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Original Research Article

Comparison of BACTEC MGIT with conventional methods for detection of *Mycobacteria* in clinically suspected patients of extra pulmonary tuberculosis in a tertiary care hospital

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ABSTRACT

Background: Tuberculosis is an important public health problem in India and globally. Extra pulmonary tuberculosis (EPTB) constitutes for approximately 15 to 20 per cent of all cases of tuberculosis in immunocompetent patients and accounts for more than 50 per cent of the cases in HIV- positive individuals. Main problem with the extra-pulmonary tuberculosis is the paucibacillary nature of the specimen, which makes the diagnosis difficult and delay the treatment. With this in background, this study aimed at the isolation of *Mycobacteria* from clinical specimens of patients suspected of extra pulmonary tuberculosis using BACTEC MGIT, Lowenstein Jensen (LJ) media and direct acid-fast bacilli smear examination.
Methods: A total of 66 samples were processed for direct AFB smear examination, and culture on MGIT and LJ media. Acid fast staining of the specimens was done using the Ziehl-Neelsen method.
Results: Among 66 specimens, MGIT gave a higher yield of *mycobacteria* (46.9%), lower contamination rate (3%) and shorter time to positive culture as compared to LJ media.
Conclusions: MGIT gives higher yield and faster results.

Keywords: *Mycobacterium tuberculosis*, MGIT, Lowenstein Jensen (LJ) media, Ziehl Neelsen (Z-N) staining

INTRODUCTION

The medical microbiology laboratory has a key role in the identification and management of infections caused by *Mycobacterium tuberculosis*. The precise and prompt analysis of suspected patients is the basis for the foundation of global strategies for tuberculosis control. Though the commonest presentation is pulmonary tuberculosis, the Extra Pulmonary Tuberculosis (EPTB) is also an important emerging clinical problem.[1] Tuberculosis is a global public health problem with significant incidence, and mortality rates predominantly in developing countries.[2] Extra pulmonary tuberculosis infections are paucibacillary in nature, hence more often smear negative than the pulmonary cases which makes the diagnosis difficult.[3] About 15 to 20 per cent of all cases of tuberculosis in immunocompetent patients

comprises of extrapulmonary tuberculosis and accounts for more than 50 per cent of the cases in HIV- positive individuals.[4] The conventional methods for the isolation of *Mycobacterium tuberculosis* is incompetent for early diagnosis as it takes 4-8 weeks and is very poor in sensitivity.

Although, the culture methods have been modified for early detection and diagnosis. MGIT (mycobacterial growth indicator tube) is a non-radioactive detection system which uses fluorochrome based method for detection and drug screening.[5,6] This system is useful in early detection (7-12 days) of mycobacterial growth and has also been useful for drug susceptibility testing but the experience is limited.[6] In this study, they compared the performance of BACTEC mycobacterium growth indicator tube (MGIT) culture (containing modified Middle Brook 7H9 broth base) against conventional Lowenstein Jensen (LJ) media and direct AFB smear examination in extra pulmonary tuberculosis specimens.

METHODS

Study design and period

The present study was carried out in the Department of Microbiology; Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Uttar Pradesh, India during January 2015 - June 2016. In this study, a total of 66 samples were collected from various parts of body including tissue, ascitic fluid, pleural fluid, cerebrospinal fluids (CSF), broncho-alveolar lavage (BAL), tracheal aspirates, urine, and pus from clinically suspected cases.

Culture medium inoculation, incubation, and test duration

All specimens were subjected to standard N-acetyl-L-cysteine NaOH (NALC-NaOH) digestion decontamination technique.[7,8] A final concentration of 4% NaOH was used for decontamination. After the centrifugation, the sediment was resuspended in 1.5 ml of sterile phosphate buffer. Following processing, AFB smear were prepared and stained with the ZN Staining method.

MGIT tube

The MGIT tube (from Becton Dickinson, U.S.) containing 7 ml modified Middle Brook 7H9 broth was used, to which an enrichment supplement (OADC) 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 kept in the BACTEC MGIT 960 system.

LJ media tubes

LJ slant was inoculated with processed sample and were incubated at 37°C for 8 weeks. They were checked once every week for 8 weeks. Typical colonies of *M. tuberculosis* are rough, buff, tough, non-pigmented (cream coloured) and slow-growers, i.e. colonies appearing after one to two weeks after inoculation on LJ media. MGIT tubes were also checked for any indicator of growth. Positive MGIT tubes were further confirmed by TBcID card.

RESULTS

Out of 66 extra pulmonary samples, Mycobacterium tuberculosis complex was found to be positive in 21 (31.8%) cases by LJ medium, 31(46.9%) by MGIT while only 10 samples (15.15%) were found to be AFB positive by Z-N staining method. The comparison of the MGIT media with the LJ medium and Z-N staining for detection of mycobacterium in 66 specimens is shown in Table 1.

The study also compared the time to detection of mycobacterium tuberculosis for smear positive and smear negative isolates in BACTEC MGIT and LJ medium depicted in Table 2. For smear-positive specimens, the mean turnaround time was 8 days by MGIT whereas on solid medium, it was 21 days. For smear-negative specimens, the same was 18 days by MGIT and 28 days by solid medium.

Table 1: Comparison of detection by different methods among extrapulmonary tuberculosis.

Sample	Total	Z-N staining positive (%)	LJ MEDIA positive (%)	MGIT positive (%)
Pleural fluid	23	07 (30.43%)	09 (39.1%)	16 (69.5%)
Cerebrospinal fluid	11	00 (0%)	01 (9.0%)	02 (18.1%)
Pus	09	01 (11.11%)	05 (55.5%)	07 (77.7%)
Ascitic fluid	09	00 (0%)	02 (22.2%)	03 (33.3%)
Gastric aspirate	08	02 (25%)	04 (50%)	03 (37.5%)
Pericardial fluid	03	00 (0%)	00 (0%)	00 (0%)
Endometrial tissue	03	00 (0%)	00 (0%)	00 (0%)
Total EPTB cases	66	10 (15.15%)	21 (31.8%)	31(46.9%)

Table 2: The time to detection (TTD) of *Mycobacteria* for smear positive and smear negative isolates in BACTEC MGIT and LJ medium.

AFB smear	Detection by solid medium		Detection by MGIT system	
	TTD (days)	Range (days)	TTD (days)	Range (days)
Positive (n=10)	21	11-35	08	7-14
Negative (n=56)	28	20-56	18	15-44

DISCUSSION

In India, Tuberculosis is a major and global public health problem. Conventional methods including smear and conventional culture methods, used in the diagnosis of pulmonary and extrapulmonary tuberculosis, have poor sensitivity in the samples with paucibacillary load. The use of less sensitive conventional methods for the diagnosis have contributed to the difficulties in managing patients with extrapulmonary tuberculosis and patients having paucibacillary load in pulmonary TB. Main problems begin with clinical specimens containing very few mycobacterium bacilli and their slow growth rate limits their detection by the conventional methods such as acid-fast staining and mycobacterial culture. The early diagnosis of tuberculosis helps in initial treatment and thus preventing the possible transmission of the infection.[9]

In current study, we compared solid media (LJ) and automated BACTEC MGIT960 system for isolation of *Mycobacterium tuberculosis* in clinically suspected cases of extrapulmonary tuberculosis.

The present study demonstrated that MGIT 960 system provided better isolation rate of mycobacteria 31 (46.9%) from a variety of clinical samples compared to solid media 21 (31.8%).

The obtained results are in agreement with those reported by Hanna et al, which showed that the BACTEC MGIT 960 system had a recovery rate higher than that in BACTEC 460 TB system and in solid media.[10] Tortoli et al. showed that the overall recovery rates with the BACTEC MGIT 960 system and BACTEC 460 TB systems were higher than those achieved with solid media.[6] Dongsi and Dunnc also found that the BACTEC MGIT 960 system consistently provided with better isolation rates of all mycobacterium species from a variety of clinical specimens than the traditional L.J. slants.[11] Hines et al. showed that the BACTEC MGIT 960 system had a better recovery rate of *M. bovis* (122/129) than the solid media (96/129), Rishi S et al reported that isolation rate in case of BACTEC MGIT system (50.6%, 253/500) was higher as compared to solid media (33.6%, 165/500).[12,13] In addition Bunger R et al also reported that the highest rate of Mycobacterial recovery was by MGIT i.e. 22 out of 24 (91.6%) as compared to LJ media 14 out of 24 (58.3%).[14] However, the study showed contamination rate to be less in case of BACTEC MGIT 960 system (3%) as compared to the solid medium (8%) which was in concordance to the less contamination rate in MGIT system as compared to solid media which was reported by Gaby E. Pfyffer (2% by MGIT and 8.0% by solid media) and Bunger R et al 6.0% by MGIT and 8.0% by solid media.[3,15] Significant reduction in the contamination rate can be attributed to the use of the N-acetyl cysteine sodium hydroxide (NALC-NaOH) method and the addition of the MGIT PANTA mixture.

In the present study, average time to detection of mycobacteria growth according to the smear positivity was noted to be shorter for BACTEC MGIT 960 (7-14 days for AFB positive and 15-44 days for AFB negative) compared to solid media (11-35 days for AFB positive and 20-56 days for AFB negative). This is further supported by Chihota VN et al, Rishi S et al and Bunger R et al.[13-15]

Thus, in this study MGIT 960 was found to be more rapid and efficient method than that of the conventional solid media. However, for maximum recovery of mycobacteria, it is important to use both types of media simultaneously.

CONCLUSION

Hence, we conclude that the automated culture system like BACTEC MGIT 960 have a higher isolation rate as compared to solid media (LJ). But highest isolation rate can be achieved by combining the two methods. Smear and culture negative specimens however, donot rule out tuberculosis infection. Hence there is a need for a better and rapid diagnostic technique for early detection and treatment of tubercular infection.

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