Little difference between minimum inhibitory concentrations of *Mycobacterium tuberculosis* wild-type organisms determined with BACTEC MGIT 960 and Middlebrook 7H10

E. Sturegård[1], K. A. Ängeby[2,3], J. Werngren[4], P. Juréen[4], G. Kronvall[2], C. G. Giske[2], G. Kahlmeter[5,6] and T. Schön[7]

1) *Medical Microbiology, Department of Laboratory Medicine, Malmö, Lund University, Lund,* 2) *Clinical Microbiology, MTC – Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden,* 3) *Department of Microbiology, The University of the West Indies, Kingston, Jamaica,* 4) *The Public Health Agency of Sweden, Department of Microbiology, Solna,* 5) *Department of Clinical Microbiology, Växjö Hospital, Växjö,* 6) *Department of Medical Sciences, Uppsala University, Uppsala and* 7) *Department of Clinical Microbiology, Kalmar County Hospital, Kalmar, Sweden*

Abstract

The MIC wild-type (WT) distribution for *Mycobacterium tuberculosis* in BACTEC 960 MGIT is not defined, which may result in poor reproducibility for drug susceptibility testing (DST), as several DST methods with different breakpoints are in use. In a comparison between MGIT and Middlebrook 7H10 medium of seven first- and second-line drugs, including 133 MIC determinations of 15 WT isolates, we found an agreement of 91.7% within ± one MIC dilution step. The results confirm the agreement in MIC testing between 7H10 and MGIT and indicate that breakpoints could be harmonized in order to avoid misclassification.

Keywords: Drug susceptibility testing, epidemiological cut off, minimal inhibitory concentration, normalised resistance interpretation, resistance, tuberculosis

Original Submission: 3 June 2014; **Revised Submission:** 18 August 2014; **Accepted:** 20 August 2014

Editor: M. Drancourt

Article published online: 29 October 2014

Corresponding author: T. Schön, Department of Clinical Microbiology, Kalmar County Hospital, 391 85 Kalmar, Sweden
E-mail: tschon@hotmail.com

Introduction

For *Mycobacterium tuberculosis*, the currently used clinical breakpoints for drug susceptibility testing (DST)—critical concentrations—are mostly based on consensus from the 1960s. This definition of clinical breakpoint is the drug concentration that inhibits 95% of susceptible strains but not strains that are cultured from patients not responding to therapy [1]. Several multicenter studies have been performed to transfer the critical concentrations initially established in Löwenstein-Jensen media to modern liquid and solid media such as BACTEC 960 MGIT (MGIT) and Middlebrook 7H10 (7H10) by using a previous method as the reference [2,3]. In those studies, no investigation of wild-type (WT) MIC distributions or large-scale direct comparisons of MICs between methods were included. This strategy that does not take WT distributions into account for the DST methods used today has, to some extent, contributed to a poor reproducibility and agreement for drugs such as ethambutol and for strains with MICs close to both the epidemiological cut off (ECOFF) and the critical concentration [4]. For other bacterial pathogens, susceptibility breakpoints are established by a combination of clinical outcome data, pharmacokinetic and pharmacodynamic as well as WT distributions, according to the standards of the European Committee on Antimicrobial Susceptibility Testing [5]. The WT distribution is used to define the ECOFF, which is the MIC of the upper part of this distribution. The ECOFF is, by definition, devoid of phenotypically detectable acquired and mutational resistance mechanisms to a certain drug, but should not be mixed up with the clinical breakpoint [5].

We have previously shown a clear separation of susceptible vs. resistant isolates based on ECOFFs for isoniazid and rifampicin, which corresponds to clinical outcome and pharmacokinetic and pharmacodynamic data [6]. In these studies we used a high-throughput replicator method to test over 100 strains on solid media. This would be challenging in MGIT, which is widely used for routine DST. Therefore, the aim was to establish WT MIC distributions in MGIT by testing a limited number of strains and correlating these to the MICs of the same strains previously obtained on 7H10 medium and to the results of mathematical modelling.

To establish the WT distribution, consecutive clinical isolates from 15 patients diagnosed by MGIT and Löwenstein-Jensen culture, as well as routine PCR at the tuberculosis laboratory at the Karolinska University Hospital, were included. All isolates were subsequently confirmed as unique by restriction fragment length polymorphism analysis as

http://dx.doi.org/10.1016/j.cmi.2014.08.021

described [6]. The strains were kept in −70°C and subcultured on Löwenstein-Jensen media. All isolates were susceptible to first- and second-line drugs [6]. The stock solutions of isoniazid (INH), rifampicin (RIF), ethambutol (EMB), levofloxacin (LEV), ofloxacin (OFL), amikacin (AMI), and capreomycin (CAP) were prepared as previously described [6–8]. A 1:1 suspension from a positive MGIT culture tube was inoculated in a fresh MGIT tube containing antibiotics, and DST was subsequently performed according to the manufacturer (BACTEC MGIT 960, Becton Dickinson, Franklin Lakes, NJ, USA), including a 1:100 diluted control. Serial twofold dilutions of the drugs were prepared and MGIT tubes were inoculated in final concentrations corresponding to two MIC steps above and below the MICs that had previously been obtained in the Middlebrook 7H10 [6–8]. In addition, for the first-line drugs, the standard DST procedure (SIRE-kit) was used [3]. *M. tuberculosis* H37Rv was included in all experiments as an internal control. In addition, MICs for all investigated antibiotics were determined for one multi-drug-resistant (MDR)- and two INH-mono-resistant strains.

For mathematical modelling, the normalised resistance interpretation (NRI) method was applied, which utilizes the fact that the lower part in an MIC distribution is not affected by resistant isolates and the WT population can therefore be mathematically reconstructed based on the shape of this population [9]. The ECOFFs were defined at 2.5 SD above the calculated means. NRI was used with permission from the patent holder, Bioscand AB, TÄBY, Sweden (European patent No 1383913) and performed as described previously [9].

In total, 735 MGIT tubes were used for the MIC determination of seven drugs including 15 WT isolates, three resistant and one control isolate (Table 1). For the first-line drugs, the classification into susceptible or resistant based on the MIC showed a perfect correlation to the SIRE-kit by the manufacturer. Further, the variation between and within runs for the control strain (H37Rv) in three separate rounds never exceeded one MIC dilution step, indicating excellent reproducibility (Table 1). The correlation between the individual MICs for MGIT compared to 7H10 was perfect for 40% (53/133) of the DSTs performed, and 91.7% were within ± one MIC dilution step (Table 2). For LEV and CAP, MICs were 0.4–0.6 mean dilution steps higher, whereas RIF and AMI were 0.75 MIC dilution steps lower in MGIT compared to 7H10. A limitation of the study is the few MIC determinations performed per drug and thus, the suggested ECOFFs for MGIT need to be repeated in other laboratories with a larger number of isolates. The performance of the NRI analysis as an objective tool to determine the ECOFF [9] was confirmed by a close correlation between the current critical concentrations and the proposed ECOFFs within one MIC dilution step, except for RIF, which was two MIC dilution steps lower (0.25 mg/L) compared to the current critical concentration. This finding could partly explain recent reports where RIF-susceptible strains exhibited

TABLE 2. Differences in MIC dilution steps for *Mycobacterium tuberculosis* drug susceptibility testing in BACTEC 960 MGIT compared to Middlebrook 7H10 (n = 19)

MIC dilution steps[a]	RIF (n)	INH (n)	EMB (n)	AMI (n)	CAP (n)	OFL (n)	LEV (n)
−2	2	1	1	5	0	0	0
−1	10	12	10	7	0	4	1
0	5	6	4	6	12	11	9
1	2	0	4	1	7	3	8
2	0	0	0	0	0	1	1

RIF, rifampicin; INH, isoniazid; EMB, ethambutol; AMI, amikacin; CAP, capreomycin; OFL, ofloxacin; LEV, levofloxacin.
[a]The numerical difference in twofold MIC dilution steps between the two methods for the tested drugs of each individual isolate using Middlebrook 7H10 as a reference for BACTEC 960 MGIT was indicated. One dilution step (1) indicates that the MIC level for MGIT was higher than 7H10, and consequently, that −1 dilution step indicates that 7H10 was higher than MGIT, whereas 0 indicates no difference between the methods.

TABLE 1. MIC distribution ranges (mg/L) in BACTEC 960 MGIT versus Middlebrook 7H10

	H37Rv	H37Rv	MDR	MDR	INH-R	INH-R	WT	ECOFF	WT	ECOFF	ECOFF - NRI	Mean ΔMIC	7H10	MGIT
	MGIT	7H10	MGIT	7H10	MGIT	7H10	MGIT	MGIT	7H10	7H10	2.5 SD MGIT	7H10 vs MGIT	CC	CC
n	3	9	1	1	2	2	15	15	15	90[a]	15	15		
INH	0.064	0.064–0.125	4	8	1;8	2;8	0.064–0.125	0.125	0.032–0.064	0.125	0.14	0.063	0.2	0.1
RIF	0.125–0.25	0.032–0.25	128	64	0.125/0.125	0.125;0.5	0.032–0.25	0.25	0.064–0.125	0.5	0.28	0.75	1	1
EMB	2–4	1–2	8	4	1;5	4;4	1.0–4.0	4	1.0–2.0	2	3.9	−0.25	5	5
AMI	0.5	0.5–1	>512	>512	0.25;0.5	1;2	0.25–1.0	1	0.5–1.0	1	1.12	0.75	ND	1
CAP	2	1–2	8	8	1;2	1;2	1.0–4.0	4	0.5–1.0	4	9.3	−0.44	10	2.5
OFL	1	0.25–0.5	0.5	0.5	0.125;0.5	0.125;1	0.5–2.0	2	0.5–1.0	1	2.8	−0.13	2	2
LEV	0.25–0.5	0.125–0.25	0.5	0.5	0.125;0.5	0.125;0.5	0.25–1.0	1	0.25–0.5	0.5	0.86	−0.56	2	2

H37Rv, *Mycobacterium tuberculosis* strain H37Rv; MDR, multi-drug-resistant isolate; INH-R, isoniazid monoresistant isolates; WT, wild-type; ECOFF, epidemiological wild-type cut-off; NRI, normalised resistance interpretation; CC, critical concentration; INH, isoniazid; RIF, rifampicin; EMB, ethambutol; AMI, amikacin; CAP, capreomycin; OFL, ofloxacin; LEV, levofloxacin.
[a]Derived from [6].

mutations in *rpoB* [10]. Whether such isolates with MICs above the tentative ECOFF (0.25 mg/L) but below the critical concentration (1 mg/L) carrying mutations in *rpoB* will respond to standard doses of RIF is unknown and remains to be shown in clinical trials. The MDR isolate was classified as susceptible for EMB and CAP in 7H10 (4 mg/L and 8 mg/L, respectively) but was resistant in the MGIT system (8 mg/L for both drugs) along with confirmed resistance mutations in the *embB* and *rrs* genes. Thus, with the same MIC level, an isolate may be classified as resistant or susceptible according to the current breakpoints, indicating a poor reproducibility between methods and a risk of misclassification of clinical isolates.

In conclusion, we investigated the WT MIC distributions in MGIT for first- and second-line drugs, which corresponded well to that of 7H10. We propose that WT MIC distributions for *M. tuberculosis* are similar regardless of whether liquid or solid Middlebrook media is used, and suggest that breakpoints could be harmonized in order to avoid misclassification.

Transparency declaration

This work was supported by Malmö University Hospital, Department of Clinical Microbiology, FORSS (The Medical Research Council of Southeast Sweden), the Swedish Society of Antimicrobial Chemotherapy (SSAC), Marianne and Marcus Wallenberg foundation, the Swedish Medical Association and the Karolinska University Hospital.

Acknowledgements

The authors would like to thank the laboratory technicians Åsa Larsson and Lena Dahlgren in Malmö, Sweden for expert technical support in MIC determinations in BACTEC 960 MGIT.

References

[1] Canetti G, Froman S, Grosset J, Hauduroy P, Langerova M, Mahler HT, et al. Mycobacteria: laboratory methods for testing drug sensitivity and resistance. Bull World Health Org 1963;29:565–78.

[2] Pfyffer GE, Bonato DA, Ebrahimzadeh A, Gross W, Hotaling J, Kornblum J, et al. Multicenter laboratory validation of susceptibility testing of mycobacterium tuberculosis against classical second-line and newer antimicrobial drugs by using the radiometric bactec 460 technique and the proportion method with solid media. J Clin Microbiol 1999;37:3179–86.

[3] Rusch-Gerdes S, Pfyffer GE, Casal M, Chadwick M, Siddiqi S. 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 2006;44:688–92.

[4] Angeby K, Jureen P, Kahlmeter G, Hoffner SE, Schon T. Challenging a dogma: antimicrobial susceptibility testing breakpoints for mycobacterium tuberculosis. Bull World Health Org 2012;90:693–8.

[5] Kahlmeter G, Brown DF, Goldstein FW, MacGowan AP, Mouton JW, Odenholt I, et al. European Committee on Antimicrobial Susceptibility Testing (EUCAST) technical notes on antimicrobial susceptibility testing. Clin Microbiol Infect 2006;12:501–3.

[6] Schon T, Jureen P, Giske CG, Chryssanthou E, Sturegård E, Werngren J, et al. Evaluation of wild-type MIC distributions as a tool for determination of clinical breakpoints for *Mycobacterium tuberculosis*. The J Antimicrob Chemother 2009;64:786–93.

[7] Angeby KA, Jureen P, Giske CG, Chryssanthou E, Sturegård E, Nordvall M, et al. Wild-type MIC distributions of four fluoroquinolones active against mycobacterium tuberculosis in relation to current critical concentrations and available pharmacokinetic and pharmacodynamic data. J Antimicrob Chemother 2010;65:946–52.

[8] Jureen P, Angeby K, Sturegard E, Chryssanthou E, Giske CG, Werngren J, et al. Wild-type MIC distributions for aminoglycoside and cyclic polypeptide antibiotics used for treatment of mycobacterium tuberculosis infections. J Clin Microbiol 2010;48:1853–8.

[9] Kronvall G, Giske CG, Kahlmeter G. Setting interpretive breakpoints for antimicrobial susceptibility testing using disk diffusion. Int J Antimicrob Agents 2011;38:281–90.

[10] Van Deun A, Aung KJ, Bola V, Lebeke R, Hossain MA, de Rijk WB, et al. Rifampin drug resistance tests for tuberculosis: Challenging the gold standard. J Clin Microbiol 2013;51:2633–40.