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ORIGINAL ARTICLE

Prothionamide susceptibility testing of *Mycobacterium tuberculosis* using the resazurin microtitre assay and the BACTECMGIT 960 system

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Abstract Resazurin microtitre assay (RMA) has been successfully used to detect minimal inhibitory concentrations (MICs) of both first-line and several second-line drugs in drug susceptibility testing (DST) of *Mycobacterium tuberculosis* (MTB). In this study, we firstly compared prothionamide (PTH) susceptibility testing of *Mycobacterium tuberculosis* (MTB) using resazurin microtitre assay (RMA) and MGIT. Overall, the sensitivity and specificity of RMA for detecting PTH susceptibility was 96.5% [95% confidence interval (CI): 91.7–100.0] and 93.2% (95% CI: 89.6–96.8) respectively. In addition, the median time to positivity was significantly shorter for RMA than for the automated MGIT 960 (RMA, 8 days [range: 8–8 days] vs MGIT, 10.1 days, [range: 5.0–13.0]; $P < 0.01$). Concordance rate for MICs between RMA and MGIT for PTH-resistant group was 64.3% (95% CI: 46.5–82.0), which was significantly lower than that of PTH-susceptible group (85.9%, 95% CI: 78.8–93.0; $P = 0.01$). In conclusion, our data demonstrated that RMA can be used as an acceptable alternative for determination of PTH susceptibility with shorter turn-around time. When compared with MGIT 960, RMA method was prone to produce higher MICs for PTH-resistant MTB strains.

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Introduction

Prothionamide (PTH) is a member of thioamide drugs that forms NAD adducts to inhibit mycolic acid biosynthesis [1]. Due to its high efficacy against multidrug-resistant tuberculosis (MDR-TB), PTH has been recommended for the treatment of MDR-TB in clinical practice [2]. The World Health Organization endorsed automated liquid culture systems as the gold standard for second-line drugs, including PTH [3]. However, the liquid culture isolation requires expensive equipment and media and a well-serviced biosafety level-3 laboratory, which are inaccessible for countries with a high TB burden and resource-limited settings [4]. As a consequence, there is an urgent need for establish a simple and inexpensive method for determining the susceptibility to second-line drugs in *Mycobacterium tuberculosis* (MTB).

Recently, a rapid method based on the oxidation-reduction indicators has been successfully used to detect MICs of both first-line and several second-line drugs in DST of MTB [5]. Previous literatures have demonstrated that this method could produce reliable drug-susceptibility results for kanamycin (KAN), capreomycin (CAP), ofloxacin (OFX), ethionamide (ETH) and para-aminosalicylic acid (PAS), while requiring short turn-around times in comparison with conventional gold standards [6]. Unfortunately, there has been no report on evaluation of the performance of the resazurin microtitre assay (RMA) to determine the susceptibility to PTH in MTB. In addition, the RMA exhibited conflicting performance across different antimicrobial agents, the detection sensitivity of which ranged from 100% for kanamycin and ofloxacin to 84% for capreomycin [6]. Considering these facts, there is an urgent need for evaluating the feasibility of RMA for detecting PTH susceptibility of MTB. In our study, we aimed to compare PTH susceptibility testing of MTB using the

resazurin microtitre assay and the BACTEC MGIT 960 system.

Materials and methods

Bacterial strains

A total of 248 MTB strains were enrolled from Guangzhou Chest Hospital for this evaluation. All the strains were stored in 7H9 broth supplemented with 10% glycerol at minus 70°C freezer. Prior to performing the drug susceptibility testing, the strains were recovered on L-J medium for 4 weeks at 37°C.

Drug susceptibility testing

The PTH susceptibility of MTB isolates was detected by the Bactec MGIT 960 automated system following the instructions from the manufacturer [7]. The concentration of PTH in the MGIT tube was 2.5 μg/ml, which followed the guideline from World Health Organization (WHO) [3]. The MICs of MTB isolates were determined by MGIT 960, and the concentrations of PTH in the MGIT tubes included 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, and 80 μg/ml.

Resazurin microtitre assay

The MICs of MTB strains were also detected by RMA as previously described [8]. After inoculation at 37°C for 7 days, the assay was stained by supplementing 20 μl of resazurin (Alamar Blue, Sigma–Aldrich, Taufkirchen, Germany) and 50 μl of sterile 5% Tween 80. The plates were re-incubated for an additional 24 h at 37°C. The color change from blue to pink indicated growth of the bacteria, and visual MICs were defined as the lowest concentration antibiotic that inhibited this color change. For differentiation between susceptibility and resistance to PTH, the critical concentration for MIC was set as 2.5 μg/ml accordingly. All MIC determinations were performed in triplicate for each strain.

Statistical analysis

The concordance rate was calculated as the proportion of strains with concordant results between two methods over the sum of strains. The kappa test was used to evaluate the consistency between these two methods to detect susceptibility of MTB to PTH. In addition, the *t*-test was used to compare the average time to positivity between these two methods. Data analysis was performed with SPSS 14.0 (SPSS Inc., USA).

Results

As shown in Table 1, of 57 PTH-resistant MTB isolates diagnosed by MGIT, 55 were identified by the RMA method, with a sensitivity of 96.5% [95% confidence interval (CI): 91.7–100.0]. In addition, 178 out of 191 PTH-susceptible MTB isolates were confirmed by RMA method, suggesting a specificity of 93.2% (95% CI: 89.6–96.8). Statistical analysis showed that kappa value of these two methods was 0.84, indicating that RMA exhibited favorable concordance with MGIT for detecting PTH susceptibility of MTB isolates. In addition, we also compared the time to positivity for detection PTH susceptibility of MTB isolates between RMA and MGIT. The median time to positivity was significantly shorter for RMA than for the automated MGIT 960 [RMA, 8 days (range: 8–8 days) vs MGIT, 10.1 days, (range: 5.0–13.0); $P < 0.01$].

In order to evaluate the performance of RMA for detecting PTH susceptibility, we randomly selected 120 MTB isolates from all the tested isolates, and detected the MICs of these isolates by two different methods. Overall, the concordance rate between RMA and MGIT 960 was 80.8% (97/120, 95% CI: 73.8–87.9). Out of 23 isolates with different MICs, 19 (82.6%) harbored higher MICs detected by RMA than MGIT. We further compared the concordance rate according to PTH-susceptible (MIC ≤2.5 μg/ml) and PTH-resistant group (MIC >2.5 μg/ml). For the PTH-resistant group, the concordance rate was 64.3% (18/28, 95% CI: 46.5–82.0), which was significantly lower than that of the PTH-susceptible group (79/92, 85.9%, 95% CI: 78.8–93.0; $P = 0.01$, Table 2).

Table 1 Comparison of DST results obtained by use of MGIT 960 and RMA

RMA[a]	MIGT 960		Total	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	PPV (%, 95% CI)	NPV (%, 95% CI)	Concordance rate (%, 95% CI)	Kappa value (±SE)
	R	S							
R	55	13	68	96.5	93.2	80.9	98.9	94.0	0.84
S	2	178	180	(91.7–100.0)	(89.6–96.8)	(71.5–90.2)	(97.4–100.0)	(91.0–96.9)	(0.80–0.88)
Total	57	191	248						

[a] RMA: resazurin microtitre assay; R: resistant; S: susceptible.CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value; SE: standard error.

Table 2 Comparison of MICs determined by MGIT 960 and RMA

Method	MIC (μg /ml)	RMA[a]											Concordance rate (%)
		0.08	0.16	0.31	0.63	1.25	2.5	5	10	20	40	80	
MIGT 960	0.08	*3*											80.8 (73.8~87.9)
	0.16		*13*		1								
	0.31		2	*36*	3								
	0.63				*15*	2							
	1.25				2	*4*	3						
	2.5						*8*	1	2				
	5							*3*	1	1			
	10								*7*		1	2	
	20									*1*		1	
	40										*1*	1	
	80											*6*	

[a] The number in italics represents that MIC values determined by MGIT 960 and RMA are same.

Discussion

We firstly performed this study to access the diagnostic accuracy of RMA for detection of PTH susceptibility of MTB isolates. Our results suggest that RMA can be used for determination of PTH susceptibility with shorter turn-around time. Notably, despite showing acceptable concordance with the MGIT 960 reference method, we observed that 13 isolates identified as PTH-resistant by RMA were PTH-susceptible, contributing to more than 85% of MTB isolates with discordant DST results. Hence, our data indicate that RMA is sufficient for determination of PTH-susceptible strains, while a small proportion of PTH-resistant results from RMA may be misdiagnosed. In line with this finding, further MIC results supported our hypothesis, which revealed that the RMA method was prone to produce higher MICs than MGIT 960, especially for MTB strains with high-level PTH MICs.

Previous literature has demonstrated that RMA shows an excellent correlation with the conventional reference method, yielding a concordance rate greater than 98% [6], which is higher than 94% for PTH from our observation. Similarly, there is strong evidence that the overall level of agreement between the DST results obtained by MGIT 960 and other methods is lower for PTH when compared with other second-line anti-TB drugs [9–11]. It is interesting to explore the potential explanation for this low agreement of the DST result for PTH. A previous study from Lefford et al. found that MIC distribution of thioamides between probable susceptible (PS) strains and probable resistant (PR) strains could not be well separated [12]. Hence, accurate PTH susceptibility results have always been difficult to obtain. To solve the dilemma for determining in vitro PTH susceptibility, van Ingen and colleagues suggested a triple division into susceptible, intermediate, and resistant status based on the different degrees of resistance [9], which may provide a better indication for the efficacy of PTH treatment.

We also realize that a major disadvantage of this assay is its biosafety. Because the plates require a liquid medium, the repeated pipetting of liquid samples could produce aerosols [6, 13]. Recently, several pieces of commercial equipment are available for dispensing liquid samples into 96-well microtitre plates automatically [14], which provides a potential solution for reducing the exposure of aerosols for laboratory staffs.

In conclusion, our data demonstrated that RMA can be used as an acceptable alternative for determination of PTH susceptibility, with shorter turn-around time. When compared with MGIT 960, the RMA method was prone to produce higher MICs for PTH-resistant MTB strains.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no competing interests.

Ethical approval This study was approved by the Ethics Committee of Beijing Chest Hospital affiliated to Capital Medical University.

References

1. Wang F, Langley R, Gulten G et al (2007) Mechanism of thioamide drug action against tuberculosis and leprosy. J Exp Med 204(1):73–78
2. Falzon D, Jaramillo E, Schunemann HJ et al (2011) WHO guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update. Eur Respir J 38(3):516–528
3. World Health Organization (2008) Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs. WHO/HTM/TB/2008.392
4. Wells WA, Boehme CC, Cobelens FG et al (2013) Alignment of new tuberculosis drug regimens and drug susceptibility testing: a framework for action. Lancet Infect Dis 13(5):449–458
5. Sethi S, Mandal J, Kumar P et al (2007) Susceptibility testing of Mycobacterium tuberculosis by broth microdilution method: a rapid alternative method. Diagn Microbiol Infect Dis 57(4):447–449
6. Martin A, Camacho M, Portaels F, Palomino JC (2003) Resazurin microtiter assay plate testing of Mycobacterium tuberculosis susceptibilities to second-line drugs: rapid, simple, and inexpensive method. Antimicrob Agents Chemother 47(11):3616–3619
7. Zhang Z, Wang Y, Pang Y, Liu C (2014) Comparison of different drug susceptibility test methods to detect rifampin heteroresistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother 58(9):5632–5635
8. Zhang Z, Wang Y, Pang Y, Kam KM (2014) Ethambutol resistance as determined by broth dilution method correlates better than sequencing results with embB mutations in multidrug-resistant Mycobacterium tuberculosis isolates. J Clin Microbiol 52(2):638–641
9. van Ingen J, Simons S, de Zwaan R et al (2010) Comparative study on genotypic and phenotypic second-line drug resistance testing of Mycobacterium tuberculosis complex isolates. J Clin Microbiol 48(8):2749–2753
10. Kruuner A, Yates MD, Drobniewski FA (2006) Evaluation of MGIT 960-based antimicrobial testing and determination of critical concentrations of first- and second-line antimicrobial drugs with drug-resistant clinical strains of Mycobacterium tuberculosis. J Clin Microbiol 44(3):811–818
11. Rusch-Gerdes S, Pfyffer GE, Casal M, Chadwick M, Siddiqi S (2006) Multicenter laboratory validation of the BACTEC MGIT 960 technique for testing susceptibilities of Mycobacterium tuberculosis to classical second-line drugs and newer antimicrobials. J Clin Microbiol 44(3):688–692
12. Lefford MJ, Mitchison DA (1966) Comparison of methods for testing the sensitivity of Mycobacterium tuberculosis to ethionamide. Tubercle 47(3):250–261
13. Gazi MA, Islam MR, Kibria MG, Mahmud Z (2015) General and advanced diagnostic tools to detect Mycobacterium tuberculosis and their drug susceptibility: a review. Eur J Clin Microbiol Infect Dis 34(5):851–861
14. Heysell SK, Pholwat S, Mpagama SG et al (2015) Sensititre MycoTB plate compared to Bactec MGIT 960 for first- and second-line antituberculosis drug susceptibility testing in Tanzania: a call to operationalize MICs. Antimicrob Agents Chemother 59(11):7104–7108