Original Article

Comparative Study of Solid Culture and Liquid Culture for the Diagnosis of Pulmonary Tuberculosis

Jahan H[1], Hasan K[2], Khanam RA[3], Bhowmik D[4], Tarana MN [5], Sarker S[6], Sarwar S[7]

Conflict of Interest: None
Received: 09-12-2018
Accepted: 09-04-2019
www.banglajol.info/index.php/JSSMC

Abstract

Background: *Tuberculosis is a highly infectious disease and has the highest burden with it. Diagnosis of tuberculosis in many countries is still dependent on microscopy. For developing countries with a large number of cases and financial constraints, evaluation of rapid and inexpensive diagnostic methods has great importance. Culture of Mycobacterium tuberculosis complex (MtbC) is the accepted reference standard for confirmation of TB infection and is necessary for drug susceptibility testing (DST). There are several methods for culturing MtbC*

using solid and liquid media. Although solid media has been used for over 100 years, liquid culture media is increasingly being introduced in low and middle income countries (LMIC).

Objective: *The purpose of the present study was to compare the efficacy of solid culture and liquid culture in the diagnosis of pulmonary tuberculosis.*

Methodology: *This cross sectional study was done in the Department of Microbiology at Sir Salimullah Medical College, Dhaka and National Institute of Chest Disease & Hospital (NIDCH), Dhaka during the period of January 2016 to December 2016 for a period of 1(one) year. Sputum samples from suspected MDR-TB patients were collected by purposive sampling technique from OPD of Sir Salimullah Medical College (SSMC) and NIDCH. Microscopy, liquid culture in liquid MGIT 960 media were done for MTB diagnosis.*

Result: *This study shows the comparison of results of microscopic examination of solid culture and liquid culture (MGIT 960). The liquid MGIT 960 method detected more positive samples than solid culture 68% vs 67%. The mean turnaround time of detection (TTD) of MTB was 34.3±5.2 days for Lowenstein-Jensen media and 17.5±3.8 days for MGIT 960 (p value <0.05). So, liquid culture gave earlier result than solid culture.*

Conclusion: *Liquid culture more positive result than solid culture under microscope in smear of sputum and also liquid culture gave earlier result than solid culture.*

Key Words:
MDR-TB, liquid culture, solid culture, MGIT

[J Shaheed Suhrawardy Med Coll 2019; 11(1): 28-31]
DOI: https://doi.org/10.3329/jssmc.v11i1.43175

1. Dr. Hosne Jahan, Assistant Professor, Department of Microbiology, Shaheed Suhrawardy Medical College, Dhaka
2. Prof. Dr. Kamrul Hasan, Department of Cardio Thoracic Surgery, NICVD, Dhaka.
3. Dr. Rashida Akter Khanam, Assistant Professor, Department of Microbiology, Shaheed Suhrawardy Medical College, Dhaka
4. Dr. Devolina Bhowmik, Lecturer, Department of Microbiology, Shaheed Suhrawardy Medical College, Dhaka
5. Dr. Mst. Naznin Tarana, Assistant Professor, Department of Microbiology, Faridpur Medical College, Dhaka.
6. Dr. Soma Sarker, Lecturer, Department of Microbiology, Shaheed Suhrawardy Medical College, Dhaka
7. Dr. Sharmin Sarwar, Lecturer, Department of Microbiology, Shaheed Suhrawardy Medical College, Dhaka

Correspondence to: Dr. Hosne Jahan, Department of Microbiology, Shaheed Suhrawardy Medical College, Dhaka, Bangladesh; Email: drhosnejahan1970@gmail.com; Cell no.: +8801711107976.

Introduction

Tuberculosis (TB) remains a major global health problem. It ranks as the second leading cause of death from an infectious disease worldwide. Tuberculosis (TB) is a deadly infectious disease caused by Mycobacterium tuberculosis. The worldwide prevalence of TB was 9.6 million in 2014, with 1.5 million deaths. China has the third highest number of incident and fatal cases of TB worldwide (930000 and 38000, respectively), accounting for 9.7% of cases.[1]

In Bangladesh, the incidence rate of tuberculosis (225) remained same in 2012 since 1990. But the prevalence (525 in 1990, 489 in 2005, and 434 in 2012) and mortality (61 in 1990, 52 in 2005 and 45 in 2012) rates are gradually being decreased.[2] Total confirmed cases of MDR-TB were 334

in 2010, 509 in 2011.[2] In 2012, total 622 cases were tested for MDR-TB among which 513 were confirmed by laboratory tests though the estimated cases of MDR-TB among notified were 42,000 (range 3,100-5,200).[2]

The prevention of tuberculosis relies on the early detection and cure of the infectious cases. So current efforts are focused upon improving the rapidity of identification of *Mycobacterium tuberculosis* (MTB), allowing prompt initiation of appropriate therapy. Although smear microscopy and solid culture on L-J media are widely used, liquid culture and nucleic acid amplification techniques are being increasingly used worldwide. It is recommended that the turnaround time for isolation and identiûcation of Mycobacterium tuberculosis complex should not exceed 21 days. Conventional solid culture systems such as Löwenstein-Jensen (LJ) slant or Middlebrook 7H11 agar plate rarely achieve these standards.[3] The purpose of this study is to compare conventional LJ solid media and BACTEC MGIT 960 for the detection of *M. tuberculosis* from sputum samples.

Methodology

This cross sectional study was done in the Department of Microbiology of Sir Salimullah Medical College, Dhaka and National Tuberculosis Reference Laboratory (NTRL), Dhaka during the period of January 2016 to December 2016. Total 100 sample was taken and technique was purposive consecutive sampling technique. Both (liquid (MGIT 960 culture and solid culture) type of culture was done from these 100 sample. Suspected cases of MDR-TB patients who were attended at the OPD and IPD of NIDCH and SSMC were selected as study population. Patients were excluded who were undergoing treatment or having extra-pulmonary tuberculosis or were new pulmonary tuberculosis cases. The sediment of processed sputum was used for microscopic examination by solid culture and liquid culture. Early morning sputum samples were collected in clean, sterile, leak proof, wide mouth containers. Before collecting specimens, each patient was interviewed and informed written consent was taken from patients or legal guardian of patients and relevant information were recorded systematically in a pre-designed data sheet.

Results

This study shows the comparison of results of microscopic examination of solid culture and liquid culture sputum smear. Both solid culture and liquid culture showed predominanta positive results. The liquid MGIT 60 method detected more positive samples than solid culture 68% vs 67%. The mean turnaround time of detection (TTD) of MTB was 34.3±5.2 days for Lowenstein-Jensen media and 17.5±3.8 days for MGIT 960. "t" test showed a p-value of 0.00001 ($p<0.05$) that means there was significant difference between the TTDs. So, liquid culture gave earlier result than solid culture.

Table I

Isolation of M. tuberculosis in Liquid (MGIT 960) Media and solid culture (n=100).

Result	Liquid (MGIT 960) media	Solid	P value
Positive	68 (68%)	67(67%)	0.897
Negative	32 (32%)	33(33%)	
Total	100	100	

Table II

Comparison of Turnaround Time of Detection (TTD) of MTB for Solid and Liquid Culture.

	LJ Media			MGIT 960		t	df	p value
TTD(Days)	Positive culture	Mean (Days)	TTD (Days)	Positive Culture	Mean (Days)			
<28	4		<7	5				
28-35	22	34.3±5.2	7-21	60	17.5±3.8	30.7	66	<0.001
>35	41		>21	3				

Discussion

Tuberculosis is a major and global public health problem. Conventional methods including smear and conventional culture methods, used in the diagnosis of pulmonary and extrapulmonary tuberculosis, have poor sensitivity in the samples with paucibacillary load. The use of less sensitive conventional methods for the diagnosis have contributed to the difficulties in managing patients with extrapulmonary tuberculosis and patients having paucibacillary load in pulmonary TB. Main problems begin with clinical specimens containing very few mycobacterium bacilli and their slow growth rate limits their detection by the conventional methods such as acid-fast staining and mycobacterial culture. The early diagnosis of tuberculosis helps in initial treatment and thus preventing the possible transmission of the infection.[4]

Bangladesh has a long history of research and demonstration projects on TB. The detection of Acid fast bacilli is often considered as the evidence of the infected stage. Thus, the laboratory plays a critical role in the diagnosis of TB.[5] In developing countries, microscopy of the specimen is by far the fastest, cheapest, and most reliable method for the detection of AFB.[6,7] However fluorescent staining has been added in Revised National Tuberculosis Control Program (RNCP) because of more sensitive and rapid results and can be used in field areas. In current study, we compared solid media (LJ) and automated BACTEC MGIT960 system for isolation of *Mycobacterium tuberculosis* in clinically suspected cases of pulmonary tuberculosis.

In this study, out of 100 samples, *M. tuberculosis* was detected in 68(68%) samples when cultured in MGIT 960 medium. Helb et al.[8] isolated 81 (76%) *M. tuberculosis* out of 107 clinical sputum samples in Vietnam, a finding similar to the current study. On the other hand, Hasan et al.[9] examined 421 specimens from TB suspects of Bangladesh and recovered 45 (10.6%) *M. tuberculosis* isolates in MGIT 960 cultures. It is much lower than present finding and may be related to difference in case selection.

Smear microscopy yielded inferior result compared to solid and liquid cultures in this study as well as in other studies. This procedure is best used to diagnose pulmonary TB from sputum, in areas with high TB infection and limited laboratory resources. Diagnosis of TB infection from tissues, faeces or other biological material with this technique is poor due to low numbers of mycobacteria and sample contamination with other acid-fast bacteria. Direct staining does not provide any information on the species of mycobacteria causing the infection or differentiate between viable and non-viable cells.[10,11]

In this study, MGIT 960 culture yielded highest (68%) isolation of *M. tuberculosis* from sputum samples. Solid culture and GeneXpert MTB assay showed equal 67% positivity. However, smear microscopy gave inferior results when compared to solid culture and liquid culture. Chien et al.[12] reported that recovery rates were 94% with BACTEC MGIT 960 and 75.8% with L-J. So, BACTEC MGIT 960 was better than L-J. Both showed superior performance to the present study. Satti et al.[13] reported that recovery rate of M. *tuberculosis* complex was 97.6% on BACTEC MGIT 960 system and 83.7% on L-J medium. Somoskovi et al.[14] showed the rates of recovery of *M. tuberculosis* were 96.4% with the BACTEC MGIT 960 liquid medium and 81.8% with the L-J medium. These were similar to previous studies. On the other hand, Rodrigues et al.[15] showed that 41% specimens were positive by MGIT 960 TB system and 24% *M. tuberculosis* complex isolates grew on the conventional L.J medium. These were less than current study findings as well as previous results. Helb et al.[8] in their study showed that positivity for Z-N staining, L-J media culture, MGIT 960 liquid culture and GeneXpert assay were 29%, 63%, 76% and 58% respectively. Scott et al.[16] in their study showed positive MGIT culture in 38% participants and GeneXpert positive result in 36.6% of participants. However, Zeka et al.[17] had 93% MGIT culture positive. These variations might be due to difference in population and mycobacterial characteristics, sampling techniques and microbiological methods applied.

Liquid culture systems have many advantages. Several studies have shown that they have a shorter time to detection and have a higher recovery rate of mycobacteria when compared to solid culture. This difference may be due to the added enrichment of the liquid culture media or the ability of bacteria within a liquid medium to spread through the media and access to all the nutrients whereas with solid media, bacteria are limited to the nutrients in the vicinity of the colony.[18] Another important thing can be mentioned. The use of solid media with liquid culture does increase the recovery rate of mycobacteria when compared to liquid culture alone. The actual improvement varies between studies from 1% - 8% depending on which culture systems are being compared.[18,19,20,21]

In the present study, liquid culture on MGIT 960 yielded much earlier (TTD-17.5±3.8 days) positive result than solid culture (TTD- 34.3±5.2 days) (Table XIV). Most (88%) of the positive results in liquid culture were found within 7 to 21 days. However, on L-J media 4 (5.96%) of positive growths were yielded in <28 days, 22 (32.84%) of positive growths were yielded within 28-35 days and 41 (61.2%) after 35 days. Somoskovi et al.[14] showed in their study the

mean time of detection of *M. tuberculosis* in smear-positive specimens was 12.6 days for BACTEC MGIT 960 medium and 20.1 days for LJ medium, and in smear-negative specimens it was 15.8 days for BACTEC MGIT 960 medium and 42.2 days for LJ medium. Bunger *et al.,* (2013) reported that for smear-positive specimens, the mean turnaround time was 8 days by MGIT 960 media whereas on LJ medium, it was 36 days. For smear-negative specimens, the same was 18 days for MGIT 960 media and 40 days for LJ medium. Helb et al.[8] showed similar results on MGIT 960 culture-6% within 7 days, 89% within 7-21 days and 3% required >21 days.

Conclusion

This study conclude that the automated culture system like BACTEC MGIT 960 have a higher isolation rate as compared to solid media (LJ). But highest isolation rate can be achieved by combining the two methods. Smear and culture negative specimens however, donot rule out tuberculosis infection. Hence there is a need for a better and rapid diagnostic technique for early detection and treatment of tubercular infection. The mean turnaround time detection was earlier in liquid than solid culture.

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