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REVIEW

Recent developments in genomics, bioinformatics and drug discovery to combat emerging drug-resistant tuberculosis

Soumya Swaminathan [a, *], Jagadish Chandrabose Sundaramurthi [b], Alangudi Natarajan Palaniappan [c], Sujatha Narayanan [d]

[a] National Institute for Research in Tuberculosis (ICMR), Chetpet, Chennai, 600031, India
[b] Division of Biomedical Informatics, Department of Clinical Research, National Institute for Research in Tuberculosis (ICMR), Chetpet, Chennai, 600031, India
[c] Department of Clinical Research, National Institute for Research in Tuberculosis (ICMR), Chetpet, Chennai, 600031, India
[d] Department of Immunology, National Institute for Research in Tuberculosis (ICMR), Chetpet, Chennai, 600031, India

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SUMMARY

Emergence of drug-resistant tuberculosis (DR-TB) is a big challenge in TB control. The delay in diagnosis of DR-TB leads to its increased transmission, and therefore prevalence. Recent developments in genomics have enabled whole genome sequencing (WGS) of *Mycobacterium tuberculosis (M. tuberculosis)* from 3-day-old liquid culture and directly from uncultured sputa, while new bioinformatics tools facilitate to determine DR mutations rapidly from the resulting sequences. The present drug discovery and development pipeline is filled with candidate drugs which have shown efficacy against DR-TB. Furthermore, some of the FDA-approved drugs are being evaluated for repurposing, and this approach appears promising as several drugs are reported to enhance efficacy of the standard TB drugs, reduce drug tolerance, or modulate the host immune response to control the growth of intracellular *M. tuberculosis*. Recent developments in genomics and bioinformatics along with new drug discovery collectively have the potential to result in synergistic impact leading to the development of a rapid protocol to determine the drug resistance profile of the infecting strain so as to provide personalized medicine. Hence, in this review, we discuss recent developments in WGS, bioinformatics and drug discovery to perceive how they would transform the management of tuberculosis in a timely manner.

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* Corresponding author. Present address: Indian Council of Medical Research, P.O. Box No. 4911, Ansari Nagar, New Delhi, 110029, India.
E-mail address: doctorsoumya@yahoo.com (S. Swaminathan).

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1. Introduction

Emergence of drug resistance in *Mycobacterium tuberculosis*) is one of the major obstacles for successful control of tuberculosis (TB). Globally, about 3.3% of new cases and 20% of previously treated cases are reported to have MDR-TB (multidrug-resistant tuberculosis: resistant to rifampicin and isoniazid); about 9.7% of the MDR-TB patients develop XDR-TB (extensively drug-resistant tuberculosis: resistant to rifampicin, isoniazid, one of the quinolones and one of the aminoglycosides) [1]. Another form of TB, namely, TDR-TB (totally drug-resistant tuberculosis: resistant to all of the first-line and second-line drugs), has been reported from Italy, Iran, India and South Africa [2]. Among 480 000 estimated MDR-TB cases in 2014, only 123 000 were detected and reported [1]. The burden of drug-resistant tuberculosis (DR-TB) is reported to be very high among HIV positive individuals than HIV negative TB patients [3,4] a fact which we have also observed in South Indian population [5]. Drugs used to treat DR-TB are more toxic, more expensive, and require a longer duration [6]. Besides, the cure rate of DR-TB is reported to be only 48%, versus 90% in drug-susceptible new TB cases [6]. Time taken to determine the drug resistance pattern is a critical factor that can lead to inordinate delay in initiating appropriate treatment.

The present situation clearly indicates that there is an urgent need to detect drug resistance rapidly and initiate personalized treatment for every patient, so as to prevent the transmission of DR-TB and effectively control TB globally. Therefore, in this review we discuss molecular mechanism of drug resistance, recent developments in the field of genomics and bioinformatics which could together potentially lead to develop a protocol to diagnose drug resistance profile in TB in a couple of days. We also highlight the improvements in drug discovery with special focus on those candidate drugs useful against DR-TB and infer how these developments together will facilitate personalized treatment for TB.

2. Drug resistance in tuberculosis

Rifampicin (RIF), isoniazid (INH), ethambutol (EMB), pyrazinamide (PZA) are used as the first line drugs for tuberculosis. Fluoroquinolones (levofloxacin, moxifloxacin and ofloxacin) and aminoglycosides (kanamycin, amikacin, capreomycin) form the major second line-drugs for tuberculosis. Majority of the drug resistance in *M. tuberculosis* is caused by mutations in genes which are either drug targets or activators of pro-drugs. Conventional methods to determine resistance to anti-tuberculosis drugs involve isolation, culture and drug susceptibility testing (DST) which take about 2 months. Even with the Mycobacteria Growth Indicator Tube (MGIT) system, the process takes about 1–2 weeks [7–9]. Shortening the delay between specimen collection and appropriate regimen selection is critical for reducing the rate of transmission of DR-TB, preventing additional resistance, and improving treatment outcome. Though molecular methods like GeneXpert (MTB/RIF) and MTBDRplus Line Probe Assay help to diagnose drug resistance early, they are limited to a few genes [10–13]. Generally, multiple mutations in one or several genes are involved in resistance to TB drugs. This makes the molecular-based prediction of resistance to all of the TB drugs difficult. In the following section we discuss various mutations that are associated with drug resistance, the knowledge gap between phenotypic and genotypic determination of drug resistance, and we also highlight the implications of these mutations in the evolution of molecular-based rapid assays.

2.1. Mechanism of resistance to first-line drugs

INH is a prodrug activated by a bacterial enzyme catalase-peroxidase (*katG*) and the activated drug inhibits the enoyl reductase (*inhA*) which subsequently block mycolic acid pathway of *M. tuberculosis*. Therefore, mutations in either *katG* or *inhA* cause resistance to INH. Based on a systematic review [14], it was found that mutations in *katG*315 explain 64.2% of phenotypic resistance while mutation *inhA*-15 explain 19.2% of INH resistance. Frequency of these mutations vary between different geographical regions; for example, the frequency of *katG*315 mutation is 55.5% in western pacific region while 73.5% in Africa and 78.4% in South East Asia [14]. Furthermore, several more mutations in *katG* and *inhA* and mutations in several other genes are also associated with INH resistance [15]. Therefore, determining INH resistance based on conventional sequencing is a complex process. By performing a meta-analysis, Bai et al. (2016) reported a pooled sensitivity and specificity of 91% and 99% respectively for MTBDR*plus* assay in rapid detection of INH resistance which include mutations from *katG* and *inhA* [16]. Having known at least 22 different genes to be associated with resistance to INH, and the mutations listed in TBDReaMDB database [15], the gap between the phenotypic and genotypic determination of INH resistance is likely to continue as long as mutations from fewer genes alone are used as diagnostic markers. However, inclusion of all the mutations from multiple genes in a rapid molecular diagnostic assay for each of the TB drugs may also be practically not feasible.

RIF is an important TB drug that inhibits an enzyme DNA-directed RNA polymerase, encoded by a gene *rpoB*. Therefore, mutations in the *rpoB* are involved in the emergence of RIF resistant *M. tuberculosis*. More than 95% of the rifampicin resistant mutations occur in a 81-bp region, known as rifampicin resistance-determining region (RRDR) that spans codons 507 to 533 [11,17,18]. Within RRDR, mutations in codon 531, 526 and 516 are reported to account for 86% RIF resistance [17]. Using a meta-analysis, it was observed that the pooled sensitivity and specificity of MTBDR*plus,* which includes mutations from *rpoB* to be 96% and 98% respectively [16]. Similarly, for Xpert® MTB/RIF, the pooled sensitivity and specificity were 89% and 99% respectively [19]. However, the sensitivity of Xpert® MTB/RIF was reported to comedown (67%) when applied on smear-negative pulmonary tuberculosis [19,20]. On the other hand, the achievement of high-quality sequencing from a smear-negative sputum sample recently [21], indicates that there is a ray of hope for improvement in precise prediction of drug resistance using whole genome sequencing (WGS) based study.

Though the definition of the MDR-TB includes only two of the first-line drugs (INH and RIF), additional resistance to PZA, EMB and streptomycin (SM) make the treatment options lesser and complex. PZA is a prodrug, activated by pyrazinamidase (*pncA*) of *M. tuberculosis* into its active form pyrazinoic acid which subsequently kills non-growing persistent *M. tuberculosis*. Therefore mutations in *pncA* are involved in resistance to PZA. However, 641 different mutations in 171 codons are reported all along *pncA* and