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Use of MGIT 960 for rapid quantitative measurement of the susceptibility of *Mycobacterium tuberculosis* complex to ciprofloxacin and ethionamide

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***Objectives*: Tentative standards for testing MICs for *Mycobacterium tuberculosis* include agar dilution and the BACTEC method. However, the conventional agar dilution method requires 3–5 weeks to complete; whereas BACTEC, although a rapid test, involves the use of radioisotopes. In contrast, the MGIT 960 system uses a fluorescence quenching based oxygen sensor that can be read automatically. This system is not only robust, safe and simple, but has been validated for susceptibility tests of first-line antituberculous agents.**

***Methods*: We evaluated 46 clinical strains of *M. tuberculosis* isolated from patients admitted to Kaohsiung Veterans General Hospital. Testing of MICs of ciprofloxacin and ethionamide was carried out by MGIT 960 and compared with the agar dilution method.**

***Results*: Good agreement was found between MGIT 960 and agar dilution. The greatest concordance between the agar dilution and MGIT assay at ±1 and ±2 dilution was 80.4% and 97.8% for ciprofloxacin, and 82.6% and 93.5% for ethionamide, respectively.**

***Conclusion*: MGIT 960 was found to be comparable to the current NCCLS standard method, agar dilution, and has the advantage of being rapid (obtaining results within 5–17 days, average 8.9 days) and easy to achieve standardization.**

Keywords: minimum inhibitory concentration, *M. tuberculosis*, susceptibility testing

Introduction

Tuberculosis (TB) in Taiwan still represents a common disease in the community, and it has been gaining increasing clinical relevance in immunocompromised patients, especially those with human immunodeficiency virus (HIV) infection.[1,2] Current chemotherapy is usually highly effective against *Mycobacterium tuberculosis*, however, there is an increasing need for alternative drugs as a result of either drug resistance or intolerance to first-line drugs. Laboratories supporting TB services must therefore be able to provide prompt, reliable susceptibility testing for antituberculous agents in addition to first-line drugs.

Tentative standard methods for the testing of MICs for *M. tuberculosis* include agar dilution and BACTEC methods (Becton Dickinson, Microbiology Systems, Sparks, MD, USA). Protocols have been described for carrying out antimicrobial susceptibility testing by the conventional proportion method on solid media and by BACTEC.[3–6] Both methods require technical expertise for the interpretation of results and the agar dilution method has the additional disadvantage of requiring 3 weeks of incubation.[7,8] BACTEC, although rapid, involves the use of radioisotopes. Alternative methods have been reported to provide rapid indirect drug susceptibility test results, such as the microplate Alamar Blue colourimetric (MAB) method.[9–11] The MAB method is cheap and rapid, however, it requires a greater level of technical expertise to provide reliable results. Furthermore, the microplates do not have a tight seal which may represent a biohazard unless handled carefully in the laboratory.

In contrast, the MGIT 960 system (Becton Dickinson) uses a fluorescence quenching based oxygen sensor that can be read automatically. The system is robust, safe, simple and has been validated for first-line

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Table 1. MIC distributions of ethionamide and ciprofloxacin by agar dilution and MGIT 960

	Ethionamide		Ciprofloxacin	
MIC (mg/L)	agar dilution	MGIT 960	agar dilution	MGIT 960
>16	0	1	1	0
16	1	5	1	1
8	2	1	0	1
4	8	7	0	0
2	17	22	7	3
1	14	7	18	10
0.5	4	3	16	29
0.25	0	0	1	2
Range	0.5–16	0.5–>16	0.25–>16	0.25–16
MIC_{50}	2	2	1	0.5
MIC_{90}	8	16	2	2

Table 2. Distribution of MICs of ciprofloxacin by agar dilution and MGIT 960

	No. of strains with MIC (mg/L) by agar dilution							
MIC (mg/L) by MGIT 960	>16	16	8	4	2	1	0.5	0.25
16	1							
8		1						
4								
2						1	2	
1					2	4	4	
0.5				1	5	13	9	1
0.25						1	1	

Table 3. Distribution of MICs of ethionamide by agar dilution and MGIT 960

	No. of strains with MIC (mg/L) by agar dilution						
MIC (mg/L) by MGIT 960	>16	16	8	4	2	1	0.5
>16					1		
16		1	2	1	1		
8						1	
4				1	4	2	
2				5	8	8	1
1				1	3	2	1
0.5						1	2

drug susceptibility tests. Reliable results showing an excellent correlation with reference methods on *M. tuberculosis* complex have been reported.[6,12–17] The MGIT technique has been thoroughly evaluated with qualitative measurement against the first-line drugs isoniazid, rifampicin, ethambutol, streptomycin, pyrazinamide,[18] and quantitative measurement against the second-line drug, kanamycin.[19] The purpose of this study is to investigate the reliability of the MGIT 960 system for the determination of MICs of ciprofloxacin and ethionamide for *M. tuberculosis* complex isolates.

Materials and methods

M. tuberculosis strains

Forty-six isolates of *M. tuberculosis* complex, collected in the period 1994–2000 were randomly selected to include 23 (50%) strains susceptible to the four first-line drugs, 17 multiple drug-resistant TB (MDRTB), and six strains with any drug resistance. All isolates were identified by BD ProbeTec ET ctb identification test using standard procedures as recommended by the manufacturer (Becton Dickinson). Testing of MICs of antituberculous drugs was carried out by both the agar dilution and MGIT 960 method in parallel.

Antimicrobial agents

The stock solutions of each drug were prepared in accordance with manufacturer's instructions and kept in aliquots at –20°C. From these stock solutions, working solutions were made in distilled water to be incorporated into the 7H11 media. Ethionamide was purchased from Sigma and ciprofloxacin was obtained from Bayer (Leverkusen, Germany).

MIC determination by the agar dilution method

The agar dilution method was carried out according to recommended standard procedures.[4,20] Briefly, 7H11 agar medium (Becton Dickinson) was prepared from a dehydrated base as recommended by the manufacturer. Some multiple drug-resistant *M. tuberculosis* strains may not grow or grow poorly on 7H10, while growing sufficiently on 7H11 agar. After the agar was autoclaved, oleic acid–albumin–dextrose–catalase (OADC) supplement (Becton Dickinson) and drugs were added at 50–56°C by doubling dilutions except for isoniazid which was five-fold diluted. Four millilitres of each concentration of antimycobacterial-containing medium was dispensed into plastic quadrant Petri dishes. As a growth control, one quadrant in each plate was filled with 7H11 agar medium with no drug. An inoculum of each isolate was prepared in Middlebrook 7H9 broth, and the absorbance was adjusted until it was equivalent to that of a McFarland No. 1 Standard. Final suspensions were carried out by adding Middlebrook 7H9 broth to prepare 10^{-2} and 10^{-4} dilutions of the standardized suspensions. Upon solidification of the medium, the plates received 0.1 mL on each quadrant of the agar plates. The inoculated plates were then incubated at 37°C for 3 weeks. The MICs of each isolate–drug pair were the lowest concentration of the antimycobacterial agents that inhibited >99% of the colonies growing on the drug-free control. *M. tuberculosis* H37Rv strain and an MDR strain (clinical isolate) were used as control strains.

MIC determination by the MGIT 960 method

Appropriate solutions of drugs were added in a volume of 0.1 mL to an MGIT (Mycobacteria Growth Indicator Tube) tube to achieve the desired final concentrations. The range of concentrations used for each drug is the same as in the agar dilution method. An inoculum was made from an initial MGIT broth culture after 1–2 days of showing positive by the instrument (Day 1 to Day 2) or diluted 1:5 for Day 3 to 5 positive tube. Half a millilitre of the inoculum was inoculated into each drug-containing tube that had been supplemented with 0.8 mL of OADC and antimicrobial. The drug-free control vial was inoculated with a 1:100 dilution of the

Table 4. Comparison of MGIT 960-determined and agar dilution-determined MICs for *M. tuberculosis* complex

	% Agreement in MGIT 960-determined MIC with agar dilution-determined MIC within dilution							% Agreement within	
	<–2	–2	–1	0	+1	+2	>+2	±1 dilutions	±2 dilutions
Ciprofloxacin	2.2	13	39.1	28.3	13	4.3	0	80.4	97.8
Ethionamide	0	2.2	19.6	30.4	32.6	8.7	6.5	82.6	93.5

inoculum to represent 1% of the bacterial population. The vials were loaded and the MIC was determined to be the lowest dilution that was negative by automated reading in drug-containing tubes when the control vial turned positive. The MIC was compared to the agar dilution method.

Results and discussion

The range of MICs, MIC_{50} and MIC_{90} of ciprofloxacin and ethionamide by agar dilution and MGIT 960 are listed in Table 1. Readings of the MICs determined by MGIT 960 were easily done, when the drug-free tube showed positive in an average 8.9 days, with a range of 5–17 days.

The MIC of ciprofloxacin determined by MGIT 960 correlated best if determined as the lowest concentration of drug-containing tubes remaining negative, interpreted at 1 day after the drug-free tube showed positive; designated as (D1, <D0). For ethionamide, the agreement within ± 2 $\log_2$ for (D1, <D0) is as good as for (D0, <D0), drug-containing tubes remaining negative, when the drug-free tubes turned positive. The distribution of MICs of ciprofloxacin and ethionamide are shown in Tables 2 and 3, respectively. There was an agreement between the MIC determined by the MGIT 960 and agar dilution methods within ± 1 $\log_2$ dilutions reaching 80.4% and 82.6% for ciprofloxacin and ethionamide, respectively. Within ± 2 $\log_2$ dilution, the agreement was increased to 97.8% and 93.5%, respectively (Table 4).

Good agreement of MIC between the agar dilution and MGIT 960 results was observed for all clinical isolates of *M. tuberculosis* tested. The agreement with the agar dilution method was over 90% for both drugs tested. The adapted criteria rendered the interpretation easy and can be integrated into the routine schedule.

MGIT 960 was found to be equivalent to the current NCCLS standard method, agar dilution and had the advantage of being reasonably fast (giving results in 5–17 days). The test was technically easy to perform, and the results were easy to interpret. Inoculum preparation and susceptibility turnaround times were satisfactory. Determination of MIC based on growth unit (GU) values obtained using the MGIT 960 is more objective, precise and less labour intensive than the agar dilution method. The latter method is prone to inter-individual variation and is more technique-dependent. Although further studies with larger isolate collections are required to confirm the optimal breakpoint concentration and the interpretation criteria, the MGIT 960 system appears to be an appropriate technique for carrying out MIC tests in areas where MDRTB is endemic, and where such tests are increasingly required.

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