testing of Mycobacterium tuberculosis strains and clinical specimens. J. Clin. Microbiol., 45, 2635 - 2640.
33. Ling D. I., Zwerling A. A. & Pai M. (2008). GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. Eur. Respir. J., 32, 1165 – 1174.
34. World Health Organization (2020). WHO consolidated guidelines on tuberculosis. Module 3: diagnosis–rapid diagnostics for tuberculosis detection [Internet]. Geneva: World Health Organization.
35. World Health Organization (2020). Molecular assays intended as initial tests for the diagnosis of pulmonary and extrapulmonary TB and rifampicin resistance in adults and children: rapid communication [Internet]. Geneva: World Health Organization.
36. Feliciano C. S., Namburete E. I., Rodrigues P. J., Peronni K. & Dippenaar A. & Warren R. M., et al. (2018). Accuracy of whole genome sequencing versus phenotypic (MGIT) and commercial molecular tests for detection of drug-resistant Mycobacterium tuberculosis isolated from patients in Brazil and Mozambique. Tuberculosis (Edinb), 110, 59 - 67.
37. Witney A. A., Cosgrove C. A., Arnold A., Hinds J., Stoker N. G. & Butcher P. D. (2016). Clinical use of whole genome sequencing for Mycobacterium tuberculosis. B. M. C. Med., 14(46).