

Comparison of the Lowenstein-Jensen Medium, the Middlebrook 7H10 Medium and MB/BacT for the Isolation of *Mycobacterium Tuberculosis* (MTB) from Clinical Specimens

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ABSTRACT

Introduction: Tuberculosis (TB) is the most common cause of death due to a single infectious agent worldwide in adults. India alone accounts for 30% of the global tuberculosis burden. There is a need for a method of cultivation of mycobacteria that is reliable and economical and has a short turnaround time.

Objective: The present study was attempted to assess the feasibility of using MB BACT and Middlebrook 7h10(MB7H10) as primary isolation media for mycobacteria. They were compared with the LJ medium, which was the gold standard.

Materials and Methods: Various clinical specimens from a total of 236 clinically suspected cases of TB were studied. All the samples were decontaminated by using the modified Petroff's method. Each sample was subjected to ZN staining and it was simultaneously inoculated onto the LJ medium, the MB7H10 medium and MB BACT. The growth from the cultures were confirmed by ZN staining and they were speciated by using biochemical reactions.

Results: Out of the 236 samples which were screened, 116 iso-

lates were obtained. All the 116 were isolated from MB BACT, 82 were isolated from the LJ medium and 62 were isolated from MB7H10. 82 isolates were obtained from MB BACT and the LJ medium, 62 were obtained from MB7H10 and MB BACT, 58 were isolated from LJ and MB7H10 and 58 were isolated from LJ medium, MB7H10 and MB bact. Neither the LJ medium nor the Middlebrook 7h 10 medium could isolate mycobacteria exclusively. It showed that the combination of media did not prove to be superior over the use of MB BACT alone. The average isolation time of LJ, the MB7H10 medium and MB BACT was 30.81 days, 31.06 days and 18.70 days.

Interpretation and Conclusion: MB BACT is a better medium as compared to the LJ medium and the MB7H10 medium, both in terms of the number of isolates and the isolation rate. The MB BACT method proved to be a very speedy method and it could isolate mycobacteria 7-10 days earlier as compared to the LJ medium and the Middlebrook 7 H10 medium.

Key Words: MB BACT, MB7H10 medium, *Mycobacterium tuberculosis*

INTRODUCTION

Tuberculosis has been for many centuries the most important of the human infections, in its global prevalence, its devastating morbidity and its massive mortality. Despite many advances in its diagnosis and treatment, the problem of tuberculosis is on its rise, both globally and in India. At present, the global incidence of this disease is increasing at the rate of 0.4 % per year [1].

It has been estimated that a third of the world's population, about 2 billion people, are infected with the tubercle bacilli. Every year, between 8 and 9 million new cases of tuberculosis appear and 3 million persons die from the disease [2].

A large majority of the cases and deaths are reported from the poor nations. India is one of the worst affected countries. More than 40 % of the population is infected and some 15 million suffer from tuberculosis in our country, of which over three million are highly infectious open cases. In 2009, out of the estimated global incidence of 9.4 million TB cases, 2 million were estimated to occur in India [3].

With the progress of the AIDS pandemic, tuberculosis has become a problem for the rich nations also. A close relationship has emerged between tuberculosis and HIV.

The worldwide spread of Multi Drug Resistant Tuberculosis (MDRTB) has added new troubles to the already existing problem. At present, 3.2 % of the world's new cases of TB are multi drug resistant [4]. In India, the incidence of MDRTB ranges from 1.3 to 3% [5].

The most effective control measure for checking the spread of TB is to detect it early and to treat it optimally at the earliest. Although ZN staining smear microscopy is most commonly employed for an early detection, it is rather insensitive and it fails to detect a large number of cases [6].

Under these circumstances, the cultivation of *Mycobacterium tuberculosis* provides a sensitive and a specific means for the diagnosis of TB. The conventional culture methods such as the use of Lowenstein Jensen medium requires 3 to 6 weeks for its isolation, plus an additional 1 to 2 weeks for its speciation. Such

a prolonged turnaround time in the diagnosis is unacceptable, as rapid detection and identification of MTB is essential both for medical and epidemiological purposes [7].

Thus, there is a need of a culture method that is reliable and which has a short turnaround time. Each method has its own advantages and disadvantages, starting from the LJ medium and the Middlebrook 7H 10 medium (MB7H10) to the present trendy and speedy automated methods like the MB/BACT 3D device [8-13]. MB/BACT is a safer and a quicker method as it is an automated method which involves liquid media and as it does not involve any radioactive material.

The MB/BACT Mycobacteria Detection System utilizes a colourimetric sensor and reflected light to monitor the presence and the production of carbon dioxide (CO_2) which is dissolved in the culture medium. If microorganisms are present in the test sample, carbon dioxide is produced, as the organisms metabolize the substrates present in the culture medium. When the growth of the microorganisms produces CO_2 , the colour of the gas permeable sensor which is installed in the bottom of each culture bottle, changes from blue-green to yellow. The lighter colour results in an increase in the reflectance units, as is monitored by the system. The bottle reflectance is monitored and recorded by the instrument every 10 minutes. MB BACT requires one person who is good at computer basics and is trained, in order to feed the data of the sample and to take the bar code reading. Even though the cost of each MB/BACT bottle is costlier as compared to the conventional LJ medium and the MB7H10 agar, it is cost effective in identifying the growth 1-2 weeks earlier and with even minimum amount of growth.

In the present study, an attempt was made to access the feasibility of using the MB7H10 medium and the MB/BACT 3D device as primary isolation media for MTB. They have been compared with the LJ medium, which is the gold standard.

MATERIALS AND METHODS

The present study was carried out at the Mycobacteriology Division of the Department of Microbiology of BLDEU's Shri B M Patil Medical College, Bijapur, over a period of one and a half years, from October 2009 to May 2011. Over 236 suspected cases of tuberculosis who attended the OPDs of Medicine, Paediatrics, Respiratory Medicine and Surgery and admitted to the wards of these departments, were included in the study. The present study was approved by ethical clearance committee of BLDEA'S Shri. B.M. Patil Medical College, Bijapur India. The informed consent of the patients was taken before the collection of the specimens. There were 225 pulmonary samples and 11 extra pulmonary samples. The extra pulmonary samples which were collected were bronchial washings and biopsy samples from lymph node swellings and bone lesions. All the specimens were examined microscopically by using Ziehl-Neelsen's staining as per the standard protocol [14].

The modified Petroff's technique was used for the decontamination of the sputum samples [15]. Two slants each of the LJ medium slope and the Middlebrook 7H10 (MB7H10) agar slope and the MB/BACT bottle were inoculated for each clinical specimen.

Commercially obtained LJ slants were used for the study (Hi Media). Two loops full of the decontaminated deposit was inoculated on the entire surface of 2 LJ slopes in a pre-sterilized inoculation hood, taking the necessary aseptic precautions. The date

of the inoculation was noted. The slopes were incubated at 37°C for a maximum period of 8 weeks. They were inspected daily for growth or for contamination.

MB7H10 agar base as well as a commercially obtained OADC supplement were used for the study (Hi Media). The medium was prepared as per the manufacturer's instructions and it was dispensed in 10ml aliquots in sterile screw capped bottles.

Two bottles of MB/BACT (procured from Biomerieux) were taken, each of which was inoculated with 500 μ l of the decontaminated deposit aseptically. The date of the inoculation was noted. The tubes were incubated at 37°C for a maximum period of 8 weeks. They were observed daily for any appearance of growth [16].

All the MB/BACT bottles were brought to room temperature along with the MB/BACT antibiotic supplement which was supplied. 0.5 ml of the antibiotic supplement which was provided was injected into the MB/BACT bottle by using a 2ml syringe. 0.5 ml of the decontaminated deposit of the sample was aspirated with the help of a syringe and it was injected into the antibiotic supplement containing MB/BACT bottle. The bottles were loaded into the MB/BACT machine and the machine was checked for a beeping sound which signalled the appearance of growth. The growth of the tuberculosis bacilli on LJ medium, the MB7H10 agar and MB/BACT was confirmed by observing the presence of acid fast bacilli in the ZN stained smears which were made from the colonies.

The mycobacteria were speciated by assessing the rate of growth, the growth at different temperatures, pigmentation, the niacin test, the nitrate reduction test and the catalase test.

An isolate was designated to be that of *Mycobacterium tuberculosis*, if it grew slowly (taking more than 7 days) and had buff coloured, non-pigmented, dry and rough colonies which were difficult to emulsify, which grew only at 37°C and not at room temperature or at 42°C and which showed positive niacin and nitrate reduction tests and a low catalase activity.

RESULTS

The present study was attempted to assess the feasibility of using MB/BACT and MB7H10 as the primary isolation media for mycobacteria. They have been compared with the LJ medium, which was the gold standard.

For the purpose of the study, we included 236 suspected cases of tuberculosis who attended the OPDs of Medicine, Paediatrics, Respiratory Medicine and Surgery and admitted to the wards of these departments.

The three media were compared with respect to the number of isolates, the rate of isolation and the type of isolates.

Two hundred and thirty six suspected cases of tuberculosis were examined, of which one hundred and sixty eight cases were smear positive. Out of the 168 smear positive patients, 51(30.35%) patients had 1(+) grading on smear microscopy, while 79(47.02%) had 2(+) grading and the remaining 38(22.61%) had 3(+) grading.

Among the 236 cases which were screened, mycobacteria were isolated in 82 cases (34.74%) by the LJ medium, in 62 cases(26.27%) by the MB7H10 medium and in 116 cases (49.15%) by the MB/BACT automated system [Table/Fig-1].

Out of the 120 isolates of mycobacteria, 116 (96.66%) were identified as *Mycobacterium Tuberculosis* (MTB), while the remaining 4

| Culture status | LJ | | MB7H10 | | MB/BacT | |
|------------------|-------------|------------|-------------|------------|-------------|------------|
| | No of cases | Percentage | No of cases | Percentage | No of cases | Percentage |
| Culture Positive | 82 | 34.74 | 62 | 26.27 | 116 | 49.15 |
| Culture Negative | 154 | 65.26 | 174 | 73.73 | 120 | 50.85 |
| Total | 236 | 100 | 236 | 100 | 236 | 100 |

[Table/Fig-1]: Distribution of cases according to Culture status

| Type of Isolate | No. of Cases | Percentage |
|-----------------|--------------|------------|
| MTB | 116 | 96.66 |
| NTB | 04 | 03.34 |
| Total | 120 | 100 |

[Table/Fig-2]: Isolation of MTB and NTB

| Duration | LJ | MB7H10 | MB/BacT |
|----------|----|--------|---------|
| 1-7 | 0 | 0 | 0 |
| 8-14 | 0 | 0 | 23 |
| 15-21 | 1 | 0 | 62 |
| 22-28 | 26 | 16 | 31 |
| 29-35 | 43 | 37 | 0 |
| 36-42 | 12 | 9 | 0 |
| Total | 82 | 62 | 116 |

[Table/Fig-3]: Comparison of LJ, MB7H10 and MB/BACT for duration of isolation of MTB

| Duration Days | Median | | | | | | | | | |
|---------------|-----------|----|-----|---------------|----|-----|---------------|----|-----|---|
| | LJ (N=82) | | | MB7H10 (N=62) | | | Mbact (N=116) | | | |
| Duration Days | + | ++ | +++ | + | ++ | +++ | + | ++ | +++ | |
| 1-7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8-14 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 2 | 17 | |
| 15-21 | 0 | 1 | 0 | 0 | 0 | 0 | 6 | 36 | 20 | |
| 22-28 | 4 | 8 | 14 | 1 | 5 | 10 | 6 | 22 | 3 | |
| 29-35 | 3 | 23 | 17 | 0 | 20 | 17 | 0 | 0 | 0 | |
| 36-42 | 0 | 9 | 3 | 0 | 9 | 0 | 0 | 0 | 0 | |
| Total | 7 | 41 | 34 | 1 | 34 | 27 | 16 | 60 | 40 | |

[Table/Fig-4]: Comparison of LJ, MB7H10 and MB/BACT for duration of isolation of MTB and its correlation with smear Microscopy grading

| Type of Isolate | LJ | MB7H10 | MBact | Between LJ & MB7H10 | | Between MB7H10 & MB BACT | | Between LJ & MB BACT | |
|-----------------|-------|--------|-------|---------------------|-----------|--------------------------|---------------|----------------------|---------------|
| | | | | Z-Value | P-Value | Z-Value | P-Value | Z-Value | P-Value |
| Mean | 30.81 | 30.18 | 18.79 | 0.816 | P < 0.420 | 13.36 | P < 0.0001 HS | 20.79 | P < 0.0001 HS |
| SD | 4.44 | 5.91 | 3.45 | | | | | | |
| N | 82 | 66 | 116 | | | | | | |

[Table/Fig-5]: Test of significance between different methods based on duration of isolation

(3.34%) were found to be Non-Tuberculous Mycobacteria (NTM). The further speciation of NTM was not attempted [Table/Fig-2].

Although the maximum incubation period for the growth was 56 days, the maximum time which was taken by any strain to grow, was 42 days.

The mean duration of the isolation on LJ, the MB7H10 medium and MB/BACT was 30.81 days, 31.06 days and 18.70 days respectively. The difference between the LJ medium and the MB7H10 medium was not significant.

Thus, the period of the maximum isolation was the 3rd week, fol-

lowed by the 4th week on MB/BACT and it was the 5th week, followed by the 4th week on the LJ medium and the MB7H10 medium.

Further analysis showed that on the LJ medium, 70 (85.36%) of the 82 strains were isolated by the 34th day of incubation i.e., by the end of the 5th week and that 53 (85.48%) of the 62 strains were isolated on Middlebrook 7H10 in this period. 85 (73.3%) of the 116 strains were isolated by the 21st day of incubation by MB/BACT i.e. by the end of the 3rd week and almost 100% were isolated by the end of the 24th day [Table/Fig-3].

Only MB/BACT could isolate mycobacteria (23 isolates) in the 2nd week. In the 3rd week, the LJ medium could isolate 1 mycobacterium which was 1(+) by the smear microscopy grading, apart from MB/BACT, which isolated 62 isolates, of which 6 were 1(+), 36 were 2(+) and 20 were 3(+) by the smear microscopy grading [Table/Fig-4].

In the 4th week, the LJ medium could isolate 26 isolates and MB7H10 could isolate 16 isolates. MB/BACT could isolate a total of 31 isolates in 4 weeks, of which 6 were 1(+), 22 were 2(+) and 3 were 3(+) by the sputum microscopy grading. In the 5th and 6th weeks, the LJ medium could isolate a total of 55 isolates and MB7H10 could isolate a total of 46 isolates. There were 5 isolates (4 MTB and 1 NTB) that were negative by sputum smear microscopy, but they were isolated by MB/BACT. Of these, three were isolated by 24 days, 1 was isolated by 14 days and 1 was isolated by 26 days by the MB/BACT automated method.

Note : HS → Highly Significant Difference

When the LJ medium was compared with the Middlebrook 7H 10 medium in terms of the number of isolates, the Z value turned out to be 0.816. The P value, according to the Z value, showed that

there was no much significant difference between the two if the maximum allowable error was 42%. When the same 2 media were compared in terms of the turnaround time for isolation, it was found that most of the isolates were detected earlier by the Middlebrook 7H 10 medium by the end of the 5th week [Table/Fig-5].

When the Middlebrook 7 H 10 medium and MB/BACT were compared with each other, the Z value turned out to be 13.36, with a P value <0.0001, thus showing that there was a highly significant difference between the 2 methods. The MB/BACT method turned out to be more superior than the MB 7H 10 medium, both in terms of the number of isolates and the isolation rate [Table/Fig-5].

When the LJ medium and MB/BACT were compared with each other, the Z value turned to be 20.79, thus giving a P value of <0.0001. This showed that there was a highly significant difference between the 2 methods. MB/BACT proved to be a superior method over the LJ medium, both in terms of the number of isolates and the isolation rate [Table/Fig-5].

DISCUSSION

The present study was attempted to assess the feasibility of using MB/BACT and MB7H10 as primary isolation media for mycobacteria. They were compared with the LJ medium, which was the gold standard. In our study, the grading of the ZN stained smears was done as per the recommendations of NTI. The percentages of the patients with the microscopy grades of 1+, 2+ and 3+ were 30.35%, 47.02% and 22.61% respectively.

Our findings were in accordance with those of Paramashivan C.N [5] and coworkers. In their study, 76.7% patients were of the 1(+) grade, while 0.5% patients were of the 3 (+) grade [17].

The grading of the smears gives an idea with regards to the bacterial load. It depends upon various factors such as the time of collection, the number of samples which are taken, the nature of the samples, the treatment with antituberculous drugs and its duration and the method of grading which was used. In our study, a large number of patients were on the antituberculosis treatment for variable time periods. This might have reduced the bacterial load. So, a majority of patients were of grade 1 (+), which ranked highest among the 3 grades.

In our study, the overall rate of isolation was 51% (120/236). It included both MTB and the Non-Tuberculous Mycobacteria (NTM) which were isolated on LJ or MB7H10 or both. Our isolation rate was comparable to that which was reported by Narang P. et al., [18]. It was higher than that which was reported by Ghatole M et al., [19]. Kothadia et al., [20]. Narang P and Mendiratta et al., [18], while it was lower than that which was observed by Damle et al., [21], Tsukamura et al., [22] Erlich et al., [23] and Jena et al., [24].

The Important Factors which are Responsible for Such a Wide Variation are as Under:

The case selection criteria are important. Damle et al., [21] (80%) included the smear positive cases in their study, while Jena et al., [24] (85.1%) included the clinically and radiologically proven cases of pulmonary tuberculosis in their study. On the contrary, Kothadia et al., [20]. (25.47%) and Chauhan et al., (21%) used the clinically "suspected" cases as their subjects. We included the clinically suspected cases of tuberculosis in our study.

The number of sputum samples which are collected and the methodology of collection exert their influence on the rate of isolation. Jena et al., (85.6%) [24] used three consecutive samples, while Narang P. et al., (56.23%) [18] used 2 samples per patient. One was a spot collection and the other was an overnight collection. The rate of isolation varies with the method of decontamination which is used. Damle and Kaundinya found that the Nassau's swab method of decontamination was superior to Petroff's method and the NALC method in giving positive cultures. The study of Claudio Peirsomoni et al., has also reported a variation in the culture yield when different decontamination methods were used [25]. We included the modified Petroff's method in our study. The previous antitubercular treatment and its duration is an

important determining factor for the isolation of MTB. Jena et al., and Panda B. et al., have conclusively proved that the rate of isolation decreases as the duration of the antituberculosis treatment increases. In our study, 34 out of the 48 smear positive and culture negative patients were on antitubercular treatment.

In our study, of the 120 isolates, 116 (96.66%) were identified as MTB, while the remaining 4 (3.34%) were identified as NTM. No attempt was made to speciate the NTM isolates further. Our observations with regards to the isolation of NTM were comparable to those of Trivedi et al., Saran et al, and Mukhopadhyaya et al. They are considerably lower than that of Pyffer G. E. et al., and Enrico Tortoli et al., [26-28].

In our study, among the 236 cases which were screened, mycobacteria were isolated in 82 cases (34.74%) by the LJ medium, in 62 cases (26.27%) by the MB7H10 medium and in 116 cases (49.15%) by the MB/BACT automated system. The sensitivity, specificity, positive predictive value and the negative predictive value of the MB7H10 medium in comparison to LJ was 69.5%, 94.3%, 95% and 66.7% respectively.

Our results were comparable to that of Claudio et al., [25], Paul I Lin et al., [11], Adler et al., [8] A.Carricajo [9] et al., and Angeby [10] K.A et al.. The sensitivity and the negative predictive value of MB7H10 in comparison to those of LJ was 69% and 63% respectively [16]. A lack of sensitivity which was observed in these two studies, makes MB7H10 a less preferred medium for the primary isolation of MTB, on its own. In our study, no isolates were detected exclusively on the MB7H10 medium. The recommendation of Martin T. to use a larger size of the inoculum did not work out in our study and in that of Bhargava A et al., The reason for getting a low yield on the MB7H10 medium Could be due to not incubating the bottles under a CO₂ atmosphere.

In our study, the mean duration of isolation on the LJ medium, the MB7H10 medium and on MB/BACT were 30.81 days, 31.06 days and 18.70 days respectively. The difference was not significant between the LJ medium and the MB7H10 medium. But there was a highly significant difference between MB/BACT and the other two media. The period of maximum isolation was the 3rd week, followed by the 4th week for MB/BACT. The period of maximum isolation was the 5th week, followed by the 4th week for the LJ medium and the MB7H10 medium.

Our findings correlated substantially with those of Bhargava A et al., [16], who found that the average time which was taken by LJ and MB7H9 for the detection of growth was 5 weeks and 6 weeks respectively. These findings may be due to a similarity in the selection criteria. Bhargava A. et al., used smear positive and negative cases as well as pulmonary and extra pulmonary cases. The duration of isolation of mycobacteria on the MB7H10 medium in our study was comparable with that which was found by Concepcion F, RMT and Myrna T. Mendoza et al., who found Middlebrook 7H 10 to have an equal isolation time as compared to the LJ medium.

MB/BACT could isolate mycobacteria at an average duration of 18.70 days, which was similar to the findings of A.Carricajo et al and Claudio Piersoni et al., MB/BACT proved to be superior to the LJ medium and the MB7H10 medium in the isolation rate, as it could isolate mycobacteria 7-10 days earlier as compared to the other two media [29-31]. This finding was similar to those of Concepcion F, RMT and Myrna T. Mendoza et al., [32].

CONCLUSIONS

The present study was attempted to assess the feasibility of using MB/BACT and MB7H10 as primary isolation media for mycobacteria. They were compared with the LJ medium, which was the gold standard.

MB/BACT is a better medium as compared to the LJ medium and the MB7H10 medium, both in terms of the number of isolates which were obtained and the isolation rate. MB/BACT proved to be a very speedy method and it could isolate mycobacteria 7-10 days earlier as compared to the LJ medium and the Middlebrook 7H 10 medium.

In our study, the MB7H10 medium could isolate mycobacteria at the same speed as compared to the LJ medium and there was not much significant difference in the number of isolates statistically, with the allowable error of 42%. The MB7H10 medium could be thought of as an alternative to the LJ medium, as it is cheaper and even easy to prepare.

MB/BACT is a safer and quicker method, as it is an automated method which involves liquid media and as it does not involve any radioactive material.

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