

TUBERCULOSIS: ADVANCEMENTS IN DIAGNOSIS AND DRUG SUSCEPTIBILITY TESTING

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INTRODUCTION

Among the communicable diseases, tuberculosis (TB) still remains one of the major public health issues. According to the World Health Organization (WHO) estimates, one-third of the world's population has been infected with *Mycobacterium tuberculosis* (MTB).¹ 95% of infections occur in the under-developed world where diagnostic and treatment facilities are limited or absent. About 9.2 million cases occurred worldwide in 2006 and with 1.7 million deaths.² It is estimated that around 70 million people will die from tuberculosis within next 20 years if adequate measures are not taken to stop the spread of tuberculosis.³ The risk of developing active disease in immunocompetent individuals is 10% in lifetime while it is 10% per year of life in immune-compromised individuals.⁴ The incidence of TB in Pakistan has been estimated to be 181 per 100,000 of population per year according to WHO estimates.⁵ Rapid emergence of multidrug resistant tuberculosis (MDR) has complicated the situation to control this entity.⁶ In Pakistan, 28% MDR strains have been from Northern areas.⁷

MDR-TB strains are emerging as a real challenge in the combat to control tuberculosis. The situation is more grave in developing and underdeveloped countries. The global prevalence of MDR-TB is 1.4% in primary cases and in previously treated persons it is 13%.⁸ MDR-TB is defined as tuberculosis resulting from organism that is resistant to both isoniazid and rifampicin. In recent years, extremely drug resistant (XDR) TB, that is a strain which is resistant to at least rifampicin and isoniazid (which is the definition of MDR-TB), in addition to any fluoroquinolone, and to at least one of the three injectable drugs used in anti-TB treatment, Capreomycin, Kanamycin and Amikacin, have posed serious threats all over the world. There are two types of resistance in case of tuberculosis that is primary and acquired. Primary drug resistance is one which occurs in a patient who has never taken anti-tuberculous treatment before and develops disease with an organism which is already resistant. Acquired drug resistance occurs in a patient already received or receiving treatment. Causes of resistance may be due to sub-

standard drugs, inadequate therapy, poor compliance, adding single drug to the failed regimen and lack of diagnostic facilities. Recently, the terminologies of primary drug resistance and acquired drug resistance have been changed to "drug resistance among news cases" and "drug resistance among treated cases" respectively.⁹

Despite continuous efforts in the field of mycobacteriology, direct microscopy for AFB in clinical specimens is still the main microbiological method used for the diagnosis/screening of pulmonary TB in under-developed world with huge burden of tuberculosis. This method though inexpensive, rapid and highly specific for the detection of AFB in sputum specimens, has sensitivity of 25 to 50%.¹⁰ The low sensitivity is a major drawback of this method as the number of tubercle bacilli must be 10^4 per mL of sputum to be visualized under direct microscopy. Another disadvantage of direct microscopy is that it fails to distinguish between tuberculous and nontuberculous mycobacteria.

Culture still remains the gold standard for the diagnosis of TB. Enormous research work has been done all over the world to develop new techniques for the culture and subsequent sensitivity of mycobacteria. Conventional egg based solid medium, Lowenstein Jenson (LJ) medium, still remains the method of choice for culture and drug susceptibility testing (DST) in most of the laboratories. However, conventional culture and DST method requires 6 to 8 weeks thus delaying the initiation of treatment. This leads to the spread of MDR strain among the community if therapy is not started promptly. There are many advancements in the field of mycobacteriology such as TB Bead test, LED microscopy, urinary nucleic acid amplification tests, mycolic acid analysis (HPLC) but these techniques are still under development. Our main focus will be on the modalities which are employed presently in various laboratories for culture and DST of mycobacteria.

Quantiferon TB Gold Test: Until recently, Tuberculin skin test was the only modality available for the detection of latent tuberculosis. However, this technique has many limitations. A recently intro-

duced test called Quantiferon TB Gold test uses whole blood as an aid in diagnosing tuberculosis. This test was approved by the U.S. Food and Drug Administration (FDA) in 2005. Interferon- γ (IFN- γ) is a cytokine produced by activated T-lymphocytes. It plays an important role in the immune response to tuberculosis. The antigens used in this test include mixtures of synthetic peptides representing two *M. tuberculosis* proteins, ESAT-6 and CFP-10. After incubation of the blood with antigens for 16 to 24 hours, the amount of interferon-gamma (IFN-gamma) is measured. If the patient is infected with *M. tuberculosis*, their white blood cells will release IFN-gamma in response to contact with the TB antigens. The advantages of this test are that it requires a single patient visit to draw blood sample, results are available in 24 hours and it is not affected by prior BCG vaccination.

Nucleic Acid Amplification (NAA) Methods: Development of Polymerase Chain Reaction (PCR) is a mile stone in the field of diagnostic mycobacteriology especially in extrapulmonary TB. Detection of *inhA* and *rpoB* genes by PCR can allow same day diagnosis of MDR. This method is costly and requires technical expertise. However, genotypic methods cannot replace the conventional modalities such as microscopy and culture. Many of the molecular methods are research tools and are not widely available. PCR has been widely used on various specimens for the detection of MTB DNA. Though the diagnostic utility of PCR in blood, other body fluids such as ascitic fluid, urine, pericardial fluid, pus from cold abscesses, and tissue biopsy specimens has been studied, available evidence is far from convincing.¹¹

Mycobacteriophage Assays: Mycobacteriophages are viruses that infect mycobacteria. Initially, phage typing was used for the speciation of mycobacteria by using mycobacteriophages.¹² Mycobacteriophages have been extensively investigated for their use in diagnostic microbiology in the last decade.

Automated Systems: The broth based BACTEC 460 system, introduced in 1980, has considerably improved the detection time of mycobacteria and it was a milestone in the advancement in mycobacteriology.^{13,14} However, this system had many drawbacks like radiation hazard, manual loading and unloading of vials, no inbuilt incubation system and lack of computer software. The fully automated, high capacity BACTEC MGIT 960 system is now being used as a rapid diagnostic system for tuberculosis in many developed countries. This system is fully automated, easy to use, non-invasive, non-radiometric and has high performance. This system has been evaluated at various laboratories in Pakistan with excellent results.^{15,16}

Combination of a solid media with one broth-based method such as MGIT 960 system is now widely accepted as "gold standard" for TB diagnosis.¹⁷

Thin layer agar (TLA) method: This novel technique uses Middle Brook 7H11 agar for the diagnosis and DST of mycobacteria. It has shortened the time period for culture of mycobacteria from 6 to 8 weeks to 1-2 weeks. This technique was described recently by Martin A.¹⁸ In Pakistan, this technique was first evaluated at Armed Forces Institute of Pathology (AFIP).¹⁹ This technique allows the observation of micro-colonies of mycobacteria under a simple microscope within two weeks. It can also give a presumptive identification of mycobacteria based on their characteristic cording and colony morphology. TLA has additional advantage to use para-nitrobenzoic acid (PNB) for the confirmation of MTB complex. Recently this technique has also been evaluated for simultaneous diagnosis and DST in one plate within four weeks.²⁰ It can be used alternatively to automated BACTEC systems in resource poor settings like ours.

Nitrate Reductase Assay (NRA): In the past decade, abundant research work has been done for the diagnosis and DST of mycobacteria by using calorimetric techniques. MTB has characteristic to reduce nitrate to nitrite in a medium containing KNO_3 . This reduction can be visualized with naked eye by adding Griess reagent as described by Musa *et al.*²¹ The advantage of this method is that it is fast, reliable, inexpensive and can easily be used in resource limited peripheral laboratories. This technique has high sensitivity and specificity and been evaluated at various places.^{22,23} More research is going on to optimize this technique for the DST of MTB directly on clinical specimens.

CONCLUSION

Recent advancements in the field of mycobacteriology have helped in reducing the time for detection and DST of mycobacteria thus providing timely information to the clinicians. Direct detection and DST of mycobacteria by TLA and NRA methods are encouraging modalities and can reliably be employed in low-income laboratories. Molecular methods allow same day detection of mycobacteria but they are expensive, need specialized equipment and technical skills.

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