

A comparative study between microscopy and culture in detection of M.tb among smear negative pulmonary and extra pulmonary tuberculosis

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Abstract

Introduction: Tuberculosis (TB) is still a leading cause of death in many parts of the world. Adept techniques of rapid detection, isolation, identification and susceptibility testing of Mycobacterium tuberculosis (M.tb) are essential for management. Our aim was to compare the sensitivity and rapidity of smear microscopy, concentration method, rapid slide culture, Lowenstein - Jensen (LJ) culture and MGIT semiautomated culture methods in detection of Mycobacterium tuberculosis among smear negative pulmonary TB and extra pulmonary TB cases and to detect the drug resistance pattern to first line drugs among M.tb isolates.

Materials and Methods: A prospective study was conducted for one year between January 2016 to January 2017 in the Department of Microbiology at a tertiary care hospital. Sputum smear negative pulmonary and extra pulmonary specimens were subjected to direct microscopy by Zeihl-Neelsen and fluorescent staining. Culture was done by Rapid slide culture, Lowenstein-Jensen media (LJ media) and BACTEC MGIT system. Positive isolates were confirmed by appropriate biochemical reactions and rapid immunochromatographic test. Drug susceptibility testing was done for first line drugs.

Results: The sensitivity of direct microscopy was 3% and after concentration it was 4%. Overall culture positivity was 12%. Detection rate by LJ method, rapid slide culture method and MGIT methods was 6%, 4% and 12% respectively. The mean detection time was 22.5 days by MGIT and 31.5 days by conventional LJ method.

Conclusion: This study highlights the importance of culturing suspected smear negative pulmonary and extra pulmonary Tuberculosis cases prior to empirical therapy. Newer automated culture methods aid in earlier detection of cases.

Keywords: Extrapulmonary tuberculosis, MGIT, Mycobacterium tuberculosis, Smear negative tuberculosis.

Introduction

TB is still a lingering global threat. India, falls under the high burden label.¹ According to WHO (2016) statistics, around 2 million people developed TB in India. Out of which 28% are smear negative cases of pulmonary tuberculosis and 19% are extra pulmonary tuberculosis, 6% of them develop drug resistance and account for 26% of the total deaths due to TB.² Smear negative TB and extrapulmonary TB being paucibacillary are important challenges in the road to achieve the "End TB strategy" and inefficient diagnosis is a major pitfall in the path. Direct smear microscopy, though cheap and rapid, lacks sensitivity. Isolation in culture requires a long time, because of which there is a need for a rapid method which has good sensitivity and specificity for the detection of M. tuberculosis.

Materials and Methods

A prospective study was conducted for a period of one year between January 2016 to January 2017 at a tertiary care hospital in Bangalore. We included the specimens from the following patients:

1. Patients having radiological lesions suggestive of active pulmonary/ extra pulmonary tuberculosis.
2. Patients having strong clinical evidence suggestive of extra pulmonary Tuberculosis and
3. Sputum smear negative for M.tuberculosis among clinically suspected pulmonary TB cases with the help of Department of Respiratory Medicine.

Sputum, gastric lavage, bronchoalveolar lavage fluid and extrapulmonary samples such as Lymphnode aspirate, Pleural fluid, Cerebrospinal fluid, Synovial fluid, Urine, Ascitic fluid, Pus aspirate or tissue specimen collected under aseptic precaution in a wide mouth container and transported to laboratory without any preservative were processed as follows:

Microscopy: Direct smear was prepared from the purulent portion of the specimen on a clean slide on an area of 2 × 1 cm. They were air-dried, heat fixed and subjected to Zeihl-Neelsen staining (ZN stain) and Fluorescent staining as recommended by National Tuberculosis Institute.³ Smears were graded according to RNTCP guidelines.⁴ Modified Petroff's method was used for decontamination of sputum samples.⁵ Smears were also made after concentration and subjected for ZN and fluorescent staining.

Modified Slide Culture/ Rapid Slide Culture Technique: From the centrifuged sediment, smear was done over the lower one-third of two slides using one half of the longitudinally cut slide. The slides were put inside a Mc cartney bottle with the smear immersed in a medium containing seven ml of citrated human blood diluted with deionized water and made selective with addition of Polymyxin B (2,00,000 units/L), carbenicillin (100mg/L), Trimethoprim (10mg/L) and Amphotericin B (10 mg/dL). The inoculated medium was incubated for seven days at 37°C. On the seventh day the slides were dipped in sterile water, dried in hot air oven at 80°C for 30 minutes, stained by ZN method

and fluorescent method. Slides were then examined under oil immersion for microcolonies of acid fast bacilli.

The growth was recorded and graded. The readings of zero grade was taken as negative and those of grade One to four were taken as positive.

Grade 0- No division of acid fast bacilli as compared to control slide.

Grade 1+ small clumps upto 4 bacilli were present but absent in the control.

Grade 2+ large clumps of bacilli

Grade 3+ large clumps with some cord formation

Grade 4+ Micro-colonies with good cord formation.⁶

Culture on LJ medium: From the sediment, one loopful each was inoculated on to two slopes of Lowenstein-Jensen medium using a 5 micron, 22 gauge, nichrome wire loop. Date of inoculation was noted. The slopes were incubated at 37°C for a maximum period of eight weeks. The slopes were inspected daily for the growth or for contamination. In case of growth of Mycobacteria, date of appearance of first colony was noted and slopes were further incubated for more growth.³

Further, each growth was identified by colony morphology, rate of growth, pigment production, Nitrate reduction test., Niacin test and Pyrazinamidase test.⁷

Culture in Mycobacterium growth indicator tube (MGIT) by semiautomated methods: Protocol of Ruhı Bunger et al was followed.⁸ Becton Dickinson (BD) BBL MGIT tubes were used for processing and the BACTEC MGIT TB system Product and Procedure manual was referred to. MICRO-MGIT system was used to look for green signal to indicate growth in the tube. Visually the Mycobacterium growth appears granular white deposit at the bottom of the tube with clear liquid above. Growth of Mycobacterium was confirmed by aseptically pipetting one or two drops of media and preparing smears which were stained by ZN and fluorescent method. A drop of the fluid was also inoculated onto 5% sheep blood agar in order to rule out contamination. Specimens positive for Acid fast bacilli in smear and showing no growth on 5% sheep blood agar were considered positive for Mycobacterial growth. Immunochromatography assay to detect MPT64 antigen using SD BIOLINE TBAG MPT64 rapid antigen detection kit was performed to confirm the positive isolates of *M.tuberculosis* complex and to differentiate from Non tuberculous mycobacteria.⁹

All confirmed positive isolated for Mycobacterium tuberculosis were subjected for drug susceptibility testing for four first line drugs i.e Streptomycin, Isoniazid, Rifampicin and Ethambutol using BD BACTEC MGIT 960 SIRE kits which is based on proportion method.¹⁰

Results were tabulated and statistically analysed. Sensitivity, specificity, positive predictive value, negative predictive value and P value were calculated and compared.

Results

A total number of 100 patients were included in the study, out of which 70 were male patients and 30 were female patients. Majority of the patients in the study group belonged to the age group 41-50yrs (20%), followed by 51-60yrs (19%) and 31-40yrs (18%). Youngest patient in the study group was of age 10yrs and eldest of age 80yrs. Fever was the most predominant symptom seen in 90 cases (90%) followed by cough in 57 cases (57%). Other symptoms were of loss of weight in (37%) cases and loss of appetite in (12%) cases. Erythrocyte sedimentation rate was raised in about (36%) cases. Radiological study was suggestive of TB in (72%) cases and negative in (28%) cases. (2%) cases were found to be HIV reactive however there was no co infection with TB. Of total 100 cases, (57%) cases were pulmonary samples and (43%) were extrapulmonary samples, most common samples processed was bronchoalveolar lavage fluid (42%) as depicted in Graph 1. (3%) cases were positive and (97%) cases were negative with ZN stain and fluorescent stain on direct smear. After concentration (4%) cases were found to be positive. In rapid/Modified slide culture method, (4%) cases were found to be positive and (96%) were negative. In LJ medium, out of 100 cases, (6%) cases were positive and (94%) cases were negative. (12%) cases were found to be positive for growth and (88%) cases were found negative for growth in MGIT method. Correlation of direct smear finding, after concentration, rapid slide culture and LJ medium with MGIT method is depicted in Table 1 and Table 2.

The sensitivity of the direct smear method was 25%, concentration method was 33.33%, rapid slide culture was 33.33%, LJ method was 50% in comparison to the MGIT method. The specificity was 100% for all the methods. However the P value was <0.05 for all the methods.

Out of 12 isolates which showed growth in MGIT method, all were sensitive to rifampicin and streptomycin (100%) and 11 cases (91.6%) were sensitive for isoniazid and ethambutol. One isolate each were resistant to isoniazid and ethambutol, as depicted in Graph 2.

Table 1: Correlation of direct smear finding, concentration method, rapid slide culture and LJ medium with MGIT method

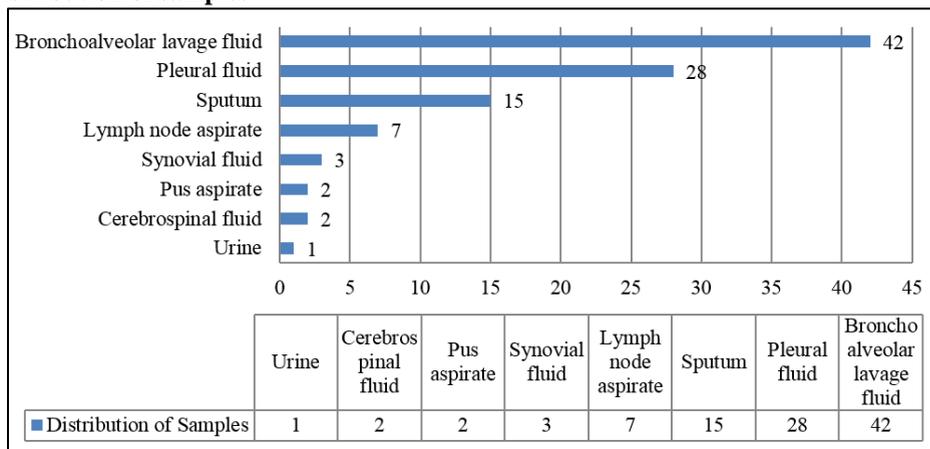
Method	Criteria	Number of patients (N=100)	MGIT method		P value
			Positive (N=12)	Negative (N=88)	
Direct smear Finding	Positive	3(3%)	3(12%)	0	<0.05
	Negative	97(97%)	9(75%)	88(100%)	
Concentration method	Positive	4(4%)	4(33.33%)	0	<0.05
	Negative	96(96%)	8(66.66%)	88(100%)	
Rapid Slide Culture	Positive	4(4%)	4(33.33%)	0	<0.05
	Negative	96(96%)	8(66.66%)	88(100%)	
LJ method	Positive	6(6%)	6(50%)	0	<0.05
	Negative	94(94%)	6(50%)	88(100%)	

Table 2: Evaluation of correlation direct smear, concentration method, modified slide culture and LJ medium in relation to MGIT method

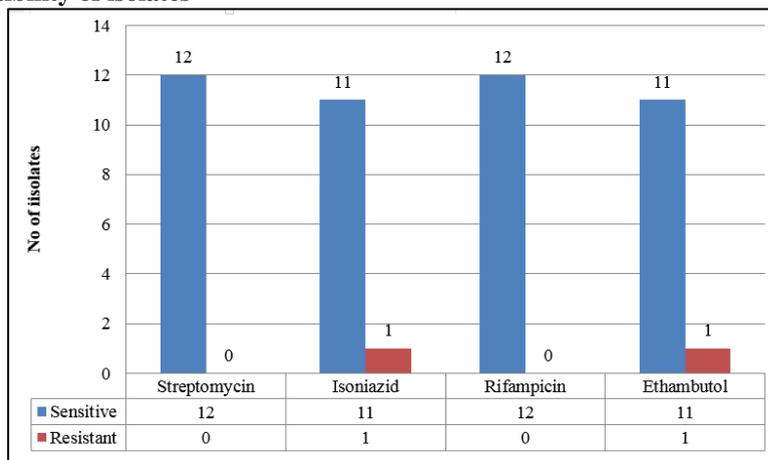
Method	Sensitivity	Specificity	PPV	NPV	P value
Direct smear Finding	25%	100%	100%	90.72%	<0.05
Concentration method	33.33%	100%	100%	91.66%	<0.05
Rapid Slide Culture	33.33%	100%	100%	91.66%	<0.05
LJ method	50%	100%	100%	93.61%	<0.05

PPV-Positive Predictive Value, NPV- Negative Predictive Value

Graph 1: Distribution of samples



Graph 2: Drug susceptibility of isolates



Discussion

Tuberculosis continues to curb the living standards of the society especially in country like India. It is important to identify and treat smear negative pulmonary and extra pulmonary tuberculosis which form the tip of the iceberg and often lead to morbidity and mortality. With the rise of MDR-TB cases, it is utmost important to determine the most cost effective and time efficient diagnostic method for proper management, better treatment outcome and to ensure successful TB control.¹¹

We compared the sensitivity and specificity of different diagnostic techniques (smear microscopy and culture) available at a tertiary care hospital for detection of M.tuberculosis and their susceptibility towards first line drugs. The study included clinically and radiologically suspected (new) cases of smear negative pulmonary and all extra pulmonary tuberculosis, also excluding patients already on antituberculosis treatment.

Majority of the patients in our study group belonged to age group 41-50yrs (20%) with a male preponderance of 70% similar to a study by Sarada Dasari et al who showed a higher prevalence among 41-60yrs (34%) and increased number of cases among males (64.7%).¹²

41-60yrs forms the larger part of the economically productive group who are exposed to open TB cases in the community. Also the onset of co-morbid conditions like diabetes in this age group increase the susceptibility to infection. Increased outdoor exposure, smoking and alcohol make males vulnerable to infection.

Out of 100 cases, 3(3%) were positive and 97 (97%) were negative i.e 3/43 (7%) extrapulmonary samples were found positive with both ZN stain and fluorescent stain. This finding is similar to a study by Bunker et al and Sarada et al who reported a direct smear positivity rate of 8.33% and 7.69% respectively among extrapulmonary samples.^{8,12} ZN method is an excellent differential stain for AFB which is rapid, simple and specific but the detection limit is at least 10⁴ bacilli per ml of sputum. The sensitivity is lower especially in paucibacillary cases. Fluorescent staining has a higher rate of detection and is more rapid as many slides can be screened in a short duration using lower magnification and the introduction of LED microscopes which are cost effective and have longer shelf life in Government run RNTCP centres has greatly improved the diagnostic facility. It is to be noted that an equivalent sensitivity was noticed for ZN and fluorescent staining in our study most probably because the cases were either smear negative pulmonary and extrapulmonary cases.^{13,14} Concentration by modified Petroff's method was found to be more sensitive than direct microscopy in our study i.e (4%).

Among the culture methods the sensitivity of culture on LJ medium and rapid slide culture was 50%,

33.33% respectively in comparison with the MGIT method and all three showed 100% specificity.

Growth on LJ medium is considered the Gold standard for culture and is more sensitive than microscopic detection of AFB. It has an ability to give positive result in specimen containing 10-100 bacilli per ml. However, the time to positivity is 6-8 weeks. In our study 6/100 (6%) cases showed growth on LJ medium with a mean duration for isolation being 31.5 days. All isolates were confirmed with TB MPT64 antigen detection immunochromatography test kit by SD Bioline. Similar to a study by Bunker et al who also showed 6.06% isolation. However, other studies like Ravish et al and Hemavathi et al have shown a higher percentage of positivity, 23% and 24.26% respectively. The low rate of positivity can be explained by the smear negative and paucibacillary nature of our samples included in the study.^{8,13,15}

Rapid slide culture is advantageous as microcolonies can be detected under ordinary bright light microscope within seven days. It is rapid, cheap, effective and more sensitive than microscopy. Drug sensitivity testing can also be done.^{16,17} In the present study, 4/100(4%) cases were positive for growth on rapid slide culture method with a sensitivity of 33.33% and specificity of 100%. However, other studies, a study by Hemavathi et al (20.71%), Purohit et al (93.5%) and Nair et al (23.8%) showed higher and varying rates of positivity.^{15,17,18}

Mycobacterium growth indicator tube (MGIT) has an oxygen quenched fluorescent sensor which detects the growth of Mycobacterium. It is a highly sensitive semiautomated method with a faster turn around time to detect growth of tubercle bacilli. In our study 12/100(12%) cases were found positive for Mycobacterium tuberculosis with a mean duration of isolation being 25.5 days. Our study correlates with studies such as Bunker et al and Jagadish Rawat et al which show a higher percentage of positivity with MGIT method as compared to LJ method and higher rate of sensitivity in case of smear negative samples. Hence, clearly MGIT is a better method of culture as compared to LJ method.^{8,19}

12/12 (100%) isolates which grew in MGIT were sensitive to rifampicin and streptomycin and 11/12 (91.6%) were sensitive to isoniazid and ethambutol. One isolate each were resistant to isoniazid and ethambutol. No MDR-TB were noted in our study.

Conclusion

We evaluated and compared the methods available at a tertiary care centre in an earnest attempt to determine the most efficient contender and found that semiautomated MGIT was highly efficient diagnostic tool for smear negative pulmonary and extra pulmonary tuberculosis samples. Although automated methods are highly sensitive and specific in diagnosis of paucibacillary tuberculosis, newer molecular methods

which are based on principle of polymerase chain reaction such as Xpert MTB/RIF (Cartridge Based-Nucleic acid amplification technique-CB-NAAT) which have a detection limit of one bacilli/ml, can also detect rifampicin resistance and have a turn around time of around two hours for Xpert MTB/RIF are more promising tools for detection and treatment outcome assessment to achieve our goal of ending tuberculosis in India.^{20,21}

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