Research Article

Factors Associated with Missed Detection of *Mycobacterium tuberculosis* by Automated BACTEC MGIT 960 System

Yu Pang,[1] Biyi Su,[2] Huiwen Zheng,[1] Zhiguo Zhang,[3] Aijing Ma,[1] Yufeng Wang,[1] and Yanlin Zhao[1]

[1]*National Center for Tuberculosis Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China*
[2]*State Key Laboratory of Respiratory Diseases, Department of Clinical Laboratory, Guangzhou Chest Hospital, Guangzhou, China*
[3]*Changping Tuberculosis Dispensary, Beijing, China*

Correspondence should be addressed to Yanlin Zhao; zhaoyanlin@chinatb.org

Despite the demonstration of excellent performance, mycobacterial growth in BACTEC MGIT 960 can go undetected. The aim of this study was to investigate the prevalence of "false-negative" culture sample in Beijing and the potential factors associated with the detection failures by MGIT 960. Of the 577 sputum samples tested, 141 (24.4%) were culture-positive for mycobacteria, of which 133 (94.3%) were automatically determined by MGIT 960 system and 8 (5.7%) were positive for visual growth (false negative by MGIT). Statistical analysis showed that positive grade of specimen had no influence on the false-negative rate by MGIT 960 system ($\chi^2 = 2.207$, $P = 0.820$). In addition, the mean time to detection (TTD) was 241.4 (range: 224–261) hours for false-negative group and 186.8 (range: 173–199) hours for positive group. The difference in TTD between false-negative and positive groups was statistically significant ($P < 0.01$). In conclusion, our data demonstrate that the automatic MGIT missed a small portion of bacteriological mycobacterial patients. In addition, the poor growth rate rather than the low grade of AFB smear is associated with the detection failure by MGIT. Our findings highlight the notion that manual inspection for all instrument-negative MGIT tubes will bring about considerable benefit to patients and clinicians.

1. Introduction

Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide. Rapid diagnosis of TB is critical for initiating effective treatment and preventing its transmission in the community [1]. Recent advances in molecular methods have shortened the turnaround time for the identification of *Mycobacterium tuberculosis* (MTB); however, culture is still essential for phenotypic drug susceptibility testing and improving the case detection of smear negative patients [1, 2]. Due to the slow growth rate, conventional solid culture systems including Löwenstein-Jensen (LJ) slant or Middlebrook 7H11 agar plate always require 8 weeks of incubation before a negative result is reported, which cannot meet the criteria of clinical practice [3].

In recent years, the BACTEC MGIT 960 system, a fully automated and nonradiometric culture system, has been recommended for faster mycobacterial isolation from clinical specimens [4]. The culture is monitored with the oxygen-quenching fluorescent sensor technology every 60 minutes, which provides a satisfactory performance in a short laboratory turnaround time when compared with conventional method [2, 4, 5]. The BACTEC MGIT 960 is therefore widely considered as the gold standard for the diagnosis of TB [3]. Despite the demonstration of excellent performance, mycobacterial growth in liquid culture can go undetected, which has been reported by several researchers [6, 7]. Similarly, we found that a small number of MGIT 960 culture tubes with an obvious mycobacterial colony in the bottom of the tubes were determined as "culture-negative" by automatic BACTEC MGIT 960 system in the clinical practice (Figure 1). The aim of this study was to investigate the prevalence of "false-negative" culture sample in Changping District, Beijing, and the potential factors
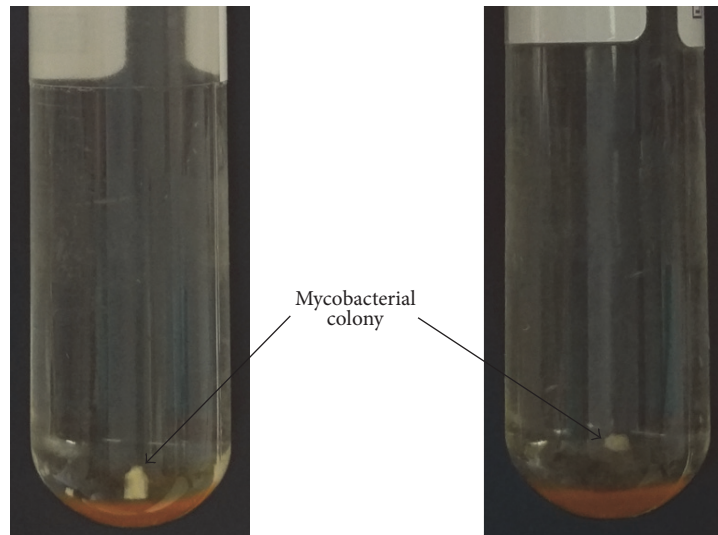
FIGURE 1: Typical appearance of mycobacterial colonies in the bottom of "false-negative" tubes.

associated with the growth detection failures by MGIT 960.

2. Materials and Methods

2.1. Specimens. Clinical sputum samples came from suspected TB patients seeking health care in a TB referral dispensary (Changping TB Dispensary) between June 2015 and January 2016, and all the patients enrolled in this study had never received TB treatment before. The specimens were digested with the sodium hydroxide and N-acetyl-L-cysteine (NaOH/NALC) method according to a previous study [8]. After decontamination, the sample was neutralized with sterile phosphate buffer (pH = 6.8) and centrifuged at 3000 ×g for 15 min. The pellet was resuspended in 2 mL of phosphate buffer.

2.2. AFB Smears. Smears were prepared by using the concentrated sediments. Then, all the smears were stained with auramine O and examined with fluorescence microscopy for acid fast bacteria (AFB). The grading of smears was determined according to the guidelines from the Chinese Center for Disease Control and Prevention, which starts with negative to scanty to 4+ [9].

2.3. BACTEC MGIT 960. The BACTEC MGIT 960 culture tube containing 7H9 broth, enriching supplement, and an antibiotic mixture was used for the culture of MTB according to the manufacturer's instructions. Briefly, 0.5 mL of the processed specimen was inoculated into the MGIT 960 culture tube, which was further incubated at 37°C in the MGIT 960 instrument. The culture was monitored automatically every 60 min for increased fluorescence with the BACTEC 960 TB System. Tubes that were classified as negative after 42 days were manually inspected for macroscopic evidence of growth. The probable "false-negative" cultures were inoculated on the Löwenstein-Jenson (L-J) medium for further species identification.

2.4. Species Identification. Colonies were scraped and genomic DNA was extracted according to previously reported techniques [10]. The genomic DNA was used for the sequencing of 16S rRNA to perform molecular species identification [11]. DNA sequences were aligned with the homologous sequences of the reference mycobacterial strains using multiple sequence alignments (https://www.ncbi.nlm.nih.gov/BLAST).

2.5. Time to Detection (TTD). The BACTEC MGIT 960 culture system was used to evaluate the growth rate of MTB isolates as previously described [12]. The fresh grown MTB colonies were harvested from the surface of L-J slants. Followed by vigorous agitation for one minute, the supernatant suspension was adjusted to 1.0 McFarland turbidity standard. The 10^{-3} dilutions of the 1 McFarland suspension were further prepared and then inoculated into the MGIT 960 culture tube supplemented with oleic acid, albumin, dextrose, and catalase (OADC). Tubes were incubated at 37°C in the MGIT 960 instrument, and time to detection was defined as the hours between the date of culture inoculation and the earliest date at which the instrument recorded positive growth.

2.6. Statistical Analysis. SPSS version 15.0 (IBM, Armonk, NY) was used for all data analysis. The Pearson chi-square test was used to analyze the proportions of "false-negative" (FN) and MGIT 960-positive MTB strains classified into different smear grades. The mean TTD between FN and MGIT 960-positive MTB isolates was compared with t-test. If the P value was less than 0.05, the difference was declared as significant.

3. Results

Of the 577 sputum samples tested, 141 (24.4%) were culture-positive for mycobacteria, of which 133 (94.3%) were automatically determined by MGIT 960 system and 8 (5.7%) were positive for visual growth (false negative by MGIT). We

Table 1: Proportion of false-negative culture results determined by MGIT among specimens belonging to different grades of AFB smear.

Grade of AFB smear	Total	Number of positive cultures (%)	
		MGIT positive[a]	MGIT false negative
Negative	494	59 (11.9)	4 (0.8)
Scanty	11	9 (81.8)	1 (9.1)
1+	23	20 (87.0)	1 (4.3)
2+	22	21 (95.5)	1 (4.5)
3+	18	17 (94.4)	0 (0.0)
4+	9	7 (77.8)	1 (11.1)
Total	577	133 (23.1)	8 (1.4)

[a]MGIT: BACTEC MGIT 960; $\chi^2 = 2.207$, $P = 0.820$.

Table 2: TTD of MTB between MGIT positive and false-negative isolates.

Classification	TTD (range)[a]	P value
MGIT positive	186.8 (173–199)	<0.01
MGIT false negative	241.4 (224–261)	

[a]TTD: time to detection (hours); MGIT: BACTEC MGIT 960.

analyzed the proportion of false-negative results of MGIT 960 system among specimens at various positive grades. As shown in Table 1, the false-negative rates of MGIT 960 system were 6.3% (4/63), 10.0% (1/10), 4.8% (1/21), 4.5% (1/22), 0.0% (0/17), and 12.5% (1/8) for negative, scanty, 1+, 2+, 3+, and 4+ sputum samples, respectively. Statistical analysis showed that positive grade of specimen had no influence on the false-negative rate by MGIT 960 system ($\chi^2 = 2.207$, $P = 0.820$).

Further molecular identification demonstrated that, out of the 141 isolates, there were 139 isolates of MTB and 2 isolates of *Mycobacterium avium* complex (MAC). All the *Mycobacterium* isolates classified as false-negative group were MTB. In addition, we compared the growth rate of MTB between "false-negative" and positive groups determined by MGIT. Eight MTB isolates were randomly selected from positive group as a control. As shown in Table 2, the mean TTD was 241.4 (range: 224–261) hours for false-negative group and 186.8 (range: 173–199) hours for positive group. The difference in TTB between false-negative and positive groups was statistically significant ($P < 0.01$).

4. Discussion

There is no doubt that BACTEC MGIT 960 provides a solution for the reliable and rapid diagnosis of tuberculosis, while the mycobacterial growth in liquid culture can go undetected by this automatic detection system [6]. In this study, we identified that the frequency of these growth detection failures was 5.7% (8/144) in the clinical practice of Changping District, Beijing, which was similar to the observations from Peña et al. (6.1%) [6]. In China, the low proportion of TB patients with bacteriological evidence is a major challenge for the TB control program. A recent national survey from China indicates that only 26% of pulmonary TB patients are bacteriologically positive cases [13]. In other words, more than 70% of TB patients are diagnosed according to the clinical symptoms and radiographic presentations, which is significantly higher than the proposed recommendation of 50% from the World Health Organization (WHO) [14]. Considering the wide use of MGIT in the microbiological laboratories of TB hospital in China, our findings indicate that the visual inspection towards all instrument-negative MGIT tubes may eliminate the potential risk of missing false-negative TB patients.

The grade of smear positivity has been considered as an important indicator for sputum bacterial load [15]. Numerous literatures have demonstrated that the time to positivity in MGIT liquid culture shows good correlation with the grade of AFB smear, which may be expected to have different bacterial load in the originating sputum samples [5]. However, based on our investigations, the grade of AFB smear had no influence on the detection failure of MGIT, while the growth rate of MTB isolates seems to be a critical factor for the failure of MGIT in the detection of MTB. The growth rate of bacteria is often used to evaluate their fitness under different environmental conditions [16]. The significantly lower growth rate of false-negative MTB isolates reflects their poor fitness in vitro, failing to reach a detection limit of MGIT by the end of 42-day incubation. In addition, the specimens are pretreated with sodium hydroxide for 15 min, which serves as an obvious stress for MTB growth. Although the MTB isolates exhibit tolerance to the base stress, we hypothesize that they will be damaged under the exposure to strong base, especially for the isolates with low fitness, thereby resulting in the detection failure by MGIT.

There were several obvious limitations in our study. First, the sample size of isolates with false-negative results was small, which may undermine the efficacy of statistical analysis. Second, the false-negative sputum samples determined by MGIT were not loaded on another medium in parallel. We therefore missed an opportunity to compare the performance of different culture systems to detect the "false-negative" MTB isolates. Third, several previous reports have revealed that MTB with resistance mutations is associated with a decrease in fitness [16–18], while the drug susceptibility profiles and mutant types conferring drug resistance of MTB isolates were not detected in this study. Hence, further analysis of MTB isolates with false-negative results will extend our knowledge regarding the relationship between specific mutations and loss of fitness.

In conclusion, our data demonstrate that the automatic MGIT missed a small portion of bacteriological mycobacterial patients. In addition, the poor growth rate rather than the low grade of AFB smear is associated with the detection failure by MGIT. Our findings highlight the notion that manual visual inspection for all instrument-negative MGIT tubes will bring about considerable benefit to patients and clinicians.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

Authors' Contributions

Acknowledgments

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