

Automated quantitative drug susceptibility testing of non-tuberculous mycobacteria using MGIT 960/EpiCenter TB eXiST

Katja Lucke†, Michael Hombach†, Ute Friedel, Claudia Ritter and Erik C. Böttger*

Institut für Medizinische Mikrobiologie, Universität Zürich, Nationales Zentrum für Mykobakterien, Zürich, Switzerland

*Corresponding author. Institut für Medizinische Mikrobiologie, Universität Zürich, Gloriastrasse 30/32, 8006 Zürich, Switzerland. Tel: +41-44-634-2700; Fax: +41-44-634-4906; E-mail: boettger@imm.uzh.ch

†These authors contributed equally to this study.

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Objectives: To assess the predictive value of *in vitro* drug susceptibility testing (DST) in slow-growing non-tuberculous mycobacteria (NTM), knowledge on quantitative levels of drug susceptibility should be available. The aim of this study was to investigate the suitability of the MGIT 960/TB eXiST system for quantitative DST of NTM.

Methods: We have assessed quantitative levels of drug susceptibility for clinical isolates of *Mycobacterium avium*, *Mycobacterium intracellulare* and *Mycobacterium kansasii* by comparing radiometric Bactec 460TB-based DST with non-radiometric DST using MGIT 960/TB eXiST.

Results: MGIT 960/TB eXiST gives results comparable to those of Bactec 460TB.

Conclusions: The MGIT 960/TB eXiST appears suitable for quantitative DST of NTM.

Keywords: drug resistance, antibiotic susceptibility, clinical isolates

Introduction

Antimicrobial therapy of infections with slow-growing non-tuberculous mycobacteria (NTM) is hampered by the lack of standardized procedures for drug susceptibility testing (DST). The radiometric Bactec 460TB instrumentation (Becton Dickinson Microbiology Systems, Sparks, MD, USA) has been used in the past for susceptibility testing of slow-growing NTM, mainly *Mycobacterium avium* complex (MAC) and *Mycobacterium kansasii*.^{1–4} In clinical laboratories the radiometric system has largely been replaced, e.g. with the Mycobacterium Growth Indicator Tube 960 system (MGIT 960, Becton Dickinson), not least because marketing of the Bactec 460TB instrumentation and associated consumables has been terminated.^{5,6} A main advantage of the MGIT 960 platform is the lack of radiochemistry, its full automation and its compatibility with computerized expert systems for interpretation. The aim of this study was to determine quantitative levels of drug susceptibility for therapeutically relevant antibiotics using the Bactec MGIT 960/EpiCenter v5.53 system (equipped with the TB eXiST module) and a representative set of clinical isolates of *M. avium*, *Mycobacterium intracellulare* and *M. kansasii*, and to compare the results with those obtained by the radiometric Bactec 460TB system.

Materials and methods

Organisms

Twenty-six clinical isolates (*M. kansasii*, *n*=10; *M. avium*, *n*=10; *M. intracellulare*, *n*=6) were investigated in this study; the strains originated from patient specimens submitted to the mycobacteriology laboratory of our institution (see Table S1, available as Supplementary data at JAC Online). Mycobacterial isolates were identified by sequence analysis of the 16S rRNA gene as described previously.⁷ Sequence analysis of *hsp65* was used to differentiate *M. kansasii* and *Mycobacterium gastri*.⁸

Antimicrobial agents

The antibiotics amikacin, clarithromycin, ethambutol, ofloxacin, rifabutin and rifampicin (Sigma Aldrich Chemie GmbH, Buchs, Switzerland) were selected based on guidelines for treatment of NTM.⁹ Ofloxacin was chosen as the class representative for the newer quinolones. Antibiotic concentrations chosen for the study are summarized in Table S2 (available as Supplementary data at JAC Online).

Susceptibility testing using Bactec 460TB and the MGIT 960 system with the EpiCenter TB eXiST software system

DST was performed as recommended by the manufacturer and test results were interpreted as described previously.¹⁰ The terms susceptible (S),

intermediate (I) and resistant (R) as used in this study describe *in vitro* growth inhibition at a given drug concentration and are not to be confused with predictability of clinical outcome. The I category indicates that the studied drug concentration significantly (>99%), but not completely, inhibits bacterial growth *in vitro*. An example is given in Figure S1 (available as Supplementary data at JAC Online). In brief, positive MGIT vials were subcultured for susceptibility testing as follows: (i) for subcultivations within 1 or 2 days after the MGIT 960 system recorded a positive growth signal, bacterial suspensions with *M. kansasii* and *M. intracellulare* were diluted 1:5 and bacterial suspensions with *M. avium* were diluted 1:25 with sterile 0.9% NaCl; and (ii) for subcultivations within 3–5 days after the MGIT 960 system recorded a positive growth signal, bacterial suspensions with *M. kansasii* and *M. intracellulare* were diluted 1:25 and bacterial suspensions with *M. avium* were diluted 1:125 with sterile 0.9% NaCl. The latter dilutions constituted the MGIT working suspensions. MGIT tubes supplemented with 0.8 mL of enrichment (BACTEC MGIT 960 SIRE Supplement; Becton Dickinson) were inoculated with 0.2 mL of drug solution and 0.5 mL of the working suspension. For the drug-free growth control, the working suspension was diluted 1:100 with sterile 0.9% NaCl and 0.5 mL of the diluted working suspension was inoculated (proportion testing). The procedure for preparing the MGIT working suspensions was determined empirically to generate a positive signal with the drug-free growth control within 4–10 days following inoculation.

Statistical analysis

To assess the accuracy of quantitative DST, the results of MGIT 960 testing were compared with those of Bactec 460TB (Figure 1). Wilson's method was used to calculate the agreement of MGIT 960 and Bactec 460TB.¹¹

Results

A total of 435 out of 520 data points showed complete agreement. The overall agreement of MGIT 960 and Bactec 460TB independent of antibiotic, drug concentration and species was 87.1% [95% confidence interval (CI) 80.2%–91.9%]. Disagreements presented as minor errors exclusively. No major discrepancies (R versus S) were observed. Ofloxacin and amikacin showed peaks of disagreements at concentrations of 10 mg/L and 4 mg/L, respectively (Figure S2; available as Supplementary data at JAC Online). The results are discussed in detail below.

Amikacin

Amikacin test results correlated well between MGIT 960 and Bactec 460TB. A total of 104 data points were obtained, 91 of which showed complete concordance. Thirteen data points of 10 isolates had minor discrepancies: (i) 3 isolates were R (MGIT 960) versus I (Bactec 460TB) at 1.0 mg/L, but resulted in S at 4 mg/L with both systems, indicating MICs between 1.0 mg/L and 4 mg/L; and (ii) for 7 isolates minor discrepancies (R versus I, I versus S) were observed at 4 mg/L (at a concentration of 10 mg/L these 7 isolates were found to be fully susceptible with both test systems, indicating MICs for the isolates of 4–10 mg/L).

Clarithromycin

For clarithromycin, a total of 104 data points were obtained, 100 of which showed complete concordance. Four data points of four isolates had minor discrepancies: two isolates

were S (MGIT 960) versus I (Bactec 460TB) at 4.0 mg/L; and a further two isolates were S (MGIT 960) versus I (Bactec 460TB) at 16.0 mg/L. These four minor discrepancies were resolved by congruent test results at the corresponding higher drug concentration, indicating MICs for the corresponding isolates close to 4 mg/L and 16 mg/L, respectively. Two isolates of *M. avium* were resistant at all drug concentrations with MGIT 960 and Bactec 460TB. Genetic sequencing was done for one of the isolates and revealed a single point mutation at 23S rRNA position 2058.¹² Clinical macrolide resistance in the MAC is almost exclusively associated with mutations in 23S rRNA, affecting nucleotide residues A2058 and A2059.^{12–15} These mutations confer high-level macrolide resistance, i.e. MICs >64 mg/L.^{12–14,16} To further substantiate our findings we studied a series of 14 *M. avium* and 4 *M. intracellulare* isolates displaying macrolide resistance, with the resistance mechanism identified at the molecular level, i.e. mutation of 23S RNA residue 2058 or 2059—including both clinical resistant strains and laboratory mutants.^{12,13} The isolates unanimously tested as resistant at all drug concentrations used in the MGIT 960 (MICs of clarithromycin >64 mg/L; see Table S3, available as Supplementary data at JAC Online).

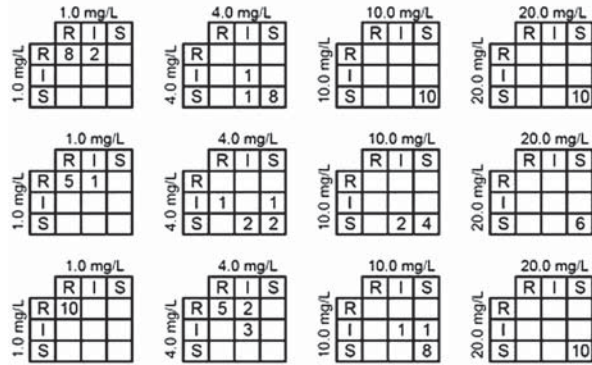
Ethambutol

For ethambutol, a total of 78 data points were obtained, 72 of which showed complete concordance. Six data points of six isolates had minor discrepancies: (i) one isolate was R (MGIT 960) versus I (Bactec 460TB) at 5.0 mg/L, one isolate was I (MGIT 960) versus S (Bactec 460TB) at 5.0 mg/L and two isolates were S (MGIT 960) versus I (Bactec 460TB) at 5.0 mg/L—all of these isolates were S at 12.5 mg/L by both methods, indicating that the MIC had to be close to 5 mg/L; and (ii) two isolates were S (MGIT 960) versus I (Bactec 460TB) at 12.5 mg/L—both resulted in S at 50 mg/L.

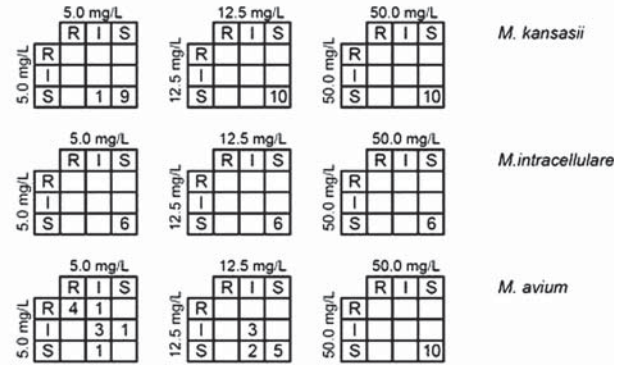
Ofloxacin

For ofloxacin, a total of 104 data points were obtained, 80 of which showed complete concordance. Twenty-four data points of 13 isolates had minor discrepancies: (i) 2 isolates were I (MGIT 960) versus R (Bactec 460TB) at 2.0 mg/L, and S (MGIT 960) versus I (Bactec 460TB) at 10 mg/L, and 1 isolate was S (MGIT 960) versus I (Bactec 460TB) at 2.0 mg/L and 10 mg/L—all three isolates were S at 20 mg/L with both methods; (ii) 6 isolates were I (MGIT 960) versus R (Bactec 460TB) at 10.0 mg/L [2 of the 6 isolates showed concordant results at 20 mg/L with both systems (I and S, respectively) and 4 of the 6 isolates were I (MGIT 960) versus R (Bactec 460TB) or S (MGIT 960) versus I (Bactec 460TB) at 20 mg/L; 5 of the 6 isolates were S at 50.0 mg/L with both test systems and 1 isolate resulted in S (MGIT 960) versus I (Bactec 460TB) at 50.0 mg/L]; (iii) 3 isolates were S (MGIT 960) versus I (Bactec 460TB) at 10.0 mg/L and 20.0 mg/L, and S at 50.0 mg/L with both methods; and (iv) 1 isolate was I (MGIT 960) versus R (Bactec 460TB) at 20.0 mg/L and I at 50.0 mg/L with both methods.

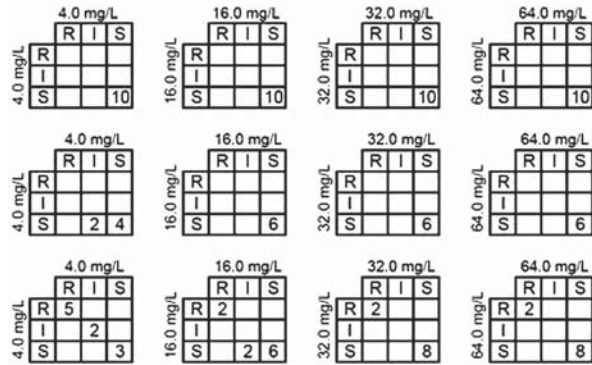
Amikacin



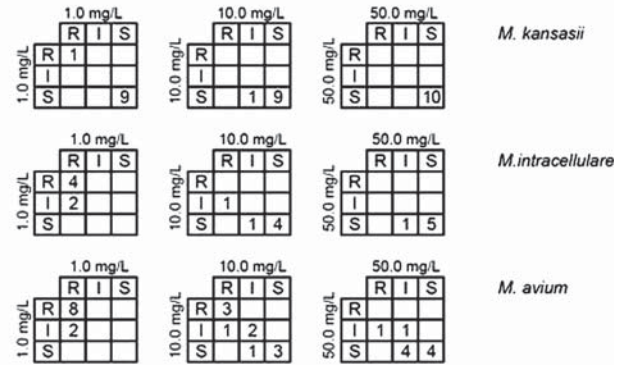
Ethambutol



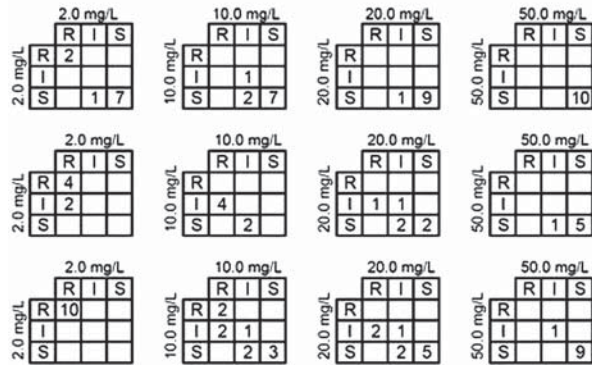
Clarithromycin



Rifampicin



Ofloxacin



Rifabutin

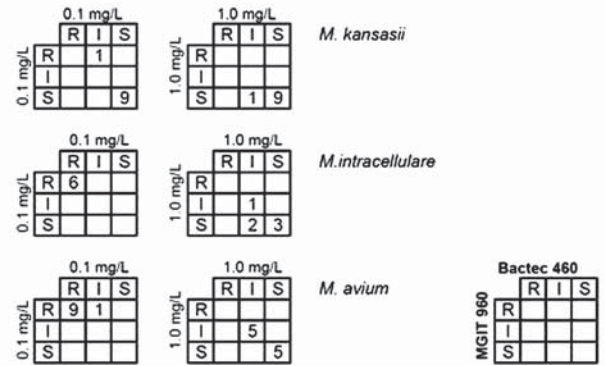


Figure 1. Comparison of Bactec 460TB and MGIT 960 quantitative DST. Total number of NTM tested for amikacin, ethambutol, clarithromycin, rifampicin, rifabutin and ofloxacin: n=26 [*M. kansasii* (n=10), *M. avium* (n=10), *M. intracellulare* (n=6)]; data separated according to species.

Rifabutin

For rifabutin, a total of 52 data points were obtained, 47 of which showed complete concordance. Five data points of four isolates had minor discrepancies: (i) two isolates were R (MGIT 960) versus I (Bactec 460TB) at 0.1 mg/L [one of the two isolates was S (MGIT 960, Bactec 460TB) at 1.0 mg/L and one isolate was S (MGIT 960) versus I (Bactec 460TB) at 1.0 mg/L]; and

(ii) two isolates were S (MGIT 960) versus I (Bactec 460TB) at 1.0 mg/L. Comparing the three isolates, which were S and I for rifabutin at 1.0 mg/L, with rifampicin testing results demonstrated that rifampicin was R at 1.0 mg/L. With rifampicin at 10.0 mg/L, two out of three isolates were S (MGIT 960) versus I (Bactec 460TB) and one isolate was I (MGIT 960) versus R (Bactec 460TB). At 50.0 mg/L, two out of three isolates were S

(MGIT 960 and Bactec 460TB) and one isolate was S (MGIT 960) versus I (Bactec 460TB).

Rifampicin

For rifampicin, a total of 78 data points were obtained, 63 of which showed complete concordance. Fifteen data points of 13 isolates had minor discrepancies: (i) at 1.0 mg/L, 4 isolates were R (MGIT 960) versus I (Bactec 460TB) and all isolates resulted in S (MGIT 960, Bactec 460TB) at 10.0 mg/L; (ii) 2 isolates were I (MGIT 960) versus R (Bactec 460TB) at 10.0 mg/L [both resulted in S (MGIT 960) versus I (Bactec 460TB) at 50.0 mg/L]; (iii) 3 isolates were S (MGIT 960) versus I (Bactec 460TB) at 10.0 mg/L and S at 50 mg/L by both methods; and (iv) 3 isolates were S (MGIT 960) versus I (Bactec 460TB) at 50.0 mg/L and 1 isolate was I (MGIT 960) versus R (Bactec 460TB) at 50.0 mg/L.

Discussion

There is an urgent need for standardized means and proper procedures for DST of NTM in the diagnostic laboratory. Only limited information is available correlating *in vitro* susceptibility testing results with clinical outcome.^{17,18} Drug susceptibility of NTM may be quite heterogeneous, with remarkable interspecies differences and intraspecies variability.^{3,4,19,20} As a result, the definition of drug susceptibility by 'critical concentration' testing is barely possible. It is generally agreed that quantitative DST may provide better correlation with clinical outcome and allows monitoring of discrete changes in drug susceptibility during treatment.^{21,22} Our results show high concordance for Bactec 460TB and automated MGIT 960 readings compared over a wide range of drug concentrations, and are in general agreement with recent data comparing Bactec 460TB with manual MGIT 960 readings.^{23,24} We observed only minor discrepancies, i.e. R versus I, I versus S (see Figure 1). If the true MIC lies near the cut-off value for two adjacent interpretative categories, method-dependent fluctuations will randomly shift interpretation to one or the other category, resulting in discrepancies. The closer the true MIC is to the cut-off for two adjacent categories, the higher will be the frequency of corresponding discrepancies.²⁵ For example, our results show minor discrepancies peaking at drug concentrations of 10 mg/L and 4 mg/L for ofloxacin and amikacin, respectively (Figure S2). Inter-assay agreement (reproducibility) of repeat testing ranged from 71% for rifampicin to 100% for clarithromycin (Figure S3, available as Supplementary data at JAC Online), and only minor disagreements were recorded.

With the exception of DST for clarithromycin the clinical relevance of *in vitro* DST is not well established for slow-growing NTM, in part because of the lack of standardized procedures for *in vitro* susceptibility testing and, until recently, the lack of correlation with clinical efficacy and outcome. As a result, the current recommendations for NTM DST only include clarithromycin.²⁶ In contrast to *M. tuberculosis*, clinical isolates of slow-growing NTM with any defined resistance phenotype other than clarithromycin and associated with a well-characterized molecular genetic alteration are largely lacking. Therefore, with the exception of clarithromycin, we were unable to study clinical NTM strains with acquired resistance to any of the other drugs

investigated in this study, i.e. amikacin, ethambutol, ofloxacin, rifampicin or rifabutin. To the best of our knowledge, corresponding series of well-characterized drug-resistant NTM clinical isolates have not been described in the literature. While this is a significant limitation and shortcoming of the study, our data are relevant, as they provide the information necessary to recognize wild-type susceptibility and any possible change thereof. In our study we included a significant number of well-defined macrolide-resistant MAC strains to validate MGIT 960/EpiCenter TB eXiST for recognition of macrolide resistance.

Future studies will be necessary to investigate the accuracy of the MGIT 960 system for recognition of drug resistance other than clarithromycin. The discontinuation of the Bactec 460TB platform makes the development of alternative testing procedures urgent. At the very least, our study demonstrates that MGIT 960 DST gives results comparable to those of Bactec 460TB. Several features of the MGIT 960/EpiCenter platform offer significant advantages compared with the Bactec 460TB system, e.g. non-radioactive growth detection, full automation, software-supported data interpretation and ease of standardization. The procedure investigated in this study is a first step in the establishment of standardized, rapid and reliable techniques for quantitative DST of NTM. Additional studies including drug-resistant clinical isolates are warranted to assess the accuracy of the MGIT 960 system for recognition of drug resistance in NTM.

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Transparency declarations

E. C. B. has a consultancy agreement with Becton-Dickinson. All other authors: none to declare.

Supplementary data

Tables S1–S3 and Figures S1–S3 are available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

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