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MICROSCOPIC OBSERVATION OF DRUG SUSCEPTIBILITY TESTING-RAPID, RELIABLE AND COST EFFECTIVE METHOD TO DETECT DRUG RESISTANCE TB

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ABSTRACT

Introduction: About one third population of the world are infected with Tuberculosis and now, it become a serious global concern of public health. Multi drug resistance tuberculosis(MDR-TB) is also global health problem. Conventional method and automated methods for the detection drug resistance tuberculosis are either slow or costly. Microscopic observation of drug susceptibility testing (MODS) is use to detect drug resistance TB directly from the sputum and this method is based on the characteristic growth of Mycobacterium tuberculosis (MTB) in liquid media. Aim: Comparison of MODS assay with Conventional culturing method with respect to: detection of Mycobacterial growth and time taken for the culture positive. Comparison Drug resistance between MODS and Conventional method. Method: Total 207 samples were enrolled in this study which were acid fast smear positive sputum among 496 suspected patient. Decontamination procedure were done on all smear positive samples and it used in culturing and Drug susceptibility test (DST) for MODS assay and for conventional DST method. In MODS assay Middle brook 7H9 broth was used as media, with and without critical concentration of Isoniazid (INH) and Refampicin (RIF). Result: Median time taken by the MODS test was 10-14 day for culture and DST where as 22 days for culture and 66 days for DST took by Conventional method. Sensitivity and specificity was 100% and 97.4% respectively. It was less expensive than Conventional DST method. positive prediction value and negative prediction value was 100%. Conclusion: MODS assay offers a rapid, simple, economical and feasible method for simultaneous culture and DST of MTB.

KEYWORDS: Mycobacterium tuberculosis, Multidrug resistance tuberculosis, Drug susceptibility test. Microscopic drug susceptibility test.

INTRODUCTION

Tuberculosis (TB) remains one of the world's deadliest communicable diseases. One third of the world's population is infected with TB. An estimated 9.0 million people developed TB from that 1.5 million died from the disease In 2013 (WHO 2014). India is the country with the highest burden of TB an estimated incidence 2.1 million cases of TB for India out of a global incidence of 9 million. (Global control of tuberculosis 2014). World Health Organization (2014) estimated that 3.5% of new cases and 20.5% of previously treated cases of TB were of MDR (Multi Drug Resistant) TB. Drug resistance surveillance data show that an estimated 4,80,000 people developed MDR-TB in 2014 and 1,90,000 people died as a result of MDR-TB. The global emergence of Extensively Drug Resistant Tuberculosis [XDRTB] has complicated the problem.

Under the Revised National Tuberculosis Control Programme [RNTCP], sputum microscopy is use for diagnosis as well as for monitoring response to therapy, But it cannot be used for assessing drug susceptibility status.[2,3]

There is an urgent need to identify methods that would rapidly detect both the presence of Mycobacterium tuberculosis [MTB] and drug resistant status. Culture based methods are considered as the gold standard[4], but it take so much time to detection and require expensive equipment and reagents.

Molecular method can detect MTB and Drug resistance TB in less time but require costly instrument and all laboratory can not afford this instrument .Molecular method is very costly.

It was therefore important to identify tests that would be economical, yet reliable. The Microscopic observation Drug- Susceptibility (MODS) assay is a novel, rapid, simple, tissue culture based direct assay which relies on formation of characteristic cord like growth of MTB in liquid culture medium.[5]

The objective of this study is early detection of drug resistance among TB patient and evolution of simple and inexpensive method.

MATERIAL AND METHOD

A total of 495 clinical samples (sputum) were obtained from patients of suspected pulmonary TB from June 2013 to august 2015. The patient were asked to take early morning sputum. when he wakes up without brushing the teeth, asked to a deep breath and expectorate sputum in given wide mouthed sterile plastic container

Microscopy

All sputum samples were processed for acid fast microscopy and reported according to RNTCP guidelines.[6]

Decontamination and concentration

Smear positive samples were digested and decontaminated using NALC-NaOH method as per standard protocol and then centrifuged at 3000g for 15 minutes.[4] Decontaminated samples were used in the MODS assay and in conventional DST method.

MODS assay

The procedure was carried out as described by Moore et al.[5]

2.3.1 **S**upplemented Middlebrook (MB) 7H9 broth and working concentration of isoniazid (INH) (4µg /ml) and Rifampicin (RIF) (10µg /ml) were prepared

Inoculation of MODS plate

500µl of decontaminated sediment was suspended in 4.8 ml of supplemented MB 7H9 broth (sample-broth mixture). This was used to inoculate 24 wells sterile tissue culture plates [Becton and Dickinson, USA] for MODS assay. For each sample, four wells in a column of a tissue culture plate were used (two drug free controls and two drug containing wells). To each of the four wells, 900µl of sample-broth mixture was added keeping 1.7 ml of sample-broth mixture as "backup". In the first two wells, 100µl of supplemented MB 7H9 broth was added. These two wells acted as growth controls. In the third well, 100µl of INH (4µg/ml) was added to obtain the final concentration of 0.4µg/ml. In the fourth well, 100µl of RIF (10 µg/ml) was added to obtain the final concentration of 1µg/ml. In each plate, column three was used as negative control containing only supplemented MB 7H9 and either INH or RIF. Any growth in this lane indicated cross contamination. On each processing day, two positive controls were run. H37RV was used as a drug sensitive control and one known MDR strain was used

as drug resistant control. Tissue culture plates were sealed in plastic zip lock bags and were incubated at 37°C.

Microscopic observation of MODS Plates

The plates were examined daily within the zip lock bags only, using an inverted microscope beginning from day three. MTB growth was reported qualitatively by visualization of the characteristic serpentine or tangled cord like growth formation. The Microscopic observation for DST was taken on the same day as culture positivity in control well. Bacterial or fungal contamination was identified by clouding of media within three days.

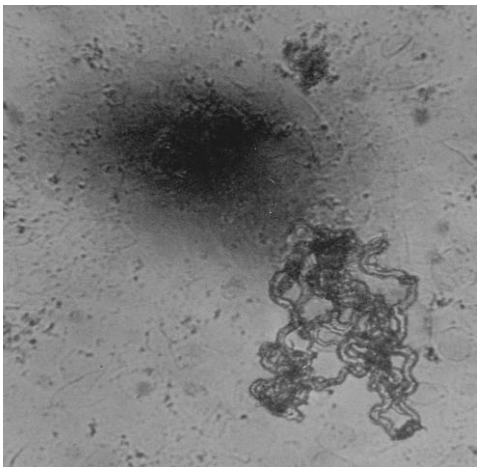

FIg 1: Cord formation

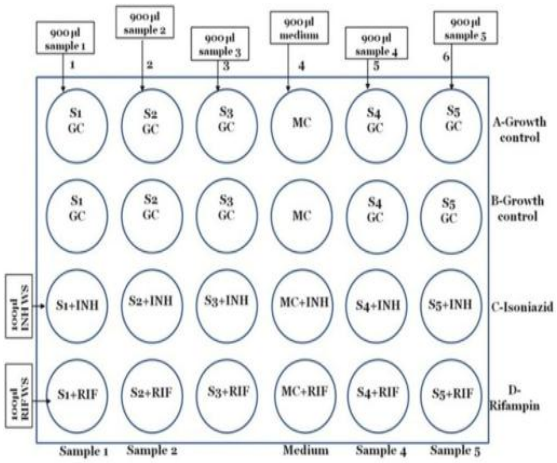

Fig 2: MODS plate

CONVENTIONAL METHOD

Culture on LJ medium

Decontaminated sputum was inoculated on LJ media and reading of culture isolates as MTB was done as per WHO protocol.[4]

Conventional DST method

DST by proportion method was as described by Canetti et al.[7]

RESULT

from 496 suspected pulmonary TB sample 207 samples were found positive by smear microscopy. All Smear positive samples saw culture positive by both MODS assay and LJ method. Total 207 samples were enrolled in this study and from that 77 found to have MDR TB, 20 were Only INH resistance and 17 were RIF resistance. When it was compare to Conventional DST method MODS had 100% sensitivity and 98.4 % specificity.

Table 1: Comparison of Both method on the basis of Time taken for the culture

Sr. no	Method	Time for culture	Time for DST	Cost
1.	MODS	10-14 days	10-14 days	50
2.	Conventional DST	20-28 day	60 days	250

DISCUSSION

In the present study in smear positive patients, the culture positivity rate was 100% for both MODS assay as well as LJ medium. Very high culture positivity rates (92%-98%) using MODS assay have been observed in studies which have included even smear negative patients(Moore DA et al.,2007; Oberhelman RA et al.,2006; Arias M et al.,2007).[9,11]

In present study shows MODS have high sensitivity (100%) and high specificity (98.4 %). High Sensitivity (100%) and specificity (100%) found by Kashmira limye et al., 2010 using MODS assay to detect drug resistance TB. High Sensitivity and specificity to detect Drug resistance TB found by many scientists using MODS method Time taken for diagnosis of drug resistance TB in present study was 10-14 days using MODS and by using Conventional method it was 60 days. MODS gave result quickly than conventional method. MODS assay detect drug resistance TB in less time than conventional method(Arias M et al.,2007; Leung E et al.,2012; Benson Kidenya et al.,2013).[11,13]

Cost for detection using MODS was 50 Rs. and by Conventional DST method it was 250 Rs. in present study. 80 Rs using MODS assay and 700 Rs. using Conventional DST method by Kashmira limaye et al., 2010. According to Leung E (2012) cost of MODS per test was $1.48 and by Benson kidenya (2013) cost was $ 4.39.

Once standardized, MODS assay is feasible to perform in resource constrained settings with trained manpower. The equipment required for MODS assay is similar to that required for culture of mycobacteria. Inverted light microscope is the only item which many laboratories lack and need to invest in.

CONCLUSION

Based on the results of the present study and available literature, it can be concluded that MODS assay can be used for early and accurate detection of MTB and MDRTB. Given its simplicity, low cost and reduced turnaround time (within two weeks), it would be an excellent method for routine tuberculosis testing in developing countries, if recommended as standard method.

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