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Guest Editorial

TB-Whole Genome Sequencing Bridging the Diagnostic Gaps for Effective Treatment

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Abbreviations

TB: Tuberculosis; WGS: Whole Genome Sequencing; WHO: World Health Organization; MDR-TB: Multidrug Resistant TB; XDR-TB: Extremely Drug-Resistant; PMDT: Programmatic Management of Drug-Resistant TB; DST: Drug-Sensitivity Test; CDST: Culture-Drug Sensitivity; RR: Rifampicin Resistance; RRDR: Rifampicin Resistance Defining Region; NGS: Next Generation Sequencing; PZA: Pyrazinamide; INH: Isoniazid; RIF: Rifampicin; MIC: Minimum Inhibitory Concentration; MGIT: Mycobacterial Growth Indicator Tube; TAT: Turn-Around-Time

Tuberculosis (TB) affects millions every year, causing great morbidity and mortality worldwide (approximately 1.4 million lives in 2021) [1]. Despite being preventable and curable, TB still remains a major challenge to public health, with a large number of patients failing to respond to standard treatment. TB dramatically affects the quality-of-life of the patients, and also raises many socio-economic issues especially in medium- and high-burden regions like India, Pakistan, China, Indonesia.

World Health Organization (WHO), with an aim to reduce TB deaths by 90% between 2015 and 2030, prevent 80% new cases and reduce the socio-economic impact of the disease at family-level, initiated the 'End TB strategy' [2]. With continual efforts worldwide, the tuberculosis global incidence has reduced substantially; however, efforts still need to be made to reach the goal. The emergence of multi-drug resistant TB (MDR-TB) has been a serious obstacle for global TB control programme, with only 1/3rd of the population with MDR-TB, getting access to treatment in 2021 [1].

The standard TB treatments involve a combination of anti-TB drugs, which are often expensive, toxic and painful injection-based regimens that can take up to six months to complete. These treatment options, despite being sub-optimal have been in use since long, with limited success rates [1]. Laboratory tests based on the 'Guidelines on Programmatic Management of Drug-Resistant TB (PMDT) in India, also have shown gaps, being targeted in their approach and with many cases having unavailability of drug-sensitivity test (DST) for all the 18 anti-TB drugs prescribed [3].

Given these concerns, early and comprehensive detection of TB and MDR-TB is the need- of- the- hour and over the recent times, several techniques have been developed to fulfill this requirement. Culture-drug sensitivity (CDST), considered as the gold standard for TB diagnosis is time-consuming and thus many efficient alternatives like (LPA) line-probe assay, real-time PCR, GenXpert were devised [4]; however, expensive equipment and skilled technicians are required for conducting real-time PCRs and abbreviation LPA. Xpert MTB/RIF on the other hand being a rapid molecular-based test endorsed by WHO, has speeded TB diagnosis, with the added advantage of detecting MTB complex and RIF resistance (RR). This test has been very efficient in tackling TB in low- and middle-income countries. Xpert-Ultra has added value, increasing the sensitivity, especially in paucibacillary TB [5] as well as in pulmonary and extra-pulmonary TB [6]. Despite these advantages, mutations associated with Isoniazid (INH) as well as those outside the RRDR (rifampicin resistance defining region) could be missed by Xpert and thus warrants other tests like CDST for confirmation.

Most of these issues can be addressed efficiently by the use of sequencing technology like next- generation sequencing (NGS), which can rapidly analyze the entire genome, allow species identification, screen for all mutations, detect drug-resistance and predict the evolutionary mechanism of the organism. Whole-Genome Sequencing (WGS), a revolutionary molecular diagnostic tool, has become increasingly important in the fight against TB, as it provides a detailed picture of the genetic make-up of the disease and helps to identify the most effective treatment [7]. It screens the entire genome using high-end sequencing technique to trace the culprit gene/mutation causing TB and drug resistance. Due to its ability for greater genome coverage (not only the target region), increased epidemiological information and understanding of new resistance mechanism for both existing and novel drugs, has turned out to be the go-to tool for TB diagnosis [4]. This technique can assist in reliable prediction of treatment outcomes and resistance profiles of all anti-TB drugs in a single run with rapid results [8]. Most often, poly-microbial infections (mixed and co-infections) can give rise to misidentification of strains and underestimation of disease transmission, resulting in poor treatment outcomes [9]. WGS helps identify such poly-microbial infections, by determining the susceptibility profile of drugs like pyrazinamide (PZA), that can facilitate shortening of treatment regimen [10].

The PMDT guidelines indicate a shorter MDR-TB regimen with high-dose INH (along with other drugs) during intensive treatment period, for patients with RR and sensitivity towards Fluoroquinolones (FQ) and second-line injectable drugs (SLIDs). With INH-resistant TB being more common compared to RR-TB, it is advisable to include DST for Isoniazid (INH) along with Rifampicin (RIF) at the initial diagnosis and regularly monitor for any development of INH resistance amongst those treated with it [3]. More so the RR and MDR-TB treatments can be drastically affected with presence of 'disputed mutations', which can result in discordance between phenotypic and genotypic DST [11]. Minimum Inhibitory Concentration (MIC) testing have shown 'undisputed' mutations associating with higher MIC values (≥ 20 mg/liter) compared to 'disputed' mutations (4 to > 20 mg/liter). Interestingly, it was seen although 'undisputed mutations' did not show any delay in time to positivity in mycobacterial growth indicator tube (MGIT), 'disputed mutations' showed a mean delay of 7.2 days. This highlights that mutations conferring low-level resistance can show delay in growth on MGIT, affecting the treatment decisions [12]. Likewise, WGS can also identify co-occurring mutations, further assisting in selection of most effective treatment regimen [3]. Thus, depending on the resources available, sequencing and/or MIC testing is recommended for better management of RR- and MDR-TB cases [11].

WGS has been greatly instrumental in understanding the evolution, genetic diversity and mechanisms involved in TB drug resistance [9] and combines current research with diagnostics, putting forth a better alternative in comparison to the combined sensitivity and specificity of all other modalities for TB diagnosis. It also aids in epidemiological surveillance investigations and can be instrumental in designing and targeting SNP-based strain-specific PCR markers, thereby making it a good candidate for deployment as routine method for epidemiological study [3]. The crucial information generated by WGS, can aid in developing effective control and preventive strategies, identify patients who might be at higher risk of developing the disease, develop new diagnostic tools and treatments. The turn-around-time (TAT) can be effectively reduced using WGS, with increasing drug testing capacity towards 18 drugs, resulting in rapid incorporation of DST for newly introduced drugs by real-time monitoring of their resistance-associated genes.

WHO in 2019, advocated the inclusion of linezolid (LZD) and Bedaquiline (BDQ) in group A drugs, for addressing extremely drug-resistant (pre-XDR TB and XDR- TB cases). However, the existing molecular tests like line-probe assay are incapable of determining the resistance profile for these drugs. In this scenario and with newer drugs being introduced into the treatment regimen, monitoring their resistance has become all the more crucial to avoid acquired resistance and prevent future outbreaks due to transmission. WGS with its robust quality control, precision analysis and simplified interpretation, promises to achieve the goal 'End TB'.

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