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Evaluation of the BACTEC MGIT 960 TB with Solid Media for Recovery of Mycobacteria from Extrapulmonary Specimens in South Tunisia

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Abstract

The slow growth of M. tuberculosis complex is a major challenge for TB diagnosis, hence the importance of using media to obtain quick results. We compared the system MGIT960 to solid media: LJ and Coletsos in the diagnosis of extrapulmonary tuberculosis. A total of 634 extrapulmonary samples were processed for direct AFB smear examination, and culture on MGIT960 and solid media. 98 strains were isolated by the three media (15.4%). The mycobacterial recovery rate was 93.8%, 77.5% and 70.4% respectively for MGIT960, LJ and Coletsos media. The mean turnaround time for mycobacterial growth for MGIT960 was 18.5 ± 7.6 days. It was respectively 44.8 ± 19.3 and 42.8 ± 19 days for LJ and Coletsos media. Unlike most studies, the MGIT960 contamination rate (1.7%) was lower than that of solid media (2.2% for LJ and 2.5% for Coletsos). The MGIT 960 offers several advantages: speed, sensitivity and ease of use.

Keywords: Extrapulmonary tuberculosis; MGIT 960; Lowenstein Jensen (LJ) media

Introduction

Tuberculosis is a major cause of morbidity and mortality worldwide. Mycobacterium tuberculosis has infected one third of world's population and causes 8.8 million new cases with approximately 1.1 million deaths each year [1]. Socioeconomic development and medical advances have contributed to the improvement of the epidemiological situation of tuberculosis over the decades, especially in developed countries. However, since the spread of HIV, there has been stagnation or even an increase in the tuberculosis incidence compound by the gradual abandonment in some countries of TB programs and the emergence of the problem of drug resistance.

Furthermore, the relative frequency of extrapulmonary TB (EPTB) has increased at the expense of pulmonary forms, both in developed countries than in developing countries. EPTB comprises 10-50% of all tuberculosis in HIV negative patients and about 35-80% in HIV infected patients [1,2]. It accounts for 13-21% of TB cases in areas of low HIV prevalence [3]. The incidence of EPTB has increased worldwide. In the United States, EPTB represented 21% of all TB cases in 2006 against 15.7% in 1993 [4]. The same situation was observed in Turkey (32.5%), in Algeria (49.4%), in Germany (16.9%), in Spain (33.4%), and in China (9.7-11.8%) [5-7]. In Tunisia, the prevalence of tuberculosis is 22.4/100.000. The relative frequency of EPTB was 44.7% in 2010 [8].

EPTB is often hard to diagnose with conventional methods, due to the longer time required for the culture, the difficulty of sampling and the paucibacillary nature of samples [6]. So there is a need for a rapid, sensitive and accurate detection system. Several new non radiometric technologies for growth and detection of mycobacteria have been developed to reduce the time to detect (TTD) and identify mycobacteria in clinical specimens; among these, the Mycobacterium Growth Indicator Tube (MGIT) system [9].

The MGIT is a liquid broth medium that is known to yield better recovery and faster growth of mycobacteria. In addition to Middlebrook 7H9 liquid media, the MGIT tube contains an oxygen-quenched fluorochrome. It detects oxygen consumption induced by growing micro-organisme [9]. There are a few published reports on the evaluation of Bactec MGIT 960 on extrapulmonary samples. The present study attempted to measure the efficacy of the Bactec MGIT 960 method for the detection of Mycobacteria in extrapulmonary specimens. The results were compared with those of direct AFB smear examination, Lowenstein-Jensen (LJ) and Coletsos media in terms of recovery and contamination rates.

Material and Methods

Study design and period

The study was carried out in the regional laboratory of hygiene tertiary care hospial Hedi Chaker in Sfax-Tunisia during the period of November 2012 to December 2013. It included suspected TB patients coming from different areas of Tunisia.

Study population

A total of 634 specimens collected from 606 patients were evaluated for detection and identification of mycobacteria. Two patients were excluded at screening as they were previously treated with anti-tubercular medications. The clinical samples included Lymph node aspirate (154), pleural fluid (156), CSF (131), pus (72), ascitic fluid (45), synovial fluid (8), tissue (41), pericardial fluid (7), bone scrapings (10), bone marrow (4), Sperm (3), and peritoneal fluid (3).

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Acid fast bacilli smears

Smears were prepared from each sample, stained by Ziehl Neelson method and examined for presence of AFB with a light microscope. The grading of smears was carried out as described by Kent and Kubica [9,10].

Decontamination and processing of the samples

All specimens were liquefied and decontaminated by the standard N-acetyl-L-cysteine, sodium hydroxide method (NaOH-NALC). After 15 min holding at room temperature, specimens were neutralized with phosphate buffer saline (PBS, pH 6.8) and centrifuged at 3000 rcf for 15 min. The pellets were resuspended in 1.5 ml of sterile phosphate buffer and collected for further analysis.

Culture system: The suspension was inoculated in parallel in both liquid (BACTEC MGIT 960) and solid media (LJ and Coletsos).

LJ and Coletsos culture: Five LJ and one Coletsos slants were inoculated with 0.1 ml of the pellet and were incubated at 37°C for 12 weeks. They were checked twice weekly for first week and then once a week until 12 weeks. Contaminated cultures were discarded.TTD was calculated as the time from the date of culture inoculation to the earliest date of visible colonies.

BACTEC MGIT 960 liquid media: The BBL MGIT tube was inoculated by 0.5 ml of the decontaminated and concentrated specimen suspension. It contained 7 mL of modified middlebrook 7H9 broth enrichment with albumin, dextrose and catalase (BBL MGIT OADC) and an antibiotic mixture consisting of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (BBL MGIT PANTA). After inoculation, the tubes were incubated up to 42 days at 37°C. Culture vials are monitored hourly by the instrument. All the positive tube was further confirmed by ZN staining, subculturing on blood agar plate and LJ slant for identification and DST. The TTD of mycobacteria was based on the date of the earliest instrumental indication of positivity.

Identification morphological and biochemical identification

Mycobacteria were differentiated as MTB and NTM based on growth rate on solid media, colony characteristics and appearance and biochemical tests using niacin test and nitrate reductase test.

Identification of mycobacterium from MGIT 960

For differentiation of M. tuberculosis complex and MNT, a commercially kit was used, the BD MGIT MTBc identification test (TBc ID). It is a rapid chromatographic immunoassay for the qualitative detection of M. tuberculosis complex antigen from AFB smear-positive BD MGIT tubes. The assay is performed according to the manufacturer's instructions. For the identification of MTBC species, we used morphological, biochemical and molecular identification by the GenoType® MTBC.

Data analysis

Results were entered into an Excel spreadsheet from individual case files. The data was analyzed using EPI info version 7. The chi square (χ^2-value) test was used to evaluate differences between recovery rates and contamination rates in different media. Student's t-test was used to compare the mean of TTD. A P value<0.05 was considered statistically significant.

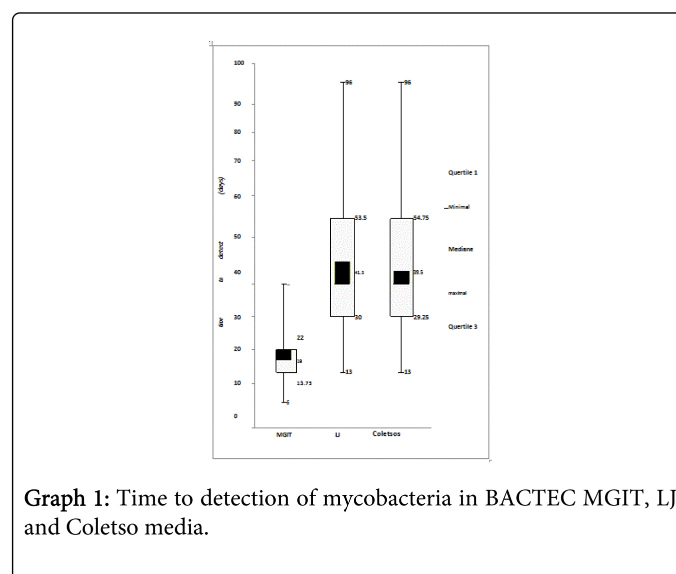
Results

Among 634 extra pulmonary specimens, M tuberculosis complex was found to be positive in 98 (15.4%) cases by any of the three media i.e. MGIT, LJ and Coletsos. Out of the 98 positive cultures, 4 were also smear positive by Z.N for AFB.

Of the 98 positive specimens, the highest rate of mycobacterial recovery was by MGIT 960 i.e. 92 out of 98 (93.8%) as compared to solid media, 77 out of 98 (78.5%). By both methods, 71 (72.4%) were positive for M. tuberculosis complex. In addition, 22 samples (22.4%) were found positive only by MGIT 960, while 6 samples (6.1%) were found positive by LJ only. The MGIT 960 has improved significantly the recovery rate of M. tuberculosis complex (p<0.05).

Mycobacteria were recovered from the different specimen types at various quantities. Recovery rates differ among Lymph node aspirate (66[42.8%]), CSF (3[2.3%]), pleural fluid (7 [4.5%]), pus (13[18%]), tissue (3[7.3%]), bone scrapings (5[50%]) and peritoneal fluid (2[66%]). The detailed analysis showed that the MGIT 960 was able to detect more MTBC isolates in each category of specimens, with the exception of tissue, where the solid media were able to recover 3 isolates in contrast to 2 isolates recovered with the MGIT 960 method. The comparison of the MGIT media with the solid media for detection of Mycobacterium is shown in Table 1. Solid media and MGIT 960 System detected 80% of isolates from smear positive samples whereas from smear negative samples they detected respectively 9.7% (61/614) and 12.3% (76/614) isolates (Table 2).

The average TTD was 18.5 ± 7.6 days for MGIT 960 with the extremity from 6 to 38 days. The median was 18 days. For solid media, the average period of positivity was 44.8 ± 19.3 days and 42.8 ± 19 days for LJ and Coletsos, respectively. The median was 41.5 days for LJ with extremes from 13 days to 96 days. Graph 1 show the average time of positivity on different culture media. The MGIT 960 has statistically significant decrease in mean time of positivity (p<0.05). Comparison of recovery rate and time taken by both the methods was described in Graph 2. In total, 73% of the clinical specimens were positive by MGIT 960 during the first three weeks of incubation (Graph 2). In contrast, only 8% were positive by LJ during first three weeks and 73% were positive between the fourth and eighth week.



Graph 1: Time to detection of mycobacteria in BACTEC MGIT, LJ and Coletso media.

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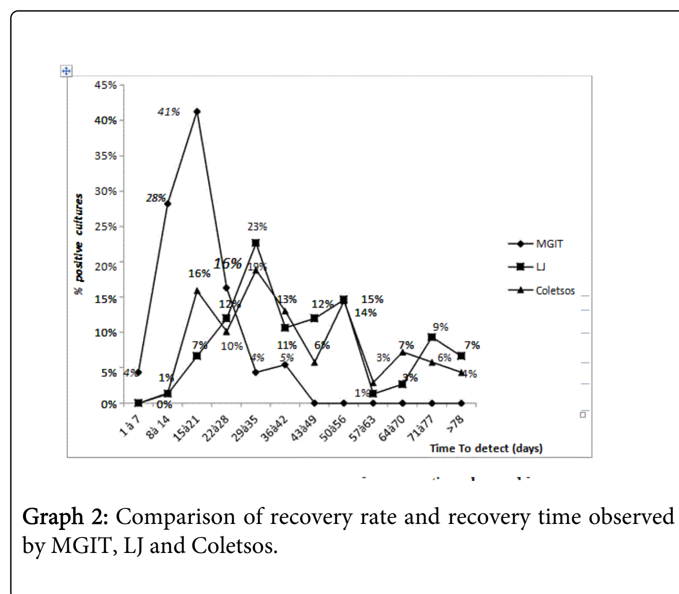

Graph 2: Comparison of recovery rate and recovery time observed by MGIT, LJ and Coletsos.

Specimen Type	No of specimens tested	No. of ZN positive specimens	All media combined	Total No.(%) recovered		
				MGIT 960	Solid media	Both method
Lymph node	154	12	66 (-42.8)	63 (95.4)	50 (75.7)	46 (69.9)
Biopsie	42	2	23 (-54.7)	21(91.3)	20 (86.9)	17 (73.9)
FNA	109	9	41(37.6)	40 (97.5)	28 (68.3)	27 (65.8)
Pus	3	1	2 (66.7)	2 (100)	2 (100)	2 (100)
Ascitic fluid	45	0	0	0	0	0
synovial fluid	8	0	0	0	0	0
Pericardial fluid	7	0	0	0	0	0
Peritoneal fluid	3	0	2 (66)	2 (100)	2 (100)	2 (100)
CSF	131	0	3 (-2.3)	3 (100)	2 (66.7)	2 (100)
Pleural fluid	156	0	7 (4.5)	6 (85.7)	3 (42.8)	3 (42.8)
Bone marrow	4	0	0	0	0	
Bone scrapings	10	2	5 (50)	5 (100)	5 (100)	5 (00)
Pus	72	6	13 (18)	12 (92.3)	12 (92.3)	12 (92.3)
Sperm	3	0	0	0	0	0
Tissue *	41	0	3	1 (33.3)	3 (100)	1 (33.3)
Total	634	20	98 (15.4%)	92 (93.8)	77 (78.5)	71 (72.4)

Table 1: Distribution of MTBC isolates recovered in each culture system. *Specimen source comprised skin, spleen, lesions and synovial tissue.

Isolate (N°)	MGIT960 (%)	Solid media (%)	All media (%)
Smear +	16 (80)	16 (80)	16(80)
Smear-	76 (12.3)	61 (9.7)	82 (13.3)
Total	92 (14.5)	77 (12.1)	98 (15.4)

Table 2: Recovery rates of mycobacteria by different systems and their combination.

For smear-positive specimens, the mean turnaround time was 17 days by MGIT 960 whereas on LJ medium, it was 37.3 days. For smear-negative specimens, the same was 18.8 days by MGIT and 46.3 days by LJ medium (Table 3). Of the 634 cultures, 11 cultures were contaminated on MGIT 960 (1.7%) and 13 contaminated cultures (2.2%) on LJ. The rate of contamination on Coletsos was 2.5%. No statistical difference was found between the rate of contamination of solid media and liquid medium (p>0.05). A relevant portion of missed mycobacteria isolates (1% for MGIT, 1% for LJ, 5.1% for Coletsos) were lost because of bacterial contamination (Table 4).

Isolate (N°)	MGIT960(days)		LJ (days)		Coletsos	
	Avr	Range	Avr	Range	Avr	Range
Négative (N=76)	18,8	18-38	46.3	29-96	46,3	16-96
<10 bacilles (N=5)	20.2	Nov-38	40.6	19-53	37,8	(19-67
Pos+ (N=9)	19.9	Aug-42	43.6	13-83	32,1	13-54
Pos++ (N=1)	7		16		16	
Pos+++ (N=1)	6		30		21	

Table 3: Time to detect for mycobacteria according AFB.

Culture		LJ				Coletsos		Total
MGIT	NTM	cc	Neg	Pos	NTM	cc	Neg	Pos
NTM	2	0	1	0	0	1	0	3
cc	0	5	5	1	4	6	1	11
Négative	4	7	511	6	7	515	4	528
Positive	0	1	22	69	5	26	61	92
TOTAL	6	13	539	76	16	548	66	634

Table 4: Comparison BACTEC MGIT 960 and solid media. NTM : Non tubercular mycobacteria; cc : contaminated culture ; neg : negative ; pos : positive MTBC.

Discussion

The MGIT 960 system has found its place as a standard method for the isolation of mycobacteria [11,12]. This system has been extensively evaluated, but very few studies have been carried solely on extrapulmonary specimen [13]. In our study, a statistically significant difference (p<0.000001) in recovery rate was demonstrated by MGIT 960 TB system compared to conventional LJ method. The system was more useful in paucibacillary specimens like Lymph nodes, FNAC, pleural fluid and CSF [11,13]. Indeed, even though the LJ and the Coletsos medium were counted together as a single system, only 77 (78.5%) isolates of MTBC were recovered, in contrast to 92 (93.8%) isolates with the MGIT 960 system. These results are in accordance with those of other studies dealing mainly with pulmonary specimens [11-15].

In our study, total positivity rate of mycobacteria was 15.4%, which was higher than Hillemann et al. (8%) [11] and Bunger et al. (12%) [13]. Rishi et al. [16] and Rodrigues et al. [15] found high rates (51.6% and 42% respectively). This can be explained by the fact that these authors had included both pulmonary and extrapulmonary tuberculosis samples. The MGIT 960 showed better recovery rate compared to solid media (93.9% vs 77.6% for LJ). This result is similar to that reported in the literature where it is 91.9% in the study of Bunger et al. [13], 90.3% in the study Hillemann et al. [11], and 91.6% in the study of Macondo [17].

The highest isolation rate MTBC complex was found with MGIT 960 mainly from lymph nodes samples (40.9%). Our results showed that the MGIT 960 was able to detect more MTBC than any sample type with the exception of tissues other than lymph node (100% for LJ media vs 33% for MGIT 960). Similar results were found by Hillemann et al. [11] who showed that the MGIT 960 was the most sensitive to all types of extrapulmonary specimens except stool specimens explained by the high frequency of breakthrough contamination (30.8%) in MGIT.

The combination of MGIT 960 and LJ method (100%) was more sensitive than the MGIT 960 alone (92 isolates) and LJ alone (76 isolates). All studies shows that none of the methods could isolate all of the mycobacteria, therefore the combined use of solid media and MGIT 960 system could be justified for the maximum recovery [13].

In this study, average TTD of mycobacteria growth according to the smear positivity noted to be significantly shorter for BACTEC MGIT 960 compared to LJ media indeed, the mean times for isolation of MTBC from LJ culture and MGIT 960 were 44.8 days (1396 days) and 18.5 days (6-38 days), respectively. For smear-positive specimens, the mean turnaround time was 17 days by MGIT 960 whereas on LJ medium, it was 37.3 days. For smear-negative specimens, the TTD was 18.8 days by MGIT and 46.3 days by LJ media. Similar results were reported by Douglas et al. [18] with TTD of 19.3 days and 35 days by BACTEC MGIT 960 and LJ media respectively. Bunger et al. reported an average TTD shorter than our results: for Bactec MGIT 960 (7-10 days for AFB positive and 8-44 days for AFB negative) and LJ media (15-34 days for AFB positive and 32-42 days for AFB negative) [13]. This may be because of the paucibacillary nature of the samples [18].

Among culture-positive samples (98), 73% were detected by MGIT in the first 3 weeks. While only 8% were detected by LJ in the first three weeks. Almost 19% of the samples were positive by LJ after eight weeks highlighting the importance of solid media incubation for a longer time (at least 10-12 weeks). For LJ medium, similar TTD were observed by Rodrigues et al. which time positivity of MGIT960 was 91% during the first 2 weeks and only 4% for the LJ medium [15].

In our study, contamination rate of MGIT 960 medium (1.7%) is lower than that of solid media (2.2% for LJ and 2.5% for Coletsos). It can be explained by the addition of PANTA mixture [13,19-21]. The high contamination rate of Coletsos media can be explained by the made that we use a single tube Coletsos against 5 tubes for LJ. This result was also reported by Bunger et al. [13]. Although, Hillemann et al. [11] found that the rate of contamination of MGIT 960 was higher than that on LJ and explained that contamination by the high contamination rate of stool [11].

Our contamination rate was the lowest compared to other studies on pulmonary and extrapulmonary samples. Contamination rates vary from one study to another. It can be explained by the final sodium hydroxide concentration ranging from 1% to 4% and the nature of the samples. Studies on extrapulmonary samples, including ours, seem to be reporting the lowest (6-7.5%) contamination rates this can be explained by the made that most samples are sterile unlike the pulmonary samples.

In conclusion, the MGIT 960 offers several advantages: speed, sensitivity, ease of use and lack of radioactivity. In our study, it has drastically shortened the detection period and decreases the workload. But this system is more expensive than solid media and requires more infrastructures, needs purchase of specific equipments and maintenance is needed.

None of the authors have a commercial or other financial interest associated with the information presented in this manuscript.

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