Susceptibility Testing for Mycobacteria

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Mycobacterial susceptibility testing is important for the management of patients with tuberculosis and those with disease caused by certain nontuberculous mycobacteria. To help standardize methods used in the clinical microbiology laboratory for testing susceptibility of mycobacteria, the National Committee for Clinical Laboratory Standards (NCCLS) recently updated NCCLS document M24-T (published in 1995), which is the tentative standard for antimycobacterial susceptibility testing of Mycobacterium tuberculosis. The second edition of the NCCLS tentative standard (document M24-T2) differs considerably from the initial document. It contains revised guidelines for the testing of M. tuberculosis complex and newly proposed guidelines for the testing of some nontuberculous mycobacteria, including the rapidly growing mycobacteria (Mycobacterium fortuitum group, Mycobacterium chelonae, and Mycobacterium abscessus), Mycobacterium avium complex, Mycobacterium kansasii, and Mycobacterium marinum, as well as Nocardia species and other aerobic actinomycetes. The recommendations for mycobacterial susceptibility testing that are outlined in NCCLS document M24-T2 are reviewed.

Mycobacterial susceptibility testing is important for appropriate patient management. It should be done on initial isolates of Mycobacterium tuberculosis from all patients and on clinically significant isolates of certain nontuberculous mycobacteria (NTM). In general, when any type of antimicrobial susceptibility testing is performed in the clinical laboratory, use of a standardized method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) is optimal. For the nonfastidious bacteria that grow aerobically (e.g., Staphylococcus aureus and Escherichia coli), such NCCLS guidelines have been available for ~20 years. NCCLS standards for mycobacteria, however, have lagged behind. In 1995, the NCCLS published its first document for mycobacterial susceptibility testing, document M24-T, which is a tentative standard that addressed only M. tuberculosis [1]. The second edition of this tentative standard (NCCLS document M24-T2) was recently developed [2]. It includes revised guidelines for the testing of M. tuberculosis complex (MTBC), in addition to proposed recommendations for the testing of certain NTM and aerobic actinomycetes. In this report, I review the recommendations for mycobacterial susceptibility testing that are outlined in NCCLS document M24-T2.

MTBC

Methods that are typically used in the United States for susceptibility testing of MTBC are based on the ability of the isolate that is undergoing evaluation to grow on agar or in broth containing a single "critical" concentration of one drug. The critical concentration of a drug represents the lowest concentration that inhibits "wild" strains (i.e., isolates that have never come into contact with antituberculous drugs) but that does not inhibit isolates from patients who are not responding clinically to therapy. For many years, agar proportion has been considered the standard method of MTBC susceptibility testing in the United States, and the NCCLS has recently proposed that it be used as the reference method for all drugs but one [2]. The exception is pyrazinamide, for which the radiometric BACTEC TB 460 (BD Biosciences, Sparks, MD) is the proposed reference method [2]. When using agar proportion, the definition of resistance is based on the fact that therapy is less likely to be clinically successful when >1% of the bacterial population being tested in vitro is resistant [3].

Although agar proportion is the proposed reference method against which the performance of newer methods must be compared, the NCCLS Subcommittee on Antimycobacterial Susceptibility Testing recognizes that, in general, it does not provide rapid results (the usual turnaround time is 3 weeks after the test is set up). To ensure the earliest possible detection of re-
sistant MTBC, the NCCLS subcommittee recommends that laboratories use a rapid method of susceptibility testing to reach the goal (proposed by the Centers for Disease Control and Prevention [CDC]) of reporting the results of MTBC susceptibility testing within 28 days of receipt of a specimen in the laboratory [4]. The recommended rapid method of testing involves the use of commercial broth-based systems that have been cleared by the US Food and Drug Administration (FDA). Two such FDA-cleared systems are currently available: the radiometric BACTEC TB 460, which is the most frequently used method for susceptibility testing of MTBC in the United States, and the more recently introduced ESP Culture System II (Trek Diagnostics, Westlake, OH) [5–7]. Other broth systems have been evaluated [8] and are expected to be available in the near future. For drugs that have not been cleared by the FDA for testing with commercial systems, the standard agar proportion method should be used.

The primary drugs that are recommended by the NCCLS subcommittee for testing against isolates of MTBC are isoniazid (at 2 concentrations) and rifampin, ethambutol, and pyrazinamide (1 concentration of each). This combination of agents was selected to provide comprehensive information related to the initial 4-drug therapy currently recommended for the treatment of most patients with tuberculosis in the United States. Secondary antituberculous drugs used for testing are capreomycin, cycloserine, ethionamide, kanamycin, ofloxacin, para-aminosalicylic acid, rifabutin, and streptomycin. The concentrations of some drugs tested are different, depending on the method or medium used; those drugs that are recommended for testing by use of the agar proportion method are listed in table 1 [2]. All of these secondary drugs, with the exception of cycloserine, plus a higher concentration of ethambutol should be tested for any isolate that is resistant to rifampin or is resistant to ≥2 primary drugs. Cycloserine remains an option therapeutically, but in vitro testing is not recommended because of the technical problems associated with the test.

State and provincial public health laboratories serve as referral centers for mycobacterial testing and therefore should provide—or should ensure—access to susceptibility testing of MTBC against the full panel of primary antituberculous drugs, as well as against secondary drugs, when indicated. In other laboratories, however, modification of the recommended panel of primary antituberculous drugs may be appropriate. For example, there is considerable pressure for hospital laboratories to reduce costs, and the recommended rapid, commercial broth systems are expensive. Therefore, in communities where resistance to pyrazinamide alone is rarely or never encountered, testing of a reduced panel of isoniazid, rifampin, and ethambutol may be an economical alternative that would provide sufficient information for patient management. If such a protocol were adopted, pyrazinamide would be tested only if the isolate was resistant to one or more of the 3 other primary agents. Decisions about testing protocols should be made in collaboration with infectious disease and pulmonary specialists. Factors to consider in the decision-making process include the patient population served, the prevalence of drug resistance in the community, access to further testing if drug resistance or intolerance is encountered, the turnaround time for those additional results [2], and the local treatment regimens recommended by those who are responsible for tuberculosis control.

As advocated by the CDC, susceptibility testing should be performed on the first isolate of MTBC obtained from each patient [4]. Testing should be repeated if the patient still has a positive culture result after 3 months of receiving appropriate therapy or if the patient fails to respond clinically to therapy.

With regard to the reporting of results, the NCCLS subcommittee suggests that, at a minimum, laboratories should report both the name of the drug tested and an interpretation of the result (e.g., “susceptible,” “resistant,” or “borderline resistant”) [the last category applies to pyrazinamide only]. In the case of isoniazid, for which the testing of 2 concentrations is recommended, if an isolate is resistant to the critical concentration

![Table 1. Antituberculous drugs and their recommended concentrations for testing by means of the agar proportion method.](image)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration, µg/mL</th>
<th>7H10 agar</th>
<th>7H11 agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoniazid</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Rifampin b</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Ethambutol</td>
<td>5.0</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Pyrazinamide c</td>
<td>NRP</td>
<td>NRP</td>
<td></td>
</tr>
<tr>
<td>Secondary d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capreomycin</td>
<td>10.0</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Ethionamide</td>
<td>5.0</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Kanamycin e</td>
<td>5.0</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Para-aminosalicylic acid</td>
<td>2.0</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>Rifabutin f</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Data were obtained from the National Committee for Clinical Laboratory Standards document M24-T2 [2]. NRP, not recommended for pyrazinamide testing. Permission to use portions of M24-T2 (Susceptibility Testing of Mycobacteria, Nocardia, and Other Aerobic Actinomycetes: Tentative Standard—Second Edition) has been granted by NCCLS. The interpretive data are valid only if the methodology in M24-T2 is followed. The current standard may be obtained from NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087, USA.

a A critical concentration (the lowest concentration that inhibits wild strains; 0.2 µg/mL) and a higher concentration (1.0 µg/mL) should be tested.

b Rifampin is the class agent for rifampine.

c The manufacturer’s directions for the radiometric BACTEC procedure should be followed.

d All secondary drugs should be tested on isolates of Mycobacterium tuberculosis complex that are resistant to rifampin or resistant to any 2 primary drugs. Testing of cycloserine, which is an option therapeutically, is not recommended because of technical problems associated with the test. In addition to the secondary drugs listed, a higher concentration of ethambutol (i.e., 10 µg/mL in both 7H10 and 7H11 agar) should be tested.

e Kanamycin is the class agent for amikacin.

f Some investigators have included a higher concentration, usually 1.0–2.0 µg/mL. The clinical significance of these concentrations, especially in the setting of resistance to rifampin, is unknown.
(0.2 μg/mL) but is susceptible to the higher concentration (1.0 μg/mL), then addition of the following comment to the report is suggested: “These test results indicate low-level resistance to isoniazid. Some evidence indicates that patients who are infected with strains exhibiting this level of resistance to isoniazid may benefit from continuing therapy with isoniazid. A specialist in the treatment of tuberculosis should be consulted regarding the appropriate therapeutic regimen and dosages” [2]. Laboratories also may choose to report the drug concentration(s) used for testing. If they do report the concentrations, the laboratory also should specify the testing medium, the testing method used, and the equivalent reference method. For laboratories that use the agar proportion reference method, the percentage of resistance may be reported. If drugs are tested at concentrations other than the critical concentrations (or at their equivalent concentrations for methods other than the agar proportion reference method), then the concentration and the method, or the reference method–equivalent concentrations should be specified.

Slowly Growing NTM

At present, there are sufficient data on which to base general recommendations for susceptibility testing of certain slowly growing NTM (i.e., Mycobacterium avium complex [MAC], Mycobacterium kansasii, and Mycobacterium marinum). The antimicrobial agents that the NCCLS subcommittee recommends for testing reflect the opinions of the American Thoracic Society (ATS) with regard to appropriate therapy [9]. However, data currently are insufficient to adequately address all issues relative to susceptibility testing.

Several other slowly growing NTM may cause human disease. Susceptibility testing of such isolates should be considered only for those believed to be clinically significant (e.g., isolates from blood, other sterile body fluids, or tissues or multiple isolates from sputum). The current ATS criteria for the determination of the clinical significance of any NTM from respiratory specimens are findings of either 3 positive sputum/bronchial wash samples with negative smear results for acid-fast bacilli (AFB) or 2 positive culture results and 1 positive smear result [9]. Alternatively, if only 1 bronchial wash is available, and it has a positive culture result or an AFB smear result of grade ≥2+, then the ATS considers this sufficient to establish clinical significance. Single positive culture results that have a negative AFB smear result and/or contain low numbers of organisms are unlikely to be clinically significant.

For Mycobacterium terrae, nonchormogenetic, Mycobacterium xenopi, and Mycobacterium simiae, the primary and secondary drugs and concentrations suggested in the section on M. kansasii should be used, and the same interpretive guidelines should be used. However, at this time, too few isolates have been studied to recommend a specific method for these species. Similarly, for the fastidious mycobacteria Mycobacteri-
of macrolides, which, in vitro, are more active under mildly alkaline conditions (pH 7.3–7.4) than under mildly acidic conditions (pH 6.8 [which is the pH of the commercially available BACTEC 12B medium]) [16, 17]. Because at this time there are no unequivocal data to support the superiority of one pH value in comparison with another, NCCLS guidelines indicate that testing the susceptibility of MAC isolates to macrolides in vitro have acquired resistance (as defined in vitro testing as shown in table 2) after a few months of macrolide monotherapy and can develop resistance at either pH value is acceptable, providing that recommendations for interpretation of the breakpoints (table 2) are followed [2].

For either method, both the MIC value and an interpretation of the testing result, which are based on the breakpoints listed in table 2, should be reported. These interpretive criteria are based, in part, on data from clinical trials of macrolide monotherapy in humans [11]. Virtually all wild-type MAC isolates are susceptible to macrolides, but they develop resistance (as defined by in vitro testing as shown in table 2) after a few months of macrolide monotherapy and can develop resistance with combination therapy. More than 95% of MAC isolates that demonstrate resistance to macrolides in vitro have acquired a point mutation in the V domain of the 23S rRNA gene [18, 19]. Therefore, clinically significant resistance may be defined as a clarithromycin MIC of >32 μg/mL at a pH of 6.8 (or an MIC of >16 μg/mL at a pH of 7.3–7.4) or an azithromycin MIC of >256 μg/mL at a pH of 6.8. In addition, a positive clinical and microbiological response can be expected if the clarithromycin MIC is <16 μg/mL for testing performed at a pH of 6.8 (or if the MIC is <4 μg/mL at a pH of 7.3–7.4) or if the azithromycin MIC is <64 μg/mL at a pH of 6.8.

Because it is highly unlikely for untreated wild strains of MAC to be intermediate or resistant to macrolides, the NCCLS subcommittee recommends that laboratories not report such results until they are confirmed by repeat testing, until the identity of the isolate is confirmed, or both [2]. A confirmed intermediate result may suggest that the patient has a mixed population of MAC organisms and thus should be followed for emerging resistance.

**M. kansasii**

Antituberculous agents that are routinely used for therapy of *M. kansasii* infections are rifampin, ethambutol, and isoniazid. Although these drugs generally are clinically active against untreated strains, treatment failure can occur [20]. In virtually all such cases, the isolate demonstrates in vitro resistance to rifampin and, occasionally, to isoniazid, ethambutol, or both, as well. Therefore, for those patients for whom therapy fails or for those who have a poor response to initial therapy, susceptibility testing should be performed [2, 9]. Untreated wild strains of *M. kansasii* generally are susceptible to the critical concentrations of rifampin and ethambutol used to test MTBC (table 1). In contrast, they are resistant to the critical concentration of isoniazid (0.2 μg/mL for the agar proportion method) and show variable susceptibility to the higher concentration (1.0 μg/mL), although isoniazid appears to be clinically active in vivo. Therefore, because in vitro results do not correlate with clinical outcome, testing of the susceptibility of *M. kansasii* to isoniazid should not be done [20].

Because treatment failure is always associated with resistance to rifampin, and because laboratories generally are not provided with drug treatment information, the ATS and the NCCLS subcommittee recommend that all initial isolates of *M. kansasii* be tested for susceptibility to rifampin only. Isolates may be tested by either the agar proportion method described for MTBC or by a broth-based method that provides comparable results [20–22]. Susceptibility testing should be repeated if the results of culture remain positive after 3 months of appropriate therapy. Isolates that are susceptible to rifampin are also susceptible to rifabutin, so testing susceptibility to the latter drug is not necessary among patients who are infected with HIV and who are receiving protease inhibitors. For isolates of *M. kansasii* that are resistant to rifampin, the NCCLS subcommittee suggests testing rifabutin (0.5 and 2.0 μg/mL), ethambutol (5 μg/mL), isoniazid (5 μg/mL), streptomycin (10 μg/mL), clarithromycin (32 μg/mL, as the class agent for newer macrolides), amikacin (10 μg/mL), ciprofloxacin (2 μg/mL, as the class agent for newer fluoroquinolones), and trimethoprim-sulfamethoxazole (TMP-SMZ) or sulfamethoxazole (32 μg/mL) [2]. In individual cases for which susceptibility testing of rifampin-resistant *M. kansasii* seems to be indicated, isolates should be sent to an experienced reference laboratory.

**M. marinum**

Routine susceptibility testing of *M. marinum* is not recommended for 2 reasons. First, the species is consistently susceptible to several clinically useful antimicrobial agents, including rifampin, ethambutol, doxycycline, minocycline, TMP-SMZ, and clarithromycin [9]. Second, the risk of acquired mutational resistance to one or more of these agents is minimal. However, susceptibility testing of *M. marinum* may be considered for patients who do not respond clinically after several months of therapy and who continue to have positive culture results. Agar proportion, agar disk elution, and broth microdilution have been used to test the susceptibility of *M. marinum* isolates to a variety of drugs, including rifampin, ethambutol, and isoniazid.

**Table 2.** Interpretive breakpoints for *Mycobacterium avium* complex.

<table>
<thead>
<tr>
<th>Drug</th>
<th>pH value</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarithromycin</td>
<td>6.8</td>
<td>&lt;16</td>
<td>32</td>
<td>≥64</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>7.3–7.4</td>
<td>&lt;4</td>
<td>8–16</td>
<td>≥32</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>6.8</td>
<td>&lt;128</td>
<td>256</td>
<td>≥512</td>
</tr>
</tbody>
</table>

NOTE. Data were obtained from National Committee for Clinical Laboratory Standards document M24-T2 [2]. Testing is performed by broth dilution. Permission to use portions of M24-T2 (Susceptibility Testing of Mycobacteria, Nocardia, and Other Aerobic Actinomycetes: Tentative Standard—Second Edition) has been granted by NCCLS. The interpretive data are valid only if the methodology in M24-T2 is followed. The current standard may be obtained from NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087, USA.
been used, but reports of susceptibility testing by any method are limited, and few comparative studies have been done. Therefore, at present, no one method can be recommended. Suggested drugs for testing are rifampin (1 μg/mL), ethambutol (5 μg/mL), clarithromycin (8 μg/mL, as the class agent for newer macrolides), amikacin (32 μg/mL), and sulfamethoxazole or TMP-SMZ (32 μg/mL) [2].

Rapidly Growing Mycobacteria

The NCCLS subcommittee recommends the standard broth microdilution method for susceptibility testing of the Mycobacterium fortuitum group (M. fortuitum, Mycobacterium pergerinum, and M. fortuitum third variant complex), Mycobacterium chelonae, and Mycobacterium abscessus [2]. The method and guidelines for interpretation of results on theoretical grounds also should apply to Mycobacterium mucogenicum, Mycobacterium smegmatis group (M. smegmatis, Mycobacterium goodii, and Mycobacterium wolinskyi), and the clinically significant, pigmented, rapidly growing mycobacteria; however, data to support this are not currently available. The NCCLS subcommittee also recommends that isolates be identified to the species level or, at a minimum, M. fortuitum group be differentiated from M. chelonae and M. abscessus.

Susceptibility testing should be performed for any clinically significant rapidly growing mycobacteria (e.g., isolates from blood, sterile body fluids, tissue, and material collected from skin and soft tissue lesions). The rapidly growing mycobacteria, especially M. abscessus, may cause pulmonary disease; however, they also may transiently colonize the respiratory tract. For this reason, not all rapidly growing mycobacteria recovered from sputum are clinically significant. Sputum isolates that are most likely to be true pathogens are those that meet the previously described ATS criteria for clinical significance. Isolates that fail to meet these criteria (e.g., only a few colonies are recovered from only one of multiple sputum specimens) are more likely to be contaminants or colonizers and thus do not warrant susceptibility testing. If the results of cultures of specimens (obtained from any site except a respiratory site) remain positive after a minimum of 6 months of appropriate antimicrobial therapy, susceptibility testing should be repeated, and tests to confirm the identity of the species should be performed.

Antimicrobial agents and concentrations (in serial 2-fold dilutions) that should be tested against the rapidly growing mycobacteria are amikacin (1–128 μg/mL), cefoxitin (2–256 μg/mL), ciprofloxacin (0.125–16 μg/mL), clarithromycin (0.06–64 μg/mL), doxycycline (0.25–32 μg/mL), imipenem (1–64 μg/mL), and sulfamethoxazole (or TMP-SMZ) (1–64 μg/mL). In addition, for isolates of M. chelonae only, tobramycin (1–32 μg/mL) should be tested. Tobramycin should not be tested (or at least results should not be reported) against isolates of M. fortuitum group or M. abscessus, because, therapeutically, the drug is superior to amikacin only for infections caused by M. chelonae. Data from several studies have shown that virtually all isolates of M. chelonae and M. abscessus are resistant to sulfamethoxazole (MIC ≥64 μg/mL), whereas all M. fortuitum group are susceptible [23, 24]. Therefore, if the species identity of the isolate is known (or if, at a minimum, M. fortuitum group has been differentiated from M. chelonae and M. abscessus), testing of a sulfonamide may not be necessary.

In general, susceptibility results with rapid growers are available within 3–4 days, rather than within a time frame of 7–10 days or even longer for the other mycobacteria. MIC values and an interpretation, which are based on the breakpoints for the rapidly growing mycobacteria listed in table 3 [2], are reported for each drug tested, with 2 exceptions. First, imipenem results should not be reported for isolates of M. chelonae and M. abscessus. This recommendation is based on the results of a recent multicenter evaluation that showed that imipenem MICs for M. chelonae and M. abscessus (which usually are at the resistance breakpoint of 8–16 μg/mL) were not reproducible.

### Table 3. Broth microdilution interpretive criteria for rapidly growing mycobacteria.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>≤16</td>
<td>32</td>
<td>≥64</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>≤16</td>
<td>32–64</td>
<td>≥128</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤1</td>
<td>2</td>
<td>≥4</td>
</tr>
<tr>
<td>Clarithromycin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≤2</td>
<td>4</td>
<td>≥8</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>≤1</td>
<td>2–8</td>
<td>≥16</td>
</tr>
<tr>
<td>Imipenem&lt;sup&gt;c&lt;/sup&gt;</td>
<td>≤4</td>
<td>8</td>
<td>≥16</td>
</tr>
<tr>
<td>Sulfamethoxazole&lt;sup&gt;d&lt;/sup&gt;</td>
<td>≤32</td>
<td>—</td>
<td>≥64</td>
</tr>
<tr>
<td>Tobramycin&lt;sup&gt;e&lt;/sup&gt;</td>
<td>≤4</td>
<td>8</td>
<td>≥16</td>
</tr>
</tbody>
</table>

<sup>a</sup> Isolates of Mycobacterium abscessus with an MIC of ≥64 μg/mL should be retested. If the repeat result is ≥64 μg/mL, the MIC should be reported with the following comment: “The MIC is greater than expected for this species. If the drug is being considered for therapy, the laboratory should be notified so the isolate can be sent to a reference laboratory for confirmation of resistance.”

<sup>b</sup> Isolates of Mycobacterium fortuitum group with a trailing end point should be considered resistant. Results for Mycobacterium chelonae and Mycobacterium abscessus should be read at 3 days (or not after 4 days). Clarithromycin is the class agent for the newer macrolides.

<sup>c</sup> Report for M. fortuitum group, M. smegmatis group, and M. mucogenicum. If the MIC is ≥8 μg/mL, the test should be repeated with an incubation period of no more than 3 days. If the repeat result is an MIC ≥8 μg/mL, the MIC should be reported with the following comment: “The MIC is greater than expected for this species. If the drug is being considered for therapy, the laboratory should be notified so the isolate can be sent to a reference laboratory for confirmation of resistance.” MICs to imipenem should not be reported for M. chelonae or M. abscessus.

<sup>d</sup> MIC is 80% inhibition of growth. Results are predictable by species; therefore, testing may not be necessary.

<sup>e</sup> Report for M. chelonae only. If the MIC is ≥4 μg/mL, the test should be repeated. If the repeat result is ≥4 μg/mL, the MIC should be reported with the following comment: “The MIC is greater than expected for this species. If the drug is being considered for therapy, the laboratory should be notified so the isolate can be sent to a reference laboratory for confirmation of resistance.”
resulting in major category changes of susceptibility [2, 24–26]. Second, as previously indicated, tobramycin results should be reported only for \( \text{M. chelonae} \). Because tobramycin is the aminoglycoside of choice for \( \text{M. chelonae} \) infections [24], amikacin results need be reported only if the isolate of \( \text{M. chelonae} \) being tested is resistant to tobramycin.

Although the broth microdilution procedure for the rapidly growing mycobacteria is not technically difficult to perform, interpretation of the MIC is not always obvious. It requires skill acquired through experience with the test and knowledge of the expected susceptibility patterns of the different species. Growth of the rapidly growing mycobacteria in microtiter trays frequently does not appear as a crisp, well-defined button in the bottom of the well, as occurs with the rapidly growing aerobic and facultative bacteria. Moreover, in broth, some rapidly growing mycobacteria have trailing end points with certain drugs, particularly \( \text{M. fortuitum} \) with clarithromycin. Given these potential difficulties, laboratories that infrequently encounter rapidly growing mycobacteria should consider referring those isolates for which susceptibility testing is indicated to a reference laboratory with demonstrated expertise in such testing. The Etest has been evaluated as a potential alternative to broth microdilution for susceptibility testing of the rapidly growing mycobacteria; however, the procedure has not yet been standardized to yield results that consistently correlate with those obtained by broth microdilution [27]. Therefore, at this time, use of the Etest is not recommended for testing the rapidly growing mycobacteria.

Summary

Use of standardized methods of mycobacteria susceptibility testing is optimal. The first NCCLS document regarding antmycobacterial susceptibility testing was a tentative standard that addressed \( \text{M. tuberculosis} \) only [1]. The second edition of the NCCLS tentative standard is much different from the initial document. It contains revised guidelines for testing MTBC and proposed recommendations for testing certain slowly growing NTM (i.e., \( \text{M. kansasi} \), and \( \text{M. marinum} \)) and the rapidly growing mycobacteria (\( \text{M. fortuitum} \) group, \( \text{M. chelonae} \), and \( \text{M. abscessus} \)). For the most part, the recommendations outlined in the recent NCCLS document that concern test methods are based on published data, and those regarding drug selection and the frequency of testing reflect the current opinions of the CDC and the ATS. The NCCLS document, however, does not answer all questions concerning mycobacterial susceptibility testing, especially those regarding methods for testing the slowly growing NTM, because the necessary data simply are not available. Those areas for which more research is needed are clearly indicated and should stimulate further investigation. As new data concerning mycobacterial susceptibility testing become available, that information will be incorporated into future editions of the NCCLS M24 document.

Acknowledgments

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