



Note

Evaluation of BACTEC MGIT 960 system for the second-line drugs susceptibility testing of *Mycobacterium tuberculosis* in China

Li-li Zhao ^a, Qiang Xia ^b, Nan Lin ^{c,d}, Binbin Liu ^{a,e}, Xiu-qin Zhao ^a, Zhiguang Liu ^a, Kang-lin Wan ^{a,*}

^a National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention/State Key Laboratory for Infectious Disease Prevention and Control/National Reference Laboratory of tuberculosis, Beijing 102206, China

^b Zhejiang Prevention and Treatment Center of Tuberculosis, Zhejiang TCM & WM Hospital, Hangzhou 310003, China

^c National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

^d The Ministry of Education Key Laboratory of Biopesticide and Chemical Biology, College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China

^e Department of Epidemiology and Health Statistics, Public Health of central South University 410078, Changsha, China

ARTICLE INFO

Article history:

Received 19 March 2012

Received in revised form 25 June 2012

Accepted 26 June 2012

Available online 2 July 2012

Keywords:

BACTEC MGIT 960 system

The proportion method

L–J media

Drug susceptibility testing

The second-line drugs

Mycobacterium tuberculosis

ABSTRACT

When the performance of the BACTEC MGIT 960 system was evaluated with the traditional proportion method (PM) on 321 clinical isolates of *Mycobacterium tuberculosis* from China, concordance values were 98.44% capreomycin, 98.75% kanamycin, 98.75% ofloxacin and 94.08% ethionamide. The turnaround time with BACTEC MGIT 960 system (7.5 ± 1.8 days) was significantly shorter than with PM (28 days or 42 days). Therefore, the BACTEC MGIT 960 system was a reliable and rapid method for second-line drug susceptibility testing of TB in China.

© 2012 Published by Elsevier B.V.

The prevalence of drug-resistant tuberculosis (TB) is a very serious problem in China. It is one of the 27 countries with the highest incidence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) (World Health Organization, 2008). Based on the national baseline survey on TB in 2007–2008, the observed prevalence of MDR-TB among pulmonary TB patients was 8.32%, the XDR-TB prevalence was 0.68%, so it was estimated there were 120,000 cases of pulmonary bacteriological confirmed MDR-TB and 10,000 cases confirmed XDR-TB. So large numbers of MDR-TB and XDR-TB cases urgently demand for accurate and rapid drug susceptibility testing (DST) for second-line drugs, ensuring timely and adequate adjustments in treatment and minimizing the transmission of drug-resistant strains. However, in the current clinical routine of China, the second-line DST of TB is performed by traditional L–J PM requiring at least 28 days before getting the report (Canetti et al., 1969).

As a rapid phenotypic DST basing on liquid media, BACTEC MGIT 960 system had been applied for the DST to the first-line drugs (Kobayashi et al., 2006; Kruuner et al., 2006; Tomita et al., 2004; Kontos et al., 2004; Johansen et al., 2004; Golyshchevskaia et al., 2003; Huang et al., 2002). It could automatically report the drug susceptibilities according to the

predefined algorithms in 4–13 days after inoculation. Recently, some researchers used this platform for the second-line drugs susceptibility testing of tuberculosis and established the second-line drugs critical concentrations and procedures of BACTEC MGIT 960 system referring to BACTEC MGIT 460 system (Kruuner et al., 2006; Rusch-Gerdes et al., 2006; Rodrigues et al., 2008; Lin et al., 2009; Sharma et al., 2011). Despite the arrival of this novel tool, it remains uncertain that if the second-line drugs susceptibility testing with BACTEC MGIT 960 system is suitable for the diagnosis of resistant-TB in China without being evaluated in comparative studies.

In light of these findings, the aim of our study is to evaluate the performance of the BACTEC MGIT 960 system for the second-line drugs susceptible testing by analyzing a total of 321 TB clinical isolates.

For comparison, traditional L–J PM was taken as the reference standard, since it has been used for the second-line DST for over ten years in China.

All TB clinical isolates in our research were obtained from different patients with TB in Beijing, Fujian, Hunan, Shanxi, Shanghai, Tibet, Liaoning, Sichuan, Guangxi, Gansu and Henan of China between 2005 and 2010. Of these isolates, 281 isolates were resistant to one or more first-line drugs (isoniazid, rifampicin, streptomycin, ethambutol) and 40 isolates were susceptible based on previous drug susceptibility testing. For the resistant isolates, we included as many isolates from our archived collections as we could verify. Susceptible isolates from the same period were randomly chosen.

* Corresponding author at: National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, P.O. Box 5, Changping, Beijing 102206, China. Tel./fax: +86 10 58900779.

E-mail address: wankanglin@icdc.cn (K. Wan).

For BACTEC MGIT 960 testing, TB clinical isolates were inoculated first into MGIT 960 vials and cultured into the instrument until positive. Susceptibility testing to the second-line drugs using the BACTEC MGIT 960 system was performed strictly according to procedure of manufacturer for the primary drugs. Notably, the second-line drugs— capreomycin (CPM), kanamycin (KAN), ofloxacin (OFX) and ethionamide (ETH) were obtained in chemically pure forms from Sigma and prepared according to the standard procedures described by Rusch-Gerdes et al. (2006) and Rodrigues et al. (2008). Then 0.1 ml of the appropriated drug solution was aseptically pipetted into each MGIT vial. Final drug concentrations were 2.5 µg/ml for CPM, 2.5 µg/ml for KAN, 2.0 µg/ml for OFX and 5.0 µg/ml for ETH (Rusch-Gerdes et al., 2006; Rodrigues et al., 2008; WHO, 2008). All inoculated drug-containing and GC tubes were placed in the DST set carrier and entered into the MGIT 960 instrument as unknown drugs using the DST entry feature. The instrument flagged the DST set “complete” when the growth control reached a growth unit (GU) value of 400. At that point, the GU values of drug-containing tubes were retrieved from the instrument by printing out the DST set report, and the results were interpreted manually. If the GU of the drug containing tube was > 100 when the GU of the growth control was 400, the results were defined as resistant. If the GU values of the drug-containing tubes were ≤ 100, the results were considered susceptible (Rusch-Gerdes et al., 2006; Rodrigues et al., 2008).

Traditional drug susceptibility testing was carried out with L–J media according to the standard PM procedure recommended by World Health Organization (WHO) guideline (WHO, 2008). The critical concentration for the L–J PM were 40 µg/ml for CPM, 30 µg/ml for KAN, 2.0 µg/ml for OFX and 40 µg/ml for ETH. Results were read 28 days or 42 days after inoculation of the media (WHO, 2008).

M. tuberculosis H37Rv (ATCC 27294) was used as a quality control by two methods. If it showed a resistance result to any drug, all tests of that batch had to be repeated.

For the discrepant results between the two methods, retesting was performed twice using both methods. If the retesting results remained the same, results obtained from two methods were considered as true and used for the data analysis.

Data was analyzed using SPSS 16.0 statistical software. Agreement between the qualitative test results was assessed using the kappa statistic. The kappa value was interpreted as follows: <0.2, poor; 0.21–0.4, fair; 0.41–0.6, moderate; 0.61–0.8, good; and ≥0.81 excellent (Altman, 1999).

Reproducibility testing with BACTEC MGIT 960 system was performed prior to testing TB clinical isolates. A panel of 10 TB isolates with known susceptibility patterns was tested at three separate cycles with BACTEC MGIT 960 system. The results were compared to the expected results. In our evaluation, the overall reproducibility testing agreement was 99.2% for all drugs in a total of 120 tests (119/120), with only 1 incorrect result for ETH (Table 1).

Three hundred and twenty-one TB clinical isolates were tested for susceptibility to the second-line drugs at the critical concentration, resulting in a total of 1284 tests. As indicated by the DST results with L–J PM, which was taken as the reference standard, there were a large amount of resistant-strains included in this study: for CPM, n = 27; for KAN, n = 33; for OFX, n = 104; for ETH, n = 76 (Table 2).

Table 1
Reproducibility testing for the second-line drugs with BACTEC MGIT 960 system.

Drugs	Strains	No. of tests performed	No. of results agreeing with L–J PM method	Agreement (%)
CPM	R	15	15	100
	S	15	15	
KAN	R	18	18	100
	S	12	12	
OFX	R	15	15	100
	S	15	15	
ETH	R	15	15	96.7
	S	15	14	

Table 2
Drug susceptibility results of TB clinical isolates by BACTEC MGIT 960 system and L–J PM.

Drugs	Results of MGIT 960	Results of L–J PM		Sensitivity (%)	Specificity (%)	Agreement (%)	Kappa value
		R	S				
CPM	R	25	3	92.59	98.98	98.44	0.901
	S	2	291				
KAN	R	33	4	100	98.60	98.75	0.936
	S	0	284				
OFX	R	102	2	98.08	99.08	98.75	0.972
	S	2	215				
ETH	R	76	19	100	92.24	94.08	0.849
	S	0	226				

Comparing the results of BACTEC MGIT 960 system with L–J PM, there were 1252 consistent results (97.51%) out of a total of 1284 tests. The discordant results were 32 (2.49%). Of these, 4 strains were sensitive according to BACTEC MGIT 960 system but resistant by L–J PM (CPM n = 2; OFX n = 2) and 28 were resistant according to BACTEC MGIT 960 system but sensitive by L–J PM (CPM n = 3; KAN n = 4; OFX n = 2; ETH n = 19). The results remained the same after repeating with two methods.

Table 2 also showed the sensitivity, specificity and agreement of BACTEC MGIT 960 system, compared with L–J PM for the four second-line drugs. Kappa statistics indicated that there were excellent agreements for CPM, KAN, OFX and ETH (kappa value > 0.8) between the L–J PM and BACTEC MGIT 960 system.

The turnaround time for obtaining susceptibility results with BACTEC MGIT 960 system was 7.5 ± 1.8 days (range, 4.0–13.0 days) for the second-line drugs. While with L–J PM, the turnaround times were 28 days or 42 days.

There are rare publications reported that the performance of BACTEC MGIT 960 system for the second-line drugs susceptibility testing was evaluated with the L–J PM. Most reports focused on the comparison between BACTEC MGIT 960 system and BACTEC MGIT 460 system (Rusch-Gerdes et al., 2006; Rodrigues et al., 2008; Sharma et al., 2011). There are also several papers available for the second-line drugs susceptibility testing between BACTEC MGIT 960 system and agar PM (AP) (Kruuner et al., 2006; Lin et al., 2009; van Ingen et al., 2010). However, AP is likely to exhibit some variations such as manufacture method and critical concentrations of some drugs compared with L–J PM.

Before adopting a new method, laboratories should validate the results by performing the main method in the current clinical routine and new method in parallel for a series of clinical isolates. Furthermore, the choice of selected TB clinical isolates should include both the resistant- and the sensitive-isolates, permitting checking for the presence of very major error (VME, false-sensitive result) and major error (ME, false-resistant result). In this study, we evaluated the performance of the BACTEC MGIT 960 system for the DST to the second-line drugs among a large amount of drug-resistant TB using the traditional L–J PM as a reference method.

To assure the data quality of this study, reproducibility testing with BACTEC MGIT 960 system was performed before testing the 321 clinical isolates. In addition, for the discrepant results between the two methods, retesting was performed twice using both methods. If the retesting results remained the same, results obtained from two methods were considered as true and used for the data analysis.

Our data indicated that the BACTEC MGIT 960 system had an excellent test performance for CPM, KAN, OFX and ETH (kappa value > 0.8). The agreement for CPM between BACTEC MGIT 960 system and L–J PM was 98.44%, in line with the previous results (Kruuner et al., 2006; Rusch-Gerdes et al., 2006; Rodrigues et al., 2008; Lin et al., 2009; van Ingen et al., 2010). The agreements were 98.75% for KAN, 98.75% for OFX and 94.08% for ETH, slightly lower than the results tested by Rodrigues (Rusch-Gerdes et al., 2006; Rodrigues et al., 2008).

There were 4 VMEs (2 with CPM and 2 with OFX), 28 MEs (3 with CPM, 4 with KAN, 2 with OFX and 19 with ETH) observed with the BACTEC MGIT 960 system. This meant BACTEC MGIT 960 system had the tendency to generate ME rather than VME for the second-line drugs susceptibility testing. This phenomenon hadn't been demonstrated in the previous reports based on the second-line drugs susceptibility testing evaluations between BACTEC MGIT 960 system and BACTEC MGIT 460 system (Rusch-Gerdes et al., 2006; Rodrigues et al., 2008; Sharma et al., 2011). However, it is not surprising that results obtained from these two liquid-medium based systems are consistent with each other because both systems are made by the same company. Since there are no other comparable studies available between BACTEC MGIT 960 system and L-J PM, it is difficult to explore the reason for discordant results between these two methods. We presumed it probably related to the difference in the methodology. Clearly, future studies are needed to verify our presumption.

So many ME with ETH also showed in the study conducted by Lin et al. (2009) whose data based on the evaluation between BACTEC MGIT 960 system and AP. The cause was probably that the current tested concentration of ETH with BACTEC MGIT 960 system (5.0 µg/ml) was not equivalent to the critical concentration of solid media (40 µg/ml) (Lin et al., 2009). There should be further work to determine if a different ETH concentration with BACTEC MGIT 960 system would improve the concordance with the L-J PM.

Turnaround time for the DST is important for the patient to receive a timely and appropriate treatment. In our research, the average turnaround time for the second-line drugs with BACTEC MGIT 960 system was about 7.5 days, similar to the previous studies (Morcillo et al., 2010). While with L-J PM, the reporting time was 28 days or 42 days. Hence, the turnaround time with BACTEC MGIT 960 system is substantially shorter than that with L-J PM.

In summary, our study demonstrated that the BACTEC MGIT 960 system and traditional L-J PM yielded largely identical results in these 4 second-line drugs susceptibility testing of *M. tuberculosis*. It also had a shorter turnaround time than that with L-J PM. Hence, The BACTEC MGIT 960 system proved to be a rapid and reliable method for the second-line drugs susceptibility testing of *M. tuberculosis* and might take place of traditional L-J PM as a clinical common DST method in China.

Acknowledgments

We thank Dr. Salman Siddiqi (Becton Dickinson Diagnostic Systems, Sparks, Maryland, U.S.A.) and Eric Zhao (Becton Dickinson Diagnostic Systems, Beijing, China) for their helpful advice on the experiments, the staff of Beijing, Fujian, Hunan, Shanxi, Shanghai, Tibet, Liaoning, Sichuan, Guangxi, Gansu and Henan for supplying clinical isolates. This work was financially supported by the Collaboration Project for Tuberculosis Control Managed between the International Health

Exchange and Cooperation Center, Ministry of Health, P. R. China and Becton Dickinson Diagnostic Systems.

References

- Altman, D.G., 1999. Inter-rater Agreement. In: Altman, D.G. (Ed.), Practical Statistics for Medical Research. Chapman & Hall/CRC, London, pp. 403–409.
- Canetti, G., Fox, W., Khomenko, A., Mahler, H.T., Menon, N.K., Mitchison, D.A., Rist, N., Smelev, N.A., 1969. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. Bull. World Health Organ. 41 (1), 21–43.
- Golyshvskaia, V.I., Irtuganova, O.A., Smirnova, N.S., Domotenko, L.V., 2003. Comparison of nitrate reductase and automatic BACTEC MGIT 960 AST techniques for determining the drug sensitivity of mycobacteria tuberculosis. Probl. Tuberk. Bolezn. Legk. 8, 34–37.
- Huang, T.S., Tu, H.Z., Lee, S.S., Huang, W.K., Liu, Y.C., 2002. Antimicrobial susceptibility testing of *Mycobacterium tuberculosis* to first-line drugs: comparisons of the MGIT 960 and BACTEC 460 systems. Ann. Clin. Lab. Sci. 32 (2), 142–147.
- Johansen, I.S., Thomsen, V.O., Marjamaki, M., Sosnovskaja, A., Lundgren, B., 2004. Rapid, automated, nonradiometric susceptibility testing of *Mycobacterium tuberculosis* complex to four first-line antituberculous drugs used in standard short-course chemotherapy. Diagn. Microbiol. Infect. Dis. 50 (2), 103–107.
- Kobayashi, I., Abe, C., Mitarai, S., 2006. BACTEC MGIT 960 system for drug susceptibility testing of *Mycobacterium tuberculosis*: a study using external quality assessment strains. Kekkaku 81 (2), 57–62.
- Kontos, F., Maniatis, M., Costopoulos, C., Gitti, Z., Nicolaou, S., Petinaki, E., Anagnostou, S., Tselentis, I., Maniatis, A.N., 2004. Evaluation of the fully automated Bactec MGIT 960 system for the susceptibility testing of *Mycobacterium tuberculosis* to first-line drugs: a multicenter study. J. Microbiol. Methods 56 (2), 291–294.
- Kruuner, A., Yates, M.D., Drobniowski, F.A., 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.
- Lin, S.Y., Desmond, E., Bonato, D., Gross, W., Siddiqi, S., 2009. Multicenter evaluation of Bactec MGIT 960 system for second-line drug susceptibility testing of *Mycobacterium tuberculosis* complex. J. Clin. Microbiol. 47 (11), 3630–3634.
- Morcillo, N., Imperiale, B., Di Giulio, B., 2010. Evaluation of MGIT 960 and the colorimetric-based method for tuberculosis drug susceptibility testing. Int. J. Tuberc. Lung Dis. 14 (9), 1169–1175.
- Rodrigues, C., Jani, J., Shenai, S., Thakkar, P., Siddiqi, S., Mehta, A., 2008. Drug susceptibility testing of *Mycobacterium tuberculosis* against second-line drugs using the Bactec MGIT 960 System. Int. J. Tuberc. Lung Dis. 12 (12), 1449–1455.
- Rusch-Gerdes, S., Pfyffer, G.E., 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.
- Sharma, M., Thibert, L., Chedore, P., Shandro, C., Jamieson, F., Tyrrell, G., Christianson, S., Soualhine, H., Wolfe, J., 2011. Canadian multicenter laboratory study for standardized second-line antimicrobial susceptibility testing of *Mycobacterium tuberculosis*. J. Clin. Microbiol. 49 (12), 4112–4116.
- Tomita, M., Takeno, H., Suzuki, K., Sakatani, M., Kinoshita, Y., Kobayashi, I., 2004. A study on inoculum density and reproducibility of drug susceptibility testing by BACTEC MGIT 960. Kekkaku 79 (11), 625–630.
- van Ingen, J., Simons, S., de Zwaan, R., van der Laan, T., Kamst-van Agterveld, M., Boeree, M.J., van Soolingen, D., 2010. Comparative study on genotypic and phenotypic second-line drug resistance testing of *Mycobacterium tuberculosis* complex isolates. J. Clin. Microbiol. 48 (8), 2749–2753.
- WHO, 2008. Policy guidance on TB drug susceptibility testing (DST) of second-line drugs. WHO, Geneva. WHO/HTM/TB/2008392.
- World Health Organization, 2008. Global tuberculosis control: surveillance p, financing. WHO report 2008. The Organization, Geneva. WHO/HTM/TB/2008.393.