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Original Research Article

MYCOBACTERIAL DIAGNOSIS-COMPARISON OF AN AUTOMATED CULTURE SYSTEM WITH THE CONVENTIONAL METHOD

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Abstract

Background: Tuberculosis is an important disease of public health importance. Drug resistant tuberculosis especially multidrug resistance poses great challenge because of prolonged and expensive treatment options and in 30% of cases treatment failure and death occurs. By automated system rapid and efficient recovery of *mycobacterium tuberculosis* as well as detection of drug resistance is possible. **Materials and Methods:** A hospital based cross sectional study was done on clinically diagnosed tuberculosis patients who were on treatment. Both pulmonary and extra pulmonary specimens collected from 243 patients which were processed by modified Petroff's method were inoculated into both MB/BacT and Lowenstein Jensen medium. Drug susceptibility tests to first line anti TB drugs were done by proportion method using automated MB/BacT system and conventional LJ medium. **Result:** Isolation rate by automated method was 30.85% and 8.64% by conventional method. For pulmonary specimen it was 41.06% and 11.9 % respectively whereas for extra pulmonary it was 14% and 3.22%. Average time taken for isolation was 9.2 days by automated method compared to 42.48 by conventional method. Mean time for detection of drug resistance was 10.24 days for MB/BacT and 27.29 for LJ medium. Out of 17 isolates 12 and 11 drug resistant strains were detected by MB/BacT method and by LJ medium respectively.11 MDR strains were detected by both methods. **Conclusion:** Higher isolation rate, faster recovery of mycobacterium tuberculosis and earlier detection of drug resistance is possible by automated culture system compared to conventional method.

INTRODUCTION

Tuberculosis (TB) is a chronic granulomatous disease caused by mycobacterium tuberculosis (MTB) complex. It remains as one of the leading causes of death from infectious diseases at global level.[1] Globally India contributes to nearly 1/3 of world's tuberculosis cases and has highest rate of new tuberculosis cases with half a million deaths.[2] Despite discovery of anti-tubercular drugs and efforts of WHO and international union against tuberculosis it continues to be an important public health problem facing mankind particularly developing countries.[3] Continuing spread of drug resistant TB is the most urgent and difficult challenge facing global TB control.[4] This has intensified the need for rapid isolation of MTB and drug susceptibility testing which is useful for initiation of timely and appropriate therapy.[5] Definite diagnosis depends on isolation and identification of MTB by laboratory cultivation which is more sensitive than microscopy and allows recovery of bacteria for drug susceptibility testing.[6] By conventional method (Lowenstein Jensen medium) it takes 4-8 weeks or more for growth. Automated system (MB/BacT) using broth-based media as compared to conventional method has reduced the time for isolation and improved recovery.[7] Drug susceptibility testing using solid medium is time consuming and takes about 4-6 weeks due to slow growth on solid medium. Automated culture system enables laboratory to determine susceptibility to first line anti TB drugs within 1-2 weeks. Multidrug resistant (MDR) and Extensively Drug Resistant (XDR)TB continues to be significant public health problem in many countries including India.[1] So earlier diagnosis, detection of drug resistance and treatment help in preventing transmission of drug resistant tuberculosis.

MATERIALS AND METHODS

A Hospital based cross-sectional study was conducted at Department of Microbiology, Govt

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Medical College Kozhikode, India for a period of one year after getting approval of Institutional Ethics Committee. Clinically diagnosed tuberculosis patients who were on treatment were included and the patients on antitubercular treatment were excluded from the studies.

Both pulmonary and extra pulmonary specimens were collected in sterile leak proof containers provided. In case of any delay in processing specimens were refrigerated and processed within 24 hours itself. Totally 243 specimens were collected and processed. Smears were prepared from the specimens and Ziehl Neelsen staining was done. Sputum smears were graded as per Revised National Tuberculosis Control Programme (RNTCP) grading. Specimens from non-sterile sites were decontaminated and concentrated by modified Petroffs method.[7] Double the volume of 4%NAOH was used for pulmonary samples whereas equal volume of 2% NAOH was used for decontaminating extra pulmonary samples. Specimens from sterile sites were not decontaminated and inoculated directly. Decontaminated specimens were centrifuged at 3000g and the deposit was taken for inoculation. Biopsy and bone marrow specimens were grounded using sterile mortar and pestle with minimum amount of sterile distilled water. Processed specimens were inoculated onto automated MB/BacT culture bottles and to two slopes of Lowenstein Jensen medium (LJ medium). MB/BacT bottles were loaded into the MB/BacT system and LJ bottles were incubated at 37^0C. Checked for growth in LJ bottles daily for first week and then weekly for 8 weeks. MB/BacT bottles which were flagged positive were taken out and Ziehl Neelsen staining was done for confirmation and sub cultured to LJ slopes. MB/BacT bottles without growth were declared negative after 41 days. Negative LJ bottles were discarded after 60 days. Identification of the isolates were done by heat stable catalase test and nitrate reductase test to differentiate between mycobacterium tuberculosis and nontuberculous mycobacteria (NTM).

Drug susceptibility test was done for the17 isolates obtained with both automated and conventional methods by proportion method using LJ medium with incorporated drugs and MB/BacT. Concentration of the drugs were 0.2mg/l, 40mg/l, 4mg/l and 2mg/l for INH, rifampicin, ethambutol and streptomycin respectively. Growth suspension prepared was inoculated onto drug incorporated and control medium and incubated at 37^0C.The first reading was taken after 28 days and second reading on 42^{nd}day.The percentage resistance was calculated as the ratio of the number of colonies on drug containing media to those on control media. If it was >1% the isolate was taken as resistant.[8]

0.5 ml of reconstituted antibiotics INH, rifampicin, streptomycin and ethambutol were added to four MB/BacT bottles and distilled water was added to two other bottles. 0.5 ml restoring fluid was added to all six bottles.0.5 ml of undiluted growth suspension was added to the bottles containing drugs and to one bottle taken as direct control.0.5 ml of 1/100 diluted growth suspension was added to sixth bottle which was proportional control. If drug incorporated bottles flagged positive before or along with proportion control bottle it was considered as resistant and bottles flagged positive after proportional control were considered sensitive.

Statistical Analysis

All the data were entered in MS excel spread sheet. Qualitative variables were expressed as frequency and percentage and statistical significance were measured by chi-squared test. Test performance was assessed by determining sensitivity, specificity, positive predictive value and negative predictive value. Agreement between the tests were assessed by Kappa value and P value of <.05 was considered as statistically significant. Comparison of nominal data was performed by paired T test.

RESULTS

Isolation rate by automated method (MB/BacT) was 30.85% whereas by conventional method (LJ medium) it was only 8.64%.[Table1]Highest isolation was obtained from sputum followed by lymph node biopsy in both MB/BacT and LJ medium.

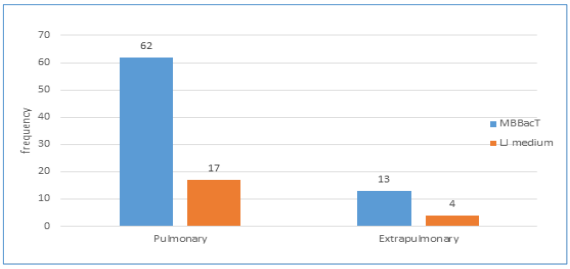

Figure 1: Pulmonary and extrapulmonary isolates by automated and conventional culture

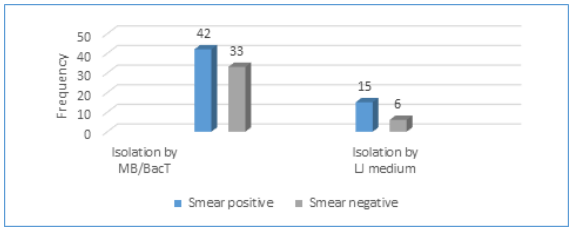

Figure 2: Smear positive and smear negative isolates by automated and conventional methods

For pulmonary specimens rate of isolation by automated and conventional method was 41.06% and 11.9% respectively whereas for extra pulmonary specimens it was 14% and 3.22%. [Figure 1]

Isolation from smear positive specimens by automated and conventional methods were 91.3% and 32.6% respectively whereas from smear negative specimens it was 16.75 % by automated method and 3.04 % by conventional method. [Figure 2]

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Table 1: Specimen wise rate of isolation of MTB by automated and conventional methods.

Specimen	Number	Isolation by MB/BacT	Isolation by LJ medium
		Frequency (%)	Frequency (%)
Sputum	117	53 (45.3)	17(14.53)
Bronchial washing	34	9 (26.47)	1 (2.94)
CSF	18	1(5.56)	0 (0)
Urine	18	2(11.11)	1(5.56)
Lymph node biopsy	14	5(35.71)	2(14.28)
Pleural fluid	10	1(10)	1(10)
Tissue	9	2(22.22)	0(0)
Pus	8	1(12.5)	1(12.5)
Bone marrow	7	1(14.29)	0(0)
Other aspirates	3	0(0)	0(0)
Pericardial fluid	2	0(0)	0(0)
Ascitic fluid	2	0(0)	0(0)
Peritoneal fluid	1	0(0)	0(0)
Total	243	75 (30.85)	21 (8.64)

Total 17 isolates were obtained by both MB/BacT and LJ medium. 58 isolates were obtained by automated method alone but not by conventional method. [Table 2] Difference in isolation rate was highly significant since P value was <.0001.

Table 2: Comparison of culture results by automated and conventional methods.

MB /BacT	LJ medium			X^2 value	P value
	Positive	Negative	Total	27.03	<.0001
Positive	17	58	75		
Negative	4	164	168		
Total	21	222	243		

From pulmonary specimens 14 isolates were obtained from both MB/BacT and LJ medium. 48 were recovered from MB/BacT alone and 3 isolates were obtained from LJ medium alone. Among extrapulmonary specimens 3 isolates were obtained from both systems.10 isolates were obtained from MB/BacT alone.The difference in isolation rates from pulmonary and extrapulmonary specimens by MB/BacT and LJ medium were statistically highly significant since P value was <.001 and .004 respectively [Table 3].

Table 3: Comparison of culture results of pulmonary and extra pulmonary specimens by automated and conventional methods

MB/BacT	LJ Medium			X^2 test	P value
Pulmonary specimens					
	Positive	Negative	Total		
Positive	14	48	62	11.64	<.001
Negative	3	86	89		
Total	17	134	151		
Extrapulmonary specimens					
Positive	3	10	13	8.0633	.004
Negative	1	78	79		
Total	4	88	92		

13 isolates were obtained from both automated and conventional method from smear positive specimens and 29 were obtained by automated alone. 2 were isolated from LJ medium alone. But the difference in isolation rate was not statistically significant since the P value was .60. Whereas in smear negative isolates 4 were recovered from both systems. 29 isolates were obtained from MB/BacT system and 2 from LJ medium alone. This difference was highly significant since P value was .006. [Table 4]

Table 4: Comparison of culture results of smear positive and smear negative specimens by automated and conventional methods

MB/BacT	LJ Medium			X^2test	P value
Smear positive specimens				0.0477	.60
	Positive	Negative	Total		
Positive	13	29	42		
Negative	2	2	4		
Total	15	31	46		
Smear negative specimens				7.667	.006
Positive	4	29	33		
Negative	2	162	164		
Total	6	191	197		

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Mean detection time for the isolates were 9.2 days by MB/BacT system and 42.48 days by LJ medium.[Table 5] By Paired T test this difference was found to be statistically highly significant since P value was<.0001.

Table 5: Comparison of mean detection time by MB/BacT and LJ medium

Mean detection time in days by MB/BacT	Mean detection time in days by LJ medium	df	T	P value
9.62	42.48	20	22.8745	<.0001

71% of isolates were obtained within 20 days by MB/BacT. But there was no isolation from LJ medium within that period. [Table 6] Minimum detection time was 3 days in MB/BacT and 30 days in LJ medium.

Table 6: Comparison of time of appearance of growth by automated and conventional methods

Time of appearance of growth	Automated method Number (%)	Conventional method Number (%)
01-10 days	13	0
11-20 days	42	0
21-30 days	12	2
31-40 days	8	7
41-50 days	0	6
51-60 days	0	6

Drug susceptibility tests for first line antitubercular drugs were done for 17 isolates obtained from both MB/BacT and LJ medium. Out of 17 isolates 4 were sensitive to all four drugs and 12 were drug resistant by MB/BacT. By LJ medium in 11 isolates drug resistance could be detected. 11MDR strains could be detected by both methods. Out of 11 MDR cases 8 were having history of treatment failure and 3 were defaulters. Resistance to INH was detected in 12 by MB/BacT and in 11 by LJ medium. Rifampicin resistance was detected in 11 by both methods. Streptomycin drug resistance was detected in 9 by MB/BacT and 10 by LJ medium. Ethambutol drug resistance was detected in 8 by MB/BacT and 7 by conventional method. [Table7]

Table 7: Drug susceptibility test result by MB/BacT system and LJ medium

Drug	Resistant by both MB/BacT and LJ medium	Resistant by MB/BacT and sensitive by LJ medium	Resistant by LJ Medium and sensitive by MB/BacT medium	Sensitive by MB/BacT and LJ medium	Total
INH	11	1	nil	5	17
Rifampicin	11	nil	nil	6	17
Streptomycin	9	nil	1	7	17
Ethambutol	7	1	nil	9	17

For all drugs kappa value was found to be >.80 and *P* value was <.0001. So almost perfect agreement between MB/BacT and LJ medium.

Table 8: Performance of MB/BacT in comparison with LJ medium for detection of drug resistance

Detection of drug resistance	Sensitivity	Specificity	PPV	NPV	Kappa value	P value
INH	100	83.33	91.67	100	.86	<.001
Rifampicin	100	100	100	100	1	<.001
Streptomycin	90	100	100	87.5	.88	<.001
Ethambutol	100	90	87.5	100	.88	<.001
INH+Rifampicin	100	100	100	100	1	<.001

Average time for detection of drug resistance were 10 .24 days for MB/BacT and 28.29 days for LJ Medium. Since P value is < .0001 the difference is highly significant.

Table 9: Mean turnaround time in days for drug susceptibility test by MB BacT and LJ medium

	MB/BacT	LJ medium	df	T value	P value
Mean turnaround time in days for drug susceptibility results	10.24	28.29	15	10.1396	<.0001

DISCUSSION

Early bacteriological diagnosis and detection of drug resistance are essential for appropriate treatment and prevention of transmission of tuberculosis.[1] Continuing global threat of tuberculosis has led to an urgent need to design more effective diagnostic methods. A positive culture for mycobacterium tuberculosis confirms the diagnosis of active disease. Despite molecular techniques definite diagnosis of tuberculosis still relies on culture of MTB. Mycobacterial culture and drug susceptibility testing

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using solid medium are the standard methods due to their simple procedures. For increasing isolation rates, reducing the period for isolation and detection of drug resistance in a short time liquid culture system is preferable.[1]

Highest isolation was obtained from sputum by both methods in our study. In a study by MirovicV.et al in 2002 also higher isolation was obtained from respiratory samples.[9] Since the bacillary load is more for sputum especially smear positive, recovery rate will also be higher in them. Tuberculous lymphadenitis is the most common form of extrapulmonary tuberculosis. Isolation rate from lymph node biopsy was highest among extrapulmonary specimens in our study. In a study done in Kerala in 2020 also isolation rate from lymph node biopsy was found to be highest among extrapulmonary samples.[10] In this study isolation rates by automated method was 30.85% compared to 8.64% by the conventional method. In the study by Mirovic V et al in 2002 also higher recovery by MB/BacT was observed.[9] Out of 75 isolates 17 were obtained both by automated and conventional methods. All isolates except 4 obtained with LJ medium was isolated by MB /BacT method.58 were isolated by MB /BacT alone and not by LJ medium. In a similar study by Oberoi and H Kaur from Ludhiana the isolation rate was found to be 16.4% by automated method and 2.2% by conventional method.[11] In the study by Seoung -Cheol Kim et al in Korea in 2016 detection rates were 70.4% in MB/BacT and 66.4% in LJ medium.[1] In the present study difference in isolation rates between MB/BacT and LJ medium was highly significant since the P value was found to be <0.0001.Reason may be MB/BacT detect mycobacterium based on metabolism rather than visible growth. Isolation rate from pulmonary specimens were 41.06% and 11.26% by automated method and conventional method respectively whereas for extrapulmonary specimens the isolation rate was 14.13% and 4.34%. Extrapulmonary isolation rate by automated method was found to be high especially from smear negative specimens in a study conducted in Pune in 2016.[12] In the study conducted by the Sagar Mali et al in Karnataka MB/BacT positivity was 14.8% and LJ medium positivity 8.45%.[13] In our study higher rate of isolation was obtained by automated method for both pulmonary and extra pulmonary specimens. Difference in the recovery rate was statistically significant and P value was<.05. Paucibacillary nature of specimens and small quantity of the samples may be the reason for low isolation rate in LJ medium. In the present study isolation rate among smear positive specimens were 91.30%by MB/BacT and 32.60%by LJ medium. In smear negative isolation was 16.75% by automated and 3.05% by LJ. In both smear positive and smear negative isolation rates by MB/BacT was definitively more. For smear negative specimens this difference was found to be statistically significant since P value was .006. In a study by Uddin MN et al conducted in Bangladesh isolation from smear positive specimens were almost equal but isolation from smear negative specimens by MB/BacT was significantly higher.[14] Oberoi and H Kaur from Ludhiana in their studies observed 63.9% isolation from smear positives by automated method and 30.5% by conventional method whereas isolation rate was 12.9% and 0.6% by automated and conventional methods respectively from smear negative specimens.[11] Compared to smear positives isolation from smear negative specimens were significantly higher by automated method in all these studies. In smear positives detection is also possible by microscopy. Smear negative tuberculosis also play a role in transmission of tuberculosis and can be diagnosed by culture only. So increase in recovery will be helpful in early diagnosis.

Mean time of detection was 9.62days for MB/BacT and 42.48 for conventional method. The study by Roggenkamp A et al showed mean detection time as 17.2 days for MB/BacT and 29.8 days for LJ medium.[15] Study by Oberoi and H Kaur from Ludhiana showed mean detection time of 16 days and 26 days by automated and conventional methods respectively.[11] In the study conducted in Korea in 2016 mean time was found to be 14 and 24 days respectively by MB/BacT and LJ medium.[1] Meantime of detection by automated method in the present study was almost similar to these studies. But mean time of detection by conventional method was less in these studies compared to the present study. Minimum time for detection was 3 days for MB/BacT and 30 days for LJ medium. 73% of growth occurred in lesser than 20 days in MB/BacT whereas in LJ medium no growth was detected during this time.

For low inoculum specimens (smear negative pulmonary and extra pulmonary specimens) automated liquid culture is more effective for recovery and provide faster results. Extrapulmonary infection with mycobacterium tuberculosis complex remains a diagnostic problem which is often difficult to establish and often misdiagnosed.[16] For detection of mycobacteria in clinical specimens smear microscopy is by far most popular among all methods. Cases of extra pulmonary infections are more smear negative. Nucleic acid amplification methods are rapid but not as sensitive as culture. Isolation by automated method still remains gold standard for diagnosing extra pulmonary infections. Microscopy by Ziehl Neelsen staining can detect bacilli when they are more than 10^4/ml in sputum. Transmission can occur before bacillary level reach 10^4/ml. During this period of unknown duration persons continue to transmit infection. Though smear positivity correlated well with infectivity, infection certainly occur from paucibacillary smear negative cases also. While smear negative patients can become smear positive later, these patients should be detected not only for his sake but also for public health reasons.[17]

Despite large scale effort for control of TB emergence of drug resistant tuberculosis present a

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major challenge for TB control in India. Preventing development of drug resistant TB should continue to be top priority for all countries. Mycobacterium tuberculosis can acquire spontaneous mutation but most of the drug resistance is manmade.[18] Drug resistance develop either due to infection with resistant strain or as a result of inadequate treatment. Ensuring adherence to full course of treatment is the key to cure TB and prevent emergence of drug resistance.

17 isolates obtained from both LJ medium and MB/BacT were tested for drug sensitivity to first line drugs. (INH, rifampicin, streptomycin, ethambutol) by proportion method in MB/BacT and LJ medium. Drug resistant cases were 70.59% and 64.7% by automated and conventional method. 11(64.7%) were MDR strains. According to global tuberculosis report 2018 India has highest number of MDR TB(26%).[19] In the present study resistance to INH, rifampicin, streptomycin and ethambutol were 70.58% ,64.7%, 52.94% and 47.0% .In a study done by Rajani Ranganath et al in Karnataka resistance to INH, rifampicin, streptomycin and ethambutol were 31.2%, 28%, 21.6% and 17.6% by MB/BacT.[20] In another study by Seoung-Cheol Kim et al from Korea INH and rifampicin resistance were 14.3% and 8.8% respectively.[1] In our study higher MDR detection and higher drug resistance to individual drugs may be due to the fact that all the patients were previously treated. No resistance could be detected in 3 extrapulmonary isolates.

There was good concordance between the results of drug susceptibility testing by automated method and LJ medium. For rifampicin there was 100% concordance with kappa value of 1. For INH one strain found resistant in MB/BacT was sensitive in LJ medium and kappa value was found to be .86. For streptomycin and ethambutol also good concordance were observed. By both MB/BacT and LJ medium 11 MDR strains were detected with 100% agreement between the two methods. In a study by Maria S. Diaz-Infantes et al 100% concordance was obtained with both INH and Rifampicin.[21] Good concordance was observed in the study by Seoung-Cheol Kim et al from Korea also with agreement of 97.7% for INH and 98.6% for Rifampicin.[1]

Mean turnaround time for drug susceptibility results by MB/BacT was found to be 10.24 days and 28.29 days for LJ medium. The difference in mean detection time was found to be statistically significant since P value was found to be <.0001.In a study by Maria S. Diaz-Infantes et al in Itali average detection time was found to be 7days and 21 days by MB/BacT and LJ medium.[21] According to the study by Deepthi Nair et al in 2009 in New Delhi average turn around time was 8 days and 20 days.[22] In a study conducted in Korea turn around time by MB/BacT and LJ medium was found to be 10 and 20 days respectively.[1] All these study results are almost similar to the present study. Early detection of drug resistance is of importance in starting treatment earlier. INH and rifampicin are keystone drugs in the management of tuberculosis. Patients with MDR TB requires prolonged treatment with drugs which are less effective and toxic. Patients with MDR TB represent an infectious hazard to the community if not managed effectively. Second line drugs if not used rationally can lead to emergence of XDR TB which is untreatable. Antitubercular drugs are double edged sword while they destroy pathogens they also select for drug resistant bacteria against which these drugs are ineffective. Results of drug sensitivity results help to select a proper treatment regimen or modify treatment for better management of patients and for surveillance and timely control of spread of drug resistant TB. There was good concordance between the results of drug susceptibility by automated method and conventional method in this study.

CONCLUSION

Automated (MB/BacT) system gave higher yield and faster results for both pulmonary and extra pulmonary specimens compared to solid medium. Compared to smear positive specimens smear negative yielded higher isolation in MB/BacT. Turnaround time for detection of drug resistance was lower for MB/BacT. But in detection of drug resistance no significant advantage was found for either method. So drug susceptibility testing can be done by both methods in combination for better results.

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