Research Paper

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Comparative evaluation of various methods for the detection of Mycobacterium tuberculosis in clinically suspected pulmonary tuberculosis patients

Medical Science

KEYWORDS : Mycobacterium tuberculosis, BACTEC MGIT 960, LJ media

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ABSTRACT

BACKGROUND: Pulmonary Tuberculosis (PTB) is one of the most common infectious diseases in the world.The transmission of PTB can be reduced by early detection and cure of infectious cases. So, there is a need for emergence of new techniques for the more precise and rapid identification of Mycobacterium tuberculosis in clinical samples. AIMS: To compare BACTEC MGIT 960 (M960) with conventional culture on Lowenstein Jensen (LJ) media and direct acid fast bacilli (AFB) smear examination for the detection of Mycobacterium tuberculosis in clinically suspected cases of Pulmonary Tuberculosis. METHODS & MATERIAL: A total of one hundred and fifty sputum samples from suspected cases of PTB were evaluated. Specimens were processed for direct AFB smear examination and culture on M960 and LJ media. STATISTICAL ANALYSIS: Student 't' test was used and p value was calculated. RESULTS: Among suspected cases, 41/150 (27.33%) samples were positive by M960 culture, 34/150 (22.67%) by LJ media and 35/150 (23.33%) by direct AFB smear examination. The mean time to detect growth was 8.9 days on M960 and 20.8 days on LJ media. CONCLUSION: M960 system is a dependable, high-capacity, compact, fully automated, non invasive and continuously monitored instrument. It considerably improves the recovery and decreases the time required to detect Mycobacterium tuberculosis from human clinical samples than the traditional LJ slant.

INTRODUCTION

Tuberculosis (TB) is a disease of great antiquity in both western and eastern hemispheres. However it became a major health problem when urbanization and industrialization in seventeenth and eighteenth centuries, resulted in crowded living conditions facilitating its spread. Since then it has caused more suffering and death than any other bacterial infection.

TB remains a major global health problem, responsible for about one third of the preventable deaths[1]. Worldwide, around 10 million new cases of tuberculosis add each year with three million deaths[2]. According to World Health Organization (WHO) Global report 2011, India accounts for nearly one fifth of tuberculosis cases worldwide and every year around 2 million people develop TB[3].

The spread of HIV/AIDS, re-emergence of TB and the appearance of multiple drug resistant TB (MDR- TB) have further worsened the impact of the disease. Since the prevention of Tuberculosis relies mainly on the early diagnosis and treatment, current efforts are focused upon improving the rapidity of identification of *Mycobacterium tuberculosis* (MBT), allowing prompt initiation of appropriate therapy[4].

Although AFB microscopy and conventional LJ culture remains the cornerstone for the diagnosis of TB, the sensitivity of these traditional methods is quite low, especially in the samples containing small number of organisms[5]. One of the most significant disadvantages of conventional culture is the time taken for detection and for sputum microscopy is the issue of accuracy which further depends on quality of stains, microscope etc along with issues of observational errors. Therefore, there is a need for rapid, sensitive and accurate detection of these organisms in clinical specimens to hasten the administration of appropriate therapy and to prevent the spread of infection in the community.

In recent years, several new radiometric and non radiometric technologies for rapid growth and detection of AFB have been introduced.This paper summarizes the results of a study carried out to compare one such automated system i.e. M960 with conventional culture method i.e. LJ media and direct AFB smear examination. M960 system is a fully automated, high capacity, non-radiometric, non-invasive instrument, which neither requires needles nor other sharp implements to incubate and monitor M960, 7 ml culture tubes[14]. Mycobacterial Growth Indicator Tube (MGIT) contains modified middle brook 7H9 broth base. The bottom of the tube contains a silicone bed in which a fluorescent compound sensitive to dissolved oxygen is embedded. When the growing *Mycobacteria* use up the oxygen, this causes the silicone to fluorescence which is then detected automatically under long wavelength ultraviolet light[15].

METHODS

Test site:

The study was carried out in the Department of Microbiology, Government Medical College (GMC), Amritsar over a period of one and a half year (January 2013- June 2014).

Specimens:

A total of 150 sputum samples received in Microbiology department of GMC, Amritsar were investigated from patients with features suggestive of Pulmonary Tuberculosis.

Specimen processing:

Within 24 hrs of receiving the samples, direct smear was made. It was stained with ZN method and graded as per Revised National Tuberculosis Control Programme (RNTCP) guidelines[1]. All specimens were digested and decontaminated using the NALC-NaOH method as recommended by the CDC's (Centre for Disease Control) Public Health Mycobacteriology: A Guide for the Level III Laboratory[16]. A maximum of 5-10 ml sample was taken in a falcon tube. An equal amount of a mixture containing 50 ml of 4% sodium hydroxide, 50 ml of 2.9% sodium citrate and 0.5% N -acetyl cysteine was added to it. The falcon tube was vortexed for 20-30 sec. and then it was kept at 37 °C for 20 minutes. After that, neutralization was done by filling the tube up to 50 ml mark with sterile phosphate buffered saline (PBS, pH 6.8). It was again vortexed for 20-30 sec. The tube was then centrifuged for 20 min at 3000g. Supernatant was discarded and the pellet was reconstituted with 1-2 ml of sterile phosphate buffer. The tube was then vortexed again[1]. Digested, decontaminated and concentrated sample was divided into four parts.

First part- was inoculated into M960 tube following manufacturer's instructions under following steps.

Culture on MGIT tube

The BACTEC MGIT 960 instrument is a fully automated system that exploits the fluorescence of an oxygen sensor to detect growth of mycobacteria in culture[17].

A lyophilized vial of BBL MGIT PANTA (Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim and Azlocillin) antibiotic mixture was reconstituted with 15ml of BACTEC MGIT ODAC (Oleic acid, Dextrose, Albumin and Catalase) Growth Supplement. Then 0.8ml of MGIT ODAC Growth Supplement-MGIT PANTA antibiotic mixture was added aseptically into the 7ml MGIT tube. The MGIT tube was labelled with specimen number. After that 0.5ml of the digested, decontaminated and concentrated specimen suspension was added to the MGIT tube. Tubes were tightly recapped and mixed well. Then the tubes were entered into the instrument by scanning the bar codes. The system indicated presumptive positive vials by audible alert sounds or visual signals such as illuminating the indicator lamp on the front of the drawer. Then the positive tube's bar code were scanned to extinguish LED light for that tube's station. Gram's and AFB smears were then prepared along with culture on blood agar from 0.1ml of aliquot removed from the bottom of the tube to rule out contaminating bacteria[18].

Second part- was inoculated on LJ media.

Inoculation on LJ Media

2-3 drops of processed specimen were inoculated on LJ media slant via hand operated pipette. The inoculated media was incubated first at 37 °C in horizontal position for 48 hours and then in vertical position for 8 weeks. The slopes were examined daily for four days and then twice weekly for 8 weeks. Culture was considered positive upon appearance of colonies on the surface and the time to detection (TTD) was based on the earliest date of detection of colonies on any of the solid media, ultimately confirmed by positive AFB smear[13]. Gram staining was also done to rule out contamination.

Third part- Was inoculated into the Blood agar plates to rule out any contamination.

Fourth part- Smear was prepared, heat fixed and stained by ZN method and examined under oil immersion lens.

ETHICS

Written & Informed consent of the patients was taken. Approval of college ethical committee was also taken.

STATISTICS

Student 't' test unpaired was used and p value was calculated.

RESULTS

Out of 150 samples processed, 41/150 (27.3%) samples were positive by BACTEC MGIT 960 culture, 34/150 (22.7%) by LJ media (Table 1) and 35/150 (23.3%) by direct AFB smear examination.

Table 1: Comparison of MGIT and LJ medium culture:

	LJ medium positive	LJ medium negative	Total
MGIT positive	34	07	41
MGIT negative	00	109	109
Total	34	116	150

The recovery rates obtained for *Mycobacterium tuberculosis* were 40/41(97.6%) isolates with M960 and 33/41(80.5%) isolates with LJ (Table 2) while the positivity of direct AFB smear examination alone was found to be 34/41 (82.9%). A total of 41/150 (27.3%) isolates of Mycobacteria were obtained by the combined use of M960 and LJ medium. This study isolated 7/41 (17.1%) strains exclusively in M960 whereas no strains were isolated only on LJ medium. In our study, out of the 41 positive cultures for *Mycobacteria,* 40/41 (97.57%) cultures were identified as *Mycobacterium tuberculosis* and 1/41 (2.43%) culture was identified as non-tuberculous mycobacteria by MPT64 antigen kit with sensitivity of 97.56%, specificity of 100%, Positive Predictive Value (PPV) of 100% and Negative Predictive Value (NPV) of 99.09%.

Table 2: Results of MPT64 Ag Kit in species differentiation:

MPT64 Ag	No. of patient with MGIT positive	Speices	Percentage
Positive	40	MTB	97.57%
Negative	01	NTM	02.43%
Total	41	-	100

Rate of isolation of *Mycobacteria* by M960 was 41/41 (100%) isolates with mean time to detection around 8.9days. Earliest growth was detected in 4 days and the maximum time taken for detection was 20 days by M960. Rate of isolation of *Mycobacteria* by LJ was 34/41(82.93%) isolates with mean time to detection of 20.794 days respectively.

Table 3: Duration for isolation by MGIT and LJ medium:

Number of days	No. of positive MGIT	No. of positive LJ
1-5	09	00
6-10	23	01
11-15	06	04
16-20	03	12
21-25	00	11
26-30	00	06
31-35	00	00
Total	41	34

The earliest growth was detected in 7 days and the maximum time taken for detection was 32 days by LJ (Table 3). In our study, statistically significant difference was found between mean time for isolation on MGIT and LJ medium with $p < 0.001$(highly significant).

DISCUSSION

In this study out of 150 samples, 41/150(27.3%) samples were positive by MGIT culture, 34/150(22.7%) by LJ media and 35(23.3%) by direct AFB smear examination. This study definitely showed increased yield on MGIT like all other studies. This increased yield on MGIT culture could be due to the combined effect of addition of nutritional supplements and antibiotics to the medium along with bigger size of the inoculums used in MGIT. The recovery rates obtained for *Mycobacterium tuberculosis* were 40/41(97.6%) isolates with M960 and 33/41(80.5%) isolates with LJ (TABLE 1). This is comparable to Somoskovi A et al[19] who found in their study that as a single medium M960 recovered 53/55 (96.4%) of *Mycobacterium tuberculosis* isolates and LJ medium recovered 45/55 (81.8%) of the MTB isolates. Our study showed 17.1% higher rate of isolation of *Mycobacterium tuberculosis* by M960 system than that by LJ media (TABLE 1). This higher rate of isolation could be because of the fact that samples that were grossly contaminated on LJ medium culture were taken as negative whereas in M960 system, since the smears were made from all instrument positive MGIT tubes, we found that there were many samples which had grown both contaminants as well as *Mycobacteria* in them. Therefore such tubes were considered positive by M960. This study isolated 7/41 (17.07%) strains exclusively in MGIT whereas no (0%) strains were isolated only on LJ medium (TABLE 1). This is in concordance with study by Somoskovi et al[19] who observed that 2/55 isolates grew only in BACTEC MGIT 960 but BACTEC12B or LJ medium did not detect any isolates alone.

The MGIT culture method showed a faster detection rate compared to the conventional LJ medium culture technique. The mean detection time by MGIT culture was 8.9 days with a standard deviation of 3.97, whereas by LJ medium culture, the mean detection time was found to be 20.8 days with a standard deviation of 5.26 (TABLE 3). Our results regarding mean time for isolation are comparable to those of Pfyffer GE et al[7] (9.9 days), Somoskovi et al[19] (12.6 days) and Tortolli et al[17] (13.3 days).

However they are considerably lower than that of Rodrigues C et al[20] (16.0days) and Bohy ME et al[21] (18.4 days). In our study we found statistical significant difference between mean time for isolation on MGIT and LJ medium with p <0.001(highly significant). This is probably because large numbers of samples were smear positive and there is high incidence of TB. Out of the 41 positive cultures for *Mycobacteria,* 40/41(97.6%) cultures were identified as *Mycobacterium tuberculosis* and 1/41(2.4%) culture was identified as non-tuberculous mycobacteria by MPT64 antigen kit (TABLE 2) with sensitivity of 97.6%, specificity of 100%, Positive Predictive Value (PPV) of 100% and Negative Predictive Value (NPV) of 99.1%. This is in accordance with study by Toihir AOS et al[22] who found that the MPT64 antigen kit had excellent sensitivity of 100% and specificity of 100% compared to that of standard biochemical detection. The advantage of this antigen assay lies in its simplicity, rapidity and ability to be performed from growth in both solid as well as liquid media. The data in our study had demonstrated the significance of this assay for the rapid clinical identification of *Mycobacterium tuberculosis.*

Our study showed that the sensitivities of BACTEC MGIT 960 system, LJ medium and Direct AFB smear were 100%, 82.5% and 87.5% which is comparable to study by El Moursy AS et al[23] and Kamel MH et al[24] who reported sensitivity of 100% by BACTEC MGIT 960 which can be explained by the use of only smear positive specimens (about 93% of all specimens) in these studies. On the other hand the low sensitivity of BACTEC MGIT were reported by Badak et al[25] and Pfyffer et al[7] , who found culture sensitivities from smear positive specimens to be 94.6%, 88.6% and 93.5%, 85.7% for MGIT and L-J medium respectively. This can be explained by low sensitivity of direct sputum smear examination.

Thus the result of this study demonstrated that the MGIT system provided better recovery of *Mycobacteria* than the traditional LJ slant. The sensitivity, specificity and positive predictive value (PPV) of MGIT compared to LJ medium was 100%, 93.97% and 82.93% respectively. However, in other studies, MGIT did not achieve 100% sensitivity for isolation of *Mycobacterium tuberculosis* complex. Dongsi Lu and co workers showed in their study that although automated MGIT system demonstrated better sensitivity than traditional L.J slant for recovery of *Mycobacteria* from clinical specimens, approximately 10% of clinically significant *Mycobacteria* species would be missed by use of this system alone. This suggest that MGIT system is not yet sufficiently sensitive to warrant elimination of supplemental solid medium[26]. Hence the use of one liquid medium and one solid medium is recommended by the Centre for Disease Control and Prevention. Nowadays the use of such a combination is acknowledged worldwide[17].

In conclusion our results showed that BACTEC MGIT 960 culture method is a simple, rapid and reliable method for mycobacterial culture requiring neither radioactive media nor sharp implements. It has a very good performance characteristic as it is easy to use and it readily fits into the customary mycobacteriology laboratory work flow requiring very limited space. The full automation eliminates the need of frequent loading and unloading of tubes and thus minimizes the risk of bottle breakage while non invasive monitoring of culture tubes eliminates the possibility of cross contamination. Some of the studies results do not go along with our study, it may be probably due to the small sample size and limited number of positive cultures obtained in our study. Further studies with a larger sample size are required to have an exact picture.

Moreover, India being the major endemic country for tuberculosis, is in need of introducing the rapid diagnostic methods more than any other country in the world. So MPT 64 TB Ag can be used as an excellent alternative for rapid identification of *Mycobacteria.* Because of its low cost, rapidity, high specificity, sensitivity, PPV & NPV, the ICT's could be used as a very useful diagnostic tool in Tuberculosis control programmes as this assay could markedly reduce the turnaround time in MTB culture & Drug susceptibility testing.

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