

Evaluation of Loewenstein-Jensen Medium Culture, MGIT 960 Culture and Different Specimen Types in Diagnosis of Bone and Joint Tuberculosis

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Abstract: Objective: The aim of this study was to evaluate L-J (Loewenstein-Jensen) medium culture, MGIT 960 culture and different specimen types in diagnosis of BJTb (bone and joint tuberculosis). Methods: Specimens of pus, caseous necrosis, tuberculous granuloma and sequestrum were collected from 52 BJTb patients. All specimens were cultured using both MGIT 960 system and L-J medium; and all pus were amplified using real-time PCR to detect the presence of *M. tuberculosis* DNA. Key Findings: A total of 191 specimens were collected. Granuloma had better chance to produce positive outcomes by L-J medium culture, while for sequestrum MGIT 960 culture had higher yield, but there was no significant difference in the recovery rates among different types of specimen either by L-J culture ($\chi^2 = 0.638$, $P = 0.888$) or by MGIT960 culture ($\chi^2 = 1.399$, $P = 0.706$). MGIT960 culture had significantly higher recovery rate than L-J culture. With a combined culture and PCR-based test, the recovery rate of pus specimen was significantly higher than that of either method alone ($P < 0.05$). Conclusion: MGIT 960 culture is superior to L-J culture in BJTb diagnosis; pus, sequestrum, granuloma and caseous necrosis are usable specimen for mycobacterial culture; combination of culture and molecular techniques can provide a better diagnostic significance.

Key words: Bone and joint tuberculosis, mycobacteria, specimen type, culture.

1. Introduction

Tuberculosis (TB) remains a major health problem in the world and about one-third of population is infected with *Mycobacterium tuberculosis* [1]. With the spread of HIV infection, various forms of EPTB (extra pulmonary tuberculosis) have been reported to be increasing at an alarming rate in recent years [2, 3]. Among EPTB cases, bone and joint involvement occurs in 5-10% of cases [4].

Although mycobacterial culturing remains the gold

standard for TB diagnosis but due to low culture-positive rate culturing does not play an important role in BJTb diagnosis. Histopathological analysis followed by an initial BJTb clinical diagnosis is often problematic since the infection site is poorly accessible and patients tend to be less compliant towards invasive procedures [5]. Currently CT and MRI are the mainstays for BJTb diagnosis [6], but they are limiting because of high cost, inaccessibility to most of the patients, and requirement of experienced image analysts.

Improvement in bacteriological identification can reduce the time required for diagnosis. Here we compared MGIT960 System (Becton, Dickinson and Company, BD) culture with L-J (Lowenstein-Jensen)

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medium culture, evaluated specimen type influencing on culture yield, and validated the PCR test of *Mycobacterium tuberculosis* DNA using specimens collected from BJTB patients.

2. Materials and methods

2.1 Ethical Statement

The ethical approvals for this study were obtained from Beijing Chest Hospital Ethics Committee. A written informed consent was acquired from each participant.

2.2 Patients

Fifty-two cases which had been confirmed as BJTB by pathological examination were recruited between June 2011 and May 2012 from Beijing chest hospital. There were 27 male and 25 female patients with a mean age of 39.4 (15-84) with 9 patients older than 60 (17.31%). The lesion of BJTB was on spine in 41 patients (78.85%), joints in 8 (15.38%) and sternum in 3 (5.77%). Specimen of pus, caseous necrosis, granuloma and sequestrum were collected from the patients during the surgical debridement.

2.3 Culture

Pus was treated with 2% N-acetyl-cysteine-NaOH for decontamination and digestion and then homogenized by vigorous stirring. Caseous necrosis, granuloma and sequestrum were homogenized by automated homogenizer Fastprep-24 (MP biomedical, USA) after adding phosphate buffer and then were treated with 2% N-acetyl-cysteine-NaOH for decontamination and digestion. Then L-J medium and MGIT 960 tube were inoculated separately with 0.1 ml of the resulting specimen, followed by incubation according to instructions. Solid media tubes were read weekly for 8 weeks and in case of no bacterial growth by end, the result was recorded as culture negative. The MGIT960 outcomes were recorded following the manufacturer's instruction.

2.4 PCR Test

For all of the pus specimens, presence of *M. tuberculosis* DNA was detected using real-time PCR amplification with a commercially available diagnostic kit (Daan gene Co.Ltd, Guangzhou, China) according to the manufacturer's instructions. Briefly, after pus was treated with 4% sodium hydroxide for 30 min, genomic DNA was extracted from the specimens. The PCR reaction parameters were as follows: 93 °C for 2 minutes, 10 cycles at 93 °C for 45 seconds and 55 °C for 60 seconds, 30 cycles at 93 °C for 30 seconds and 55 °C for 45 seconds followed by an additional elongation step at 72 °C for 5 minutes in ABI GeneAmp 5700. Positive control (dilution of *M. tuberculosis* DNA) and negative control (0.9% sterile saline) were utilized as instructed. The target genes for the kit was IS6110 insertion sequence, and reaction that produced fluorescent signal before 30 cycles was considered as positive outcome, else considered as a negative result.

2.5 Statistical Analysis

The statistical analysis was carried out using χ^2 test, with a p value of < 0.05 indicating significance. Analyses were performed using SPSS version 11.0.

3. Results

3.1 Specimen Constitution and Related Culture Recovery Rates

A total of 191 specimens were collected from 52 BJTB patients, including 52 pus, 44 caseous necrosis, 51 granulomas and 44 sequestrum specimens. For L-J culture, granuloma yielded highest positive rate (19.61%, 10/51), followed by pus (17.31%, 9/52), sequestrum (15.91%, 7/44) and caseous necrosis (13.64%, 6/44); for MGIT960 culture, sequestrum specimen yielded highest positive rate (40.91%, 18/44), followed by pus (38.46%, 20/52), caseous necrosis (31.82%, 14/44) and granuloma (31.37%, 16/51) (Table 1). Whereas no statistical difference was

observed regarding the yield of different type of specimen either by L-J culture ($\chi^2 = 0.638, P = 0.888$) or by MGIT 960 culture ($\chi^2 = 1.399, P = 0.706$). Combining different types of specimens from the same patient increased the recovery rate both by L-J culture and by MGIT 960 culture, the outcomes are summarized in Table 1.

3.2 Media Performance

MGIT 960 system had significantly higher recovery rates than L-J culture both when single and combined specimen types were considered (Fig. 1). MGIT 960 culture also had higher diagnostic efficacy (48.08%, 25/52) than L-J culture (26.92%, 14/52) ($\chi^2 = 11.08, P < 0.05$) in accordance with the standard that at least one sample produced positive outcome for each patient. Whereas combining the two culture methods had the same yield (48.08%, 25/52) as MGIT 960 culture alone among the 52 patients.

3.3 Real-time PCR Results

The positive rate in the pus specimen was 48.08% (25/52) by real-time PCR amplification. Among these 25 real-time PCR positive pus samples, 11 were

culture-positive by MGIT 960 system and 6 were culture-positive by L-J culture (Table 2). When bacteriological culture and PCR methods were integrated, the culture-positive rate in the pus specimen reached 67.31% (35/52), which was significantly higher than those acquired by either method alone ($P < 0.05$).

4. Discussion

Bone and joint TB is a serious infectious condition confronted frequently by clinicians. The definitive diagnosis of BJTB is usually established by CT-guided needle aspiration cytology-biopsy and culture on LJ medium, or by histological examination. However, even when an invasive procedure like biopsy was performed, bacteriological evidence could not always be acquired because BJTB is a paucibacillary condition [7, 8]. Prolonged diagnosis process for BJTB was commonly seen with a reported mean delay of diagnosis from 12 to 32 weeks [5, 8-10]. As bone and joint TB mimics other infective conditions, such as acute bacterial osteomyelitis and septic arthritis [4], improving the recovery rate of the laboratory diagnostics is of great importance for BJTB diagnosis.

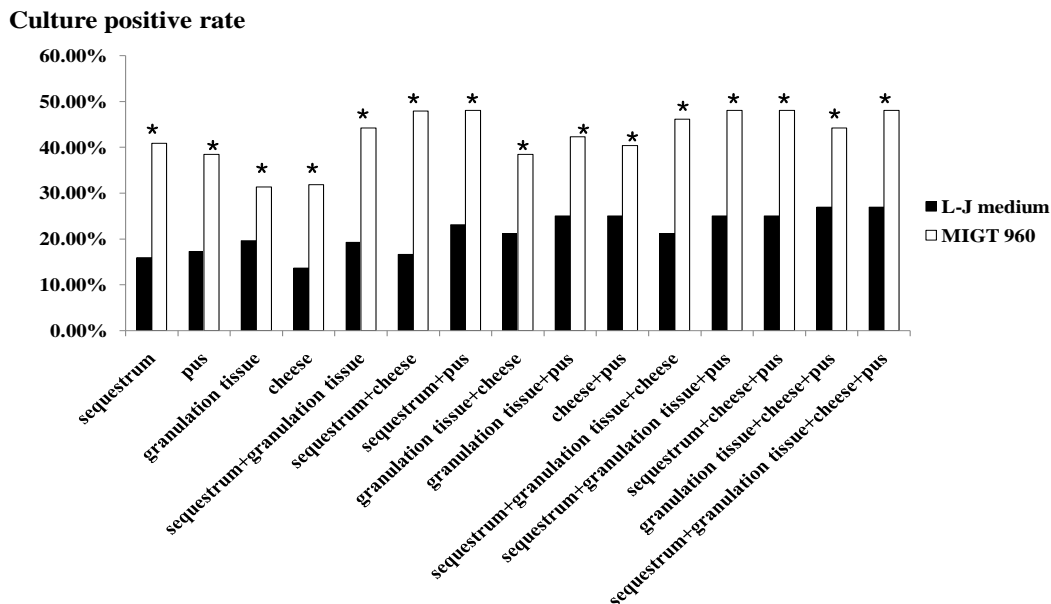


Fig. 1 Comparative analysis of the culture positive rates for *M.tuberculosis* of different samples by L-J medium and MGIT 960 system.

*Significantly higher than L-J medium group ($P < 0.05$).

Table 1 Culture positive rates for *M.tuberculosis* of different sample by Lowenstein-Jensen medium and MGIT 960 system.

Sample type	No. of sample with positive results/total no. of sample tested (%)	
	L-J medium	MGIT 960
Sequestrum	7/44 (15.91)	18/44 (40.91)
Pus	9/52 (17.31)	20/52 (38.46)
granulation tissue	10/51 (19.61)	16/51 (31.37)
Cheese	6/44 (13.64)	14/44 (31.82)
Sequestrum + pus	12/52 (23.08)	25/52 (48.08)
Sequestrum + granulation tissue	10/52 (19.23)	23/52 (44.23)
Sequestrum + cheese	8/48 (16.67)	23/48 (47.92)
Granulation tissue + cheese	11/52 (21.15)	20/52 (38.46)
Granulation tissue + pus	13/52 (25.00)	22/52 (42.31)
Cheese + pus	13/52 (25.00)	21/52 (40.38)
Sequestrum + granulation tissue + cheese	11/52 (21.15)	24/52 (46.15)
Sequestrum + granulation tissue + pus	13/52 (25.00)	25/52 (48.08)
Sequestrum + cheese + pus	13/52 (25.00)	25/52 (48.08)
Granulation tissue + cheese + pus	14/52 (26.92)	23/52 (44.23)
Sequestrum + granulation tissue + cheese + pus	14/52 (26.92)	25/52 (48.08)

Table 2 The outcomes of MGI960 culture, LJ medium culture and real-time PCR of pus specimens from 52BJTB patients.

Culture	Outcomes	Real-time PCR	
		+	-
MGIT 960	+	12	8
	-	13	19
L-J medium	+	6	3
	-	19	24

In the present study, two commonly used culture methods were performed simultaneously to find the best solution for mycobacteria recovery. We found that MGIT 960 culture had significantly higher recovery rate than L-J culture irrespective of specimen types i.e. single or combined. Among the recruited cases, combining L-J culture with MGIT 960 culture did not increase the total yield, which indicates that MGIT 960 culture is efficient than L-J culture for BJTb diagnosis.

All the specimens we recruited in this assay, including pus, sequestrum, granuloma and caseous necrosis produced significant yield for mycobacterial culture. Our study also found that granuloma was most likely to produce positive culture outcome by LJ medium, and sequestrum was most likely to be positive by MGIT 960 system, although there was no difference in culture-positive rate between different type of sample either by L-J culture ($\chi^2 = 0.638, P =$

0.888) or MGIT 960 system ($\chi^2 = 1.399, P = 0.706$). It was a surprise to us that the sequestrum specimens produced relatively high recovery rate. We assume that a full homogenization step performed by an automated homogenizer might account for this achievement, since our own experience on sequestrum culture without this step was not so successful.

Combining different type of specimens from same patient increased the recovery rate either by L-J culture or by MGIT 960 culture, and our data showed that after combining four types of specimen together, the culture-positive rate reached to 26.92%(14/52) for L-J culture, 48.08% (25/52) for MGIT960 culture. Our outcomes demonstrated that collecting multiple types of specimen can increase the chance of culture positive outcomes.

Pus specimen is the most commonly collected from BJTb patients. PCR amplification of pus specimens proved to be a very efficient measure for BJTb

diagnosis in our assay (48.08%, 25/52). Additionally, when culture and PCR test were combined, the recovery rate of the pus specimen reached 67.31% (35/52), which was significantly higher than that of either methods alone ($P < 0.05$).

BJTB cases were often treated empirically on clinical and radiological grounds, however, bacteriological culturing should be performed whenever possible. Besides confirming the diagnosis, culture positivity could also be used as a base for drug susceptibility test by phenotypic method. In our assay, drug susceptibility test was performed on 10 *M. tuberculosis* isolates, 5 of them were categorized as MDR (multidrug-resistant) of which 3 were extensively drug-resistant strains. The presence of MDR-TB resulted in a less efficient and a prolonged and complicated course of treatment. As for BJTB patient, most of them also need a full course of anti-tuberculous chemotherapy after operation, determining the identity and sensitivity of the mycobacteria is very important not just from a public healthy and epidemiological viewpoint but also from a treatment management viewpoint.

To our knowledge, this is the first study performed a parallel evaluation on the yields of different specimen types collected from BJTB patients.

5. Conclusion

MGIT 960 culture is superior to L-J culture in BJTB diagnosis; pus, sequestrum, granuloma and caseous necrosis are usable specimen for mycobacterial culturing for BJTB diagnosis, and it is important to collect different types of specimen from BJTB patient to pursue higher recovery rate; and combination of culture and molecular techniques may have a better diagnostic significance.

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