Evaluation of *Mycobacterium avium* Complex (MAC) Susceptibility Testing for Clarithromycin using SLOWMYCO Sensititre® Panels and JustOne® Strips

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Key words: *Mycobacterium avium*, clarithromycin, MIC

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Abstract

The SLOWMYCO Sensititre® panel and the custom JustOne® strip (both from TREK Diagnostic Systems, Cleveland, OH) were evaluated for susceptibility testing of *Mycobacterium avium* complex isolates against clarithromycin. Seventy one archived and prospectively collected isolates were tested using both the SLOWMYCO panel and the JustOne strip with the results compared to those obtained using the BACTEC™ 460 (BD, Sparks, MD) radiometric method and a broth microdilution reference method. Results obtained by the SLOWMYCO panel and the JustOne strip agreed with the BACTEC 460 method for 64/71 isolates (90%). Similarly concordance with the broth microdilution method was 40/43 isolates (93%) for both test systems. The effect of source medium on inoculum preparation was evaluated and there were no differences noted in MICs regardless of whether the inoculum was prepared from isolates grown in Middlebrook 7H9 medium, on Middlebrook 7H10 agar or in VersaTREK broth culture bottles (Trek Diagnostics). Clarithromycin testing for MAC using the SLOWMYCO panel and the JustOne strip methods is easy to setup, simple to read and is readily incorporate into the clinical laboratory. These systems offer advantages over the BACTEC 460 system, including the lack of need for radioactive substrates, sharps, or costly instrumentation.
INTRODUCTION

Nontuberculous mycobacteria (NTM) are increasingly recognized as a cause of pulmonary disease (1) and some of the most common NTM species isolated in the clinical laboratory are members of the *Mycobacterium avium* complex (MAC). In addition to pulmonary disease, MAC infection can result in lymphadenitis, and disseminated disease in both immunocompromised as well as immunocompetent patients (9). The American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) recommend that a three-drug regimen be utilized for treatment of MAC including a macrolide (clarithromycin or azithromycin), ethambutol and a rifamycin such as rifampin (5).

The ATS/IDSA recommend performing susceptibility testing of MAC isolates only against the macrolides based on the results of the limited number of available, well-controlled clinical trials correlating *in vitro* susceptibility data and clinical outcomes (2, 4, 6). Both the ATS/IDSA and the Clinical and Laboratory Standards Institute (CLSI) recommend that susceptibility testing of MAC be performed for the initial clinical isolate, for isolates from patients previously on macrolide therapy, for isolates from patients failing macrolide therapy, for isolates from AIDS patients developing bacteremia while on macrolide prophylaxis, and for isolates from patients with positive blood cultures after 3 months of therapy for disseminated MAC (3, 5). The CLSI guideline also suggests that clarithromycin can serve as a class drug for azithromycin and therefore only clarithromycin needs to be routinely tested. Although some experts disagree, testing of drugs other than clarithromycin is not recommended at this time since no correlation has been established between *in vitro* susceptibility testing and clinical response to agents.
other than the macrolides. The CLSI recommends the use of either the radiometric
BACTEC 460 method, broth-based macrodilution or broth microdilution methods for
susceptibility testing of MAC against clarithromycin (3).

Recently, two products, the SLOWMYCO Sensititre® panel (formerly called the
MAISLOW Sensititre® panel) and the custom JustOne® clarithromycin strip were
introduced by TREK Diagnostic Systems (Cleveland, OH). Both products are labeled as
research use only (RUO) products. The SLOWMYCO panel is a standard order
microbroth dilution panel, which can be used for testing of slowly growing mycobacteria
against amikacin, ciprofloxacin, clarithromycin, doxycycline, ethambutol, ethionamide,
isoniazid, linezolid, moxifloxacin, rifabutin, rifampin, streptomycin and
trimethoprim/sulfamethoxazole. In this study, we evaluated the performance of the
panel for predicting MAC resistance to only clarithromycin since this is the single drug
recommended for in vitro testing as discussed earlier. Similarly, we also evaluated the
performance of the JustOne clarithromycin strip, a custom-manufactured microbroth
dilution strip containing only clarithromycin. Both the SLOWMYCO panel and the JustOne
strip contain antimicrobial agents lyophilized in microtitre plate wells with each well
containing a different concentration of drug. Drug-free control wells are also provided
for each plate or strip.

MATERIALS AND METHODS

_Mycobacterium avium/intracellulare complex isolates._ Forty six archived MAC
isolates with known clarithromycin MICs, as determined previously by the authors using
the radiometric BACTEC-460 method, were used in this study. Twenty one of the 46
isolates were resistant to clarithromycin (MIC $\geq 32$ µg/ml). These isolates consisted of clinical, proficiency and reference strains that were originally identified by 16S rDNA sequencing, MAC AccuProbe nucleic acid hybridization probe analysis (Gen-Probe, San Diego, CA) or PCR-restriction endonuclease analysis (PRA) of the 65 kDa hsp gene (7, 8). The archived isolates were stored as frozen stocks at -70°C. In addition to the archived specimens, 25 consecutive, recent MAC clinical isolates were collected prospectively for this study. A random subset of 43 isolates (archived and recent) was also tested using a second comparison method, broth microdilution. An Institutional Review Board of the Mayo Clinic approved the use of all isolates.

Inoculum preparation. Inocula for this study were prepared from three different media sources (Middlebrook 7H9 medium, Middlebrook 7H10 agar, and VersaTREK broth) to determine if there was any effect of source media on test performance. Archived MAC isolates from frozen stocks were subcultured to a Middlebrook 7H9 broth bottle and a Middlebrook 7H10 agar plate and the inoculum for the SLOWMYCO panel and the JustOne strips was prepared according to CLSI M24-A and the manufacturer’s package insert guidelines (3). Briefly, confluent growth from the 7H10 agar plate was swept with a loop, emulsified in sterile water, and the concentration adjusted to 0.5 McFarland. Fifty microliters of the emulsified suspension from either the agar plate or the diluted broth were added to 10 ml of Mueller Hinton broth with OADC (TREK Diagnostics, Cleveland, OH). In addition, isolates were subcultured to VersaTREK MYCO broth bottles containing Middlebrook medium, growth supplement and 0.5 ml of a 0.5 McFarland suspension of the MAC isolates. The inoculated VersaTREK bottles were
placed on the VersaTREK 528 instrument, an FDA-approved platform for mycobacterial culture, and the cultures were incubated at 35°C until they signaled positive (usually 2.5 days). Susceptibility testing was performed within 3 days of the positive MYCO bottle signal. Inoculum from the VersaTREK bottles was prepared as described for the 7H9 broth.

Susceptibility testing. The original MAISLOW panel contained clarithromycin ranging from 0.5-64μg/ml and this was the concentration range that we tested in our study. Upon introduction of the SLOWMYCO name, the concentration range of clarithromycin was expanded to 0.06-64μg/ml. The concentration range for clarithromycin tested in the JustOne strip was 0.12-128 μg/ml. The SLOWMYCO panel and the JustOne Strip wells were inoculated with 100 μl of the Mueller Hinton broth suspension described above and incubated in a non-CO₂ incubator at 35-37°C until the growth control showed sufficient growth (7-14 days). The MICs were determined visually using an inverted mirror and read as the lowest concentration of clarithromycin showing 100% inhibition of growth. MICs obtained using the SLOWMYCO panel and the JustOne strip were compared to results obtained using either the BACTEC 460 radiometric method or the broth microdilution reference method (4). Isolates with discordant results were retested with the same methods as used in the first MIC determination. Those isolates with repeatedly discordant results were classified as either very major errors (defined as an isolate resistant (R) by the reference method but susceptible (S) by the test method), major errors (S by the reference method and R by the test method), or minor errors (Intermediate (I) by one method but S or R by the other method). Interpretative criteria were per CLSI
guidelines with an MIC of ≤ 4 µg/ml deemed S, an MIC of 8-16 µg/ml is I and an MIC of ≥ 32 µg/ml is R for the BACTEC 460 method at pH 7.3-7.4. For the broth microdilution method, an MIC of ≤ 8 µg/ml is S, an MIC of 16 µg/ml is I, and an MIC of ≥ 32 µg/ml is R. The agreement between the various methods was expressed as percent concordance and the strength of the agreement between methods was determined using kappa scores.

Precision studies. Reproducibility studies evaluating intra-day (10 replicates in a single day) and inter-day (20 replicates over 10 days) precision were performed using M. avium quality control strains ATCC 700898 (clarithromycin-susceptible) and ATCC 700897 (clarithromycin-resistant). Four technologists performed the reproducibility studies to test inter-operator variability.

RESULTS

Comparison of the SLOWMYCO panel and JustOne strip MICs to the radiometric BACTEC 460 method. The overall agreement between the SLOWMYCO panel and the BACTEC 460 method was 90% (64/71 isolates) with a kappa score of 0.79, indicating good agreement (Table 1). The very major error rate was 4.8% (1/21 isolates) and the major error rate was 0%. The most numerous discordant results were observed primarily in the I category where 5/71 isolates (7%) that were I by the BACTEC 460 method were called S by SLOWMYCO panel and 1/71 isolates (1%) was R by the BACTEC 460 method and I by the SLOWMCYO panel. Identical results were obtained regardless of the culture media used for the inoculum preparation (Middlebrook 7H9 broth,
Middlebrook 7H10 agar or VersaTREK broth). The agreement between the JustOne strip and the BACTEC 460 method was identical to that found for the SLOWMYCO panel.

Comparison of the SLOWMYCO panel and JustOne strip and to the broth microdilution method. The overall agreement between the SLOWMYCO panel and the JustOne strip with the broth microdilution method results was 93% (40/43 isolates) with a kappa score of 0.87 indicating a very good agreement (Table 2). A single isolate was called R by the microbroth reference method and S by both the SLOWMYCO panel and the JustOne strip giving a very major error rate of 5%. The major error rate was 0%. As with the BACTEC 460 comparison, discordance occurred most often in the intermediate category with 2 isolates (5%) deemed I by the microbroth reference method and S by the SLOWMYCO panel and the JustOne strip. Again, the culture medium used for inoculum preparation (7H9 broth, 7H10 agar and VersaTREK broth) had no effect on the results.

Precision. Reproducibility studies evaluating intra-day (10 replicates in a single day) and inter-day (2 replicates for 10 days) precision using M. avium quality control strains ATCC 700898 (clarithromycin-susceptible) and ATCC 700897 (clarithromycin-resistant) had 100% correlation with expected results for both the JustOne strip and the SLOWMYCO panel. No inter-operator variability was noted.

DISCUSSION
Susceptibility testing of MAC isolates against clarithromycin is recommended for initial isolates from significant sources or when therapy appears to be failing (5). Methods currently recommended for MIC determination include the radiometric BACTEC 460 and the broth microdilution methods (3, 5). In this study, we evaluated the performance of the SLOWMYCO microtiter panel and a custom JustOne microtiter strip for susceptibility testing of MAC isolates against clarithromycin. The concordance between the SLOWMYCO panel or the JustOne strip and the BACTEC 460 method was 90% with the kappa score indicating a good agreement between the methods. One isolate was deemed a very major error for both the panel and the strip when compared to the radiometric method. Interestingly, this isolate was found to be susceptible by the broth microdilution reference method. The % agreement between the panel or the strip and the broth microdilution reference method was 93% and the kappa score indicated a very good agreement between the methods with a single very major error detected (1/20 resistant isolates). A limitation of our study is that clarithromycin resistant MAC isolates are rare and therefore we were only able to obtain a total of 21 resistant isolates.

The radiometric BACTEC 460 method is a well-established assay for clarithromycin susceptibility testing of MAC isolates. However, the assay is laborious, and uses radioactive substrate. The JustOne strip and the SLOWMYCO panel are both broth microdilution methods and they demonstrate ≥90% correlation with both the radiometric method as well as a broth microdilution reference method. The SLOWMYCO panel and JustOne strip have the added advantage of being commercially available, and thus standardized, and they are easy to set up and interpret. There were no significant medium-dependent effects when the isolates were setup from different source
media (7H9 broth, 7H10 agar and VersaTREK broth). Susceptibility testing can therefore be performed without additional subcultures if the source culture is less than 5 weeks old from 7H9 broth or 7H10 agar or \( \leq 3 \) days after signaling positive in the VersaTREK MYCO bottle. Finally, the susceptibility results obtained using the SLOWMYCO panel or the JustOne strip are often available within approximately 7 days, which can have a positive impact on patient care since this is faster than the typical turnaround time for the BACTEC 460 method. Therefore, the JustOne strip and SLOWMYCO panels are alternatives to the radiometric method for clarithromycin susceptibility testing of MAC isolates.

Potential Conflict of Interest Disclosure. This study was funded by the Mayo Clinic and the University of Texas Health Sciences Center at Tyler, TX. No financial or other form of support was received from TREK Diagnostics, the manufacturer of the SLOWMYCO plate and the JustOne Strip.


Table 1. Comparison of clarithromycin susceptibility results from the SLOWMYCO panel and JustOne strip with the BACTEC-460 method.

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<th>BACTEC 460, pH 7.3-7.4</th>
<th>% agreement (kappa)</th>
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<tr>
<td></td>
<td>Susceptible ≤4.0 µg/ml</td>
<td>Intermediate 8-16 µg/ml</td>
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<td>SLOWMYCO panel</td>
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<td>Susceptible ≤8 µg/ml</td>
<td>45</td>
<td>5</td>
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<tr>
<td>Intermediate 16 µg/ml</td>
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<td>0</td>
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<tr>
<td>Resistant ≥32 µg/ml</td>
<td>0</td>
<td>0</td>
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<tr>
<td>JustOne strip</td>
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<tr>
<td>Susceptible ≤8 µg/ml</td>
<td>45</td>
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<tr>
<td>Intermediate 16 µg/ml</td>
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<tr>
<td>Resistant ≥32 µg/ml</td>
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Table 2. Comparison of clarithromycin susceptibility results from the SLOWMYCO panel and JustOne strip with the broth microdilution method

<table>
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<th>Broth microdilution</th>
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<td>Susceptible ≤8 µg/ml</td>
<td>Intermediate 16 µg/ml</td>
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<td>Intermediate = 16 µg/ml</td>
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<td></td>
<td>Resistant ≥32 µg/ml</td>
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<td>JustOne strip</td>
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<td></td>
<td>Intermediate = 16 µg/ml</td>
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<td>Resistant ≥32 µg/ml</td>
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Figure Legend

Figure 1. Photographs of the SLOWMYCO panel and JustOne strip showing susceptible and resistant isolates of *M. avium* complex. Growth control are labeled and shown in the single boxed well for each panel or strip.  

a) SLOWMYCO panel containing 0.5-64µg/ml of clarithromycin in doubling dilutions in the boxed row. The isolate is fully susceptible to clarithromycin with an MIC of $\leq 0.5$µg/ml.  
b) SLOWMYCO panel containing 0.5-64µg/ml of clarithromycin in doubling dilutions in the boxed row. The isolate is fully resistant to clarithromycin with an MIC of $> 64$µg/ml.  
c) JustOne strips containing 0.125-128µg/ml of clarithromycin in doubling dilutions in each individual strip. The isolate on the left is fully susceptible to clarithromycin with an MIC of $\leq 0.125$µg/ml. The isolate on the right is fully resistant to clarithromycin with an MIC of $> 128$µg/ml.
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MIC ≤ 0.5 µg/mL

resistant
MIC > 64 µg/mL

susceptible
MIC ≤ 0.12 µg/mL

resistant
MIC > 128 µg/mL