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Editorial

Rapid Molecular Drug Susceptibility Test is Essential to Control the Menace of Drug Resistant Tuberculosis

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Tuberculosis (TB) occurs globally and remains a significant cause of morbidity and mortality. The rise of drug resistant tuberculosis (DR-TB) is the most important menace to global Tuberculosis control. Several forms of DR-TB exist. Isoniazid(H) resistant TB is defined as TB cases whose *Mycobacterium tuberculosis* (MTB) strains are resistant to H whereas TB cases who are resistant to additional first-line anti-TB drugs, other than H and Rifampicin (R) together are known as Poly drug resistant tuberculosis. Rifampicin resistant tuberculosis (RR-TB) is referred as R resistance diagnosed by means of genotypic or phenotypic tests with or without resistance to additional anti-TB drugs. RR-TB is used as an indicator for multi drug resistant tuberculosis (MDR-TB). MDR-TB is due to resistance of MTB strains to at least H and R. Extensively drug resistant tuberculosis (XDR-TB) refers to resistance to any fluoroquinolones (FQ) and second-line injectable drugs (SLID) along with MDR-TB. In 2021 WHO reorganized the description which states that TB caused by MTB strains that fulfil the definition of (MDR/RR-TB) as well as resistant to any FQ and TB caused by MTB strains that fulfil the definition of MDR/RR-TB as well as resistant to any FQ with at least one additional Group A drug (bedaquiline or linezolid) are pre-XDR and XDR respectively [1]. The Global Tuberculosis Report 2020 estimated that out of 4,65,000 cases of RR-TB, 78% had MDR-TB. Out of estimated 4,65,000 cases of RR/MDR-TB, 44.3% were detected and only 38.1% patients were enrolled with a treatment success rate of 57.1% globally. Total 6.2% of MDR-TB cases were XDR-TB globally. It has been estimated that in India, 1,24,000 cases of RR/MDR-TB emerge every year of which 53.5% were detected and notified and 45.5% were started on treatment with a treatment success rate of 49% [2]. Although Culture based Phenotypic drug susceptibility testing (DST) is regarded as gold standard for diagnosis of DR-TB. Culture and DST by means of solid media takes 8 - 12 weeks and liquid-based culture despite being claimed to be a faster procedure still takes 4 - 6 weeks [3]. The hinderance produced by delay in culture-based DST will affect the prognosis as well as tuberculosis spread. The End TB Strategy demands worldwide access to DST to curb the spread of DR-TB [4]. Rapid and early diagnosis of DR-TB is very crucial. The world requires rapid molecular DST that can diagnose resistance to first and second line drugs to control the menace of DR-TB.

Rapid molecular drug resistance testing is frequently mentioned as nucleic acid amplification tests (NAATs), these tests depend on amplification of a targeted genetic region of the MTB complex, characteristically by Polymerase Chain Reaction (PCR). Molecular assays can detect TB and resistance to key anti-TB drugs, such as rifampicin (R) and isoniazid (H), fluoroquinolones (FQ) and second-line injectable drugs (SLID) more quickly than conventional Culture and DST. They are also accessible at various levels of health-care systems. These assays however cannot be used for determining response to treatment [5]. The Xpert MTB/RIF is a cartridge-based NAAT (CB-NAAT) for simultaneous detection of TB and RR-TB. It detects deoxyribonucleic acid (DNA) sequences specific for the MTB complex and mutations in

the Ribonucleic acid (RNA) polymerase beta (rpoB) gene, which is associated with RR. Outcomes are attained from unprocessed sputum samples in 90 minutes, with minimal biohazard and very limited technical training required to operate [5]. Truelab real-time quantitative micro PCR system by Molbio, Truenat MTB and Truenat MTB-Rif Dx are chip-based, micro real-time PCR-based NAAT for TB detection and RR detection respectively. Truenat MTB assays detect MTB in sputum after DNA extraction, results are obtained in 1 hour and Truenat MTB-Rif Dx is used sequentially for RR detection. It doesn't require air conditioning and high maintenance and therefore is ideal for primary health care setup [5]. The Xpert MTB/XDR detects mutations associated with resistance towards H, FQ, SLID such as amikacin, kanamycin, capreomycin and ethionamide (Eto) in a single test. The test uses a semi quantitative nested PCR followed by high resolution melt technology. Outcomes are attained in less than 90 minutes. Easy-to-use procedure as Xpert MTB/RIF and can be operated on existing GeneXpert machines prepared with 10-color module. The Xpert MTB/XDR assay is intended to be used as a reflex test for a specimen that is determined to be MTB positive and to serve as an aid in the diagnosis of the main types of resistance that exist in MDR/XDR-TB when used in conjunction with clinical and other laboratory findings [6].

Line probe assays (LPA) uses PCR and reverse hybridization methods for detection of drug resistance. First line (FL-LPA) detects mutations in the *rpoB* gene for R resistance; in the *KatG* gene and the *InhA* promoter region for H and ethionamide (Eto) resistance. Second line (SL-LPA) detects mutations in *gyrA* and *gyrB genes* for FQ resistance and *rrs* and *eis* for SLID resistance [7].

To curtail the emergence and spread of DR-TB, a universal, comprehensive, culture free DST can only be obtained by sequencing. Whole Genome Sequencing (WGS) is the method of understanding the whole DNA sequence of an organism's genetic material. WGS of MTB means decoding the precise arrangement of all the nucleotides that form the bacterial genome, consequently reading all the data that it comprehends. *M. tuberculosis* WGS is frequently achieved on fresh or stored frozen cultured isolates to acquire adequate decontaminated mycobacterial DNA [8]. Next Generation Sequencing (NGS) is fixated on sequencing a select set of genes or gene regions that have acknowledged or alleged links with a specific pathogen (e.g. *Mycobacterium tuberculosis*) or a specific phenotype (e.g. drug resistance). The NGS method overpowers many of the major challenges by providing fast, complete sequence indication for numerous gene regions or whole genomes of interest. NGS may be used for finding the genomic sequence variants to forecast TB drug-resistance phenotypes, recognition of strain lineage and resistance mechanisms for TB surveillance, and acknowledgement of genetically linked strains for determination of transmission chains [9]. Genetic sequencing despite being a rapid molecular test has its own limitations such as intricacy of workflows, difficult interpretation of outcomes, high cost and maintenance of equipment's have been the main concern for its wide acceptance specifically in low- and middle-income nations.

Rapid molecular DST is essential to curb the emergence and control of DR-TB globally. These various tests should be adopted quickly by high TB burden countries to end the menace caused by DR-TB. These rapid molecular tests will lead to immediate and appropriate management of DR-TB without much of time lapse.

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