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Commentary

Early detection of multi-drug resistant tuberculosis in India using GenoType MTBDR*plus* assay & profile of resistance mutations in *Mycobacterium tuberculosis*

The global burden of tuberculosis (TB) particularly with multi-drug-resistance (MDR) is increasing and has become a major health challenge[1,2]. The disease caused by *Mycobacterium tuberculosis* resistant to two primary anti-tubercular drugs, rifampicin (RIF) and isoniazid (INH), is known as MDR-TB. Any patient can be infected with MDR-TB but it is most commonly seen amongst the clinical relapse cases. It has been reported that *M. tuberculosis* resistant to RIF are more likely to have concomitant resistance to INH, making RIF resistance as a surrogate marker of MDR-TB[2]. Early diagnosis and rapid detection of RIF resistant TB is important for proper management of MDR-TB[1,3].

Timely identification of MDR-TB cases and adequately administered treatment regimens are essential to stop primary transmission of MDR-TB. WHO endorsed thrice weekly anti-tubercular treatment regimen, administered under the directly observed treatment - short course (DOTS), after its use for two decades, was questioned for its effectiveness in the treatment of MDR-TB and it was realized that the treatment of MDR-TB cases is very complex. Therefore, Revised National Tuberculosis Control Programme (RNTCP) of India introduced the internationally recommended programmatic management of drug resistant tuberculosis (PMDT) services since 2007. PMDT is an integrated programme which encompasses diagnosis, treatment and management of MDR-TB, previously known as DOTS plus. Major emphasis is given on registering, monitoring and reporting of MDR-TB cases, under new guidelines of PMDT. This programme is run jointly by WHO-Stop TB and Green Light Committee (GLC). Hence reaching to remotest area of the country, providing rapid diagnosis and appropriate category of treatment are integral objectives of this programme. For PMDT to be successful, special attention is laid on the following: *(i)* efficient and timely identification of patients who require drug susceptibility testing (DST); *(ii)* quality-assured laboratory capacity (smear, culture-DST, rapid molecular test); *(iii)* efficient drug procurement and supply chain management; *(iv)* adherence to difficult-to-take regimens for long periods; *(v)* prompt identification and management of side-effects; *(vi)* recording and reporting; and *(vii)* human and financial resources. Thus, it is a comprehensive programme, which ensures that all essential elements of the PMDT strategy are included[4].

Accurate and timely diagnosis is the backbone of PMDT activities and that MDR-TB must be diagnosed correctly before commencement of treatment. Hence, quality assured culture and DST are indispensable[5]. For this reason, in the last seven years, efforts have been made to improve and develop rapid diagnostic tools and DST methods including the line probe assay (LPA). These molecular methods are developed to target the *rpoB* gene which consists of a 81bp hot-spot region of codons 507 to 533 responsible for RIF resistance; called rifampicin resistance determining region (RRDR)[6]. So far, more than 50 mutations have been characterized within this region by DNA sequencing but only point mutations at codons 526 or 531 are known to cause high level of RIF resistance. In contrast, mutations in codons 511, 516, 518, 522 and 533 cause low-level resistance to RIF[6,7] and mutations outside RRDR associated with RIF resistance are reported very infrequently. Only rarely resistance can occur due to mutations in other regions of *rpoB* gene[6].

In this issue, Singhal *et al*[8] report prevalence of multi-drug resistant TB in samples received from North-Eastern States of India during the year

2012. The authors characterized genetic mutations responsible for drug resistance in these isolates by GenoType MTBDR*plus* assay hereafter as LPA**.** They studied 553 sputum samples from seven states of North-Eastern India. These States (and samples) were Arunachal Pradesh (121), Assam (95), Manipur (75), Meghalaya (97), Mizoram (89), Nagaland (46) and Tripura (3). Majority of their patients were males. Of the 553 samples, 372 (67.2%) were smear positive. Fifty six scanty positive samples were not included for direct LPA. The authors mention that the total number of samples subjected to LPA was 339. However, the total of smear positive (≥1+) samples (316) and MGIT culture positive isolates (43) becomes 359. The details of 20 samples which were not subjected to LPA, should have been provided. Their culture isolation rate from scanty positive samples was very poor. There were 181 smear negative and 56 scanty smear positive samples, but only 43 (18.1%) of these (237) samples were culture positive. It was expected that in addition to all smear positive (372) samples some additional cultures from smear negative samples, should have been positive. In criterion A of PMDT, only smear positive samples with treatment failure are sent for LPA, hence these patients have higher chances of culture positivity. One possibility is that the local DMCs reported these samples as falsely smear positive and wrongly labelled as treatment failure. Another possibility could be that some of the smear positive samples had non-tuberculous mycobacterial infections. But if these samples were true smear positive cases of TB infection at the local DMCs, and MGIT960 missed 8.9 per cent (33/372) of these samples, sample processing protocols need to be re-examined in their laboratory. This aspect should have been discussed. But wrong identification of these patients is unlikely when one finds extremely high drug resistance rate in these patients. In fact, one would expect several smear negative samples to become culture positive in these highly suspected MDR-TB patients. Kumar *et al*[7] reported 96.3 per cent culture positivity in smear positive samples, thus missing only 3.7 per cent smear positive cases by MGIT 960 culture. In smear negative or scanty positive samples they reported 22.6 per cent culture yield. Even though, samples processed by Kumar *et al*[7] were from patients followed under criterion B of PMDT, where one would expect lesser culture yield.

Singhal *et al*[8] report very high (61.7%) rifampicin resistance rate ranging from 40.3 per cent in Mizoram to 86.4 per cent in Meghalaya. The multi drug (RIF+INH) resistant TB was seen in 30.6 per cent (Mizoram) to 78.8 per cent (Arunachal Pradesh) cases. Seemingly, these authors used incorrect denominators while calculating these drug resistance rates. This means that their reported drug resistance was higher than the actual value. However, the RIF mono resistance was reported only in 6.0-13.3 per cent. In another study[7] carried out in Punjab, the RIF mono resistance was reported as high as 22.2 per cent, while MDR-TB was found only in 21.8 per cent . It could possibly be explained by the fact that in Punjab study, the patients were followed under criterion B and in North-Eastern States under criterion A of PMDT. In criterion B, patients are not necessarily failure cases but previously treated cases and are likely in the transition phase of drug resistance development[9] while the patients included from North-East India were confirmed failure cases, hence fully transformed resistance cases.

Singhal *et al*[8] also reported that of the known RIF mutations (78.9%), commonest mutation was in the codon S531L (65.1%) in their patients, while Kumar *et al*[7] found known RIF mutations in 81.8 per cent samples of which S531L mutation was 33.3 per cent in Delhi samples and 22.8 per cent in Punjab samples. Prevalence of other mutations was variable in both the studies. Though S531L mutation is expected to be the commonest[10], but reasons of finding solely this mutation in 65.1 per cent cases from North-Eastern States need to be investigated, if it was due to high prevalence of Beijing strain in these States (Singh *et al*, unpublished data), difference in patients characteristic or due to other reasons.

There are only three studies from India on correlating drug resistance and genotypes. One study was carried out from Mumbai but the sample size in this study was very small[11]. They found that heteroresistance was more common than MDR and suggested that these heteroresistance cases were future MDR cases. In another study carried out in Punjab by our team, it was reported that heteroresistance and MDR rates were almost similar in this State and these authors cautioned that Punjab may be the next high MDR-TB burden State soon[7]. Data on drug resistance and various genotypes circulating in North-Eastern States of India is scarce. Hence this study provides useful important information from this region. The alarming drug resistance rates reported here, even

if adjusted with correct denominators, are bothering and steps to contain the spread of drug resistant TB to neighbouring areas are urgently required.

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