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RESEARCH ARTICLE

EVALUATION OF BACTEC MICRO MGIT SYSTEM FOR ISOLATION OF MYCOBACTERIA IN A TERTIARY CARE SETTING

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ABSTRACT

Background: Tuberculosis (TB) is a significant infectious disease in many parts of the world, which is of great concern. Prompt detection, isolation, identification and susceptibility testing of Mycobacterium tuberculosis from clinical specimens is essential for appropriate management of patients with tuberculosis. This study aims to compare the sensitivity of BACTEC MGIT method in detection of Mycobacterium tuberculosis among various clinical samples and to detect the drug resistance pattern to 1st line drugs among Mycobacterium tuberculosis isolates.

Materials and Methods: A three year cross sectional study was done among 300 patients in the department of Microbiology, KIMS, Bangalore. Study group included cases with clinical or radiological evidence suggestive of tuberculosis. Specimens were subjected to direct microscopy by Ziehl-Neelsen staining and fluorescent staining. Culture was done by semi automated BACTEC MGIT system. Rapid antigen detection by SD TBAg MPT64 kit was performed to confirm the positive isolates. Isolates confirmed as Mycobacterium tuberculosis were subjected to drug susceptibility testing using MGIT method for 1st line drugs ie Streptomycin, Isoniazid, Rifampicin and Ethambutol.

Results: The sensitivity of direct microscopy was 12.33% by Ziehl-Neelsen staining and 14.33% by fluorescent staining .Overall culture positivity was 18.6% (56). 55 isolates were Mycobacterium tuberculosis and one was nontuberculous mycobacterium. The mean detection time was 20.75 days by MGIT method. A sensitivity of 96.3% for streptomycin, 89% for isoniazid, 98.1% for rifampicin and 96.3% for ethambutol was noticed.

Discussion and Conclusion: This study highlights the importance of culturing the suspected tuberculosis cases prior to empirical therapy. Newer automated culture methods aids in earlier detection of cases and drug susceptibility testing of isolates and helps in selection of appropriate treatment for tuberculosis.

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INTRODUCTION

Tuberculosis (TB) is a significant infectious disease in many parts of the world, which is of great concern. Prompt detection, isolation, identification and susceptibility testing of *Mycobacterium tuberculosis* from clinical specimens are essential for appropriate management of patients with tuberculosis. According to RNTCP annual report 2015, India accounts for nearly one fourth of tuberculosis cases worldwide and every year around 2.1 million people develop TB (http://www.tbcindia.nic.in/showfile.php?lid=3166). Direct microscopy has been the main stay in diagnosis of TB, but is less sensitive when compared to various culture methods, which is considered the gold standard, especially when the bacillary load is less, as in smear negative pulmonary TB and extra pulmonary TB. The estimated proportion of Multidrug resistant TB (MDR-TB) in India is 2.1% of all new TB cases and 15% of all previously treated cases (https://extranet.who.int/sree/Reports?op=Replet&name=%2FWHO_HQ_Reports%2FG2%2FPROD%2FEXT%2FTBCountryProfile&ISO2= IN&LAN=EN&outtype=html). Underlining the importance of drug susceptibility testing of isolates. Hence there is a need for early diagnosis of all cases and prompt treatment after assessing the sensitivity to drugs. This goes a long way in reducing patient morbidity and mortality. The standard recommendation is that all new diagnostic systems to be used in screening patients for tuberculosis are to be properly evaluated and must be able to isolate *M tuberculosis* easily, quickly and accurately.

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The conventional methods of culture such as Lowenstein Jensen (LJ) medium will take about 4 to 6 weeks for the recovery of the organism, which can significantly prolong the initiation of treatment. Newer automated systems as BACTEC MGIT system has proved to reduce this time to as short as 1 to 2 weeks. It has an oxygen sensitive fluorescent sensor embedded in silicone base to serve as an indicator of mycobacterial growth. The tube contains 7 ml of modified Middlebrook 7H9 broth enriched with 0.5 ml of a nutritional supplement and 0.1 ml of an antibiotic cocktail to suppress growth of contaminating microorganisms. As the actively growing and respiring mycobacteria consume the dissolved O2, the sensor glows indicating mycobacterial growth. So a pilot study was done to evaluate the sensitivity of MGIT system to detect *Mycobacterium tuberculosis* in a tertiary care setting. The parameters evaluated were recovery rate and rapidity of growth and drug susceptibility of Mycobacteria among various specimens.

MATERIALS AND METHODS

Study design and period

A cross sectional study was carried out during the period from November 2012 to October 2015 in Department of Microbiology, Kempegowda Institute of Medical science and Research centre, Bangalore. Total of 300 suspected cases of pulmonary and extrapulmonary tuberculosis were included in this study. Cases included were new cases of Tuberculosis who were not on antitubercular treatment. Clinical samples included were : Bronchoalveolar lavage fluid – 156 ,Pleural fluid – 66, Sputum – 33, Pus aspirate – 20, Tissue – 8, Lymphnode aspirate – 4, Ascitic fluid – 4, Cerebrospinal fluid – 3, Synovial fluid- 3, Urine -2, Pericardial fluid – 1.

Sample processing

All specimens were subjected for direct microscopy by Ziehl-Neelsen staining and fluorescent staining. Smears were graded according to RNTCP guidelines. Specimens were then processed by modified Petroff's method using N-acetyl-L-cysteine NaOH (NALC-NaOH) digestion decontamination technique. After centrifugation, the sediment was resuspended in 1.5 ml of sterile phosphate buffer.

Inoculation in MGIT tube

The BBL MGIT tube (from Becton Dickinson) containing 7 mL modified middle brook 7H9 broth was used, to which an enrichment supplement as well as a mixture of antibiotics consisting of Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim, and Azlocillin were added. After inoculation, the tubes were incubated at 37°C. Readings were taken daily for the first three weeks and once a week thereafter for culture positivity until the end of six weeks using the BBL Micro MGIT system. All the positive tubes were further confirmed by ZN staining, fluorescent staining and a sub culturing on blood agar plate. All Positive isolates were confirmed as *Mycobacterium tuberculosis* by performing immunocromatographic test SD TB MPT64 kit for antigen detection by SD Bioline.

The time to detection (TTD) of Mycobacteria was based on the date of the earliest instrumental indication of positivity.

*All sterile fluids such as CSF, pleural fluid and synovial fluid were directly inoculated into BD BACTEC Myco/F Lytic culture vials and incubated inside automated BACTEC 9050 system and followed up as above.

Drug susceptibility testing

All confirmed isolates of *Mycobacterium tuberculosis* were subjected to drug susceptibility testing by four first line drugs, ie Streptomycin(SM), Isoniazid (INH), Rifampicin (RIF) and Ethambutol (EMB). BD BACTEC MGIT 960 SIRE kits were used. Final drug concentrations were 1.0 mg/ml for SM, 0.1 mg/ml for INH, 1.0 mg/ml for RIF, and 5.0 mg/ml for EMB. For each isolate, a growth control (GC) tube with growth Supplement but without drug was included. These tubes were incubated at 37 ^{0}C and readings were taken daily up to 13 days.

RESULTS

A total of 300 cases were included in the study out of which 204 were males and 96 were females. Division of cases according to age group is shown in Table 1. Youngest case included was 3 years of age and oldest case belonged to 85 years. A total of 5 cases were associated with HIV infection. (Table 2) Direct smear microscopy was positive in total of 43 cases. (Figure 1) Out of 300 cases, 56 were positive by culture and 55 were confirmed as *Mycobacterium tuberculosis* and one was nontuberculous Mycobacterium (Figure 2). The comparison between the grading of smear microscopy, culture positivity and mean time to detection in MGIT method was as per Table 3. The comparison between the culture positivity among various specimens and mean TTD was as per Table 4. Drug susceptibility pattern of *Mycobacterium tuberculosis* for 1st line drugs is shown in Table 5. A combined resistance to Streptomycin and Isoniazid was noticed in 1 case. A contamination rate of 6% was noticed in MGIT culture method.

Table 1. Age distribution of cases studied

Age	No. of cases	Percentage
<10 yrs	4	1.33%
11-20 yrs	12	4.00%
21-30 yrs	55	18.33%
31-40 yrs	48	16.00%
41-50 yrs	60	20.00%
51-60yrs	56	18.66%
61-70 yrs	52	17.33%
>70 yrs	13	4.33%

Table 2. HIV seropositivity among cases studied

HIV positivity	No. of cases	Percentage
Positive	5	1.66%
Negative	295	98.33%

DISCUSSION

Tuberculosis, for many centuries, has been the most important of human infections, in its global prevalence, devastating morbidity and massive mortality.

Table 3. The comparison between the grading of smear microscopy, culture positivity and mean time to detection in MGIT method

Smear Microscopy Grading	No. of cases	No .of positives for mycobacterium tuberculosis culture in MGIT	Mean TTD in MGIT
Scanty	17	17	25.17
1+	8	8	18.37
2+	6	6	14.5
3+	12	11	9.83
Total positives	43*	42	19.32
Total negatives	257	13	28.46

*One case was of Nontuberculous Mycobacteria

Table 4. The comparison between the culture positivity among various specimens and mean TTD

Specimen	No. of Cases	No. of. Positives	Percentage of positivity	Mean TTD in MGIT
BAL fluid	156	26	16.66%	21.46
Sputum	33	17	51.51%	17.41
Pleural fluid	66	6	9.09%	28
Pus aspirates	20	4	20%	18.75
Synovial fluid	3	1	33.33%	27
Lymph node aspirate	4	1	25%	21
Urine	2	1	50%	17

Table 5. Drug susceptibility pattern of the cases positive Mycobacterium tuberculosis for 1st line drugs

Drug	Streptomycin	Isoniazid	Rifampicin	Ethambutol
Susceptible	53	49	54	53
Resistant	2	6	1	2
Percentage of susceptiblity	96.3%	89.0%	98.1%	96.3%

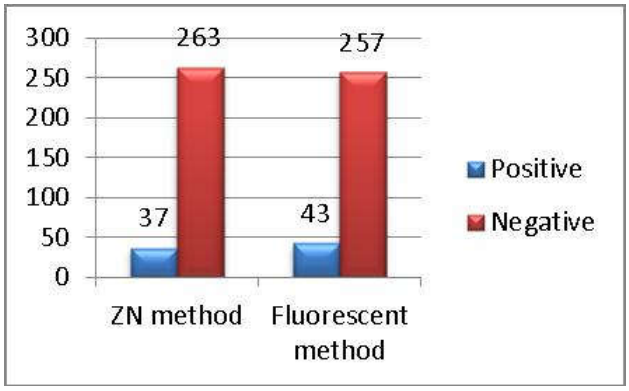

Figure 1. Comparison of direct smear microscopy by ZN and fluorescent method

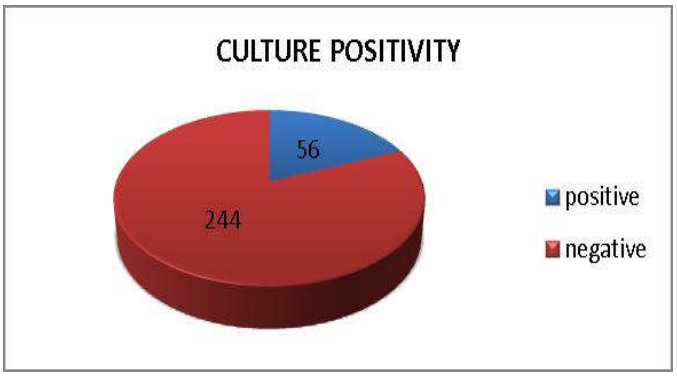

Figure 2. Culture positivity among cases studied

It is estimated that a third of the world's population is infected with the tubercle bacilli. Each year more than eight million new cases and three million deaths due to tuberculosis is being reported worldwide. (Sanjeev *et al*., 2012) A thorough surveillance in the community of suspected cases is required for the early diagnosis and treatment of this contagious disease, especially in an endemic country like India. Tuberculosis is known to infect any age group. The common age group affected in our study of was of the middle and elderly age group, the working class of the community. This might be because of increased exposure and the onset of co-morbid conditions. A similar trend is noticed in the studies by Giri Prasad *et al*. and Chakraborthy *et al*. (Giri Prasad *et al*., 2013; Chakraborty, 2004) An increased incidence was noticed among male population and the male to female ratio is 2.1:1. The on-going HIV pandemic has added increasing incidence of tuberculosis these days as both the diseases are known to go hand in hand. A total of 5 HIV positive cases were included in our study out of which one case of cold abscess was positive for the culture and was found to have developed Isoniazid monoresistance. Detection of Acid Fast Bacilli (AFB) in sputum samples constitutes the mainstay of diagnosis in this disease. However this method has low sensitivity and has little value in patients who do not expectorate significant amount of sputum spontaneously. It has been reported that approximately 25-30% of adult patients with suspected pulmonary tuberculosis does not produce sputum spontaneously or have negative AFB smears. With increase in the prevalence of TB-HIV co-infection, a rise in the proportion of sputum-negative PTB patients is anticipated. Though pulmonary form is the commonest presentation, the Extra Pulmonary Tuberculosis is also an important emerging clinical problem. Extra pulmonary tuberculosis infections are more often smear negative than pulmonary cases which makes its diagnosis difficult to establish (Bunger *et al*., 2013). In our study the fluorescent staining

method, advised by RNTCP was found to be more sensitive (14.33%) for direct microscopy when compared to the routine Ziehl-Neelsen staining method (12.33%). An increased sensitivity of fluorescent staining technique over Ziehl-Neelsen method has been reported in literature. Masood *et al* reported reported a sensitivity of 57% for fluorescent staining method when compared to 51% by ZN method (Masood Ziaee *et al*., 2008).

Culture for growth of *Mycobacterium tuberculosis* is more sensitive when compared to microscopy. It has an ability to give positive results on a clinical specimen containing as low as 10-100 bacilli per ml. An array of manual and automated systems has been developed for the faster and more accurate detection of acid fast bacilli from various clinical samples. The present study demonstrated that recovery of *Mycobacterium tuberculosis* is indeed faster in the BACTEC MGIT method compared to conventional methods. A recovery rate of 18.66% was demonstrated by MGIT method among various specimens; both smear positive pulmonary, smear negative pulmonary and extrapulmonary cases. A higher recovery rate has been reported by Chitra *et al*., 2001 and Rishi *et al*. (2007) This might be because in present study the majority of cases were constituted by paucibacillary cases (257 cases) against 43 smear positive cases.

The recovery rate of *Mycobacterium tuberculosis* was comparable against the smear positivity. The mean duration for isolating M. tuberculosis considerable reduced according the higher grade of smear positivity. A mean time to detection of less than 10 days was noticed among cases with 3+ grade smear positivity where as in cases of scanty grade smear positivity it was 25 days. Similar findings were reported by Rishi *et al*. (2007) Smear negative cases took longer upto 29 days. Highest mean time to detection was noticed among the pleural fluid samples of 28 days. Earliest culture positivity was noticed on day 6 in a Bronchoalveolar lavage fluid and latest was on day 35 in a smear negative sputum sample. The emergence of Multi drug resistant TB (MDR TB) in India is at an alarming rate. Hence it is necessary to determine the drug susceptibility of all tuberculosis cases in order to decrease the morbidity and mortality. Although treatment default has been attributed to be the major reason behind the MDR and XDR (Extensively drug resistant) TB in our country, several Primary MDR TB cases are being reported these days. The drug susceptibility pattern obtained in our study was comparable to that by Ping Zhao *et al*. (2014) In our study no MDR TB cases were reported. But an increased INH monoresistance was noticed of 10.9%. This increasing incidence of Isoniazid resistance alone has been documented by World Health Organisation as INH-resistance, alone or in combination with other drugs now the second most common type of resistance worldwide with current estimates at 10.3% for new cases and 27.7% for previously treated cases. (World Health Organization, 2008)

Conclusion

Since tuberculosis still remains a major global health problem, there is a need for a rapid, sensitive and accurate detection system like BACTEC Micro MGIT for culturing the microorganism and drug susceptiblity testing in clinical specimens. This would hasten the administration of appropriate antimycobacterial therapy thereby decreasing morbidity and mortality as well as preventing the spread of infection in the community.

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