Clinical Evaluation of Automated BACTEC MGIT 960 System for Identification, Recovery and Drug Susceptibility Testing of *Mycobacterium tuberculosis* Clinical Isolates

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Abstract

Background and Objectives: The objective of this study was to evaluate the clinical applicability of BACTEC Mycobacterial Growth Indicator Tube 960™ system with Lowenstein-Jensen (LJ) culture for routine recovery and drug susceptibility test analysis of mycobacteria from clinical specimens. **Method and Statistical Analysis:** One hundred and seven clinical specimens were processed by routine laboratory procedures like p-nitro benzoic acid test and PCR amplification of IS*6110* and inoculated in LJ medium and MGIT for mycobacterial growth recovery and DST procedures. **Findings:** Ninety four (87.8%) samples were demonstrated reproducible result by MGIT, in which 89 (83.1%) were smear positive and all the specimens were confirmed as *M. tuberculosis* complex by MGIT PNB and IS*6110* PCR procedures. The observed mean time for mycobacterial detection was 9 days for primary culture and 11 days for DST in MGIT system. **Applications/ Improvements:** The use of BACTEC MGIT 960 system was found to be a sensitive, rapid mycobacterial recovery and culture system and offers precise identification and detection of drug resistance from clinical isolates at earliest.

1. Introduction

Tuberculosis (TB) is one of the most prevalent infectious diseases in the world[1,2]. In addition, the emergence of multidrug-resistant TB is becoming increasingly common and is a major health concern in many regions of the world, particularly in developing countries[1-4]. Due to the long generation time of *Mycobacterium tuberculosis*, (Mtb) delays the rapid diagnosis and drug susceptibility analysis impairs the treatment regimen. Rapid, accurate diagnosis and drug susceptibility are a key factors to optimize treatment and prevent transmission[5]. It is important to focus rapid culture methods that are more than conventional solid culture procedures time not exceeding 21 days for the isolation and identification of Mtb[6,7]. The commonly followed methods to test TB drug susceptibility is standard proportion method on solid media which relies on the culture of tubercle bacilli but takes long time[6,8]. The automated systems like radiometric BACTEC 460TB and fluorescent BACTEC MGIT 960™ (MGIT) have shown a significant correlation with conventional methods[8-10].

The MGIT system is an advanced, automated and uninterrupted-monitoring instrument that can test up to 960 MGIT 7-ml vials for the presence of mycobacteria using fluorescence technology[11,12]. Therfore we established MGIT as a reference system along with conventional solid

LJ media for the routine culture of mycobacteria. The purpose of this study was to evaluate the performance of fully automated 7-ml BACTEC MGIT 960™ system in terms of recovery and drug suceptibility rate of mycobacteria in terms of mean time to detection, contamination rate and comparing it with Lowenstein-Jensen (LJ) solid medium in clinical specimens.

2. Materials and Methods

2.1 Study Settings and Clinical Samples

As per the routine laboratory procedures, 107 total samples consisting sputum samples, tissue and other bodily specimens were obtained from enrolled in-patients with suspected TB symptoms.

2.2 Specimen preparation

4% NaOH processed clinical specimens were ground in a sterile mortar and pestle with one volume of tissue lysis buffer (Tris 10 mM; NaCl 400 mM; di-Na-EDTA 2 mM; pH 8.2) as described[1,2]. The resultant purified pellet samples were carried out for further processes.

2.3 Primary Culture Inoculation and Identification of Mycobacterial Species

Processed clinical samples were initially inoculated in duplicates in MGIT tubes and screened for positive when growth reached 400 units according to manufacturer's recommendations (Becton Dickinson, Sparks, MD)[13] and also in LJ solid medium slants. Organisms grown on LJ medium were used for PNB test and genomic DNA isolation procedures followed with amplification of IS*6110* element. Part of the processed specimens were refrigerated for further use and to rule out discrepant results. Species identification was done based on observation of cultural characteristics and biochemical tests. Procedures were adopted to differentiate mycobacteria at species level only[14].

2.4 Solid Culture LJ Medium Drug Susceptibility Test

Drug susceptibility test was performed for all samples as described earlier[14,15] and duplicates were maintained. Slopes were incubated at 37°C and were observed for the presence of colony formation every week for two months. *M. tuberculosis* H37Rv (ATCC 27294) strain was used as control in all the procedures.

2.5 Liquid Culture BACTEC MGIT 960™ System

Positive inoculum from MGIT was subjected to PNB test (p-nitrobenzoicacid) by inoculating the culture into two MGIT tubes with and without PNB (p-nitrobenzoicacid) and incubated in the MGIT 960 system. *M. tuberculosis* complex (MTB) strains are susceptible to PNB at 500 μg/ml concentration whereas Non tuberculosis mycobacteria (NTM) are resistant to PNB. Primary culture tubes were used for the DST as described previously[13]. Negative tubes were again subjected to IS*6110* PCR procedure to look for positivity[16].

2.6 Extraction of Genomic DNA and Amplification of IS6110

One loopful of culture was homogenized in 100μL of sterile distilled water. Genomic DNA was extracted as described[16,17] and refrigerated till further analysis. Amplification with specific primers was performed in an automated thermal cycler (Eppendorf Gradient Cycler) as described and the amplified products were run in 2% agarose gel, stained with ethidium bromide and documented.

3. Results

Among the 107 specimens tested, 89 (83.1%) were identified to be smear positive, 78 (72.8%) were identified to be positive in LJ medium and 94 (87.8%) specimens were identified to be positive by MGIT. The culture positivity rate of sample is summarised in Table 1.

All the samples were found to be Mtb species and the biochemical results such as niacin production and nitrate

Table 1. Total culture positivity according to the specimen type.

S. No	Specimen (N=107)	BACTEC MGIT 960 (%) N=94	LJ (%) N=78
1.	Sputum (64)	62 (66)	57 (73.1)
2.	CSF (16)	13 (13.8)	11 (14.1)
3.	Lymphnode/Tissue (11)	10 (10.6)	3 (3.8)
4.	PUS (3)	2 (2.1)	1 (1.3)
5.	Aspirates (6)	3 (3.2)	2 (2.6)
6.	Biopsy (7)	4 (4.2)	4 (5.1)

reduction were also produced the concordant result. PCR targeting of IS*6110* element in clinical specimens were succefully amplified. A faint band was observed in agarose gel from scanty specimens when compare than 2+, 3+ smear grade samples[18,19] due to low bacilli load in corresponding sample. A clear PCR product band at 123-bp was observed on a 2% agarose gel confirming the MTB strains in clinical samples (Figure 1.).

Positive tubes with colony growth were monitored on LJ medium slants and liquid culture tubes. The mean time for detection in MGIT was 9 days whereas it was 24 days in LJ. Mean time to detect DST in MGIT was 11 days but for LJ it was 35 days. The distribution of primary culture and DST of processed specimens by MGIT is summarized in Tables 2 and 3 respectivley. Cumulative detection times for 2+ to 3+ smear grade samples were revealed higher positivity in first week of incubation in MGIT and 3 to 4 weeks in LJ slants than for smear scanty grade samples (Figure 2.). Overall MGIT showed a reproducible result for 94 (87.8%) samples and were evaluated with solid culture system. Totally 6 (5.6%) samples were reported as contamination in MGIT, 12 (11.2%) were in LJ slants. Remaining 7 (6.5%) samples were flagged as error report in the MGIT instrument. The stored processed samples of each contamination samples were further tested with PCR. MGIT showed highest positive cumulative percentage than LJ cuture (Figure 3.). Of the 107 clinical strains,
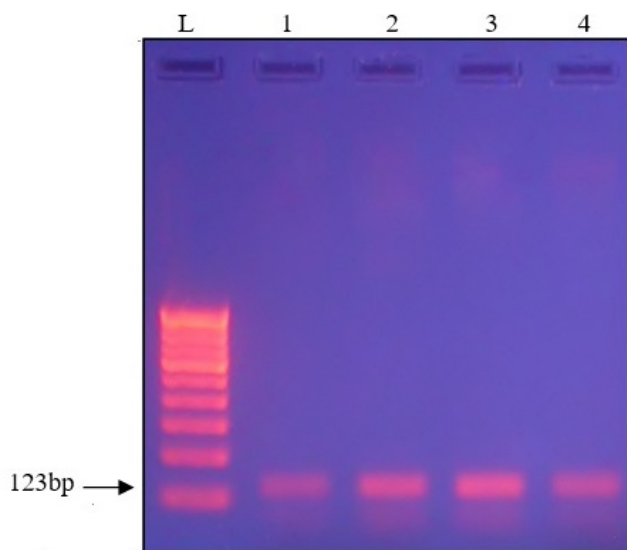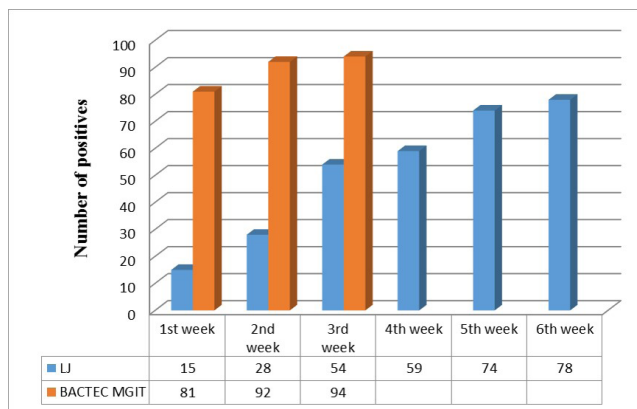

Figure 2. Cumulative percentage of detection time for of mycobacteria from clinical specimens by individual methods BACTEC MGIT 960 system and Lowenstein-Jensen (LJ).
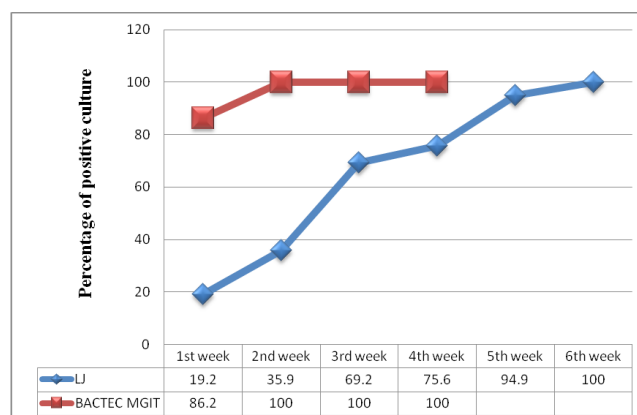
	1st week	2nd week	3rd week	4th week	5th week	6th week
LJ	15	28	54	59	74	78
BACTEC MGIT	81	92	94			

Figure 3. Cumulative percentages of detection of mycobacteria from clinical specimens by individual methods BACTEC MGIT 960 system and Lowenstein-Jensen (LJ).

	1st week	2nd week	3rd week	4th week	5th week	6th week
LJ	19.2	35.9	69.2	75.6	94.9	100
BACTEC MGIT	86.2	100	100	100		

Figure 1. Lane L. 100bp DNA ladder, Lane 1-5. 123bp PCR amplified product of *Mycobacterium tuberculosis* clinical isolates.

123bp →

18 (16.%) were resistant against all drugs, 32 (29.9%) were resistant against at least one drug and 49 (45.8%) were all susceptible isolates. We also observed that the primary resistance to INH and RIF was 17 (15.9%) and 23 (21.5%) samples respectively.

4. Discussion

BACTEC MGIT 960™ system was compared with a conventional LJ for setting up primary culture recovery and DST for MTB clinical isolates. The results of this study therefore demonstrated that the number of mycobacteria recovered in BACTEC MGIT 960TM system was greater than those recovered using conventional culture method

(LJ). Methodological differences may explain some of the discrepant results. The MGIT method, for example, use of MacFarland standard rather than a pipetting, may collect large mycobacterial clumps and make inoculum standardization difficult[5,6,8,15,20]. MGIT system is a liquid based system hence bacteria can grow and spread more easily in liquid media than solid media[21]. Solid media has low recovery rates because the bacteria can use the nutrients only in the vicinity of the colony. Due to low bacilli load (no bacilli/scanty) in the processed clinical specimens the positive rate was drastically decreased in LJ slants (no colonies/contamination). In addition, the low positivity rate shown by conventional culture method (LJ) in these studies could be because of the fact that sample slants were grossly contaminated and considered negative, whereas in MGIT system, since the smear were made from instrument positive MGIT tubes, it was found that there were samples which had mixed condition as mycobacteria growth contamination. Such tubes were considered positive by the MGIT system[22,23].

Besides higher isolation rate, the time to detection of mycobacteria was shorter in MGIT (9 days) than the conventional culture method (24 days) (Table 2). This study is highly corroborated with the findings of Tortoli et al.[11] who showed the mean detection time to be significantly shorter for methods used a liquid medium. Moreover, Hines et al.[20] recorded that the MGIT had a significantly lower mean time to detect (15.8 ± 0.8) days than BACTEC 460 TB (28.2 ± 1.0) days and solid media (43.4 ± 1.0) days. Rishi et al. 13 (2007) found that the time to detect mycobacteria was shorter in MGIT than the LJ medium; as 9.66 days with MGIT and 28.81 days with LJ medium respectively. Our study is much correlated with Rishi et al.[22] in terms of primary culture and DST by performing MGIT system.

Table 2. Days to detect MGIT-positive cultures.

AFB Smear Grade	No of positive in days				Total no of positive samples	Average no of days
	1st Week	2nd Week	3rd Week	4th Week		
3+	44	1	0	0	45	8
2+	15	4	0	0	19	9
1+	13	2	1	0	16	9
Scanty	9	4	1	0	14	10
Total	81	11	2	0	94	9

Table 3. Days to report drug susceptibility results by MGIT.

AFB Smear Grade	DST results in days				Total no of DST positive samples	Average no of days
	1st Week	2nd Week	3rd Week	4th Week		
3+	41	4	0	0	45	10
2+	12	7	0	0	19	10
1+	8	5	3	0	17	11
Scanty	3	4	7	0	14	13
Total	64	20	10	0	94	11

The highest contamination rate was found in LJ as 12 (11.2%) specimens than MGIT as 6 (5.6%) specimens. It is lower than that reported by Hanna et al.[24] and Rishi et al.[22] who found higher contamination with solid media as 21.1% and 27.2% respectively. But Somoskovi et al.[12] found higher contamination in MGIT medium. Lack of addition of antibiotics must be reason for the high contamination rate in solid medium whereas antibiotic cocktail was added to the liquid media[20,25,26]. Therefore, our resuts reported a low contamination level than previous studies and have a good correlation in recovery of mycobacteria from clinical samples with other studies.

5. Conclusions

The BACTEC MGIT 960™ system has been proven a valuable alternative to the use of radiometric procedures and is more accurate and provides rapid detection of mycobacteria than concventional solid system. However, in order to achieve better recovery and identification of Mtb from clinical specimens it is suggested to use of both solid (LJ) and automated liquid BACTEC MGIT 960™ culture media is indispensable.

6. References

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