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Performance of the BacT Alert 3D System Versus Solid Media for Recovery and Drug Susceptibility Testing of *Mycobacterium tuberculosis* in a Tertiary Hospital in Korea

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Background: Tuberculosis (TB) is a major health problem, and accurate and rapid diagnosis of multidrug-resistant (MDR) and extended drug-resistant (XDR) TB is important for appropriate treatment. In this study, performances of solid and liquid culture methods were compared with respect to MDR- and XDR-TB isolate recovery and drug susceptibility testing.

Methods: Sputum specimens from 304 patients were stained with Ziehl-Neelsen method. *Mycobacterium tuberculosis* (Mtb) isolates were tested for recovery on Löwenstein-Jensen (LJ) medium and the BacT Alert 3D system. For drug susceptibility testing of Mtb, isolates were evaluated on M-KIT plates and the BacT Alert 3D system.

Results: The recovery rates were 94.9% (206/217) and 98.2% (213/217) for LJ medium and the BacT Alert 3D system, respectively (kappa coefficient, 0.884). The rate of drug resistance was 13.4% for at least one or more drugs, 6.0% for MDR-TB and 2.3% for XDR-TB. M-KIT plate and BacT 3D Alert 3D system were comparable in drug susceptibility testing for isoniazid (97.7%; kappa coefficient, 0.905) and rifampin (98.6%; kappa coefficient, 0.907). Antibiotic resistance was observed using M-KIT plates for 24 of the total 29 Mtb isolates (82.8%).

Conclusion: The liquid culture system showed greater reduction in the culture period, as compared with LJ medium; however, drug susceptibility testing using M-KIT plates was advantageous for simultaneous testing against multiple drug targets.

Keywords: Tuberculosis; Drug Susceptibility; *Mycobacterium tuberculosis*; Tertiary Care Center; Korea

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Introduction

Tuberculosis (TB) is a major public health problem, with 8.6 million new cases and 1.3 million deaths per year[1]. Multidrug-resistant (MDR)-TB is defined as TB resistant to at least isoniazid (INH) and rifampin (RIF), and extended drug-resistant (XDR)-TB is TB caused by MDR plus any fluoroquinolone (FQ) and at least one of the injectable second-line drugs, such as amikacin (AMK), kanamycin (KM), or capreomycin[2]. MDR-TB and XDR-TB are major challenges for TB control worldwide and are of great concern because they are difficult to cure with existing drugs and are associated with high mortality[2,3]. The estimated global burden of MDR-TB is 450,000, and 3.6% of all new TB cases and more than 20% of those with a previous history of TB treatment are MDR-TB[1]. In South Africa, 1.8% of new cases and 6.7% of those with a previous history of TB treatment were MDR-TB[1]. On the other hand, 32.2% of new cases and 75.6% of those with a previous history of TB treatment were MDR-TB in Belarus[4]. The percentage of XDR-TB cases among MDR-TB cases was 9.6%, and XDR-TB cases were reported in 92 countries by the end of 2012[1]. The high proportion of XDR-TB among MDR-TB cases was reported in Azerbaijan (Baku City, 12.8%), Belarus (11.9%), Latvia (16%), Lithuania (24.8%), and Tajikistan (21.0%)[1].

Early diagnosis of MDR-TB or XDR-TB is essential for the treatment and prevention of transmission. Laboratory diagnosis of TB depends on microscopic examination of sputum stained for acid-fast bacilli (AFB) and on the culture of sputum[5]. The appearance of drug-resistant TB requires isolation of *Mycobacterium tuberculosis* (Mtb) and drug susceptibility testing, which are now recommended for all new pulmonary TB patients in response to the emergence of MDR- and XDR-TB[6].

Mycobacterial culture and drug susceptibility testing have been performed using solid egg- or agar-based medium in many countries, including Korea, because they are inexpensive and can be performed easily[7]. However, these methods require long periods to yield results[8]. The liquid medium culture method has been utilized to reduce the culture period of Mtb. Several studies have demonstrated that the liquid medium culture method is superior to the solid medium culture method, yielding quicker results and more mycobacterial isolates[9]. The World Health Organization recommends the adoption of mycobacterial culture and drug susceptibility testing using liquid culture systems[10]. Automated liquid culture systems, such as the MGIT 960 system (Becton Dickinson, Sparks, MD, USA), have been adopted in many countries and used in routine diagnosis in many diagnostic laboratories in Korea[10]. Drug susceptibility testing using the automated liquid culture system is possible for first-line drugs[11], but methods have yet to be established for second-line drugs for the diagnosis of MDR-TB and XDR-TB.

There have been few studies comparing the performance of solid and liquid culture methods for mycobacterial culture and drug susceptibility testing in high-burden MDR-TB and XDR-TB cases. In Korea, 2.7%–3.9% of new cases and 14.0%–27.2% of those with a previous history of TB treatment were MDR-TB[10,12].

In the present study, the performances of solid and liquid culture methods for mycobacterial culture and drug susceptibility testing were compared in the high burden setting of MDR-TB and XDR-TB cases in National Masan Tuberculosis Hospital in Korea. This is the largest dedicated TB national tertiary hospital in the public sector of Korea and has been responsible for the management of MDR-TB and XDR-TB cases.

Materials and Methods

1. Study sites and subjects

The study was performed at the National Masan Tuberculosis Hospital, a referral TB hospital in Korea focusing especially on MDR-TB management, and at the International Tuberculosis Research Institute, Masan, Korea. Sputum specimens were obtained from subjects visiting the National Masan Tuberculosis Hospital between April 2009 and June 2011. New and previously treated TB cases were enrolled in this study. The inclusion criteria resulted in the enrollment of adults 20 years of age or older who had clinical signs or symptoms suggestive of TB. All subjects provided written informed consent to participate, and the study was approved by the Institutional Ethics Committee of National Masan Tuberculosis Hospital.

2. Specimen processing and AFB smear

Sputum specimens were decontaminated in an equal volume of *N*-acetyl-L-cysteine–3% sodium hydroxide citrate (final NaOH concentration was 1.5%) for 15 minutes at room temperature. After decontamination, the specimens were neutralized using sterile phosphate-buffered saline (PBS, pH 6.8) and centrifuged at 3,000 ×g for 20 minutes. After decanting the supernatant, the pellet was suspended in sterile PBS (pH 6.8) and used for smear preparation and inoculation into both Löwenstein-Jensen (LJ) medium (Union Lab., Seoul, Korea) and the BacT Alert 3D system (BioMérieux, Saint-Vulbas, France).

Smears were prepared using the suspended pellet and stained by the Ziehl-Neelsen method. The presence of AFB was examined using a light microscope, and the results of the AFB smears were graded according to the American Thoracic Society/Center for Disease Control and Prevention as follows: grade 0, no bacilli in 399 fields; trace, 1–2 bacilli in 300 fields; grade 1, 1–9 bacilli in 100 fields; grade 2, 1–9 bacilli in 10 fields; grade 3, 1–9 bacilli in 1 field; and grade 4, > 9 bacilli in 1 field[13]. Smears were recorded as positive if at least 10 AFB per 100

fields were observed according to the guidelines.

3. Mycobacterial culture

Mycobacteria was cultured according to the Clinical and Laboratory Standards Institute guidelines (2003)[14] and the World Health Organization (2007)[10]. Following decontamination and sedimentation, sputum specimens were subjected to LJ media and the BacT Alert 3D system, an automatic liquid culture system for continuous monitoring of mycobacterial growth. Mycobacterial growth on LJ media was checked twice a week for 8 weeks. The time to detection was calculated as the time from the date of inoculation to the earliest date of visible colonies.

The BacT Alert 3D system, an automated liquid culture system for continuous monitoring of mycobacterial growth, was used for mycobacterial culture from sputum specimens. Aliquots of 500 μL specimen suspensions were inoculated into mycobacterial culture tubes containing modified Middlebrook 7H9 with an antibiotic supplement (amphotericin B, nalidixic acid, trimethoprim, polymyxin B, and vancomycin). Mycobacterial culture tubes were incubated at 37°C in the BacT Alert 3D system for up to 40 days. Specimens were monitored hourly for increases in the level of carbon dioxide by the BacT Alert 3D system, which gave audible and visible alerts for the presence of mycobacteria in the tubes. Time to detection was calculated as the time between the date of inoculation and the earliest date that positive growth was recorded by the instrument.

4. Identification of mycobacteria

All specimens identified as positive from any culture were examined by Ziehl-Neelsen staining and the SD TB Ag MPT64 rapid test (SD Inc., Youngin, Korea). DNA extraction was performed as described previously[13]. Briefly, three or four colonies grown on LJ slants were suspended in sterile distilled water. Suspended bacteria and mycobacterial cultures from the BacT Alert 3D system were heat-inactivated at 95°C for 30 minutes. Cell debris was precipitated by centrifugation for 2 minutes at 10,000 ×g, and the supernatant was used for the identification of Mtb and mycobacterial species. The DNA concentration was measured using the NanoDrop NT-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Identification of Mtb and species of mycobacterial species was conducted using the Accuprobe Identification Test (Gen-Probe, San Diego, CA, USA), and REBA Myco-ID (M&D Inc., Wonju, Korea), according to the manufacturer's instructions[15].

5. Drug susceptibility testing

Drug susceptibility testing was performed by the absolute concentration method with Mtb H37Rv as a reference using M-KIT plates (The Korean Institute of Tuberculosis, Osong, Korea) containing 10 anti-mycobacterial drugs. The critical concentrations of INH, RIF, ethambutol (EMB), KM, tuberlactin, ethionamide, cycloserine, *para*-aminosalicylic acid (PAS), ofloxacin, and streptomycin (SM) were 0.2, 40, 2.0, 30, 40, 40, 30, 1.0, 2.0, and 4.0 μg/mL, respectively[14]. Pyrazinamide (PZA) resistance was determined by the PZA test (PZA concentration, 50 μg/mL).

Drug susceptibility testing using the BacT Alert 3D system was conducted for INH and RIF. The drug concentrations used for the BacT Alert 3D system were 1.0 μg/mL for INH and 1.0 μg/mL for RIF. Mtb isolates showing inconsistent results were subjected to an additional drug susceptibility test.

6. Statistical analysis

Statistical analysis was performed using the GraphPad Prism program version 5.0 (GraphPad Software, La Jolla, CA, USA). Categorical variables were analyzed using the Pearson's chi-square test or Fisher exact test and continuous variables were analyzed using the t test. A Wilcoxon test for paired samples was used to compare medians of the time to detection. A kappa correlation statistical analysis was performed to assess concordance of the results from both techniques. In all analyses, $p<0.05$ was taken to indicate statistical significance.

Results

1. AFB smears

Sputum specimens collected from 304 patients who visited the National Tuberculosis Hospital were processed for detection of AFB. Of 304 sputum specimens, 210 (69.1%) were AFB smear-positive and 94 (30.9%) AFB smear-negative.

2. Recovery rates and identification of mycobacteria

Overall, the detection rates were 66.4% on LJ medium and 70.4% in the BacT Alert 3D system (Table 1). In smear-positive specimens, detection rates were 90.9% and 94.3% on LJ medium and in the BacT Alert 3D system, respectively (p=0.053). In the smear-negative specimens, the detection rate (17.0%) in the BacT Alert 3D system was higher than that (11.7%) on LJ medium, but this difference was not significant. Overall, the contamination rates were 3.6% and 4.6% for LJ medium and the BacT Alert 3D system, respectively; 3.3% of cultures were contaminated in both culture methods (Table 1). The contamination rate was similar between AFB smear-positive and AFB smear-negative specimens on LJ medium, while the contamination rate was slightly higher for AFB smear-negative than AFB-positive specimens in the BacT Alert 3D system, albeit

Table 1. Recovery rates on LJ medium and the BacT Alert 3D system based on AFB smear status

AFB smear	No. of specimens	LJ medium			BacT Alert 3D system		
		Positive	Negative	Contamination	Positive	Negative	Contamination
Negative	94	11 (11.7)	79 (84.0)	4 (4.3)	16 (17.0)	72 (76.6)	6 (6.4)
Positive	210*	191 (91.0)	11 (5.2)	7 (3.3)	198 (94.3)	3 (1.4)	8 (3.8)
Total	304*	202 (66.4)	90 (29.6)	11 (3.6)	214 (70.4)	75 (24.7)	14 (4.6)

Values are presented as number (%).
*One AFB smear-positive culture was observed, which was identified as *Mycobacterium abscessus*.
LJ medium: Löwenstein-Jensen medium; AFB: acid-fast bacilli.

Table 2. Culture results of sputum specimens on LJ medium and the BacT Alert 3D system

Variable		BacT Alert 3D system		Total
		Positive	Negative	
LJ medium	Positive	202	4	206
	Negative	11	86	97
Total		213	90	303

LJ medium: Löwenstein-Jensen medium.

Table 3. Time for recovery of *Mycobacterium tuberculosis* isolates and drug susceptibility test on LJ medium and the BacT Alert 3D system

Variable		LJ medium (day)	BacT Alert 3D system (day)	p-value
Culture	AFB smear-negative	28 (14–32)	15 (13–17)	<0.001
	AFB smear-positive	24 (20–28)	14 (12–15)	<0.001
	Total	24 (21–28)	14 (12–16)	<0.001
Drug susceptibility testing		20 (18–24)	10 (8–12)	<0.001

LJ medium: Löwenstein-Jensen medium; AFB: acid-fast bacilli.

without statistical significance (p=0.377).

A total of 218 isolates were recovered by at least one culture method. Most of the isolates (99.5%) were determined as Mtb, and only one isolate was identified as *Mycobacterium abscessus* (Table 2). Of 217 Mtb isolates, 206 (94.9%) were detected on LJ medium and 213 (98.2%) in the BacT Alert 3D system (p=0.11). On the other hand, four (1.8%) were detected only on LJ medium and 11 (5.1%) only in the BacT Alert 3D system. There was good concordance in the culture results between both culture methods, with an agreement rate of 94.8% (kappa coefficient, 0.884; 95% confidence interval [CI], 0.8269–0.9413).

3. Time to detection of mycobacterial culture and drug susceptibility testing

For the 217 Mtb isolates that grew on both LJ medium and in the BacT Alert 3D system, the median detection time for the latter was significantly less than that for former (p<0.001); 14 days (interquartile range [IQR], 12–16) versus 24 days (IQR, 2–28) for all Mtb isolates (Table 3). The detection time using the BacT Alert 3D system was significantly less than that using LJ medium for both AFB smear-negative and -positive specimens (p<0.001 in both comparisons). The median reporting times of drug susceptibility testing were 10 (IQR, 8–12) days for the BacT Alert 3D system and 20 (IQR, 18–24) days for LJ medium (p<0.001).

4. Drug susceptibility

The results of drug susceptibility testing of the 217 Mtb isolates for the four first-line drugs and seven second-line drugs using M-KIT plates and the BacT Alert 3D system are listed in Table 4. Of the 217 Mtb isolates, 29 (13.4%) were resistant to at least one or more drugs; four were mono-resistant (3 for INH and 1 for PAS), 13 were MDR (2 for INH and RIF; 2 for INH, RIF, and other second-line drugs; 1 for INH, RIF, and EMB; 3 for INH, RIF, EMB, and the second-line drug SM; 2 for INH, RIF, PZA, and second-line drugs; and 3 for four first-line drugs, and other second-line drugs), and five were XDR (2 for INF, RIF,

Table 4. Results of drug susceptibility testing of *Mycobacterium tuberculosis* isolates

	First-line drug	Second-line drug	No. of strains
Fully susceptible (n=188)	-	-	188
Mono-resistance (n=4)	INH	-	3
	-	PAS	1
Poly-resistance (n=7)	INH	SM	1
		PAS	1
		PAS, OFX	1
	EMB	SM	1
	PZA	SM	1
	INH, EMB	PAS	1
		PAS, SM	1
MDR (n=13)	INH, RIF	-	2
	INH, RIF	PAS, OFX	1
		PAS, SM	1
	INH, RIF, EMB	-	1
	INH, RIF, EMB	SM	3
	INH, RIF, PZA	CS	1
		KM, SM	1
	INH, RIF, EMB, PZA	OFX, SM	2
		ETH, PAS	1
XDR (n=5)	INH, RIF	KM, ETH, PAS, OFX, SM	1
		KM, CS, PAS, OFX, SM	1
	INH, RIF, EMB	PAS, OFX, SM	1
		KM, ETH, CS, OFX	1
	INH, RIF, EMB, PZA	KM, ETH, PAS, OFX, SM	1
Total			217

INH: isoniazid; PAS: *para*-aminosalicylic acid; SM: streptomycin; OFX: ofloxacin; EMB: ethambutol; PZA: pyrazinamide; MDR: multidrug-resistant; RIF: rifampin; CS: cycloserine; KM: kanamycin; XDR: extended drug-resistant; ETH: ethionamide.

and other second-line drugs; 2 for INH, RIF, EMB, and other second-line drugs; and 1 for four first-line drugs and other second-line drugs).

Drug susceptibility testing on INH and RIF were performed with 217 Mtb isolates using the M-KIT plates and the BacT Alert 3D system (Table 5). Of these, 30 (13.8%) and 31 (14.3%) isolates showed resistance to INH on M-KIT plates and the BacT Alert 3D system, respectively. Further, 16 (7.4%) and 19 (8.8%) isolates were identified as resistant to RIF by M-KIT plates and the BacT Alert 3D system, respectively. Two isolates (0.9%) showed resistance to INH only on M-KIT plates, and three isolates (1.4%) showed resistance to INH only with the BacT Alert 3D system. Three isolates (1.4%) were resistant to RIF in the BacT Alert 3D system alone, while no RIF-resistant isolates were detected using M-KIT plates alone.

There was good concordance between the results of drug susceptibility testing using the two methods with an agreement of 97.7% (kappa coefficient, 0.905; 95% CI, 0.822–0.987) for INH and 98.6% (kappa coefficient, 0.907; 95% CI, 0.802–1.012) for RIF, respectively.

Discussion

Early detection of drug resistance in Mtb is essential for appropriate treatment and prevention of transmission[11]. Mycobacterial culture and drug susceptibility testing using solid medium are the standard methods due to their simple procedures and economic feasibility. In addition, drug susceptibility testing against several anti-TB drugs can be performed simultaneously using M-KIT plates[16]. However, this method has a disadvantage in that it takes a long time to obtain results. To

Table 5. Results of drug susceptibility testing of *Mycobacterium tuberculosis* isolates for INH and RIF using LJ medium and the BacT Alert 3D system

Drug resistance		INH (n=217)	RIF (n=217)
LJ media	BacT Alert 3D system		
Sensitive	Sensitive	184 (84.8)	198 (91.2)
Resistant	Resistant	28 (12.9)	16 (7.4)
Resistant	Sensitive	2 (0.9)	0 (0)
Sensitive	Resistant	3 (1.4)	3 (1.4)
Agreement, %		97.7	98.6

Values are presented as number (%).
INH: isoniazid; RIF: rifampin; LJ medium: Löwenstein-Jensen medium.

reduce the culture period of Mtb, liquid culture systems have been adopted and show superior performance in reducing the culture period compared with solid medium.

In the present study, we evaluated the performance of the BacT Alert 3D system, a liquid culture system, for mycobacterial culture and drug susceptibility testing by comparison with mycobacterial culture using LJ medium and drug susceptibility testing with M-KIT plates in the situation of a high MDR-TB and XDR-TB burden. In our study population, the prevalences of MDR-TB and XDR-TB in our pool of isolates were 6.0% and 2.3%, respectively. These results were slightly different from those of Choi et al.[12] who reported a rate of resistance to at least one or more drugs of 17.6%, with MDR-TB seen in 3.9% of new cases and 27.2% of previously treated cases (8.5% in total) in a private referral center in Korea. In addition, the rate of XDR-TB was 0.8% in new cases and 4.0% in previously treated cases (1.4%). On the other hand, Bai et al.[16] reported that the rate of resistance to at least one or more drugs was 10.9%–12.8% and that of MDR-TB 1.6%–2.7% in a survey conducted during 1994–2004 at 245 health centers in Korea[14]. These differences may be related to the study populations, i.e., the larger number of previously treated cases in the referral hospitals may have resulted in the higher rate of drug resistance[7]. The study by Bai et al.[16] was performed in public health centers, a public form of primary health care in Korea that cares mostly for new cases, while our study and that reported by Choi et al.[12] were performed in a public referral TB hospital and a private referral medical center, respectively, in which the target population included more previously treated cases.

The BacT Alert 3D system, a liquid culture system, detected the growth of Mtb at an earlier time point than did LJ culture medium (14 days vs. 24 days, respectively; p<0.001) as reported previously[9]. The detection time was significantly reduced in the liquid culture system compared with LJ culture medium in both AFB smear–positive and –negative specimens. In addition, the liquid culture system showed similar results for drug susceptibility testing against the first-line drugs, INH and RIF, compared with drug susceptibility testing using M-KIT plates. The recovery rates of Mtb isolates in the BacT Alert 3D system was slightly higher than that in the LJ medium, albeit without statistical significance (p=0.34). These results were consistent with previously published studies[17,18], but our recovery rates were slightly higher[19,20].

The agreement rates of drug susceptibility testing between the M-KIT plates and the BacT Alert 3D system were 97.7% (kappa coefficient, 0.905) and 98.6% (kappa coefficient, 0.907) for INH and RIF, respectively (Table 5). These results indicate no difference in drug susceptibility testing against INH or RIF using the M-KIT plates versus the BacT Alert 3D system. These results were in accordance with previous reports[21,22]. In addition, the majority of drug-resistant Mtb isolates showed INH or RIF-related resistance. Only 17.2% of Mtb isolates were INH mono-resistant or INH and RIF dual-resistant, while the remaining Mtb isolates (82.8%) showed resistance against other first-line drugs (EMB, PZA) or six second-line drugs. These observations indicated that drug susceptibility testing against INH and RIF is essential, but that testing for the remaining drugs should not be excluded.

The liquid culture system has advantages in reducing the mycobacterial culture period and drug susceptibility testing. However, the results of drug susceptibility testing using the liquid culture system did show good concordance with that using solid medium for some drugs, such as EMB, SM, and KM. Although drug susceptibility testing using solid medium requires a longer culture time, it can be performed simultaneously for all target drugs in the case of the absolute concentration method using M-KIT plates. Therefore, drug susceptibility testing using liquid culture systems must be optimized further for other anti-TB drugs, but the combination of solid and liquid culture systems should be considered for effective and economic mycobacterial culture and drug susceptibility testing, especially in situations with a high burden of drug resistance.

In conclusion, the BacT Alert 3D system, a liquid culture system, showed superiority in reducing the culture period compared with the solid medium culture method, but drug susceptibility testing using M-KIT plates still has advantages for simultaneous testing against multiple drug targets, especially in the cases of MDR-TB and XDR-TB high burdens.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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