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IJPBS |Volume 3| Issue 2 |APR-JUN |2013|108-116

Review Article
Biological Sciences

ADVANCE TOOLS FOR DETECTION OF MYCOBACTERIUM TUBERCULOSIS: A Review

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ABSTRACT

Mycobacterium tuberculosis is a pathogenic organism, which causes high morbidity and mortality in human being. The disease tuberculosis is commonly seen in immunocompromised patients. M. tuberculosis takes time to grow on culture medium (Lowenstein-Jensen medium) for drug susceptibility testing to avoid delay, now a day molecular techniques are introduced in many developing countries including India, the new technique i.e. line probe assay it gives the results of drug susceptibility within 2-3 hours. It can be minimizing the rate of morbidity and mortality due drug resistance strain. The techniques have good specificity and sensitivity.

KEY WORDS

Mycobacterium tuberculosis, diagnosis, line probe assay, PCR, ELISA.

INTRODUCTION

Tuberculosis (TB) is an ancient disease which most commonly seen in immunocompromised individuals and leads to morbidity and mortality of patients, it is necessary to detect the organism in patients and their drug susceptibility testing and start the anti-tuberculosis drug for saving the life of peoples.

TB is an ancient disease that still remains a major global health problem. Mycobacterium tuberculosis infection is around 32% i.e. approximately 1.9 billion people. According to the world health organization 8.8 million new cases of pulmonary tuberculosis was estimated. Around 1.9 million people died of tuberculosis in immunocompromised patients. [1] Incidence of TB in India i.e. more than 40% population infected, around 15 millions are suffering and approximately half a million die every year with this disease.

TB especially multidrug-resistant TB (MDR-TB) is a global problem and a prevalence of MDR-TB as high as 26.8% has recently been reported.[2-4] MDR-TB strains are generally resistant to at least isoniazid, rifampin 2, 33% M. tuberculosis has resistant to one or more Antituberculous drug, 26% resistant to at least isoniazid and 19% resistant to both rifampin, isoniazid was reported. [5]

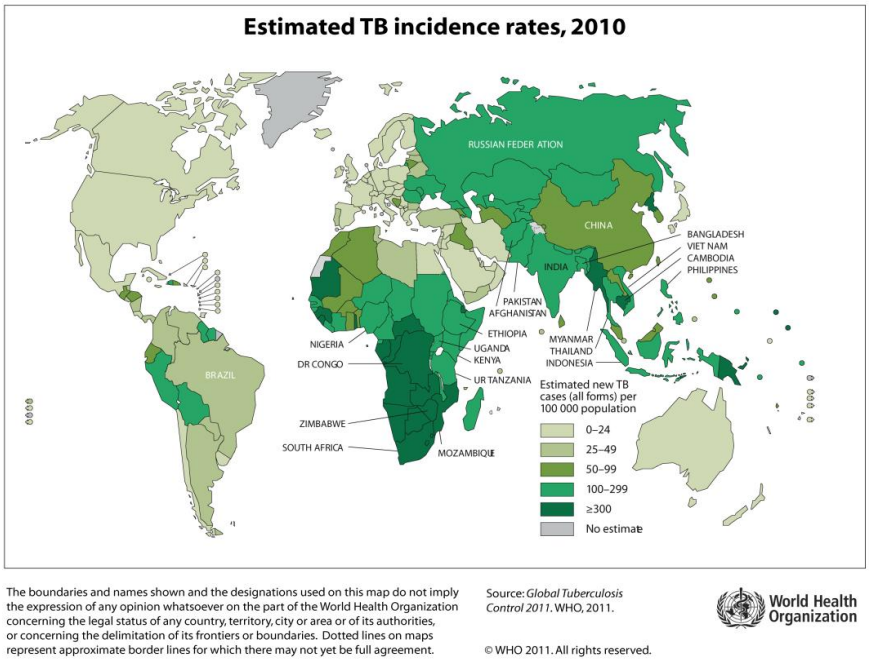

Fig. 1: The incidence rate of tuberculosis disease in 2010.

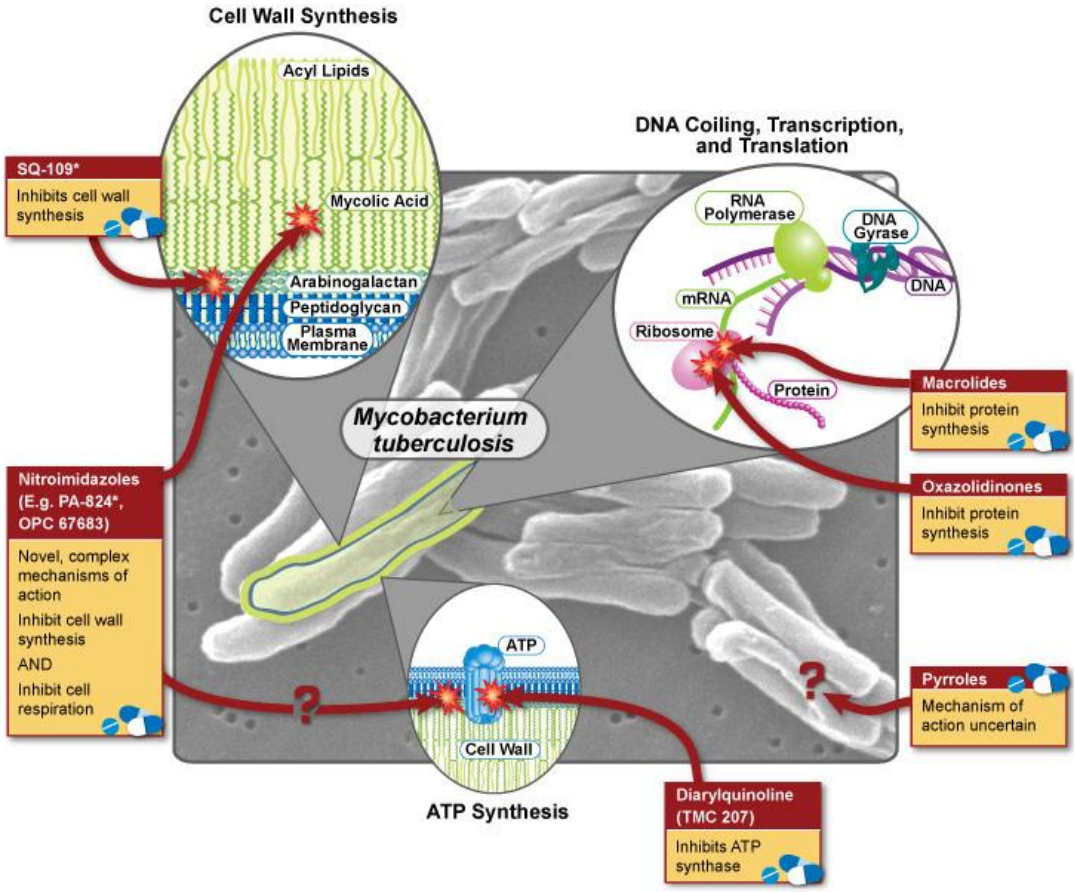

Fig. 2: Drugs action in case of tuberculosis disease.

TECHNIQUES FOR DIAGNOSIS OF MYCOBACTERIUM TUBERCULOSIS

Geno Type MTBDR*plus* assay

Geno Type MTBDR*plus* strip (Hain Life Science, Nehren, Germany) is a test which allows to simultaneous detection of *Mycobacterium tuberculosis* and their first line and second line drug susceptibility pattern and species characterization of Mycobacterium. The performance of MTBDR*plus* molecular assay was useful for rapid detection of MDR TB directly from sputum[6-7]. Several study reported that the Geno Type MTBDR*plus* assay has good results directly from positive smear and give good results of drug resistance in Mycobacterium tuberculosis[8-11]. Now a day this techniques is very useful and many tertiary care centre established the technique at their laboratory.

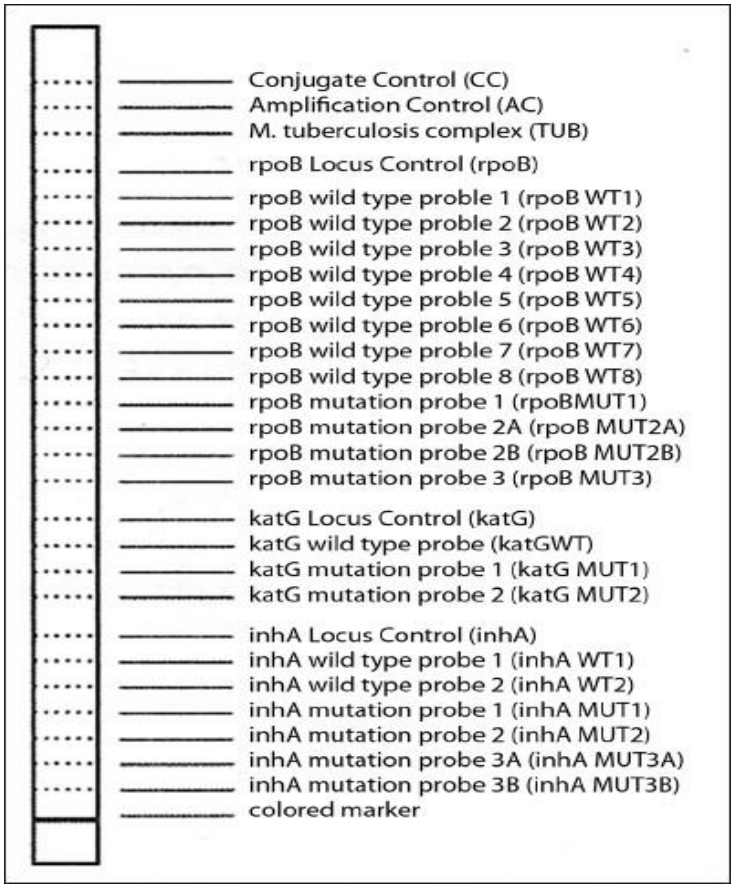

Fig. 3: Geno Type MTBDR*plus* strip (Hain Life Science, Nehren, Germany)

Septi-chek AFB

Septi-chek AFB (Roche Diagnostic Systems, Nutley, N.J.) is a test which allows simultaneous detection of Mycobacterium tuberculosis and Non-tuberculosis Mycobacteria (NTM), Septi-chek AFB can be used for the detection and isolation of Mycobacteria from various clinical specimens i.e. pulmonary and extra-pulmonary. The unique advantage of this technique is the simultaneous detection of MTB, NTM, other respiratory pathogens and even contaminants. [13]

Microscopic observation broth-drug susceptibility assay (MODS)

The Microscopic observation broth-drug susceptibility assay (MODS) technique required only a strings and tangles by simple light microscopy. This MODS assay, unlike other rapid detection systems, does not require either

radioactive isotopes or fluorescent indicators. It is particularly well suited for use in developing countries, as it is rapid, reliable, and inexpensive method that give the results in 9 days within inoculation. MODS has good alternative for susceptibility testing in developing countries. Drug susceptibility test of MDR TB if not timely diagnosed, it may cause morbidity and mortality in patients. Susceptibility tests are not performed in many areas where TB is endemic, often because rapid testing systems are expensive, solid culture tests often renders the results clinically irrelevant. Susceptibility results on solid medium are generally takes 4 to 5 weeks after inoculation, even when done directly from sputum concentrates. MODS give the result of drug susceptibility testing directly from the patient sample within 2 weeks. [14]

BACTEC 460TB

BACTEC 460TB (Becton Dickinson, Sparks, MD, USA) is the best method for rapid drug susceptibility testing of *M. tuberculosis* i.e. rifampicin, isoniazid, ethambutol, pyrazinamide and streptomycin. [15] The level of agreement between results from the MB/BacT system and those from the BACTEC 460TB method was 96%. Concordance values for isoniazid (96.3%), rifampin (98.8%), and ethambutol (98.8%) were good. The level of agreement for streptomycin was (90.2%).[16] Study showed that the BACTEC 460TB radiometric method was give 87% of the positive results within 7 days and 96% within 14 days of inoculation. The BACTEC 460TB method is considered to be inexpensive and provide fast result in countries which endemic for tuberculosis[17].

MB/BacT

MB/BacT (Organon Teknika, Turnhout, Belgium) is a non-radiometric continuous monitoring system which is designed for the isolation of mycobacteria from various clinical specimens. It utilizes a colorimetric sensor and reflected light to continuously monitor the CO2 concentration in the culture medium. [18] A study showed that the mean time for the detection of M tuberculosis from sputum, cerebrospinal fluid (CSF) and urine samples was 17.5 (±6.4) days for MB/BacT, 14.3 (±8.2) for BACTEC and 24.2 (±7.5) days for egg-based media cultures.[17] The study concluded that MB/BacT is an acceptable alternative for BACTEC 460 despite some minor disadvantages such as increased contamination and slightly longer time for detection of growth[18].

Bactek Mycobacteria Growth Index Tube (MGIT)

The MGIT is a nonradiometric, flourescence, manual or automated system for rapid detection of mycobacterium species from various clinical samples. It consist of a glass tube with modified 7H12 broth base, enriched with basic nutrients for growth of mycobacteria and a mixture of antibiotics to inhibit the growth of contaminating bacteria. A fluorescent compound is embedded at the bottom of the tube. The flourescent compound does not flourence in the presence of oxygen, but following depletion of oxygen as a result of mycobacteria growth there is fluorescence which can be detected visually under UV light. In the automated version (MGIT 960 system), fluorescence is detected by a sensor. The system holds 960 tubes which are continuously monitored[19].

Chromatographic technique

The technique is based on the difference in length of mycolic acid residue on the cell wall of different species of mycobacteria. It is used for species detection from colonies isolated on cultural medium. Reference laboratories used this technique for epidemiological studies.

Chromatographic techniques have changed as technology has evolved. Column chromatography and thin layer chromatography has been replaced by gas chromatography and most recently by high pressure liquid chromatography (HPLC). Chromatography is rapid test and time taken is 2 hours. The technique is more yieldable in a small quantity of bacterial colony. [20] The disadvantage of the technique is expensive. The use of negative-ion mass spectrometry to detect tuberculostearic acid in clinical specimens is also gaining ground and hold promise for the rapid detection of mycobacteria [21, 22]

Xpert MTB/RIF

The method introducing as Xpert MTB/RIF which is an automated molecular test for Mycobacterium tuberculosis and their resistance pattern to detecting for rifampin, the method is based on Cepheid GeneXpert system. The machine is hemi nested real time PCR is uses for assay to amplify a specific sequence of the rpoB gene, which is then probed with molecular beacons for mutations within the rifampin-resistance determining region, providing a result within two hours. [24]

Nucleic acid amplification (NAA)

Nucleic acid amplification is the best technique that allows for both detection and identification of Mycobacterium tuberculosis with the help of enzymatic amplification of bacterial deoxyribonucleic acid (DNA). Now a day the most widely used technique is PCR, but transcription mediated amplification (TMA) and strand displacement amplification (SDA) are commercially available which also be used to alternative of PCR. The sensitivity of Nucleic acid amplification test is significantly greater than smear microscopy but it is slightly low specificity than culture techniques. [25] NAA test is commonly done for confirmation and comparison with ZN staining and microscopy. This technique gives good result and it takes less time. it is expensive method and also have complexity due this region this method are commonly used in developing countries. [18,25]

Polymerase Chain Reaction (PCR)

The PCR is a molecular techniques that makes sequences of DNA which is amplified and make thousands of copies from very few DNA of mycobacterium tuberculosis, the process are based on three different temperature that yield number of DNA copies from a single DNA, when the amount of amplified DNA high it can be rapidly visualized and identified by the PCR machine. [17] Studies reported that the most common target used for PCR is insertion sequence IS6110.[17, 26] This sequence is specific for Mycobacterium tuberculosis and it facilitate multiple targets for amplification, it can be up to 20 times in the genome.[17] A study reported that the PCR was positive in 95% from positive culture, the sensitivity is high of the PCR for detection of Mycobacterium tuberculosis in positive culture. [27]

Ligase chain reaction (LCR)

Ligase chain reaction is another alternative for DNA amplification; it is based on the ligation of two adjacent synthetic oligonucleotide primers which hybridize to one strands of DNA. [23] A second pair of oligonucleotides hybridizes to the complementary DNA in the same region. [17] When the nucleotides are present, the DNA polymerase and the ligase create a gap between the adjacent primers, which was then filled by nucleotides which leads to ligation of the primers. [23]

QuantiFERON-TB Gold

QuantiFERON-TB Gold (Cellestis Limited, Carnegie, Victoria, Australia) is based on the principle of ELISA test that detects the release of IFN-gamma in fresh heparinized whole blood from individual after incubation with synthetic peptides simulating ESAT-6 and culture filtrate protein-10 (CFP-10). The test procedure for QuantiFERON-TB Gold it involves the blood sample collection, add the antigen which stimulate the IFN-gamma and then incubated at 37ºC for a period of 16-24 hours, harvesting of plasma and addition of conjugate solution. The samples are then incubated for two hours at room temperature, the plates are washed at least six times and then the substrate is added. The samples are then incubated for 30 minutes, adding stop solution, reading absorbance at 450 nm and calculating results using dedicated software. The patient only needs to visit once, for samples collection, and results can be obtained in 48 hours. [18]

QuantiFERON-TB Gold In-Tube

The QuantiFERON-TB Gold In-Tube (Cellestis Limited, Carnegie, Victoria, Australia) was developed to overcome the limitation of QuantiFERON-TB Gold, which could only be used in facilities where blood testing could begin within a few hours of its collection. [29]

T-SPOT.TB test

T-SPOT.TB (Oxford Immunotec Limited, Abingdon, United Kingdom, 2008), the peripheral blood mononuclear cells are mix with peptides (ESAT-6, CFP-10) and enzyme-linked immunospot assay (ELISpot) are used to detect the increases number of cells which produces IFN-gamma (spots in each test well). [29,18] A study showed that the QuantiFERON-TB Gold In-Tube, T-SPOT.TB and tuberculin skin test (TST) in 373 HIV-infected patients,54,55 reported that IGRAs were more sensitive than TST for the diagnosis of Mycobacterium tuberculosis. [30]

ELISA

A study reported that the indirect ELISA tests in which monoclonal antibodies are used against the purified Ag 85 complex. In a study the blood samples were collected from 197 patients in plane tube containing no anticoagulant and centrifuged the samples and serum was collected. The test showed that sensitivity and specificity was 82% and 86%. [31] For antigen detection a number of affinity-purified antibodies have proven to be useful in the diagnosis of Mycobacterium tuberculosis infections. Among these, the excretory-secretory protein ES-31 was one of the first antigens to be detected. A number of antigens which can be detected when present at a concentration of 3-20 ng/mL include mycobacterial sonicates, tuberculin purified protein derivative (PPD) and antigens 5, A60, P32 and LAM, detected through sandwich or inhibition ELISA, latex agglutination or reverse passive hemagglutination (RPHA) tests.[18] The reported sensitivity of these tests were decreased i.e. 40-50% and specificity was 80-95%. [31]

Adenosine deaminase (ADA)

Adenosine deaminase (ADA) has been proposed to be a useful surrogate marker for tuberculosis in pleural, pericardial and peritoneal fluids. The results of a study performed in India revealed a sensitivity of the test was 100%, a specificity of the test was 94.6%, and a cutoff value of 40 U/L for ADA in pleural, peritoneal and pericardial fluids are reported. [28,18]

ZN Staining

ZN Staining it is first described by Paul Ehrlich and then modified by Zeihl and Neelsen, after modification it is known as ZN stain. Smear for

AFB is still the most widely used diagnostic tests in the laboratory with low sensitivity (50-600/0) but very high specificity. The concentration of specimens greatly influences the outcome of AFB smear results. For the digestion and decontaminant of specimens are concerned, no effort has been made to improve the old sample processing techniques. A new cytocentrifugation method has been reported but its role in increasing the sensitivity of AFB smear remains uncertain. [12]

Culture on LJ medium

Culture is still the gold standard for diagnosis of mycobacterial infection in general and for TB in particular. Culture for AFB is going to remain in use for a long time despite the introduction of molecular and other techniques. Most of these new tests would be add-on tests for quite some time. It is known that about 40-60% of positive culture specimens showed smear negative. That means that if AFB smear is used for diagnosis of TB, such a large number of TB patients are going to be missed. These TB patients have less than 10,000 bacteria per ml of specimen and are considered less infectious but are important to be diagnosed and treated. [12]

CONCLUSION

We concluded that the advance method for detection of Mycobacterium tuberculosis has many advantages over old method for TB detection. The Geno Type MTBDRplus strip (Hain Life Science, Nehren, Germany) is widely used and available the results within 2-3 hours. The septi-chek AFB method is available which can differentiate Mycobacterium tuberculosis from Non-Mycobacterium tuberculosis. The MODS are given the result of drug susceptibility testing directly from the patient sample within 2 weeks. For culture of mycobacteria MB/BacT (Organon Teknika, Turnhout, Belgium) and BACTEC 460TB (Becton Dickinson, Sparks, MD, USA) are available for better results. Molecular method like PCR and RT-PCR which give the accurate result even the microscopy is negative because PCR is highly sensitive and specific method that can provide a better result Xpert MTB/RIF is an automated molecular test for Mycobacterium tuberculosis and their resistance pattern to detecting for rifampin, the method is based on Cepheid GeneXpert system. Few studies are also reported that the Adenosine deaminase (ADA) has been useful surrogate marker for tuberculosis in pleural, pericardial and peritoneal fluids.

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