



## Viewpoint

### Occult drug resistance in tuberculosis: Emerging issues, upcoming challenges & possible solutions

Multidrug-resistant tuberculosis (MDR-TB) is one of the most pressing and problematic challenges being faced globally. The dynamic transmission microsimulation model of TB in India estimates that 42 per cent of the new MDR cases are spread by transmission and this proportion continues to rise over time, assuming equal transmissibility of MDR-TB and drug-susceptible TB<sup>1</sup>. In India, of the 2,264 MDR-TB patients from 15 centres catering a population of 227 million residing in seven States, treated under Programmatic Management of Drug-Resistant TB, who were treated with a standard 24-month regimen under daily-observed treatment, only 34.5 per cent patients had treatment success, 28.4 per cent died, 29.6 per cent were lost to follow up and 7.5 per cent patients experienced treatment failure or were changed to extremely drug-resistant (XDR)-TB treatment<sup>2</sup>. Globally, in 2018, there were about half a million new cases of rifampicin (RIF)-resistant TB (RR-TB), of whom 78 per cent had MDR-TB. India, China and the Russian Federation accounted for almost half of the world's cases of MDR/RR-TB<sup>3</sup>.

Detection of MDR-TB often targets the detection of resistance to the drug RIF due to its association with isoniazid (INH) resistance in more than 90 per cent of cases<sup>4</sup>. Mutation in the RIF resistance determining region (RRDR) of *rpoB* gene is the common target for the detection of RIF resistance as >95 per cent of RIF resistance occurs due to mutation within this region<sup>4</sup>. Therefore, the detection of MDR-TB in the present scenario primarily depends on the detection of RIF resistance based on mutation in *rpoB* gene. The Xpert MTB/RIF assay (also called as CBNAAT in India), endorsed by the World Health Organization (WHO) and widely used all over India by the Revised National TB Control Programme [RNTCP, now renamed as National

Tuberculosis Elimination Programme (NTEP)], detects *Mycobacterium tuberculosis* DNA and RIF resistance based on mutation in the 81 base pair (bp) RRDR of *rpoB* gene and thus would not be able to detect mutation outside RRDR. Various studies and a large multi-country meta-analysis of five randomized controlled trials have established uncertainty of the impact on treatment outcome of patients with TB even after eight years of use of Xpert MTB/RIF and were unable to confirm the Xpert MTB/RIF associated reduction in mortality among outpatients tested for TB despite great reduction of turnaround time for diagnosis and RIF resistance<sup>5,6</sup>. Possible reasons can be: Xpert MTB/RIF is unable to detect all MDR cases as it targets only RRDR.

#### Is 81 bp RRDR of *rpoB* gene enough to detect all types of RIF resistances?

RIF resistance attributed to mutation outside RRDR has been reported earlier from various countries across the world and was limited to <5 per cent<sup>7</sup>. High percentage of RIF resistance (>20%) due to mutation outside RRDR was reported for the first time from Vietnam in 2012<sup>8</sup>. In 2015, >30 per cent of RIF resistance due to mutation outside RRDR was reported from another high TB burden country Swaziland (present name eSwatini) from Southern Africa. Mutation at site 491 of *rpoB* gene (Ile491Phe) was responsible for RIF resistance in Swaziland strains<sup>9</sup>. Three years later, another study was carried out in South Africa, a high TB burden neighbouring country of Swaziland in search of existence of similar *M. tuberculosis* strains with Ile491Phe mutation<sup>10</sup>. This group of workers started their study by including only INH-resistant among those having RIF-sensitive strains as reported by Xpert MTB/RIF, line probe assay (GenoType MTBDRplus) and MGIT (Mycobacteria Growth Indicator Tube) liquid culturing (BACTEC

MGIT 960) first-line drug susceptibility testing. On subjecting to deep sequencing and whole genome sequencing (WGS), 15 per cent of INH mono-resistance strains were found to bear Ile491Phe mutation in *rpoB* gene and thus were actually found to be MDR. On further including the strains from Swaziland, all those MDR strains with Ile491Phe mutation were resistant to all first-line anti-tubercular drugs<sup>10</sup>. Hence, these patients who were initially diagnosed only as INH mono-resistant, when received the RIF, pyrazinamide and ethambutol for six months with fluoroquinolone generated possibility of emergence of resistance to fluoroquinolone and/or second-line injectable. The development of resistance to bedaquiline in some of their cases who probably had exposure to the drug cautioned for total drug resistance<sup>10</sup>.

The failure to detect RIF resistance due to mutation outside RRDR has not only missed the true MDR strains but also has shown the possibility of evolving in to XDR and its capability to spread across the border<sup>10</sup>. Considering the increasing importance of mutation outside RRDR, the following recommendations are suggested.

#### Detection of occult MDR strains

The occult MDR strains need to be made overt. This can be made by expanding the target 81 bp hotspot RRDR of *rpoB* gene and including the 491 codon. Inclusion of 491 was suggested by Siu *et al*<sup>11</sup> in 2011 when found Ile491Phe mutation as an important contributor of RIF resistance. The feasibility of 491 codon inclusion has been hypothesized due to its proximity to RRDR<sup>11</sup>.

Detection of INH resistance has often been underrated due to high percentage of mono-resistance and not acting as surrogate marker of MDR. However, the fact which is known but not acted upon is, among all first-line anti-tubercular drugs resistance to INH occurs first<sup>12</sup>. The largest ever whole genome analysis of *M. tuberculosis* strains from across five continents has shown that the *katG* Ser315Thr mutation that confers INH resistance arises before RIF resistance in a substantial number of strains across geographic regions, all MTB lineages and time period<sup>12</sup>. This indicates the inclusion of *katG* 315 in the molecular diagnostics to improve the detection of pre-MDR strains as well as to keep vigil on these strains for the presence of Ile491Phe mutations in *rpoB* gene. It is time to carry out such studies in India analogous

to South Africa to look for such strains and its prevalence.

#### Is pan susceptibility from WGS the next answer?

The pan susceptibility from WGS that fulfils the WHO standards for sensitivity and specificity has been already calculated<sup>13</sup>. Based on the results, the countries such as England, The Netherlands and USA (NY) have discontinued phenotypic drug susceptibility testing of isolates. The drug resistance is to be predicted by sequencing the genome to see pan susceptibility to four first-line drugs<sup>13</sup>. This policy change has though added incremental cost to susceptibility prediction but also time-saving benefits. This is need of the hour and likely to be a more effective method than just expanding the base pairs of RRDR.

On the other hand, the ongoing Comprehensive Resistance Prediction for TB: An International Consortium (CRyPTIC). has already developed and validated a phenotypic drug susceptibility testing quantitative assay for a panel of 14 anti-TB drugs including the drugs, bedaquiline and delamanid. This should be used to improve the patient outcomes of MDR-TB by knowing the susceptibility even to the newer drugs as these drugs are increasingly used in India to treat MDR-TB<sup>14</sup>.

There have been no major policy modifications other than introduction of Xpert MTB/RIF (CBNAAT) since its endorsement by the WHO. Considering India's target to eliminate TB by 2025, expansion of hotspot to detect occult RIF resistance and inclusion of INH resistance detection system have become the need of the hour<sup>15</sup>. Strengthening of peripheral remote laboratory capacities can be a fundamental component of the TB control strategy. However, better coordination between the diagnostic and treatment services in the health system is essential and needs to be ensured.

Patient-centred individualized therapy based on the predictions of susceptibility or at least susceptibility testing of two key anti-TB drugs, INH and RIF (given throughout the course of treatment), is required for all patients with confirmed TB, including those with drug-resistant TB. This will be challenging to use in large scale for high-burden country like India but essential in view of its goal of TB elimination<sup>15</sup>.

There is a need to detect MDR-TB more precisely, and to find better ways to detect occult drug resistances to make the TB control more effective. Thus, it is time to

be pro-active and to detect pre-MDR. Time has ripened to think about the measures to prevent the development of MDR in such strains than to wait and act on MDR. It is essential to note that all patients of TB, irrespective of resistance profiles, have a high possibility of cure if the desired diagnostic and therapeutic tools are employed on time.

**Conflict of Interest:** None.

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### References

1. Suen SC, Bendavid E, Goldhaber-Fiebert JD. Disease control implications of India's changing multi-drug resistant tuberculosis epidemic. *PLoS One* 2014; 9 : e89822.
2. Parmar MM, Sachdeva KS, Dewan PK, Rade K, Nair SA, Pant R, *et al.* Unacceptable treatment outcomes and associated factors among India's initial cohorts of multidrug-resistant tuberculosis (MDR-TB) patients under the Revised National TB Control Programme (2007-2011): Evidence leading to policy enhancement. *PLoS One* 2018; 13 : e0193903.
3. World Health Organization. *Global tuberculosis report 2019*. Geneva : WHO; 2019.
4. Yam WC, Tam CM, Leung CC, Tong HL, Chan KH, Leung ET, *et al.* Direct detection of rifampin-resistant *Mycobacterium tuberculosis* in respiratory specimens by PCR-DNA sequencing. *J Clin Microbiol* 2004; 42 : 4438-43.
5. Di Tanna GL, Khaki AR, Theron G, McCarthy K, Cox H, Mupfumi L, *et al.* Effect of Xpert MTB/RIF on clinical outcomes in routine care settings: Individual patient data meta-analysis. *Lancet Glob Health* 2019; 7 : e191-9.
6. Agizew T, Boyd R, Auld AF, Payton L, Pals SL, Lekone P, *et al.* Treatment outcomes, diagnostic and therapeutic impact: Xpert vs. smear. A systematic review and meta-analysis. *Int J Tuberc Lung Dis* 2019; 23 : 82-92.
7. Zaw MT, Emran NA, Lin Z. Mutations inside rifampicin-resistance determining region of *rpoB* gene associated with rifampicin-resistance in *Mycobacterium tuberculosis*. *J Infect Public Health* 2018; 11 : 605-10.
8. Minh NN, Van Bac N, Son NT, Lien VTK, Ha CH, Cuong NH, *et al.* Molecular characteristics of rifampin- and isoniazid-resistant *Mycobacterium tuberculosis* strains isolated in Vietnam. *J Clin Microbiol* 2012; 50 : 598-601.
9. Sanchez-Padilla E, Merker M, Beckert P, Jochims F, Dlamini T, Kahn P, *et al.* Detection of drug-resistant tuberculosis by Xpert MTB/RIF in Swaziland. *N Engl J Med* 2015; 372 : 1181-2.
10. Makhado NA, Matabane E, Faccin M, Pinçon C, Jouet A, Boutachkourt F, *et al.* Outbreak of multidrug-resistant tuberculosis in South Africa undetected by WHO-endorsed commercial tests: An observational study. *Lancet Infect Dis* 2018; 18 : 1350-9.
11. Siu GK, Zhang Y, Lau TC, Lau RW, Ho PL, Yew WW, *et al.* Mutations outside the rifampicin resistance-determining region associated with rifampicin resistance in *Mycobacterium tuberculosis*. *J Antimicrob Chemother* 2011; 66 : 730-3.
12. Manson AL, Cohen KA, Abeel T, Desjardins CA, Armstrong DT, Barry CE 3<sup>rd</sup>, *et al.* Genomic analysis of globally diverse *Mycobacterium tuberculosis* strains provides insights into the emergence and spread of multidrug resistance. *Nat Genet* 2017; 49 : 395-402.
13. Cox H, Mizrahi V. The coming of age of drug-susceptibility testing for tuberculosis. *N Engl J Med* 2018; 379 : 1474-5.
14. Rancoita PMV, Cugnata F, Gibertoni Cruz AL, Borroni E, Hoosdally SJ, Walker TM, *et al.* Validating a 14-drug microtiter plate containing bedaquiline and delamanid for large-scale research susceptibility testing of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2018; 62. pii: e00344-18.
15. Pai M, Bhaumik S, Bhuyan SS. India's plan to eliminate tuberculosis by 2025: Converting rhetoric into reality. *BMJ Glob Health* 2016; 2 : e000326.