Multi-drug resistant tuberculosis: Current status and emerging tools for its management in India

Pushpendra Singh* and VM Katoch*

ABSTRACT

Drug resistance in tuberculosis is a global problem and India is no exception. However, this rise is mainly among the previously treated cases, generally reported from the hospitals (majority of which are the tertiary health care centres). Primary resistance figures are still static and are mostly below 5% level. Detection of drug resistance in tuberculosis is conventionally performed by comparing the growth inhibition on the drug containing medium as compared to the drug free control (phenotypic methods). Since these methods are dependant on appearance of visible colonies of M. tuberculosis, these methods take longer time to provide susceptibility testing results and are labor-intensive. Therefore, alternate phenotypic methods based on the bacterial metabolism (CO₂ production, Oxygen uptake, ATP bioluminescence etc) and thus the viability of organisms, have been tried and found promising in overcoming this hindrance of longer time requirement. These systems, besides having good sensitivity and impressive specificity as compared to the conventional methods, reduce the total turn around times for isolation as well as susceptibility testing of M. tuberculosis. Recent advances in our understanding of the molecular basis of drug resistance have led to the development of genotypic methods for detection of mutations/mechanisms associated with resistance. However, most of these phenotypic and genotypic advancements require expertise and expensive equipments/technologies, which limit their wider application. This article reviews current status of multi-drug resistance in tuberculosis (MDR-TB), the recent developments for its rapid and reliable detection and effective management with special reference to India.

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INTRODUCTION

“If preventable, why not prevented?” was the question raised by George V, British Emperor in 19th century while enquiring about management of tuberculosis problem in his kingdom. The question has gained more and more relevance in due course of time with our ever increasing understanding of the biology and physiology of the causative agent *Mycobacterium tuberculosis*. Enormous mortality and morbidity associated with this “curable killer” which kills 5000 people every day, justifies the declaration of tuberculosis as the “global emergency” by World Health Organization (WHO) in 1993. Two billion people, (equal to one third of the human population) have been estimated to have latent infection with *Mycobacterium tuberculosis*, 8.8 million new cases and nearly 2 million deaths are attributable to the disease every year. Developing countries account for the 95% of TB cases and 98% deaths related to the disease. Of these, more than half the cases occur in five South-East Asian countries. India alone accounts for the 23.24% (3,394,040) of global burden of tuberculosis. Disease affects people in the prime time of their age and thus its socio-economic impact is also worrisome.

Tuberculosis became a treatable disease when streptomycin was discovered in 1944 by Selman Abraham Waksman for which he received the Nobel Prize in Physiology or Medicine in 1952. Later several other drugs were identified and combination therapy was considered better to reduce relapses. The drugs currently in use are Rifampicin (RIF), Isoniazid (INH), Pyrazinamide (PZA), and Ethambutol (EMB)/ Streptomycin (STR). However, the success in therapy gets limited due to increase in incidence of drug resistance.

Drug resistance can be defined as temporary or permanent capacity of organisms and the progeny to remain viable or to multiply in the presence of the concentration of drug that would normally destroy or inhibit the growth of their cells. Resistance of *M. tuberculosis* to anti-tuberculosis drugs is usually the result of a spontaneous genetic events and, worse, “a man-made amplification of the natural phenomenon”. The situation has been worsened by the deadly allies, the HIV pandemic and emergence of drug resistant strains of tuberculosis. 450,000 new Multi-drug resistant-Tuberculosis ((MDR-TB) cases are estimated to occur every year. Drug resistance in tuberculosis is a global problem. Drug resistance can be broadly classified as primary drug resistance (when a patient who has never taken anti-tuberculosis treatment develops tuberculosis after being infected by another patient who has resistant bacilli) and acquired drug resistance (when a patient develops resistance to one or more antitubercular drugs as a result of inadequate therapy).

The adequate and rationale use of our limited anti-tubercular arsenal is the most important concern for the management of drug resistant tuberculosis. The rapid and accurate diagnosis of the drug resistance is, therefore, particularly important both for
In acquired drug resistance, the prevalence of resistance to drugs like INH and STR is quite high and even MDR levels are high (30.2% in Gujarat during 1983-86, 10.9% in Wardha during 1988-89, 17.1% in Raichur during 1988-89 and 33.3% in New Delhi during 1990-91). Possibly due to misuse of drugs like INH and EMB, concurrent resistance to these drugs has been observed. Very high prevalence of acquired MDR has been observed in some of the recent studies, some of them based on limited number (less than 20) of isolates (69% in North Arcot during 1999, 78% in Wardha during 2000, 100% in Raichur during 1999). Though, these figures cannot be considered as useful parameter from epidemiological viewpoint, these are very important from clinical point of view for instituting the most appropriate and individualized treatment at the earliest.

Diagnosing Threat of Drug Resistance: The Sooner the Better

Conventional methods for detection of drug resistance and their limitations:

Determination of drug resistance in tuberculosis is conventionally performed by culturing *M. tuberculosis* in presence of drug (phenotypic methods) and comparing the growth inhibition with that of the drug free control. These methods have been well standardized in egg based (Lowenstein Jensen medium etc) and agar based (Middlebrook 7H10, 7H11) medium and have been evaluated in large clinical trials. Conventional microbiological methods for drug
effective and have been applied worldwide for drug resistance surveillance programmes, these methods are time consuming, cumbersome, and tedious. Development of various rapid phenotypic methods and their performance evaluations has attracted considerable attention in recent past. Table-2 summarizes important phenotypic methods/techniques described for drug susceptibility testing.

**Other phenotypic Methods**

Several other phenotypic methods proposed for rapid detection of drug resistance in tuberculosis have been published and include: ATP biolumine-
Table 2: Utility of various phenotypic methods for drug susceptibility testing.

<table>
<thead>
<tr>
<th>Method/Technique</th>
<th>Principle</th>
<th>Av. TAT</th>
<th>Remark: (Major advantages; limitations)</th>
<th>References</th>
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<tbody>
<tr>
<td>BACTEC 460</td>
<td>Radiometric (based on production of labeled $^{14}$CO$_2$ by utilization of radiolabeled palmitic acids as carbon source)</td>
<td>5-7 days</td>
<td>Rapid, sensitive and specific, Reliable for sensitivity testing to first- and second line drugs; semi-automated, involves concerns about radioactive waste and invasive reading system.</td>
<td>9-10</td>
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<tr>
<td>MGIT 960</td>
<td>Fluorimetric {fluorescence quenching-based oxygen sensor (silicon rubber impregnated with ruthenium pentahydrate) embedded at the bottom of each tube measures the consumption of the dissolved oxygen}</td>
<td>6-8 days</td>
<td>Rapidity and sensitivity comparable to BACTEC, Automated, good agreement for first line drugs.</td>
<td>11-13</td>
</tr>
<tr>
<td>MB BACT-Bact/ ALERT 3D system:</td>
<td>Colorimetric (based on the colour change of the CO$_2$ permeable sensor embedded in the bottom of the bottle measured by the reflectometric unit)</td>
<td>6-9 days</td>
<td>Rapidity and sensitivity shown to be comparable to BACTEC, fully automated, can be connected to the Laboratory Information System (LIS), Experience with second line drugs is limited.</td>
<td>14-18</td>
</tr>
<tr>
<td>E test</td>
<td>Strips containing gradients of impregnated antibiotics are applied on the surface of an agar medium inoculated with the test strain.</td>
<td>7-10 days</td>
<td>Easy and simple method.</td>
<td>19-21</td>
</tr>
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<td>Alamar Blue assay</td>
<td>Oxidation-reduction dye, alamar blue, which in the oxidized state is blue but is pink when reduced. The color change can be detected visually, or can be measured spectrophotometrically or fluorometrically.</td>
<td>7-10 days</td>
<td>Easy and inexpensive method, require lesser amount of medium and laboratory space to store plates.</td>
<td>22</td>
</tr>
<tr>
<td>Nitrate reductase assay</td>
<td>Based on reduction of nitrate to nitrite as a measure of viability of the organisms.</td>
<td>7-10 days</td>
<td>Inexpensive, simple and rapid, no requirement of expensive equipment or expertise.</td>
<td>22-24</td>
</tr>
<tr>
<td>Pha $\beta$ assay</td>
<td>Replication of infecting Mycobacteriophage (D29) in viable $M. tuberculosis$ followed by detection in rapidly growing mycobacteria.</td>
<td>2-3 days</td>
<td>Low cost assay, rapid.</td>
<td>25-30</td>
</tr>
<tr>
<td>Luciferase reporter phage</td>
<td>Viable mycobacteria are infected with genetically engineered reporter phages expressing firefly luciferase gene, which produces quantifiable light.</td>
<td>2 days</td>
<td>Simple and rapid.</td>
<td>31-32</td>
</tr>
<tr>
<td>$\beta$-galactosidase assay</td>
<td>Utilizes recombinant $M. smegmatis$ mc$^{155}$ expressing $E. coli$ lacZ gene as test organism and is based on production of $\beta$-galactosidase in presence of drug.</td>
<td>3 days</td>
<td>Can be applied for screening of compounds/drugs for their in-vitro activity.</td>
<td>33</td>
</tr>
</tbody>
</table>

Av TAT- Average turn around time, MGIT 960 - Mycobateria Growth Indicator Tube (MGIT) 960, Pha B-Phage amplified-biologically.
“DOTS Plus”. Salient features of this strategy are:

- individualized rather than standardized treatment,
- facility for providing on-site culture and susceptibility testing,
- reliable supplies of a wide range of expensive second-line drugs,
- operational studies to monitor/evaluate the effect of this shift, and
- adequate financial and technical support from international organizations.

Use of immunomodulators like Mw and M. vaccae as an adjunct to the chemotherapy is a promising possibility for the management of MDR-TB. Role of immunotherapy in modulating host's immune response from a Th-2 dominant to Th-1 dominant pathway leading to less tissue destruction and reduced mycobacterial replication has been postulated and efforts are underway to confirm these findings by randomized controlled clinical trials. Similar findings were not confirmed for M. vaccae in controlled clinical trials.

Genotypic methods

Recent advances in molecular biology and elucidation on the molecular mechanisms of drug resistance in *M. tuberculosis* have provided new tools for the rapid detection of drug resistance. Some of the important and widely used genotypic methods are briefly mentioned below in Table 3.

Several other genotypic methods have been proposed for detection of mutations associated with drug-resistant tuberculosis. These methods include: dideoxy fingerprinting, hybridization protection assays, PCR-heteroduplex formation, denaturing gradient gel electrophoresis. However, limited experimental studies are available with the applicability of these methods, and therefore, additional evaluations are required.

Management of MDR-TB

The emergence of drug resistant strains is known to reduce the efficacy of treatment. The outcome of treatment of patients infected with organisms resistant to rifampicin and isoniazid (MDR) have a high rate of treatment failure. These unacceptable failure rates and possibility of induction of resistance to additional drugs alarms for providing additional services to such MDR cases through strategy known as “DOTS Plus”. Salient features of this strategy are:

- individualized rather than standardized treatment,
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Strategies to face challenges of MDR-TB in India

Indian Government has taken several measures to counter the threat of increasing drug resistance. Still these efforts are largely preventive measures to avoid the problem and efforts to treat the MDR cases have also been initiated by including alternative drugs like fluoroquinolones, aminoglycosides (kanamycin, amikacin) in DOTS Plus programme. The efforts made in this
Table 3: Utility of various genotypic methods for drug susceptibility testing

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<tr>
<th>Method/Technique</th>
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<tbody>
<tr>
<td><strong>Automated DNA Sequencing</strong></td>
<td>Based upon Sanger's dideoxy chain termination method.</td>
<td>16 hrs.</td>
<td>Most widely used, Unambiguous characterization of mutation positions associated with resistance; Not all mutations are related to resistance.</td>
<td>37-39</td>
</tr>
<tr>
<td><strong>CR-SCP</strong></td>
<td>Based on different electrophoretic mobilities of single-stranded DNAs differing in the sequences due to their property of acquiring different three dimensional structures.</td>
<td>7-10 hrs</td>
<td>Simple and easy to perform, low cost, no expensive equipment required; not unambiguous,</td>
<td>40-42</td>
</tr>
<tr>
<td><strong>Line probe assay:</strong></td>
<td>Based on reverse hybridization between amplicon and immobilized membrane-bound probe encompassing overlapping sequences of the wild-type sequences and the most frequent mutation.</td>
<td>5 hrs.</td>
<td>Cost effective, no requirement of expensive equipment.</td>
<td>43-45</td>
</tr>
<tr>
<td><strong>Real Time PCR</strong></td>
<td>By performing melting curve analysis of the labeled hybridization probes/molecular beacons hybridized to the amplified DNA.</td>
<td>1/3-2 hrs.</td>
<td>Rapid, on line monitoring of amplification, mutation analysis of different mutations can be performed simultaneously in closed glass capillaries that minimize the post amplification carry over contamination, can be applied directly to the clinical specimens.</td>
<td>46-47</td>
</tr>
<tr>
<td><strong>DNA-Microarray</strong></td>
<td>Based on high density oligonucleotide arrays on a glass slide.</td>
<td>16-18 hrs</td>
<td>Single experiment can detect many mutations on same slide; High cost, complexity, skill required.</td>
<td>48-50</td>
</tr>
</tbody>
</table>

**PCR-SSCP:** PCR- single strand conformational polymorphism.
direction are:

(i) **Increasing the coverage with DOTS:** Over 1.28 million patients annually or more than 1,00,000 people with active tuberculosis per month, have been put on TB treatment in India alone during the past four quarters in 2004 and 2005. Currently available data show that the 85% treatment success target has already been met\(^5\). Entire country has been covered under DOTS.

(ii) **Operational research to monitor the trends in drug resistance:** Government of India through several National Institutes (TRC/NTI/JALMA) have organized surveys for prevalence of anti-tuberculosis drug resistance in different parts of country specially in fresh untreated cases to determine the primary drug resistance. JALMA has been entrusted with such a study in two districts (Kanpur Nagar and Agra) of Uttar Pradesh. Exact magnitude will be known only after the completion of the survey, available results of these studies indicate that the primary MDR is undetectable or very low in the rural population of Kanpur and Agra districts with overall MDR rate of 3.3%.

(iii) **Application of and research in methods for rapid detection of drug resistance:** The experience in use of the novel genotypic and phenotypic methods of diagnosis of disease and drug resistance, molecular fingerprinting methods is emerging from many research centres/ laboratories applying these technologies/methods. Many of these technologies, developed in other parts of the world and found to be successful there, have been adapted/modified or need modification according to the strains/ genotypes prevalent in Indian population\(^4\).

(iv) **Improving methods/regimens for prevention/ management of drug resistant cases:** Recent studies at the TRC, Chennai have shown promising results with the use of added ofloxacin in the regimens in treating MDR-TB. While interim reports appear promising, a long-term follow up is needed to draw valid conclusions (TRC, unpublished observations)\(^5\). The fluoroquinolones have been shown to have marked antimycobacterial activity and are being increasingly used in the treatment of MDR-TB. However, this class of drugs is also widely used for a variety of respiratory and other infections, due caution need to be exercised to prevent their indiscriminate use, which, invariably, will lead to the development of resistance to this class of drugs also. PAS or thioacetazone used earlier or other drugs like clofazimine, ansamycins, aminoglycosides (like kanamycin, amikacin) etc have shown to be promising in managing MDR cases. Adjunct immunotherapy is another good strategy to manage drug resistant tuberculosis. Such trials using *M. w* immunotherapy are already underway at several centres in India, like AIIMS, JALMA, TRC, LRS etc.

**CONCLUSION**

Initial drug resistance in India is still below 5% level. The magnitude of drug resistance problem to a large extent is due to acquired resistance. The prevalence of MDR-TB also is found to be at a low level in most of the regions of India. Detection
of drug resistance through novel phenotypic and genotypic methods has helped in reducing the turn around times of detection of resistance. However, these methods are still very costly and can't be applied on mass scale or for routine use. Any test that will be broadly accepted by the global tuberculosis diagnostic community needs to be cost effective, accurate, simple to perform, and easy to implement within the current infrastructure. Therefore, conventional methods and inexpensive alternatives need to be applied for early detection and management of MDR-TB.

As our understanding about the molecular basis of drug resistance mechanisms in tuberculosis is improving, it is being discovered that a lot new is to be unraveled. Recent approaches to meet these unanswered questions/issues/mechanisms about drug resistance are basically targeted towards applying the transcriptomic, proteomic, and metabolomic approaches which promise many opportunities for addressing these unsolved mysteries and to widen our understanding of this intriguing but equally interesting problem.

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REFERENCES


