Original Paper

COMPARISON OF CONVENTIONAL AND AUTOMATED CULTURE SYSTEM FOR ISOLATION OF MYCOBACTERIUM TUBERCULOSIS

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Abstract

This was a prospective comparative study between conventional and automated culture system for isolation of *Mycobacterium tuberculosis.* Sputum samples from 138 clinically diagnosed cases of pulmonary tuberculosis from Institute of Disease of the Chest & Hospital, Dhaka, Bangabandhu Sheikh Mujib Medical University, Dhaka & Combined Military Hospital, Dhaka were collected and culture was carried out in both conventional Lowenstein-Jensen (L-J) media & MB-BacT automated culture system to compare the recovery rate, mean detection time and contamination rate. The study was carried out at the Armed Forces Institute of Pathology, Dhaka Cantonment, between March 2000 and January 2001. Out of the 138 specimens, 87 were culture positive. Among these, 83 cases (95.40%) were detected by MB/BacT and 74 cases (85.06%) by L-J media. The difference was statistically significant ($p<0.05$). Statistically significant difference ($p<0.05$) was also observed for smear negative culture positive cases (41 & 32 respectively). Of the total 87 culture positive cases, 70 were detected by both the systems but among the rest, MB/BacT alone detected 13 cases & L-J media alone detected only 04 cases. The difference was statistically significant ($p<0.05$). The mean detection times for smear positive culture positive cases (41 sample) on an average were 9.24 days by MB/Bac T system and 20.6 days by L-J media. While for smear negative culture positive cases (45 sample), the figures were 18.50 and 27.50 days respectively. All the differences were statistically significant ($p<0.05$). MB/BacT system showed less contamination rate, 05 cases (3.62%) in contrast to the 07 cases (5.07%) for L-J method. This difference also reached statistically significant ($p<0.05$). The MB/BacT system thus found to be superior method of choice especially for handling large number of specimens.

Key Words: Automated culture system, Mycobacterium tuberculosis.

Introduction

Tuberculosis is a disease of great antiquity. In the past, tuberculosis has been referred to as the captain of death[1]. It caused one billion deaths in the last 200 years[2]. In Europe it was responsible for one in ten deaths in the last century[3]. The worldwide magnitude of the modern tuberculosis is so great that in April 1993 the World Health Organization (WHO) declared tuberculosis to be a global emergency[4]. About one third of the world's population have been infected with *Mycobacterium tuberculosis*[5]. Nearly three million cases of tuberculosis and one million deaths occur each year in South East Asian region. Everyday more than 1,500 people in the region die from tuberculosis. The situation is expected to worsen due to the emergence of multi-drug resistant tuberculosis and HIV-TB co-infection [6].

Many systems have been developed in the recent years apart from the traditional microscopy and culture on Lowenstein-Jensen (L-J) medium. MB/ BacT system is a non-radiometric TB-culture system having comparable results with other automated systems[7]. In different studies, the automated systems proved to yield increased sensitivity and specificity, significant reduction in mean detection time and contamination rate compared to traditional system. In this study, the automated MB/BacT system with Lowenstein-Jensen media in respect to recovery rate, mean detection time and contamination rate was compared and evaluated.

Materials and Methods

A total of 138 clinically diagnosed cases of pulmonary tuberculosis of both sexes were included in this study. The patients were selected from the Institute of Disease of the Chest and Hospital (IDCH), Dhaka; Combined Military Hospital, Dhaka Cantonment and Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, between March 2000 and January 2001. Patient's history, clinical examination, other laboratory parameters were recorded carefully in a pre-designed data sheet.

One sputum specimen was collected from each patient. Some patients required postural drainage. All the samples were carried to the Department of Microbiology and Immunology, Armed Forces Institute of Pathology (AFIP), where further works were done. The sputum samples were processed by N-acetyl-L cystine-sodium hydroxide method (2% NaOH, 2% Na-citrate and 0.5% NALC) and microscopic reporting carried out as per Kent and Kubica[8].

The sediment of the processed sample was inoculated immediately into the supplemented MB/ BacT bottles and two tubes of Lowenstein-Jensen media. Culture was carried out for 9 weeks for L-J slopes & 6 weeks for MB/

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BacT bottles after which slopes/bottles failing to show any growth were discarded as negative. On the other hand, any bottle detected and displayed as positive was taken out of the instrument and subsequent identification was carried out by different biochemical test.

Results and Observations

Among 138 clinically diagnosed cases of pulmonary tuberculosis 99 (71.74%) were male and 39 (28.26%) were female. Out of these, 46 (33.33%) were smear positive. The rate of smear positivity was almost same in case of male and female (Table-I).

Among the smear positive 46 cases, 41 (89.13%) were culture positive for Mycobacteria in both L-J media and MB/BacT system (Table-II).

Among the smear negative 92 cases, 45 (48.91%) were culture positive. MB/ BacT and L-J media detected growth of Mycobacteria in 41 (44.56%) and 32 (34.78%) specimens respectively. (Table-III).

Among 87 culture positive cases 59 (67.82%) were male and the remaining 28 (32.18%) were female. The male to female ratio was 2.11:1. The majority 70 (80.46%) of them were in the age group of 15 to 44 years with peak incidence (36.78%) in 25-34 years of age (Table-IV).

Table-V shows the recovery rate of mycobacteria by the two systems. Among 87 culture positive cases 86 (98.85%) were *M. tuberculosis* and one case (1.14%) was non-tuberculous Mycobacterium (NTM) and it was a non-photochromogen. The MB/ BacT detected a total of 83 (95.40%) and L-J media yielded 74 (85.06%) cases. Among the smear negative and culture positive cases (45) MB/ BacT and L-J media detected growth of

Table-I: Results of microscopic examination of sputum for AFB (n=138).

Sex	Total number of patients	Smear positive for AFB	Smear negative for AFB
Male	99 (71.74)	34 (34.34)	65 (65.66)
Female	39 (28.36)	12 (30.77)	27 (69.23)
Total	138 (100)	46 (33.33)	92 (66.67)

Figures within parenthesis indicate percentages.
AFB: Acid fast bacilli

Table-II : Results of culture of smear positive cases (n=46).

Results of culture	MB/BacT	L-J media
Growth of Mycobacteria	41 (89.13)	41 (89.13)
No growth of Mycobacteria	5 (10.87)	5 (10.87)
Total	46 (100)	46 (100)

Figures within parenthesis indicate percentages.

Table-III : Results of culture of smear negative cases (n=92).

Results of culture	MB/BacT	L-J media
Growth of Mycobacteria	41 (44.57)	32 (34.78)
No growth of Mycobacteria	51 (55.43)	60 (65.22)
Total	92 (100)	92 (100)

Figures within parenthesis indicate percentages.

Table-IV : Age and Sex distribution of culture positive cases (n=87).

Age group (years)	Male	Female	Total
5-14	02	0	02 (2.29)
15-24	15	8	23 (26.44)
25-34	20	8	28 (32.18)
35-44	10	5	15 (17.24)
45-54	09	6	15 (17.24)
*>55	03	1	04 (4.60)
Total 59 28 87 (100)			

Figures within parenthesis indicate percentages.
Maximum age was 74 year.

Table-V : Recovery rate of Mycobacteria by the two systems (n=87).

Mycobacterial species	No. (%) positive specimens detected by	
	MB/BacT	L-J medium
M. tuberculosis (n=86)	82 (95.34)*	73 (84.88)*
Smear positive culture positive (n=41)	41 (100)	41 (100)
Smear negative culture positive(n=45)	41* (91.11)	32* (71.11)
NTM (n=1)	01 (1.15)	01(1.15)

Figures within parenthesis indicate percentages. * p<0.05

Table-VI : Comparison of the number of specimens from which Mycobacterium could be isolated with MB/BacT and L-J media (n=87).

Species identified	Total no of specimens	No. of specimens positive in		
		MB/BacT and L-J media	Only MB/BacT system	Only L-J media
M. tuberculosis	86	69	13*	4*
NTM	01	01	00	0
Total	87	70	13	4

* p<0.05

Table-VII : Detection times of Mycobacteria in clinical specimens by the two culture systems.

Micobacterial Isolation	Mean detection time (days)			
	MB/BacT		L-J medium	
	Average	Range	Average	Range
M. tuberculosis Smear positive and culture positive(n=41)	9.24*	5-36.2	20.6*	08-42
Smear negative culture positive (n=45)	18.5*	6-41.5	27.5*	12-42
NTM (n=1)	18.2	--	21	--
Total (n=87)	14.73		24.17	

* p<0.05

Table-VIII : Rate of contamination by the two systems (n=138).

Culture systems	Number (%)
MB/BacT	5 (3.62)
L-J media	7 (5.07)
Only MB/BacT	0 (0)
Only L-J	2 (1.45)

Mycobacteria in 41 (47.13%) and 32 (36.78%) specimens respectively reaching statistically significant difference (P<0.05). Among these the number of samples not showing any growth in MB/ BacT and L-J media were 04 (4.60%) and 13 (14.94%) respectively.

When it was the only medium positive (Table-VI) MB/BacT and L-J media alone detected 13 and 4 species of *M. tuberculosis* respectively. The remaining 70 Mycobacteria grew in both the system. The MB/BacT system recovered more mycobacteria reaching statistical significance (P<0.05).

Table-VII shows the detection times of Mycobacteria by the two systems. For smear positive and culture positive 41 specimens, MB/BacT and L-J media detected on an average of 9.24 and 20.6 days respectively. For smear negative and culture positive cases these were 18.5 and 27.5 days respectively. The differences reached statistical significance (P<0.05).

Table-VIII shows the rate of contamination by the two systems. Out of total 138 specimens, the overall contamination rate of L-J media stood 7 (5.07%) and that of MB/BacT system was 5 (3.62%). The most frequent contaminations in both media were Gram-positive organisms including Staphylococci and some Gram positive bacilli.

Discussion

Technical development for the detection of Mycobacteria always received much attention. The BECTEC 460 system in conjunction with the Middlebrook 12B liquid medium has become a reference system and widely used for the detection of Mycobacteria in industrialized countries[9]. Despite all of its advantages, the BACTEC 460 system has certain limitations like high cost, high workload, radioactive reagents and possible cross-contamination[10].

The most important disadvantage of Egg based media like Lowenstein-Jensen (L-J) is the prolonged incubation period. Considering this problem certain improvements have been reported with manual systems, such as the Septi-Chek system (Becton Dickinson)[11,12] and the Mycobacterial Growth Indicator Tube (MGIT)[13]. However all these systems require much manual handling. The recently developed MB / BacT system is one of the first fully automated systems for culture of Mycobacteria.

In this study, the majority (75.86%) of the cases were within the age group 14-44 years. This correlates with Mashrek et al[14] (75%), but not with that of Oliver and Hanvey from Australia, who reported the majority in older ages (after 70 years). The difference may be correlated with the different socio-economic background. In Bangladesh, tuberculosis is endemic and most active mobile elements of the society (14-44 years) are at increased risk of contacting the disease.

Out of 87 culture positive cases 59 (67.82%) were male and 28 (32.18%) were female with male to female ratio of 2.11:1. This correlates closely with 'Review of the National Tuberculosis Programme of Bangladesh[15] where the ratio was 2.5: 1 and Siddique et al[16] (2.44:1). The higher rate of male cases may be the real reflection of tuberculosis in the community or may be due to lower hospital attendance of female patients.

Among the 138 sputum samples of pulmonary tuberculosis, 46 (33.33%) were smear positive. This result correlates with Mashreque et al (28.70%)[14] and Wadud et al (29%)[17] in Bangladesh. In another study in Bangladesh, Huq et al[18] found 40% positivity but in this case, the samples were collected from the admitted patients only.

While among 45 smear negative culture positive cases of *M. tuberculosis,* MB/BacT alone detected Mycobacteria in 13 samples and L-J media in only 4. The difference of 9 (20%) out of 45 smear negative and culture positive samples is statistically significant (P<0.05). Out of the total 86 culture positive cases of *M. tuberculosis* MB/BacT and L-J media detected 82 (95.34%) and 73 (84.88%) respectively with statistically significant difference(p<0.05). These figure correlates closely with Rohner et al[19] who showed the percentage of 93.65 and 84.13 respectively. Among the smear negative culture positive cases 04 samples failed to show growth in MB/BacT. This needs further evaluation. It may be the inherent limitation of the system.

Out of 46 smear positive cases, 41 (89.13%) yielded pure growth of Mycobacteria in both L-J and MB/BacT. This explains that load or quantum of the organism is an important determinant. Five samples of smear positive cases showing no growth may be explained by effects of antitubercular agents. For these treated cases addition of antimicrobial agent inactivating substances such as charcoal, Fuller's earth, or resins to culture media for Mycobacteria may provide more reliable results[20]. The isolation rate correlates with other studies[21].

Centre for Disease Control and Prevention recommends that the reports of isolation and identification of *M. tuberculosis* complex species should be available within 10 to 14 days or 21 days of specimen collection[22]. With the two systems the mean differences of detection time was estimated. That was 11.36 days for smear positive cases, 9 days for smear negative cases and 2.8 days for NTM. Pair-wise comparisons of mean detection time and the total average detection times were statistically significant. Reported data are in agreement with previous study of 11.8 to 21 days for MB/BacT and from 16.7 to 31 days for L-J media[19]. Some recent studies showed comparable results[23,24] .

In regards to contamination, 5 same specimens (3.62%)

proved to contaminate both systems with additional 2 (1.45%) specimens contaminating L-J media alone. This explains that the predominant contamination probably originated during specimen collection. Additional 2 (1.45%) contaminations of L-J media might have occurred subsequent handling of the L-J tubes. The lower rate (3.62%) of contamination in MB/BacT system may be related to its closed incubation and monitoring system and use of PANTA which was not used in the earlier version of the instrument where the rate was >9% [19]. The overall 3.62% of contamination rate fulfils the CDC requirement (5%). Similar rate (4.6%) was observed by Claudio et al[23]

The present study seems to be the first one on the comparison between an automated system and Egg-based media for the TB diagnosis in Bangladesh. This study evaluated the recovery rates and the detection time of Mycobacteria from 138 clinical specimens by each system alone and also by system combination. The MB/BacT proved to be a superior method. As no individual system correctly detected all mycobacteria, it is suggested to employ a combination of liquid and solid media. This information agrees with other recent studies [19].

The advantages of MB/BacT system includes it's high level of automation with reduced risk of transcription or vial inversion error. It is a closed system so cross-contamination risk is minimum. It has data management capabilities and the cost of maintenance is low. But most important advantage is the improved and fast detection of Mycobacteria compared to L-J media.

On the other hand L-J media is still a useful media for the growth of most clinically significant Mycobacteria. It has long shelf life and is ideal for small scale laboratories. The disadvantages are long incubation period, increased contamination rate, inability to perform drug susceptibility test. Although combination of the two methods as shown earlier is ideal, MB/BacT system may be considered to be an effective alternative to L-J system and even BACTEC 460 system for the culture of Mycobacteria in large capacity laboratories.

Conclusion

This study tried to focus attention towards the technical developments for the detection of Mycobacterial species. Bangladesh is endemic with tuberculosis and with introduction of HIV infection the situation may aggravate further. This may create burden on different laboratories for the prompt detection and reporting of Mycobacteria. The MB/BacT system is a non-radiometric, fully automated, closed monitoring system, which may face this challenge effectively especially for large capacity laboratories. Lowenstein-Jensen media is still very useful but it is time consuming and involves more manpower and has increased contamination rates and ultimately delays the prompt detection and reporting system. Even then, combination of the two methods are proved to increase the overall output.

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