Correlation Between Susceptibility of *Mycobacterium tuberculosis* by Microtiter Plate Alamar Blue Assay and Clinical Outcomes†

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Abstract

A rapid, inexpensive, and reliable antimycobacterial susceptibility assay is needed to effectively deal with the increasing prevalence of resistant strains of *M. tuberculosis* in Thailand. The microtiter plate Alamar blue assay (MABA) appears to be promising but requires additional data regarding correlation with clinical outcome. The correlation between the susceptibility by MABA and clinical outcomes was studied. There were 123 specimens obtained from extra-pulmonary sites of 108 patients at Ramathibodi Hospital in 1999. The authors found that susceptibility of *M. tuberculosis* isolates by the MABA correlated with the clinical outcome; patients with isolates sensitive to isoniazid, rifampicin, and ethambutol had a better clinical outcome than patients with isolates resistant to at least one of these drugs (p=0.004). Studies to determine this correlation in pulmonary tuberculosis are still needed.

Key word : *Mycobacterium tuberculosis,* Tuberculosis, Susceptibility, Correlation, Clinical Outcome

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Tuberculosis is a major infectious disease in Thailand and the incidence is still high due to the increasing number of AIDS patients[1]. The number of multidrug-resistant tuberculosis (MDR-TB) cases is increasing with respect to both initial and acquired resistance[2]. At present there is no definite standard regimen for MDR-TB. However, the regimen comprises of 3 to 4 sensitive/new anti-TB drugs often used in clinical practice. Obtaining rapid diagnosis and drug susceptibility patterns of *M. tuberculosis* are essential for improving therapeutic outcomes. This rapid assay should be inexpensive, and also reliable. The microtiter plate Alamar Blue assay (MABA) was previously reported as simple, rapid, and low-cost[3-5]. In addition, the accuracy of this test is 93.6 per cent[4]. This test is also used to adjust drug regimen and assess disease progression. However, only a few studies have reported the association between susceptibility and clinical outcome[4]. The authors therefore conducted this study to determine this association.

MATERIAL AND METHOD

Patients

Patients diagnosed to be tuberculosis with positive culture of *M. tuberculosis* at Ramathibodi Hospital from January 1999 to December 1999 were included in this study. Inclusion criteria were as follows: i) susceptibility of *M. tuberculosis* performed by MABA; ii) patients were treated with the standard short-course antituberculous drug regimen which comprised of 2 months of isoniazid (I), rifampicin (R), ethambutol (E), and pyrazinamide (Z), and 4 months of I and R); iii) patients had been followed-up and complied with treatment for at least 4 months. Most specimens used to perform the susceptibility were from lymph node, pleural and pericardial fluid, and blood. Specimens from the respiratory and gastrointestinal tract were performed at the tuberculosis center and were not included in this study.

In vitro susceptibility testing

In vitro drug susceptibility testing of *M. tuberculosis* isolates to I, R, and Ewas was performed by the MABA as described by Franzblau, et al[4]. Briefly, broth serial dilutions of four drugs were set up in 96-well, clear flat bottomed microtiter plates. Inoculum was prepared from colonies grown on the Loewenstein Jensen media, homogenized with 0.5 ml of 10 per cent Tween 80, and diluted with sterile saline to a turbidity equivalent to that of a McFarland No.1.0 standard. The suspension was then further diluted 1:50 with 7H9GC medium and the amount of 100 µl was inoculated into 100 µl of 7H9GC containing the test drug. After incubation for 7-8 days, 12.5 µl of 20 per cent Tween 80 and 20 µl of Alamar blue were added to the cultures. Growth of the organism was determined by visual determination of a color change from blue to pink. Minimum inhibitory concentration (MIC) was determined as the lowest concentration of drugs that had completely inhibited visible growth of the organism. The criteria used to interpret the MIC as susceptible and resistant was used as previously described[4] and demonstrated in Table 1. Susceptibility patterns of *M. tuberculosis* isolated to I, R, and E were determined. Data was categorized into 2 groups: class I; susceptible to I, R, and E; class II; resistant to at least one drug.

Clinical assessment

Clinical signs and symptoms were observed after patients received treatment for 4 months by investigator (SS), an expert in infectious diseases. Improvement of clinical signs and symptoms was the outcome of interest, and graded into two categories, good and poor outcome. A good outcome was defined according to each specific organ involvement as follows: resolution of fever and blood culture was negative for prolonged fever and mycobacteremia; lymph node decreased in size and inflammation for lymphadenitis; decrease of fluid for pleural, pericardial effusion or ascites; imaging showed improvement of bone lesion for osteomyelitis; resolution of joint fluid and inflammation for arthritis; absence

Table 1. Criteria used to interpret the MIC as susceptible and resistant.

Drugs	MIC (µg/ml)	Susceptibility determination
INH	≤0.5	Susceptible
	1.0	Partial resistant
	≥2.0	Resistant
Rifampicin	≤1.0	Susceptible
	>1.0	Resistant
Etambutol	≤2.0	Susceptible
	4.0	Partial resistant
	≥8.0	Resistant
Streptomycin	≤2.0	Susceptible
	4.0	Partial resistant
	≥8.0	Resistant

of pyuria and urine culture was negative for tuberculous pyelonephritis; decrease in size and inflammation for parotid abscess, orchitis, and ovarian abscess. No improvement or worsening of these signs and symptoms was categorized as poor outcome.

Statistical analysis

General characteristics of patients were described using mean and standard deviation for continuous data, frequency and percentage for categorical data. Association between the classes of susceptibility and clinical outcome was determined using Fisher-exact test. Analyses were performed using STATA version 7.0[6]. P-value less than 0.05 was considered as statistically significant.

RESULTS

One hundred and twenty three isolates from 108 patients were eligible. Fifty-five of 108 (50.1%) patients were male and mean age was 36.2 ± 21.6 years old. Forty-six (42.6%) patients were HIV-infected patients. Eighty-eight patients had positive culture at only one site; 16 patients had positive culture at 2 different sites; and 1 patient had positive culture at 3 different sites. Hence, 123 specimens were included in the analysis. The majority sources of specimens were lymph node (52.0%), followed by pleural/pericardial fluid (15.4%), and blood (10.6%), as in Table 2. One hundred and two specimens (82.9%) were susceptible to all I, R, and E drug (class I) and 21 (17.1%) specimens were resistant to at least one drug (class II).

The associations between susceptibility and clinical outcome were determined, Table 3. There were 102 and 21 isolated in class I and class II, respectively. Among these, 97.1 per cent and 76.2 per cent sites of class I and class II had good clinical outcome. Fisher exact test was used to determine this association and found that the susceptibility was statistically associated with clinical outcome (p-value =0.004), i.e. class I had a better clinical outcome than class II did.

For susceptibility class II, 14 isolates were resistant to only I, E or both and all of them had good clinical outcome, whereas 7 isolates were resistant to R with or without resistance to I/E and only 1 isolate had a good outcome. This showed that resistance with R was associated with poor clinical outcome (p-value<0.001). The authors also found that resistance with only I/E had a chance of good clinical outcome 7 (95% CI: 1.1-42.9) times relative to resistance with R with or without resistance with I/E.

Table 2. Sites of specimens.

Class	Clinical specimens (isolates)									
	Blood	Lymph node	Pleural, pericardial fluid	CSF	Ascites	Bone, joint fluid	Urine	Subcut abscess	Others*	Total
I	11	55	14	5	2	8	1	3	3	102
II	2	9	5	1	-	-	-	4	-	21
Total	13	64	19	6	2	8	1	7	3	123

* parotid gland, testis, ovarian mass

Table 3. Association between susceptibility and clinical outcomes.

Susceptibility patterns Class	Clinical outcomes, Isolates					
	Good	%	Poor	%	Total	P-value
I	99	97.1	3	2.9	102	0.004
II	16	76.2	5	23.8	21	
Resistant to:						
I/E/IE	14		0		14	<0.001
R/IR/RE/IRE	2		5		7	

DISCUSSION

In the present study, the isolates were divided into 2 classes according to the susceptibilty patterns as described. The cases with isolates in class I had a better clinical outcome than class II. The authors also found that among class II isolates, the worst clinical outcome was caused by resistance with R.

Espinal et al found that rifampin resistance was strongly associated with treatment failure particularly in countries where drug-resistant tuberculosis cases are high[7]. The authors disregarded the HIV status because most previous studies failed to identify an influence of positive HIV serology on treatment outcome[8-11].

Current therapy for tuberculosis involves multi-drug combinations such as isoniazid, pyrazinamide, rifampin, and ethambutol or streptomycin. Outcomes of treatments are influenced by many factors such as drug compliance, the time taken from patient presentation to the diagnosis, disease severity, and the degree of resistance[2,12,13].

Susceptibility testing by MABA is inexpensive, simple, and has a rapid turn-around time. In addition, the accuracy and reliability of this test are high[4]. It could be an alternative method for countries where there is limited resource for performing the standard method.

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การศึกษาความสัมพันธ์ระหว่างความไวของเชื้อวัณโรคต่อยาโดยวิธี Microtiter Plate Alamar Blue Assay และผลการรักษาทางคลินิก†

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การตรวจความไวต่อยาของเชื้อในกลุ่มวัณโรคที่ได้ผลรวดเร็ว ไม่แพงและเชื่อถือได้ ยังเป็นที่ต้องการมากในเวชปฏิบัติ เนื่องจากมีจำนวนผู้ป่วยวัณโรคดื้อยาเพิ่มขึ้นเรื่อย ๆ การตรวจความไวต่อยาโดยวิธี microtiter plate Alamar blue assay (MABA) มีข้อมูลว่าได้ผลเช่นเดียวกับวิธีมาตรฐาน แต่ยังขาดข้อมูลการศึกษาความสัมพันธ์ของการตรวจความไวต่อยาและผลการรักษาทางคลินิก ผู้วิจัยจึงได้ทำการศึกษาความสัมพันธ์ดังกล่าว ใน 123 ตัวอย่างเชื้อจากสิ่งส่งตรวจที่ไม่ใช่เสมหะจากผู้ป่วย 108 รายที่โรงพยาบาลรามาธิบดีในปี พ.ศ. 2542 พบว่าจากผู้ป่วยที่มีตัวอย่างเชื้อที่ไวต่อยาทั้งหมดมีผลการรักษาดีกว่าผู้ป่วยที่มีตัวอย่างเชื้อที่ดื้อต่อยาตัวใดตัวหนึ่ง (p=0.004) การตรวจความไวต่อยาของเชื้อวัณโรคโดยวิธี MABA มีความสัมพันธ์ทางคลินิก ควรมีการศึกษาเพิ่มเติมเกี่ยวกับความสัมพันธ์ทางคลินิกและการตรวจวิธีโดยเฉพาะในสิ่งส่งตรวจที่เป็นเสมหะ

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