Chapter from the book *Understanding Tuberculosis - Global Experiences and Innovative Approaches to the Diagnosis*

Diagnostic Methods for *Mycobacterium tuberculosis* and Challenges in Its Detection in India

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1. Introduction

Tuberculosis (TB) is one of the world’s oldest and most important disseminating infectious diseases that still accounts for a high morbidity and mortality among adults. Despite high prevalence, case detection rates are low, posing major hurdles for TB control in developed and developing countries. Traditional diagnosis of TB bacilli depends upon smear positivity in sputum samples, culture and chest radiography. All these tests have known limitations. Conventional tests for detection of drug resistance are slow, tedious and difficult to perform in field conditions. For rapid diagnosis, new methods include newer versions of nucleic acid amplification tests, immune-based assays, skin patch test and rapid culture systems. For drug resistance analysis line-probe assays, bacteriophage-based assays, molecular beacons and microscopic observation drug susceptibility assay are available. An ideal test for TB is still not available and fast emergence of drug resistant tubercle strains aided by the ever-increasing HIV AIDS-epidemic in third-world countries has stressed the need of rapid diagnostic test(s) to show the presence of mycobacteria in the clinical samples. Microscopy and culture are still the major backbone for laboratory diagnosis of tuberculosis; new methods including molecular diagnostic tests have evolved over a period of time. The majority of molecular tests have been focused on: (i) detection of nucleic acids both DNA and RNA, which are specific to *Mycobacterium tuberculosis*, by amplification techniques such as polymerase chain reaction (PCR) focusing on detection and molecular epidemiology of *M. tuberculosis*; and (ii) detection of mutations in the genes which are associated with resistance to anti-tuberculosis drugs by sequencing or nucleic acid hybridization. The development and use of rapid diagnostic tools become increasingly important in addressing the emergence and treatment of multi-drug resistant (MDR) and extreme-drug (XDR) resistant *M. tuberculosis* strains.

Tuberculosis remains one of the most challenging bacterial diseases in spite of development of a realm of antibiotics and diagnostic molecular biology techniques. The tubercle bacillus was discovered more than two hundred years ago and substantial advancements have been made in our knowledge about the development of tuberculosis in human. The organism seems to evolve over a period of time in terms of its ability to survive the action of front line...
anti mycobacterial antibiotics by developing appropriate antibiotic resistant mechanisms. The estimated mortality by World Health Organization reports over 1.7 million deaths in 2006 and 9 million new cases of tuberculosis [WHO 2008]. The economic burden of management of disease in patients in the prime of their age is enormous because of prolonged antibiotic treatment. In spite of availability of anti mycobacterial drugs, tuberculosis remains one of the major health problems facing mankind particularly in developing countries. Presently, about one third of word’s population is infected with *Mycobacterium tuberculosis*. Currently, the number of people dying of tuberculosis is more than any other infectious diseases. Death from tuberculosis comprises 25% of all avoidable deaths in developing countries [Ramachandran and Parmasivan 2003]. Nearly 95% of all tuberculosis cases and 98% of deaths due to tuberculosis are in developing countries and 75% of tuberculosis cases are in the economically productive age group. Currently, more people die of tuberculosis than from any other infectious disease. In India, out of a total population of more than 1 billion, approximately 2 million develop active disease and up to half a million die of tuberculosis. It also imparts a financial burden on the economy in terms of out-put losses because of premature deaths and ill health. To add to the existing cost burden, the cumulative effect is seen because of ever increasing number of new TB cases associated with HIV patients and about 1.8 million of these are co-infected with TB [Ramachandran and Paramasivan 2003].

2. Traditional methods of tuberculosis detection, management and limitations

Robert Koch discovered the tubercle bacillus in 1882, and there after methods of staining these microorganisms were developed to assist the diagnosis of the disease. Early diagnosis of the tuberculosis in the patients is a challenging task especially in the pauci-bacillary and extra-pulmonary forms. The conventional methods that are still the mainstay of the diagnosis of TB like Tuberculin test/ Montoux test, radiological examination and other imaging methods and sputum smear microscopy have their own limitations. Sputum smear microscopy requires 10,000 to 1,00,000 organisms/ ml and acid fast bacilli could be any pathogenic or saprophytic mycobacteria. Although smear microscopy may be made more convenient by using various fluorochromes (auramine, rhodamine, FITC etc.) but the scarce presence of tuber bacilli in the sputum has its own disadvantage. The smear positivity has to be supplemented with the culture positivity that has its own limitations because of failure of bacilli to grow or often become contaminated with other microbes. The slow growth of the tubercle bacilli on medium lingers on the confirmation of the causative organism. Histopathology is characteristic but there could be problems to get representative specimen, and non-specific features. Immunoassay based approaches are doubtful as the antibodies and the antigens may persist for some time after control of the clinical or sub-clinical disease. Thus Acid Fast Bacilli (AFB) staining of clinical material followed by smear microscopy remains the most cost effective, frequently used microbiological test for detection of TB. The major drawback of sputum smear microscopy is its poor sensitivity, especially to be ~70% in a recent review [Steingart et al., 2006]. Although the AFB staining is easy to perform in the field settings especially in the poor third world countries as well in the developing countries but the sensitivity of sputum smear microscopy is clearly less in many settings and may be sometimes as low as ~35% in some situations with high rates of TB and HIV co-infection [Khatri and Frieden 2002]. Compounding the poor test sensitivity is
in adequate or absent test quality assurance in some recourse-constrained settings, further cut down the over all yield of the microscopy, driving up the laboratory workload as more sputum tests per patient are performed in an effort to reach a diagnosis, and increasing delay in diagnosis and patient’s compliance to repeated follow-up [Dorman 2010]. Moreover drug-susceptibility status cannot be determined from the smear microscopy.

Unfortunately, the world’s largest democracy India has over 1.2 billion people and this overpopulated country also has the highest burden of tuberculosis in the world. India accounts for about 20% incidence of tuberculosis besides a high incidence of global occurrence of multi drug resistant (MDR) tuberculosis. The poor sanitation conditions, thickly populated urban and rural area, scanty medical services in villages and rural area, malnourishment, insensitivity of private sector towards quick diagnosis, treatment and management of TB positive patients and higher cost of non-standard methods of diagnosis of TB are some of the important reasons of concern. It is obvious that any global effort towards control and eradication of TB and fast emerging MDR-TB is invariably dependent upon success of concerted efforts to contain the spread of this contagious disease. In India, the National TB Programme (NTP) was initiated in 1962. However, the poor infrastructure, inadequate funding, administrative lack-luster approach, irregular drug supply, non-standard and multiple anti-tuberculosis drug therapy, irregularity or non-compliance of patients to the clinician and a long treatment period had little effect on containing the spread of TB and controlling the emerging MDR-TB strains. The management and control of TB was further compounded and complicated with low rates of TB case detection, compliance of treatment regimen (30%), high rate of default (40-60%) and continuing high mortality (1: 2000) the NTP programme had little success rate. To overcome the deficiencies of NTP a Revised National Tuberculosis Control Programme (RNTCP) was launched by the Government of India in 1997, based on the global DOTS (Directly Observed Treatment, Short Course) approach that was used to exert an epidemiological impact by achieving 70% case detection and 85% cure rate. It was an encouraging sign that by 2002, 100% of the Indian population was covered by the India’s own drug-delivery model - the DOTS programme, making this extended coverage as India’s most significant public health accomplishment. The RNTCP thus achieved a pronounced success in cure rates (>80% in new infectious cases), substantial decline in mortality with low rate (<10%) of default [Khatri and Frieden 2002; TB India 2009; Bhargava et al., 2011].

3. Tuberculosis and HIV epidemic

In spite of improvement in public health system, participation of private sector in TB detection and management still the sputum smear microscopy test is most commonly used in public health settings to detect pulmonary TB. This method has roughly 50% chance of detection. Unfortunately, a significant number of people outside the public health sector, where the most common test is the serological (various types of ELISA for detection of M. tuberculosis antigens; or anti-M. tuberculosis IgG or IgM antibodies) that are expensive and means nothing. The global impact of converging dual epidemics of tuberculosis and human immunodeficiency virus (HIV) is one of the major challenges of the present time. In India, there are 2.5 million people living with HIV and AIDS at the end of 2007 while the incidence of TB was `1.8 million cases per year [WHO 2008, WHO Global Tuberculosis Program 1992].
In a survey carried out among new TB patients by RNTCP in 2007, HIV sero-prevalence varied widely and ranged from 1-13.8% across the 15 districts [Swaminathan and Narendran 2008]. Pulmonary involvement occurs in about 75% of all HIV-infected patients with TB [Devivanayagam et al., 2001; Ahmad and Shameen 2005]. Moreover, the interaction between HIV and TB in persons co-infected with HIV and TB is bi-directional and synergistic. As HIV progresses, there is cutaneous anergy as well as impaired tissue containment of mycobacteria leading to widespread dissemination of mycobacteria. While TB can develop at any CD4 count, extra-pulmonary and disseminated forms of the diseases are more common as immunodeficiency increases. Thus HIV infection is associated not only with an increased incidence of TB but also with altered clinical manifestations especially in the advanced stages of the disease. The cost management of anti-HIV and anti-TB therapy in the patients becomes a daunting task that compromises the success rate of containment and spread of TB from HIV infected patients. Current guidelines recommend that irrespective of HIV status, TB management require a minimum of 6 months of treatment with four drugs (including rifampin) in the intensive phase and two drugs in the continuation phase. In India, under the RNTCP, patients with newly diagnosed TB receive a 6-month thrice-weekly regimen 9cat I – 2EHRZ/4RH) while those with relapse, default or failure receive an 8-months regimen (cat II – 2EHRZ/1SEHRZ/5EHR). The lifetime risk of TB in immunocompetent persons is 5% to 10%, but HIV positive individuals; there is a 5% to 15% annual risk of developing active TB diseases [Swaminathan et al., 2000]. WHO estimated 9.2 million new cases of TB globally in 2006 (139 per 100,000); of whom 7,09,000 (7.7%) were HIV positive (WHO 2008). India, China, Indonesia, South Africa and Nigeria rank 1st to 5th in terms of incident TB cases.

The first and foremost step in the diagnosis of TB is its accurate and early detection. To achieve this objective a number of methods have been developed and reported (Table 1) that achieve early growth of M. tuberculosis [Katoch and Sharma 1997; Katoch 2004].

4. Anti-TB drug resistance

The overall pattern of drug resistance to first line anti TB therapy is similar in HIV positive and negative patients; however, MDR-TB is marginally higher (3-4%) in HIV positive patients with newly diagnosed tuberculosis status [Swaminathan 2005]. Rifampicin mono-resistance is more common in HIV infected patients and arises independently from mutations in drug susceptible strains. Treatment of MDR-TB should employ at least 3-4 new drugs. The regimen should include an aminoglycoside and be given under direct observation. Extensively drug resistant (XDR) TB strains have emerged and have been reported from India [Singh et al., 2007; Thomas et al., 2007]. Such strains appeared to be as an outcome of the mismanagement of TB. It seems that XDR-TB is practically untreatable and thus an attempt may be made to limit its spread by strengthening the TB control programme. Presently, there is no national policy regarding TB preventive therapy for HIV positive patients in India [Swaminathan and Narendran 2008]. A clinical trial conducted at the Tuberculosis Research Center, Chennai investigated two different regimens; a 6-month regimen of ethambutol and isoniazid vs. a 3-year regimen of isoniazid alone, in order to establish ideal duration of therapy. In a TB-endemic country like India, consideration shall be given to provide preventive therapy to HIV-infected persons. Line probe assays, a family
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Method</th>
<th>Concept</th>
<th>Reference(s)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>BACTEC system</td>
<td>Generation and detection of radioactive CO\textsubscript{2} from substrate palmitic acid. Used world-over, detection of growth in 5-7 d. Inclusion of (NAP: beta nitro alpha acetylamine beta hydroxy propiophenone) distinguishes \textit{M. tuberculosis} [inhibition] from other mycobacteria.</td>
<td>Venkataram et al., 1998; Bemer et al., 2002</td>
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<td>2</td>
<td>Mycobacteria growth indicator tube (MGIT)</td>
<td>Developed by Becton Dickinson, growth detection by non-radioactive fluorochrome detection; useful in drug screening, early detection of mycobacterium growth in 7-12 d.</td>
<td>Bemer et al., 2002; Tortoli et al., 1999</td>
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<td>3</td>
<td>MB/BacT system</td>
<td>Developed by Organon Technika; colorimetric detection of bacterial growth in, cultures; also useful for drug susceptibility testing</td>
<td>Brunello and Fontana 2000</td>
</tr>
<tr>
<td>4</td>
<td>TK Medium</td>
<td>Developed by Salubris, Inc., MA, USA is a novel colorimetric system that indicates growth of mycobacteria by changing its color, also discriminates between mycobacteria and contamination, and enables drug susceptibility testing. Test is low cost and simple. Sensitivity of TK medium is comparable to the LJ-medium.</td>
<td>Kocagoz et al., 2004; Salubris, Inc.</td>
</tr>
<tr>
<td>5</td>
<td>Septi-Check</td>
<td>Bi-phase system developed by Roche. Consists of enriched selective broth and a slide having non-selective Middlebrook agar on one side and two sections on other side: one with NAP + egg-containing agar, and second with chocolate agar for detection of contaminating microbes.</td>
<td>Isenberg et al., 1991</td>
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<tr>
<td>S. No.</td>
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<td>6</td>
<td>Reporter phages/ Bronx box</td>
<td>Use of mycobacterium-specific phage(s) and a reporter gene (luciferase) for detection of growth and drug-susceptibility to anti-TB drugs. Viability detection by either emission of light from microbe due to activation of luciferase gene or production of plaque on an indicator strain of mycobacteria; results availability in 2 d.</td>
<td>Riska et al., 1999; Wilson et al., 1997; Krishnamurthy et al., 2002</td>
</tr>
<tr>
<td>7</td>
<td>E-test</td>
<td>Use of gradient of drug on a paper-strip; useful for drug susceptibility testing of M. tuberculosis.</td>
<td>Kirk et al., 1998</td>
</tr>
<tr>
<td>8</td>
<td>Flow cytometry</td>
<td>Use of FACS for drug susceptibility testing, high cost of equipment and trained operator are the drawbacks.</td>
<td>Kakkar et al., 2000</td>
</tr>
<tr>
<td>9</td>
<td>Line-probe assay</td>
<td>A novel DNA strip-based test that uses PCR and reverse hybridization methods for rapid detection of mutations associated with drug resistance. Designed to identify M. tuberculosis complex and simultaneous detection of mutations associated with drug resistance.</td>
<td>Morgan et al., 2005</td>
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Table 1. Methods of early detection of M. tuberculosis.

of novel DNA strip-based tests use PCR and reverse hybridization methods for the rapid detection of mutations associated with drug resistance. These kits [INNO-LiPA Rif TB kit, Innogenetics NV, Gent, Belgium; GenoType MTBDR assay; Hain Life-science GmbH, Nehren, Germany] are not currently FDA approved for use in USA. Line-probe assays are designed to identify M. tuberculosis complex and simultaneously detect mutations associated with drug resistance. In June 2008, WHO announced a new policy statement endorsing the use of line probe assays for rapid screening of patients at risk of MRD-TB (http://www.who.int/tb/en/). However, the line probe assays are not recommended as a complete replacement for conventional culture and drug susceptibility testing. Culture is
still required for smear-negative specimens and conventional drug susceptibility testing is still necessary to confirm XDR-TB.

5. Ineffective TB diagnostics in India

Ineffective TB diagnostics are a lucrative market in India. Patients seeking TB care in the private medical institutes are commonly subjected to diagnostic tests i.e. the anti-body-based blood tests, including ELISA that are completely ineffective at detecting TB. This is because a large number of the world’s population has anti-TB antibodies, though only 10% of them do develop the active form of the disease. Obviously if patients who do not have TB are misdiagnosed, they could undergo 6-months of nasty toxic anti-TB chemotherapy. If patients have active TB and the test misses it, the disease may worsen and they may continue to spread the disease in their community. According to a preliminary analysis of over 80 labs in India, it is estimated that patients undergo more than 1.5 million useless TB antibody tests each year (WHO recommends against inaccurate tuberculosis tests by Kelly Morris; www.thelancet.com vol 377 Jan 8, 2011). The absence of any regulatory mechanisms results in the import of these dubious diagnostics from France, UK, USA and other countries, where these tests are not approved for TB diagnosis. These tests generate at least US $ 15 million. In a country that has ~100,000 labs this is probably a fraction of the enormous total market. Accurate diagnose is critical to the control of tuberculosis in India, particularly in view of the fact that India has set new targets as a part of RNTCP, which includes early detection of 90% of all TB cases by year 2015.

6. Molecular diagnosis of TB in Indian context

In India various institutes working on TB have sufficient technical expertise and financial affordability to use molecular diagnostic methods for detection of tubercle bacilli in the samples. For a laboratory with good sample load and which is using rapid methods for early growth detection the additional cost of sample analysis shall not be significant. If 10-25 isolates/ growths are assessed for identity simultaneously, additional cost for each isolate using a non-radioactive detection system like digoxigenin (DIG) should not be more than 200-250 rupees (~US $ 4-5). Similarly for a PCR system using primers, which are not patented, cost should be in similar range as prices of primers and reagents have considerably been reduced during the last couple of years [Katoch 2004]. The nucleic acid amplification tests (NAATs) are designed to amplify nucleic acid regions specific to the Mycobacterium tuberculosis complex. Such tests may be used directly on clinical samples/ sputum samples. Nucleic acid amplification test (NAAT) commercial kits are available under various brands like Amplified M. tuberculosis Direct Test (MTD; Gen-Probe Inc., CA, USA), the Amplicor M. tuberculosis (MTB) tests (Roche Molecular Diagnostics, CA, USA and the BD ProbeTec ET assay (BD Biosciences, MD, USA. Besides in-house lab developed PCR assays vary widely in their protocols and vary from lab to lab. In-house NAAT are cheap and are often used in research in developing countries where commercial NAATs are quite expensive to test large number of samples and thus in-house PCR technique/ protocol(s) provide a cheap option (Pai 2004). However, all the detection methods including the conventional AFB-staining, skin tuberculin test and new generation NAAT tests have some advantages as well as limitations (Table 2).
<table>
<thead>
<tr>
<th>Method</th>
<th>Use</th>
<th>Intended use</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB smear microscopy</td>
<td>Rapid tubercle bacilli detection</td>
<td>Community</td>
<td>Needs moderate training, microscope and low investment</td>
<td>Low sensitivity</td>
</tr>
<tr>
<td>Culture on solid media</td>
<td>Mycobacterial growth and drug</td>
<td>Referral lab</td>
<td>Good sensitivity; gold standard</td>
<td>Long time to detect growth of bacteria</td>
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<tr>
<td></td>
<td>susceptibility assay</td>
<td></td>
<td></td>
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<tr>
<td>Chest radiography</td>
<td>Pulmonary TB detection</td>
<td>Referral by</td>
<td>Indications and use not restricted to TB</td>
<td>Low specificity &amp; sensitivity, trained clinician needed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>clinician</td>
<td></td>
<td></td>
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<tr>
<td>Tuberculin skin test</td>
<td>Detection of <em>M. tuberculosis</em></td>
<td>Community</td>
<td>Extensive clinical and published experience</td>
<td>Sensitivity decreases in immunocomprised persons, positive reaction in BCG vaccinees</td>
</tr>
<tr>
<td>γ-Interferon release assay</td>
<td>Detection of <em>M. tuberculosis</em> infection</td>
<td>Referral to</td>
<td>Highly specific for <em>M. tuberculosis</em></td>
<td>Trained manpower, poor sensitivity especially in immunocompromised hosts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reference lab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Automated, non-integrated NAAT</td>
<td>Pulmonary TB detection</td>
<td>Reference lab</td>
<td>High sensitivity, rapidity and detection of mutations in MDR-TB strains.</td>
<td>Moderately trained personnel and equipment, laborious and possible cross-contamination among specimens</td>
</tr>
<tr>
<td>Culture in liquid media [MGIT; BacT/ Alert and others]</td>
<td>TB detection and as a prerequisite to drug-susceptibility testing</td>
<td>Referral lab</td>
<td>High sensitivity (more sensitivity than liquid media)</td>
<td>Long time for detection; less than solid medium but high contamination rate in some settings</td>
</tr>
</tbody>
</table>
Table 2. Tuberculosis diagnostic methods in use, recently endorsed by WHO and under development.

### 7. Challenges in the TB care and control

At present a vigorous approach is needed to proactively detect the TB cases under RNTCP. The DOT service providers may be actively involved in identifying fresh potential TB patients in their community and getting them diagnosed for TB. Another possibility is contact tracing of both adults and children diagnosed to have TB. Such approach is currently being followed in HIV programs and could be considered to improve upon the case detection rate. Further prevalence studies in different parts of India may be conducted systematically to determine the existing burden of TB. The district level data will be quite helpful in achieving a realistic figure of TB cases.

**Lab strengthening:** In most cases among the poorest strata of people living in slums or rural areas the sputum transportation is difficult to reach populations is a major consideration and TB is quite commonly said to be poor man’s disease. Thus inadequate number of microscopic centers/ labs put the burden on existing microscopic centers that causes a delay in the reporting of the results of the sputum samples. Most labs conduct AFB testing and are ill equipped to perform culture of tubercle bacilli.

**Migrant populations:** Presently, there is no national level strategy and guidelines for tuberculosis care and control for the migrant population in India who move from one state or place to another one as a part of their jobs or in search of jobs. Millions of migrants are currently working in unorganized job sectors with no health facilities, insurance facilities and work place policy for disease care and control like TB, HIV etc. Such workers are solely dependent on the relatively expensive private health sector for their healthcare. Accessing those migrants at their residences and working places with the key messages of TB, DOTS
and RNTCP is extremely challenging because of the geographically scattered areas and huge number of migrants. Also women engaged in unorganized job sectors are particularly prone to tuberculosis due to continuous exploitation by the employers. The migrant workers should be mapped in the urban and peri-urban areas (construction sites, street dwellers, illegal residents along the railway tracts, brick kilns etc.) and should be provided RNTCP services (like sensitization on TB, identification of suspected TB cases, referral and tracking) through community-based programs as part of the extended TB monitoring and care program and activities. Moreover, the TB component may be introduced into the existing HIV programs for migrant workers after collaboration with National AIDS Control Programs (NACO).

New TB testing tools: For 100% detection of the TB cases new technologies and techniques shall be establishes that are reasonably cheap, rapid and easily available. Recently a new PCR based diagnostic kit has been developed through a partnership between Cepheid and Foundation for Innovative New diagnostics, the University of Medicine and Density of New Jersey, the Bill and Melinda Gates Foundation and national Institute of Health (U.S.). The study demonstrated high sensitivity and specificity, identifying 98% of patients with TB and correctly identified 98% of bacteria resistant to rifampin.

Besides PCR, some novel tests involving use of beacons for the rapid detection of mutations associated with drug resistance have been reported [Varma-Basil et al., 2004]. Employing Xpert/RIF kit 1,700 patients were screened at five sites across the world including Mumbai, and using this PCR 98% of patients with TB and resistant to rifampin were correctly identified. This PCR needed about 100 minutes compared to current tests that may take up to 3 months to have results. Unfortunately, the PCR NAATs are performed in a few national labs and specialized private hospitals only. Facilities for culture and drug susceptibility testing of TB cultures in India are grossly inadequate [Bhargava 2011]. As of 2008, only 17 accredited facilities were doing culture and drug-susceptibility testing (~0.1 facility/ 10 million residents against 1/ 10 million). Efforts are underway to enhance the number of labs/ facilities to 43 to perform drug-susceptibility testing. The RNTCP has started to include rapid diagnostic methods to perform culture and drug-susceptibility testing so as to prevent delay in management of MDR-TB patients.

8. Conclusion

TB one of the most communicable diseases is still evading accurate diagnosis because of lack and development of cheap, less labor/ equipment intensive, highly specific and sensitive methods. Culture positivity in sputum positive samples is still considered to be a gold standard as this method provides a further lead in accessing the drug susceptibility of the grown mycobacterial cultures. Currently, most of the tools/ techniques in demonstration or late-stage validation are sputum based and thus are likely to result in incremental gains in rate of TB detection. Still there is an urgent need to develop and validate a mycobacterial culture based or NAAT-based technique that is close to 100% specificity and sensitivity. The need to fast develop such techniques is urgent because of development of MDR mycobacterial strains in third-world countries as these countries are also experiencing an increased burden of HIV-positive patients. The previous decade has shown how despite ‘100% coverage’ and impressive case detection and cure rates, TB continues to be an
epidemic of magnanimous magnitude in India. Thus in India a staunch collaboration between RNTCP, NACO and private partners is the need of the hour to contain the fast spread of MRD \textit{M. tuberculosis} strains.

9. References


Mycobacterium tuberculosis is a disease that is transmitted through aerosol. This is the reason why it is estimated that a third of humankind is already infected by Mycobacterium tuberculosis. The vast majority of the infected do not know about their status. Mycobacterium tuberculosis is a silent pathogen, causing no symptomatology at all during the infection. In addition, infected people cannot cause further infections. Unfortunately, an estimated 10 per cent of the infected population has the probability to develop the disease, making it very difficult to eradicate. Once in this stage, the bacilli can be transmitted to other persons and the development of clinical symptoms is very progressive. Therefore the diagnosis, especially the discrimination between infection and disease, is a real challenge. In this book, we present the experience of worldwide specialists on the diagnosis, along with its lights and shadows.

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