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COMPARISON OF TIME FOR *MYCOBACTERIUM TUBERCULOSIS* GROWTH ON L. J. MEDIUM & BACT/ALERT 3D SYSTEM

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ABSTRACT

Main problem for *Mycobacterium tuberculosis* growth is time. This study evaluates the performance of Bact/Alert 3D system for isolates and identification of mycobacteria. 100 clinical specimens from the chest and tuberculosis department were included. All were positive by Lowenstein Jensen (LJ) media in different time. All samples were also tested by Bact/ALERT 3D system for time management study. Samples were microscopically positive for Ziehl Neelsen staining. 25 negative controls were also studied. Maximum 76% samples showed positivity in 3rd week and Minimum 10% positivity reported in 5th week of incubation in BacT/ALERT 3D system. On the other hand only 31% positivity reported in 3rd week by LJ media. BacT/ALERT 3D system was suitable, less time consuming method over LJ media culture. It may be very useful not only to quick detection but also to provide faster treatment and a better prognosis.

KEY WORDS: BacT/ALERT 3D system, Tuberculosis, Lowenstein Jensen media

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INTRODUCTION

Tuberculosis is second most common infectious disease and third leading cause of death worldwide[1]. Roughly one-third of the world's population has been infected with *M. tuberculosis* with new infections occurring in about 1% of the population each year. As per WHO Global TB report 2013, About 9 million people develop TB and 1.5 millions died from TB (including 360000 deaths among HIV positive people). India alone accounted for 2.0-2.5 million cases in 2010, thus contributing approximately 26% of all cases worldwide. According to National Tuberculosis Control Programmes, 2.6 million new cases of sputum smear positive pulmonary tuberculosis; 2.0 million new cases of sputum smear negative pulmonary tuberculosis were observed in 2010 worldwide[2].Since TB is directly related to people's health status and work capability, therefore, the control of TB prevalence has been of priority both in the public health and in the economic development of country. Early detection of tuberculosis by conventional method is time consuming because culturing on LJ Media can take 4 -8 weeks for colonies to detectable. Even then, the process may require further subculture for definitive identification. The combination of solid and liquid media is currently regarded as "gold standard"[3]. For primary isolation of mycobacteria in Direct staining and microscopy has low sensitivity and specificity and can provide only a preliminary diagnosis. Mycobacterial diagnosis should be fast and effective to prevent infection and management of appropriate antimicrobial therapy. Polymerase chain reaction is fast detection technique, but it takes huge initial investment, technical expertise and false positive results. So, automated liquid culture system based on different technologies, such as colorimetric methods that detect bacterial CO_2 production like BacT/ALERT 3D system, radiometric detection methods, like BACTEC 460 system, others use pressure sensors or fluorometric methods to detect bacterial O_2 consumption, such as ESP culture system II and BACTEC MGIT 960 system, respectively. The purpose of this study was to evaluate BacT/ALERT 3D automated system for recovery and identification of mycobacteria from clinical samples.

MATERIALS AND METHODS

A total of 100 culture positive sputum samples were included in this study. Along with patient's samples, 25 negative controls of healthy individuals were also included in this study. All samples were decontaminated and concentrated by the Petroff's method[4]. Pellet was re-suspended in phosphate- buffer saline to a final volume of 2 ml and were cultured on the following media in duplicate.

Inoculation on Lowenstein Jensen solid medium

After processing 1 ml samples were inoculated on L J media slopes and LJ tube with PNB (Para nitro benzoic acid) as per the standard protocol and incubated at 37^0C for 8 weeks and reading of culture were done weekly for 8 weeks. No growth after eight weeks of incubation was treated as negative. If growth was present in the presence of PNB, isolate was treated as Mycobacteria Other Than Tuberculosis (MOTT). If growth on LJ media slopes were detected, it was verified by Ziehl Neelsen stain for Acid Fast Bacilli (AFB). The final identification was done by conventional biochemical tests (niacin and thermo-stability of catalase enzyme at 68^0C).

Inoculation on MP Bottle for BacT/ALERT 3D

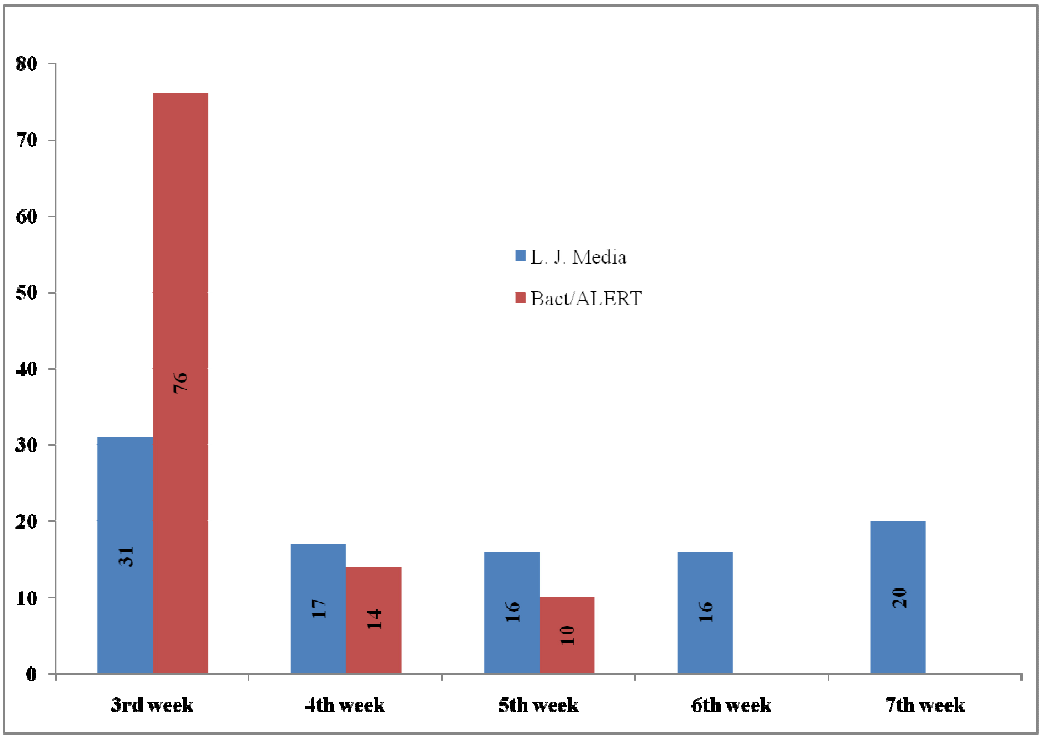
The MP bottle contained 10 ml of liquid medium (7H9 Middebrook) with casein, serum bovine albumin and catalase and was added 0.5 mL of antibiotic supplement MB/BacT (amphotericin B, azlocillin, nalidixic acid, polymyxin B, trimethoprim, vancomycin) to reduce the incidence of other bacteria contamination. After that was inoculated 0.5 ml of the digested and decontaminated sample in a bottle and incubated in the BacT/ALERT 3D system. After sample inoculation, the MP bottles were introduced into the BacT/ALERT 3D instrument for 6 weeks (predetermined time by the laboratory instrument). For this method, the

equipment used the detection algorithms to determine the presumptive positives and alerting the operator for the presence and location of positive bottles. All samples were identified as positive by the instrument BacT/ALERT 3D were reconfirmed by the ZN staining. If ZN staining confirmed AFB the result was considered positive (true positive by the instrument). If staining did not reveal AFB, 0.2 ml was transferred to LJ medium (subculture) and re-incubated at 37˚C in an incubator for 4 weeks. If the growth was AFB positive in LJ followed by ZN staining, was considered true positive by the instrument. Subsequently we realize the conventional biochemical tests for final identification. Any sample initially identified as positive by the instrument but not showing the AFB presence by staining and also not revealed mycobacterial growth in LJ subculture, was considered negative (false positive by the instrument).

RESULTS

All 100 samples showed positivity by BacT/ALERT 3D system by the end of 5th week. Maximum 76% samples showed positivity in 3rd week, while 14% in 4th week. Minimum 10% positivity was reported in 5th week. On the other end LJ medium also showed maximum positivity 31% in 3rd week followed by 20% in 7th week. 16% positivity was observed in 5th and 6th week each. 17% positive results were observed in 4th week (Fig 1.). Considering the culture method used, the average time for growth by LJ medium and BacT/ALERT 3D system is statistically significant ($p < 0.05$).

Figure
Percentage of growth on different weeks on LJ media & BacT/ALERT 3D system



DISCUSSION

Solid media culture still plays an important role in the mycobacteria isolation from sputum, but it remains a slow and intense procedure due to contamination of samples, which required decontamination treatment before culture. Mycobacterium takes several weeks to detect visible colonies on solid media. Now, more rapid and automated systems for mycobacteria

recovery from clinical samples are available. One of the important priority is sensitive detection and automation of culture process. BacT/ALERT 3D system is a non radiometric and totally automated system for mycobacterial culture. This instrument is approved by Food and Drug administration in 1996[3]. Despite considerable improvement of commercially available tests and their advantage in shortened turnaround time for diagnosis not replaced the usefulness of culture. Our aim is to evaluate performance of BacT/ALERT 3D system for recovery and identification of mycobacteria from samples. Although both methods were equal for isolation and identification, but total time taken for growth is important, which is less with BacT/ALERT 3D system. In our study, the time detection for mycobacteria in BacT/ALERT was statistically significant lower than the LJ media. Our results also supported by others[5,6]. All 100% mycobacteria samples were showed growth. Digestion and decontamination of clinical samples is required for optimal recovery of mycobacteria., which can be quickly overgrown by contaminating bacteria. Commercially available 7H9 broth contains lyophilized antibiotics, which are reconstituted and used for supplementation of standard broth media to decrease bacterial contamination[7].

CONCLUSION

We concluded that BacT/ALERT 3D system is superior over LJ medium method for recovery of tuberculosis bacteria. Time taken for growth in liquid culture method is also less, which provides faster treatment and better prognosis. The results are obtained with pretreatment of clinical samples using Modified Petroff Method for pulmonary samples. So, an additional step could be used for mycobacteria recovery with BacT/ALERT 3D system.

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