SCALING UP ANTI-MYCOBACTERIAL DRUG SUSCEPTIBILITY TESTING SERVICES IN INDIA: IT IS HIGH TIME

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Approximately nine million people develop active tuberculosis (TB) every year and this dreaded disease kills 1.7 million per year. With these figures one can understand that TB is far from under control. Human immunodeficiency virus (HIV) infection has further complicated the disease burden as it dramatically increases the risk of developing active tuberculosis and is driving the TB epidemic in Africa and India. HIV renders tuberculosis more difficult to diagnose (due to higher incidence of sputum negative disease), and treat (due to interactions and side-effects). Directly Observed Therapy short course (DOTS), as promoted by the World Health Organization (WHO) is demanding for patients and labour-intensive for health staff but aimed to improve compliance of the otherwise cumbersome and prolonged treatment regimen. However, the programme is compromised in settings where health services are poorly accessible. The increasing spread of multi-drug-resistant TB (MDR-TB) and the recalcitrant nature of persistent infections pose additional challenges to treatment with currently available anti-TB drugs. MDR-TB is more complex and expensive to treat, and in developing countries treatment is limited to a few projects with limited numbers of patients. The situation is exacerbated by the emergence of extensively drug-resistant tuberculosis (XDR-TB).[1] Patients with XDR-TB are virtually untreatable with available drugs and carry rapid and extremely high fatality rate.

Directly Observed Therapy Short Course Programme

The DOTS programme is one of the most successful and inexpensive programmes and is a highly effective means of treating TB patients and preventing new infections. Between 1995 and 2003, more than 17.1 million patients were treated under the DOTS strategy. Worldwide, 182 countries were implementing the DOTS strategy by the end of 2003, and 77% of the world’s population was covered by DOTS programme. WHO aims to achieve a 70% case detection rate and a cure rate of 85% through this programme (http://www.who.int/tb/dots/en/). The major emphasis has been on the fact that it does not mandate for mycobacterial culture and drug susceptibility testing, and only smear microscopy is considered the main tool to diagnose the pulmonary tuberculosis. However, the flip side of the programme is in several studies that smear negative pulmonary tuberculosis cases are increasing and most often these are drug resistant cases. These studies have raised concerns that smear negative pulmonary tuberculosis cases could spread drug resistant tuberculosis more efficiently than smear positive cases, especially in AIDS patients.[2,3]

Drug Resistance in India

A recently released WHO report suggests that prevalence of primary multidrug-resistant tuberculosis in India is approximately 5% but the surveillance report also admits sampling biases and the lack of sub national surveys as major limitations of this data. The report also highlights that average prevalence of acquired multidrug resistance is more than 17%. The range is wide and in some regions of India, the acquired MDR is reported in up to 58% cases[4].

In India, even though the prevalence of tuberculosis is high, most cases are empirically treated without performing culture and anti-mycobacterial drug susceptibility testing. The revised national tuberculosis control program (RNTCP) guidelines recommend susceptibility testing only for those patients who have previously been treated for TB or fail to respond to treatment after two months of TB treatment, the point at which, there is a high rate of treatment interruption. Because of not performing culture and drug susceptibility assays, the non-responsive tuberculosis cases are usually labelled as MDR cases, but there could be several other reasons of not responding even to the highly compliant directly observed therapy (DOTS). One is of course the development of drug resistance, however, an equally important fact which is not given due importance is the occurrence of non-tubercular mycobacterial (NTM) aetiology of the disease and co-infection of more than one clones of M. tuberculosis or co-infection of NTM with M. tuberculosis.[5] In a recent study, carried out at the All India Institute of Medical Sciences, New Delhi, 17 (30.9%) out of 55 clinically suspected MDR strains were finally identified as non-tubercular mycobacteria, which have intrinsic resistance to the standard antitubercular drugs.[6]

Need for Anti-mycobacterial Drug Susceptibility Testing

Mycobacterial culture isolation and drug susceptibility testing (DST) is an urgent need of the day. It is felt that
the routine sputum culture and DST is the most effective prerequisite tool to manage tuberculosis, in particular MDR cases. Although these measures seem to have higher direct cost for the government but the combined direct and indirect costs will be much lower to the programme and the concerned states. It will help contain the emergence and spread of multi-drug resistant (MDR) and extensively drug resistant (XDR) strains in the society. This will also empower the infected person with their fundamental rights of correct diagnosis and treatment. Drug-susceptibility testing is widely used as a tool for the selection of effective treatment regimens (particularly multi-drug-resistant tuberculosis), as well as for evaluation of programme efficiency and development of strategies to cope with the problem of drug-resistant tuberculosis.\(^{[1,2,6]}\)

**Limitations of Existing Infrastructure**

The paucity of the laboratories, which can carry out DST, is the major reason for the lack of sub national drug resistance surveys.\(^{[3]}\) India faces an unfortunate distinction where 3.4 million patients suffer from tuberculosis and is a country with a population of 1.1 billion harbouring the maximum TB patients in the world. In a country where every district should have at least culture facility and every medical college and tertiary care hospital should be equipped with primary drug susceptibility testing, it is quite distressful to mention that only four national laboratories are officially qualified for performing the anti-mycobacterial drug susceptibility testing. India has another distinction of having maximum patients co-infected with HIV and TB, a deadly combination. The liaison between the two most important pathogens makes the situation even worse and many patients having resistance to almost all available drugs are now being reported.\(^{[4]}\) Therefore, drug susceptibility testing of all isolates, especially in HIV-TB co-infected patients holds the key to effective management of MDR and XDR tuberculosis and government of India must act immediately on these lines.

**Recent Developments and Future Directions**

Traditionally drug susceptibility testing of mycobacterial isolates is performed on agar or Lowenstein Jensen (LJ) media using the proportion method which considered as a ‘gold standard’ but it is cumbersome, technically demanding and lacks reproducibility. However, in last two decades automation has revolutionized the microbiology services. The automated systems have dramatically reduced the time for isolation, increased the isolation rate and improved the drug susceptibility testing services. The only limitation of such systems is the requirement of infrastructure and the cost of consumables and thus, their use mainly remains limited to high resource settings. Hence there remains a need for rapid, easy to perform and cost-effective method for determining the drug susceptibility, preferably the minimal inhibitory concentration (MICs). Mshana et al.,\(^{[7]}\) in 1998 employed an easy-to-perform colorimetric method for drug susceptibility testing of *M. tuberculosis* based on oxidation-reduction indicator tetrazolium bromide. Later on Martin et al.,\(^{[8]}\) evaluated its cost-effectiveness and reported promising results. Historically, the principle of the test was first applied by Mosmann way back in 1983 for determining the growth and survival of cell lines.\(^{[9]}\) He used MTT (Microplate Tetrazolium Test) \[3-(4,5-dimethylthiazol-2-yl) - 2,5 diphenyl-tetrazolium bromide\] in the culture medium. The colour change from yellow to purple indicated the growth of cells. This method is highly desirable for resource limited countries like India. In this issue of IJMM Raut et al, describe its utility for rapid detection of MDR tuberculosis.\(^{[10]}\) The authors compared the assay with proportion method and the concordance was 90% by visual and 94% by RODU method. Sensitivity and specificity rates are also reported to be 86.8% and 100% respectively by visual method, and 95.2 and 87.5% respectively, by RODU. They conclude that MTT assay is a rapid and cheap method for performing drug sensitivity of *M. tuberculosis*. In our setting we have developed a similar but more simplified and quantitative tetrazolium microplate assay (TEMA) for determining MICs of streptomycin (STR), rifampicin (RIF), ethambutol (ETH), ciprofloxacin (CIP), ofloxacin (OFL), azithromycin (ATH) and clarithromycin (CLA) against clinical isolate of NTM.\(^{[5]}\) The microplate assay is also more useful for screening more number of strains in a single plate. However, the test should be done only in level-III bio-safety laboratories because of higher risk of occupational exposure. Besides the above mentioned tests, rapid phage based assay for detecting rifampicin resistance is also reported promising and may give results within three days. Recently, yet another single microscopic-observation drug-susceptibility (MODS) assay has been reported, in which broth cultures are examined microscopically to detect characteristic growth culture. This assay can also be used directly on the clinical samples including the sputum sample and offers more rapid and sensitive detection of multi-drug-resistant tuberculosis than the existing gold-standard methods. It has an agreement of 99% for detection of multi-drug resistance with standard methods. However, the problem of contamination and need of Level-III laboratory limit its application in resource poor settings.

In India there is an urgent need to strengthen the laboratories by ensuring a sound routine quality control program in test procedures, reagent performance, and personnel proficiency. These guidelines need to be formulated by keeping in mind the constraints pertinent to Indian settings. The involvement of different laboratories need to be encouraged by the Government. It is also possible that the laboratory heads come together proactively to form a network and participate in an external proficiency programme including the sharing of MTC strains having low, moderate and high levels of resistance to specific drugs.

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Conclusions

Directly Observed Therapy-short course (DOTS) is a highly successful programme but its limitation in smear negative pulmonary TB patients is being realised. Drug resistance to second line drugs is also being reported from India, which is a serious concern especially in AIDS patients and warrants inclusion of culture isolation and DST under the DOTS programme. With the advent of rapid tetrazolium microplate assay (TEMA) and single microscopic-observation drug-susceptibility (MODS) assay, it is high time that drug susceptibility services should widely be scaled up throughout India.

References


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