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Evaluation of Solid and Liquid Medium for the Diagnosis of Mycobacterium Tuberculosis in Brochoalveolar Lavage Samples in a Tertiary Care Hospital in North India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: Tuberculosis is a global health problem. To control the disease, timely diagnosis is necessary. Both solid and liquid cultures have been used for diagnosis. Brochoalveolar lavage is used as a specimen for diagnosis of smear negative tuberculosis and in patients who are unable to expectorate sputum. The study was done to evaluate the performance for the detection of *M. tuberculosis* from BAL in terms of detection rates, time to positivity and contamination.
Study Design: This was a descriptive cross sectional study.
Methodology: BAL samples of suspected patients were collected in the Department of Microbiology, Government medical college, Srinagar. Samples were processed, decontaminated and subjected to smear microscopy by ZN staining, culture on solid (LJ) and liquid media (BacT Alert.).
Results: Out of 283 BAL specimens, 23 (8.13%) specimens were AFB smear positive and 260 (91.87%) were AFB smear negative. The detection rate for Mycobacterium tuberculosis was 8.83% (25/283) on LJ and 12.37% (37/283) on BacT Alert. Overall, detection rate was comparable on smear positive samples but BacT Alert was more sensitive (7.7% detection rate) than LJ (3.46%) in smear-negative specimens. Although detection rates using BacT Alert were greater

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than LJ, 6 samples were positive exclusively on LJ medium. Time to positivity was significantly lower in liquid media but contamination rates were higher.
Conclusion: BacT/Alert affords rapid diagnosis of infection and therefore prompt institution of treatment for M. tuberculosis. Although BacT/Alert has higher sensitivity particularly in smear negative cases, there still remains a proportion of MTB specimens that may go undetected if not complemented with the solid media. Therefore it would be prudent if both the tests are used simultaneously to improve diagnostic yield.

Keywords: Bronchoalveolar lavage fluid; liquid culture; solid culture; turnaround time.

1. INTRODUCTION

Tuberculosis despite being an ancient disease continues to be a major public health problem. Although efforts are on to eliminate the scourge, it is top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS) [1]. The problem has been compounded with the emergence of drug resistant strains. One fourth of the world's population is infected with tuberculosis. The disease typically affects the lungs (pulmonary TB) but can also affect other sites (extrapulmonary TB). Tuberculosis is caused by the bacillus *Mycobacterium tuberculosis* complex. The organism is slow growing and its diagnosis and treatment poses a major challenge in its control. Efforts are being continuously made to shorten time to diagnosis and initiate prompt treatment. Traditionally, acid fast bacilli smear microscopy has been used as a mainstay of diagnosis for the treatment of pulmonary tuberculosis despite its suboptimal sensitivity. Many patients with suspicion of tuberculosis are unable to expectorate sputum. In such cases, bronchoalveolar lavage fluid has been accepted as a diagnostic modality. Also the bronchoalveolar lavage fluid has been increasingly used for the diagnosis of smear negative pulmonary tuberculosis [2-5]. Culture remains an acceptable method for diagnosis of tuberculosis despite the advent of numerous new technologies. A number of media, both solid and liquid have been used for the culture of *M. tuberculosis*. Traditionally, solid medium such as Lowenstein Jenson medium has been used for the culture of *Mycobacterium tuberculosis* as it best provides the growth of the organism, is economical and ensures easier visibility of colony morphology. The disadvantage with this medium is that it takes 4-8 week for the growth to occur. A number of automated culture systems have been developed with the aim to improve sensitivity and time to detection. BacT Alert 3D Mycobacteria Detection System is a colorimetric based, automated test system that utilizes liquid medium MB 7H9 for growth of mycobacteria. The instrument incubates and monitors culture bottles for microbial growth. During bacterial growth within the tube, the free oxygen is utilized and is replaced with carbon dioxide. As CO_2 is produced, the concentration of hydrogen ions increases and the pH falls causing the sensor to change to lighter green or yellow which is also detected by the photodetector [5-7,8]. The purpose of this study was to compare the BacT Alert and LJ medium for the detection of M. tuberculosis from bronchoalveolar lavage fluid in terms of detection rates, time to positivity and contamination.

2. MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, Government Medical College (GMC), Srinagar over a period of 6 months. Bronchoalveolar lavage samples of patients were received from Chest Disease Hospital, an associated hospital of the GMC, Srinagar. On receipt of samples in the laboratory, samples were transferred into a 50 ml conical falcon tube and centrifuged, thereafter the samples were decontaminated with N- acetyl-L cysteine-sodium hydroxide method (Kubica method). The supernatant was discarded and the sediment resuspended in 1.5ml of phosphate buffered saline (pH 6.8). The sediment was used for AFB microscopy and culture on solid and liquid media.

2.1 AFB Microscopy

The smear was prepared from the sediment, air dried and stained with Ziehl-Neelson method. Smears were graded according to the RNTCP guidelines.

2.2 Culture on LJ Media

Decontaminated samples were cultured onto the LJ medium. Cultures were examined after 3 days

of inoculation to exclude the growth of contaminants. Thereafter the cultures were examined weekly for the growth of mycobacteria. Growth was confirmed as *Mycobacterium tuberculosis* by observing the type of growth and biochemical testing. Culture were incubated for 8 weeks before being declared as negative.

2.3 Culture on BacT Alert

Decontaminated samples were inoculated in the BacT Alert MP bottles supplemented with OADC and antibiotics. The bottles were introduced into the machines and observed for positive signal. Once the instrument beeped positive, the bottle was taken out, AFB smear was made, subculture made on LJ medium and growth of Myco-bacterium tuberculosis confirmed by MPT60 test (SD Bioline).

2.4 Statistical analysis

Data was entered in a Microsoft excel sheet. Categorical variables were summarized as frequency and percentage. Continuous variables were summarized as mean and standard deviation

3. RESULTS

283 BAL specimens collected from patients at Chest Disease hospital, were received and processed for detection and identification of mycobacteria. 199 of these patients were males and 84 were females. Mean age of the patients was 50 years (range 10- 85 years). All of them were negative for HIV.

23 (8.13%) specimens were AFB smear positive and 260 (91.87%) were AFB smear negative. A total of 45 (15.9%) isolates were recovered and identified as *M. tuberculosis* from both solid and liquid cultures. Overall, the detection rate for Mycobacterium tuberculosis was 8.83% (25/283) on LJ and 12.37% (37/283) on BacT Alert. 2 isolates that were AFB positive were subsequently found to be non- tubercular mycobacteria on culture (Table 1). In smear positive specimen, detection rate was 95.25% (20/21) on solid culture where as it was 90.48% (19/21) on liquid culture. 2 smear positive specimen came contaminated on liquid culture whereas one came contaminated on solid culture. BacT Alert was more sensitive than LJ for detection of *Mycobacterium tuberculosis* in smear-negative specimens. The rate of smear negative but culture positive specimen were 3.46% (9/260) on solid culture and 7.7% (20/260) on liquid culture. Detection rates using BacT Alert were 2.22% greater than LJ. 6 samples were positive exclusively on LJ medium while 12 samples were positive exclusively on BacT Alert. 3 samples that came positive on LJ medium had contaminated liquid cultures. Overall, the contamination rate was 7.77% for LJ and 11.66% for BacT Alert; 2.83% of cultures were contaminated on both media.

3.1 Time to Positivity

For all the MTB samples that grew on both LJ and BacT Alert, the average time to grow on liquid media was significantly lower the solid media. The average time to detection for smear positive specimen was 8 days on liquid media whereas it was 25 days on solid media. Similarly, the average time to detection for smear negative specimen was 18 days on liquid media and 30 days on solid media.

Table 1. Recovery rates of Mycobacterium tuberculosis on LJ and BacT Alert by AFB smear status

	AFB POSITIVE (n=21)		AFB NEGATIVE (n=260)	
	n	**%**	**n**	**%**
Total no.	21	100	25	9.62
LJ	20	95.25	9	3.46
MGIT	19	90.48	20	7.7
Both media	19	95.25	5	1.9
Solid media only	2	4.8	6	2.3
Liquid media only	1	9.52	12	4.6

4. DISCUSSION

Tuberculosis is among the major public health concerns globally [9,10]. Early case detection remains a critical step in TB control and lab confirmation of *Mycobacterium tuberculosis* remains the only unambiguous diagnostic means for detection of this infection. Despite a multitude of advanced diagnostic laboratory tests including molecular methods having become available, culture methods remain the gold standard for diagnosing tuberculosis with World Health Organisation reaffirming this in their 2013 Global Tuberculosis Report [11]. The traditional solid culture medium (Lowenstein Jensen) is a time tested reference Gold Standard with high specificity but requires a long time for detection leading to unacceptable diagnostic delay [8]. A wide array of automated or semi-automated liquid culture media have become available that circumvent such delays substantially with high sensitivity.

We compared recovery rates and detection time of mycobacteria in solid based LJ medium vs. a liquid based system (BacT/Alert MP) and found higher recovery rates from LJ medium than BacT/Alert (95.25% and 90.48%, respectively) in smear positive cases. However, this was a statistically insignificant observation (*p=0.307*). The reason for lower recovery rate in liquid media was due to its high rate of contamination. Results similar to ours were also obtained by S. Levidiotoul et al. in their study [10]. In case of smear negative specimens, higher recovery rates were obtained with BacT/Alert than LJ media (7.7% and 3.46%, respectively. *p=0.018*). Similar results were also obtained by H. P. Chien et al., S. Levidiotou et al. in their study [12,13].

The average time to detection was significantly lower on liquid media than solid media in both smear positive (8 days and 25 days, respectively p=) and smear negative cases (18 days and 30 days, respectively p=). Detection times 2-3 times longer for solid media than liquid media have also been demonstrated by earlier studies [14,15,16].

Contamination rates have been reported as a major disadvantage in the use of liquid culture medium. The instrument may give false positive results due to bacterial/fungal contamination [17,18,19] as well as false negative as the rapid growth of such bacteria/ fungi may limit the growth of slow growing mycobacteria. This fact has been reiterated in this study. We also experienced higher contamination rates in liquid media than LJ media in our study (11.66% and 7.77%, respectively). Similar contamination rates were observed in other studies.

Although BacT/Alert has higher sensitivity particularly in smear negative cases, there still remains a proportion of MTB specimens that may go undetected. We found a total of 17.8% (8 of 45) positive samples that were missed on BacT\Alert but were subsequently detected by LJ medium. There was also a considerable level of agreement between the two media. The lower detection time of *Mycobacterium tuberculosis* on BacT/Alert affords rapid diagnosis of infection and institution of treatment as necessary. The costly equipment required for running liquid cultures, however, make it less accessible on large scale in developing nations with high prevalence of tuberculosis. Also detection of Mycobacterium tuberculosis from liquid culture requires the use of a rapid immunochromatographic test (such as SD MPT60 in our study). LJ medium on the other hand is cheaper with high specificity owing to lower contamination rates but has higher false negatives (28.9% in our study) as compared to liquid media.

5. CONCLUSION

The two media i.e solid and liquid media complement each other when used in combination. It is only prudent therefore, that for rapid and accurate diagnosis of tuberculosis, both the tests should be deployed simultaneously wherever possible to improve diagnostic yield and avoid false results.

ETHICAL APPROVAL AND CONSENT

The ethical clearance was sought from the institutes' ethical clearance committee. Since the study involved the samples that were received for routine diagnostic purposes, consent to publish the data was taken from the ethical clearance committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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