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# Antimicrobial Susceptibility Testing, Drug Resistance Mechanisms, and Therapy of Infections with Nontuberculous Mycobacteria

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## INTRODUCTION

Reports of nontuberculous mycobacterial (NTM) infections have been increasing worldwide (31, 132, 152, 199, 235) over the past 2 decades. Within the United Sates, the *Mycobacterium avium* complex (MAC), *Mycobacterium kansasii*, and *Mycobacterium abscessus* are the most frequently reported clinically significant species (132).

A recent joint publication by the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) of diagnostic criteria for the evaluation of patients suspected of having NTM lung disease has been important, as it has contributed to the ability to recognize NTM and has enabled clinicians to institute appropriate treatment regimens (132). Although there is still insufficient information about NTM other than the MAC and *M. kansasii*, these diagnostic criteria are universally applicable for all NTM pathogens, especially in the setting of respiratory disease (132).

A publication by the Clinical and Laboratory Standards Institute (CLSI) has defined criteria for antimicrobial susceptibility testing (AST) of NTM and has established recommendations for the antimicrobial susceptibility test method, including breakpoints for antimicrobials used in the treatment of NTM infections (451).

Prior to and concurrent with the ATS/IDSA and CLSI documents, studies of antimicrobial resistance have elucidated mechanisms of antimicrobial resistance for macrolides, aminoglycosides, linezolid, quinolones, sulfonamides, and tetracyclines (53, 237, 239, 260–266, 279, 300, 415, 419–421, 430), which were previously established as being important antimicrobials for the treatment of NTM (49–51, 53, 55, 56, 88, 109, 402, 406–413, 415, 416, 419, 421, 426, 429–432).

This article will review and update information on AST, drug resistance mechanisms, and treatment regimens for infections with NTM.

## PRINCIPLES OF ANTIMICROBIAL SUSCEPTIBILITY TESTING

In 2003, the CLSI published the first set of recommendations for AST of NTM. The document addressed susceptibility issues based mostly on clinical data, organism population distribution, and the experience of experts in the field of NTM (451, 452).

For AST of MAC isolates, the CLSI has recommended the testing of (i) clinically significant isolates from patients who previously received treatment with macrolides, (ii) isolates from blood cultures of patients who become bacteremic when receiving macrolide prophylaxis, and (iii) isolates from patients who relapse while on macrolide therapy. Additionally, if baseline testing is not performed, isolates should be saved for future testing if needed (451, 452).

Clarithromycin results are predictive of MICs of azithromycin. Because the testing of the latter antimicrobial can be problematic due to poor solubility at high concentrations, clarithromycin has been selected as the class agent for macrolide AST (451, 452).

### Definitions

The CLSI has reported detailed lists of terms needed to comprehend the mechanics of AST (451, 452). The gold standard method for AST of NTM is broth microdilution. The MIC, expressed in μg/ml and typically indexed to base 1 (e.g., 1, 2, 4, 8, 16, and 32 μg/ml), represents not an absolute value but the "true value" (the closest agreement between the average value obtained from a large series of test results and an accepted reference value). This value is somewhere between the lowest test concentration that inhibits the organism's growth (i.e., the MIC) and the next lower test concentration. Under optimum test conditions, the acceptable reproducibility of the test result should be within one 2-fold dilution of the actual endpoint. To ensure that this occurs, dilution tests should be standardized, and quality control measures should be implemented (451, 452).

As stated above, the MIC is the minimum concentration of antimicrobials at which the organism is inhibited. When there is no growth with the lowest concentration of the antimicrobial, the MIC can be determined only to be lower than or equal to the lowest concentration in the test series. Likewise, if there is growth at the highest concentration of the antimicrobial tested, the MIC can be determined only to be higher than the highest concentration in the test series.

### Evaluation and Interpretation

**Laboratory qualifications.** A major caveat for laboratories that elect to perform AST is that testing should be performed only by experienced, qualified technologists who encounter sufficient numbers of organisms to establish and maintain proficiency with NTM (114, 451, 452). Technologists should be able to recognize potential problems such as contamination, mixed cultures, unusual growth rates, or aberrant results.

**Clinical significance of isolates.** Decisions concerning the performance of AST should take into consideration the clinical significance of the isolates, the clinical relevance of the isolate as related to the pathogenicity of the species, and patient factors such as the patient's immunologic status, the site of infection, and general clinical findings, such as unexplained fever, granulomas, and inflammatory lesions, etc. Since almost all NTM species are ubiquitous in the environment (soil and water), the determination of the clinical significance of the species recovered is the first step in the AST process (103, 114).

The pathogenic potential of mycobacterial species is influenced by the immunologic status of the patient and the site from which the culture was taken. For example, the recovery of NTM in a clinical specimen from immunosuppressed hosts in conjunction with HIV or medications such as corticosteroids or tumor necrosis factor (TNF) inhibitors such as infliximab increases the possibility of the clinical relevance of the isolate. Moreover, the site of collection is also important. The finding of NTM in sterile body sites such as blood, tissue, cerebrospinal fluid, pleural fluid, and brain, etc., is almost always clinically significant. The isolation of *Mycobacterium mucogenicum* from a respiratory sample usually indicates contamination of the sample, since this species is frequently encountered in tap water. However, the isolation of the same species from a blood culture or central venous catheter is usually associated with mycobacterial sepsis (19, 54, 114).

*M. abscessus* is the most common pathogenic rapidly growing mycobacterium (RGM) isolated from cultures of pulmonary sites (131, 132, 141). However, other RGM, such as *M. fortuitum*, which is rarely a respiratory pathogen except in cases of achalasia or lipoid pneumonia, may also be recovered from pulmonary specimens (54).

The recovery of species such as *M. gordonae*, *M. mucogenicum*, or *M. terrae* from respiratory cultures is almost never clinically significant, as these species are prevalent in tap water and rarely cause lung disease (114, 132). Other newly described species such as *M. botniense*, *M. cookii*, *M. chlorophenolicum*, *M. frederiksbergense*, *M. hodleri*, and *M. murale* have been identified solely in environmental samples and have not yet been identified as human pathogens (114, 385).

In contrast, NTM species often associated with clinical disease include the MAC, *M. abscessus*, and *M. kansasii* from respiratory samples and the *M. fortuitum* group, *M. chelonae*, *M. haemophilum*, *M. ulcerans*, the *M. terrae* complex, and *M. marinum* from skin, soft tissue, or bone (132).

The likelihood of pathogenicity of NTM in the respiratory tract is related to the number of positive cultures and the number of CFU present in the sample. Isolates recovered from multiple specimens in large numbers and/or with positive smears are almost always clinically significant, in contrast to isolates recovered in low numbers or which are acid-fast bacillus (AFB) smear negative in a single sample (114).

For cultures that remain positive after 6 months of appropriate antimicrobial treatment, repeat AST is warranted (according to the CLSI). Periodic AST is important to monitor the development of mutational drug resistance, which may occur with the extended therapy prerequisite for the adequate treatment of NTM disease (132).

The performance of AST on nonsignificant clinical isolates is a waste of time and patient and laboratory finances, and results may be misleading and detrimental for patient care (114). Ultimately, a careful evaluation of the clinical setting and host factors should be the responsibility of the clinician (although, unfortunately, the decision to order AST on an NTM isolate may often fall on the laboratory). Thus, laboratory communication of clear and accurate laboratory data, such as the quantification of colonies, results of direct specimen smears, and the number of positive cultures, is also of paramount importance to the clinician's decision (114).

**Limitations.** Generally, the recommendations for susceptibility testing made by the CLSI follow the guidelines set by the joint publication of the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) (132). The criteria for AST are best applicable with MAC, *M. kansasii*, *M. marinum*, and RGM isolates, as not enough data are available regarding other species of NTM (451, 452).

AST has been performed for many years to try to predict the clinical effectiveness of specific antimicrobials against isolates of NTM. However, for some species, such as those of the *M. avium* complex, *in vitro* susceptibility testing of standard antituberculous agents, including ethambutol, rifampin, and rifabutin, does not predict the clinical response (132). Although multidrug therapy is required for the treatment of MAC infection, routine susceptibility testing of these first-line antituberculous agents should not be performed (Table 1).

**TABLE 1** Antimicrobials used for treatment of commonly encountered species of nontuberculous mycobacteria

| Rapidly growing species | Type(s) of infection and/or disease | Antimicrobials[j] |
|---|---|---|
| *M. abscessus* subsp. *abscessus* | Chronic respiratory infection, localized posttraumatic wound infection, catheter infection, disseminated cutaneous infections, eye infection | Linezolid (~50%), moxifloxacin (~15%), ciprofloxacin, levofloxacin (<5%), doxycycline (<5%), clarithromycin-azithromycin (~20%)[a] (oral); amikacin, tigecycline, cefoxitin (70%), imipenem (~50%),[b] linezolid (50%) (parenteral) |
| *M. abscessus* subsp. *bolletii* (formerly *M. massiliense*) | Chronic respiratory disease, localized posttraumatic wound infection, postsurgical wound infection, catheter infection | Clarithromycin-azithromycin,[c] linezolid (~50%), moxifloxacin (~15%), ciprofloxacin (<5%), doxycycline (<5%) (oral); amikacin, tigecycline, cefoxitin (~70%), imipenem (~50%), linezolid (~50%) (parenteral) |
| *M. chelonae* | Disseminated cutaneous infection, localized posttraumatic wound infection, sinusitis, eye infection | Clarithromycin-azithromycin, linezolid, moxifloxacin (~25%), ciprofloxacin (~20%), doxycycline (~20%) (oral); tobramycin, linezolid, amikacin (~50%), imipenem (~60%),[b] tigecycline (parenteral) |
| *M. fortuitum* | Localized posttraumatic wound infection, catheter infection, surgical wound infection, cardiac surgery, augmentation mammaplasty; rarely a respiratory pathogen (achalasia, lipoid pneumoniae) | Ciprofloxacin, levofloxacin, moxifloxacin, trimethoprim-sulfamethoxazole, linezolid, doxycycline (~50%), clarithromycin-azithromycin (~20%)[a] (oral); imipenem, tigecycline, linezolid, amikacin, cefoxitin (~50%) (parenteral) |
| *M. neoaurum-M. bacteremicum* | Catheter sepsis/bacteremia, localized posttraumatic wound infection, postsurgical wound infection | Ciprofloxacin, levofloxacin, moxifloxacin, doxycycline, linezolid, trimethoprim-sulfamethoxazole, clarithromycin-azithromycin[d] (oral); amikacin, tobramycin, linezolid, imipenem, tigecycline, cefoxitin (parenteral) |
| *M. avium* complex | Chronic respiratory infection (including cystic fibrosis), disseminated infection (usually associated with AIDS), lymphadenitis, localized cutaneous infection with tenosynovitis | Clarithromycin-azithromycin,[e] rifampin-rifabutin, ethambutol, moxifloxacin (<50%), ciprofloxacin (<25%) (oral); amikacin, streptomycin, linezolid (<50%) (parenteral) |
| *M. kansasii* | Chronic respiratory infection, disseminated disease in AIDS | Clarithromycin-azithromycin, rifampin-rifabutin,[f] trimethoprim-sulfamethoxazole, ethambutol, isoniazid, moxifloxacin, ciprofloxacin, linezolid (oral); amikacin, linezolid (parenteral) |
| *M. marinum*[g] | Localized posttraumatic wound infection, tenosynovitis | Clarithromycin-azithromycin, rifampin-rifabutin, ethambutol, ciprofloxacin (~50%), trimethoprim-sulfamethoxazole, moxifloxacin, linezolid, doxycycline (~50%) (oral); amikacin, linezolid (parenteral) |
| *M. simiae*[h] | Chronic respiratory infection | Clarithromycin-azithromycin, moxifloxacin (~60%), trimethoprim-sulfamethoxazole (oral); amikacin (parenteral) |
| *M. xenopi*[i] | Chronic respiratory infection, joint and soft tissue infection | Clarithromycin-azithromycin, rifampin-rifabutin, ethambutol, moxifloxacin (oral); amikacin, streptomycin (parenteral) |

[a] Many strains of the *M. fortuitum* group and *M. abscessus* subsp. *abscessus* contain functional *erm* genes, so extended incubation shows clarithromycin MICs to be resistant, while with a routine 3-day incubation, the MICs may appear to be susceptible.
[b] Susceptibility testing with imipenem with the *M. abscessus-M. chelonae* group is known to be problematic (lack of reproducibility).
[c] Isolates of *M. abscessus* subsp. *bolletii* do not a contain functional *erm* gene; thus, macrolide MICs remain susceptible even with extended incubation.
[d] There is a bimodal distribution of isolates that are resistant/susceptible to macrolides with extended incubation. Testing for functional *erm* genes in these species has not been performed.
[e] Clarithromycin is recommended as the class agent for the testing of the newer macrolides because clarithromycin and azithromycin share cross-resistance and susceptibility. The macrolides are the only antimicrobials for which the clinical presentation can be correlated with the *in vitro* results. There is currently no recognized value for the testing of the first-line antituberculous agents with MAC isolates.
[f] Previously untreated strains of *M. kansasii* should be tested *in vitro* only for rifampin and clarithromycin. Isolates with susceptibility to rifampin will be susceptible to rifabutin. Isolates resistant to rifampin should be tested against a panel of secondary agents, including ethambutol, fluoroquinolones, amikacin, and trimethoprim-sulfamethoxazole.
[g] Isolates of *M. marinum* do not require *in vitro* susceptibility testing unless the patient fails appropriate antimicrobial therapy after several months.
[h] The antimicrobial treatment for *M. simiae* can be difficult, and the clinical response may not correlate with *in vitro* susceptibility.
[i] The optimal antimicrobial treatment for *M. xenopi* has not been established, but most experts recommend a combination of macrolide, rifampin, and ethambutol with moxifloxacin. The clinical response may not correlate with *in vitro* susceptibility. This poor correlation may relate to the difficulty in testing for this species by current standardized methods. Some strains have been reported to have variable antimicrobial susceptibilities, and testing may need to be performed at 45°C for optimal growth in broth.
[j] Percentages indicate percent susceptibility of an organism to a given drug. Drugs without percentages listed correspond to 100% susceptibility.

## Methods for Susceptibility Testing of Rapidly Growing Mycobacteria

**Antimicrobial susceptibility patterns.** AST can also help in the differentiation of MAC isolates from other slowly growing NTM such as *M. simiae*, which is typically more drug resistant than MAC strains. Unlike MAC strains, isolates of *M. simiae* have high MICs of rifabutin (generally >4 μg/ml), providing a clear differentiation of the two species (101, 132).

However, AST as a taxonomic tool is probably most useful for the identification of rapidly growing mycobacteria (RGM) rather than the slowly growing NTM. Although molecular methods are required for the definitive species identification of RGM, antimicrobial susceptibility patterns provide useful taxonomic help for commonly encountered species, including isolates of the *M. fortuitum* group, *M. chelonae*, and *M. abscessus*. For taxonomic purposes, agar disk diffusion testing may be useful in addition to broth microdilution (54, 417, 425, 427).

The *M. fortuitum* group is easily differentiated from the *M.*

*chelonae-M. abscessus* group by agar disk diffusion susceptibility to polymyxin B (54). Isolates of the *M. chelonae-M. abscessus* group exhibit no complete or partial zone of inhibition around this drug, in contrast to the *M. fortuitum* group, which shows zones of inhibition around polymyxin B (54, 427).

Another agent to which the *M. fortuitum* group almost always shows susceptibility is the sulfonamides, represented by trimethoprim-sulfamethoxazole (TMP-SMX) as the class agent of this group (54, 420, 430, 451, 452). Less than 10% of the isolates of the *M. chelonae-M. abscessus* group exhibit susceptibility to the sulfonamides. However, almost 100% of the *M. fortuitum* group is sulfonamide susceptible (54).

Susceptibilities to both cefoxitin and tobramycin can be a useful screen for the isolates typical of the *M. chelonae-M. abscessus* group. Isolates of *M. chelonae* have MICs of cefoxitin of ≥128 μg/ml and MICs of tobramycin of ≤4 μg/ml. Conversely, isolates of *M. abscessus* typically have cefoxitin MICs in the 16- to 64-μg/ml range and tobramycin MICs of ≥8 μg/ml (54, 409, 410).

Isolates of *M. immunogenum* are similar to those of *M. chelonae*, with resistant cefoxitin MICs. However, unlike isolates of *M. chelonae*, they also have high tobramycin MICs (≥8 μg/ml) (441). By agar diffusion, the zones of inhibition for amikacin and kanamycin are equivalent with those of *M. immunogenum*, in contrast to isolates of the *M. chelonae-M. abscessus* group, which have larger zones of inhibition with kanamycin than with amikacin (54, 441).

Only rarely in cases of pretreatment with quinolones will isolates of the *M. fortuitum* group be resistant to quinolones, including ciprofloxacin and moxifloxacin, making quinolone susceptibility another marker for the *M. fortuitum* group, along with the previously discussed susceptibility to polymyxin B and sulfonamides (54, 406, 427, 430).

**Broth microdilution method.** The CLSI has recommended broth microdilution as the standard for susceptibility testing of RGM (450, 452). The broth microdilution format is traditionally set up as 2-fold dilutions, and as discussed above, the MIC does not represent as absolute value; for example, if the MIC is reported to be 32 μg/ml, the "true" MIC would fall between the lowest concentration that inhibits the growth of the organism (the "reported" MIC) and the next-lowest concentration. For example, the "true" MIC for the above-described example would be between 32 and 16 μg/ml. Thus, generally, the acceptable values are within one 2-fold dilution of the actual endpoint, and standards that help to ensure quality results of the test have been proposed (451, 452).

Among the RGM, there is a paucity of susceptibility data for species other than the most commonly encountered species (*M. fortuitum*, *M. chelonae*, and *M. abscessus*). This includes accepted pathogenic species such as *M. mageritense*, *M. porcinum*, *M. mucogenicum*, and *M. senegalense* (54, 170, 412, 414, 416, 423). Thus, currently, MIC standards that apply to the commonly encountered RGM have also been applied to other less frequently recovered RGM species until more data prove these standards to be nonapplicable.

A proper inoculum is critical for the determination of valid MICs. A too-heavy inoculum may result in falsely resistant MIC readings, whereas a too-light inoculum may give falsely susceptible MIC readings due to the inadequate growth of the organism in broth. A suspension in broth or water should be prepared from an agar medium with adequate growth of the RGM, and the inoculum should be standardized for AST to a turbidity equivalent to a 0.5 McFarland standard (451, 452). Suspensions are then diluted and inoculated into 96-well microtiter plates to achieve a final organism concentration of $1 \times 10^5$ to $5 \times 10^5$ CFU/ml. Recommended antimicrobials include amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline-minocycline, imipenem-meropenem, linezolid, moxifloxacin, tobramycin, and trimethoprim-sulfamethoxazole. Further procedural details may be found in CLSI document M24-A2 (451).

Quality control of the MIC method is performed by the testing of CLSI-recommended reference strains, including *M. peregrinum* ATCC 700686 and bacterial strains *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus faecalis* ATCC 29212. The goal of these tests is to reproduce MICs of each antimicrobial within the acceptable ranges (451, 452).

Incubation for 3 to 4 days at 30°C is preferred for the RGM, since some strains of *M. chelonae* may not grow at 35°C to 37°C. For strains that do not grow within 5 days, repeat susceptibility testing should be performed by using sterile pestles to prepare a more homogeneous broth suspension. Incubation in $CO_2$ should be avoided, as macrolide MICs will be adversely affected (452).

It should also be noted that MIC results for some antimicrobials, such as tigecycline (which has yet to be addressed by the CLSI), imipenem-meropenem, and the tetracyclines, may be invalidated due to prolonged (more than 5 days) incubation. Clarithromycin MICs should be examined after 72 h of incubation. If susceptible, the preparations should be reincubated for up to 14 days to ascertain inducible macrolide resistance (451). Inducible macrolide resistance is due to the presence of an rRNA methylase *erm* gene. The presence of this gene has been documented for most pathogenic RGM species. (See "Macrolides and ketolides" below.)

**Agar disk elution.** Prior to the CLSI recommendations, the agar disk elution method was a useful procedure, especially for small-volume laboratories. Briefly, the method uses commercial antimicrobial disks eluted into molten agar in round-well tissue culture plates. The interpretation of susceptibility is performed similarly to the method of proportion used for isolates of the *M. tuberculosis* complex (MTBC) (364, 451, 452).

Currently, the method may still be used for isolates of less commonly encountered and/or more fastidious species, such as the slowly growing species *M. haemophilum* (397, 451, 452). The use of this method for RGM is not recommended (451). An organism suspension is added over the surface of the agar (the type of agar is variable depending upon the species), and plates are incubated for the time and/or temperature required, depending upon the species being tested. Potential problems with this method include the control of the inoculum and difficulties in the reading of the endpoint with some antimicrobials. Additionally, quality control of the method can be tedious, although an organism and an agar control should be included when the test is performed (451, 452). For *M. haemophilum*, supplementation with iron or hemin is necessary. In the clinical laboratory, this can be accomplished by the addition of an X-strip to each well (397).

**Agar disk diffusion.** The current agar disk diffusion method for RGM is a modification of the Kirby-Bauer method used for bacterial AST (54). The test organism suspension is prepared as described above for broth microdilution. This suspension is swabbed over the surface of cation-adjusted Mueller-Hinton agar after the addition of oleic acid dextrose catalase (OADC). Following this procedure, commercial antimicrobial disks are placed

onto the surface. As for broth testing, the plates are incubated in a 30°C ambient atmosphere for 72 h or up to 5 days if inadequate growth occurs at 72 h. Zones of inhibition around each of the disks are read. Growth inhibition is defined as zones of complete inhibition for all agents other than the sulfonamides, for which the inhibition of the colony size is 80% compared to the size of the colonies of the control. The primary advantages of the disk method include its ease of setup and the ability for the recognition of mixed cultures that may not be obvious in broth cultures (417).

Limitations of the method include the difficulty in the interpretation of zones of inhibition, especially when the amount of drug in the disk is near the breakpoint of the drug. This is particularly noteworthy for isolates of *M. abscessus* with the 30-μg cefoxitin disk, in which the modal breakpoint for *M. abscessus* is near 32 μg/ml. The result is a fine haze of growth within the zone of inhibition, which can be difficult to interpret (417, 425).

Just as the inoculum can affect the results of broth microdilution tests for sulfonamides, too heavy an inoculum can also cause falsely resistant interpretations of the disk zones (425, 451). In this case, as with other methods, careful attention to the turbidity of the organism suspension is critical.

The agar disk diffusion method has not been standardized by the CLSI, and thus, it should be used only as an adjunct to broth microdilution testing of the RGM or as a taxonomic tool to assist with or provide a preliminary identification of some species.

**Etest.** The Etest (AB Biodisk, Piscataway, NJ) combines the ease of the agar disk diffusion method with the use of an exponential gradient of antimicrobial concentrations to yield an MIC. Plates are inoculated similarly to the agar disk diffusion method, and the Etest strip is applied onto the surface (54, 449).

Early studies with the Etest tested small numbers of isolates, with overall agreements by interpretive category from 76% for cefoxitin to 100% for amikacin and ciprofloxacin. Not all of the currently recommended agents were tested (36), and breakpoints were different from the currently accepted values, making comparisons of the recent CLSI study with prior studies difficult (449). Furthermore, the inoculum turbidities reported by some of the early studies were inconsistent. Biehle et al. used a 0.5 McFarland standard (36), while other investigators, including the recent CLSI study, used a 1.0 McFarland standard (159). Temperature incubation conditions (ambient versus $CO_2$) and the types of media used were also variable.

Although many laboratories continue to use the Etest system for AST of the RGM, a multicenter study by the CLSI in 2000 revealed significant interlaboratory discrepancies among MICs of several antimicrobials (449). Trailing endpoints, as exemplified by diffuse elliptical edges, with agents such as clarithromycin, doxycycline, and trimethoprim-sulfamethoxazole and/or higher MICs (notably of amikacin, cefoxitin, and imipenem) than those determined by the broth microdilution method along with a lack of reproducibility suggested that this method was inferior to the broth microdilution method. Similarly to the agar disk diffusion method, the determination of MICs was difficult, especially when the MIC was near the breakpoint for some of the antimicrobials. Because no standard RGM strain that exhibited reproducibility for all of the antimicrobials tested was found, no strain could be recommended for the quality control of the method. Thus, the CLSI concluded that further investigation including the clarification of endpoint determinations was necessary before the Etest method could be recommended for AST of the RGM (54, 449, 451, 452).

### Methods for Susceptibility Testing of Slowly Growing Mycobacteria

***Mycobacterium avium* complex.** The CLSI recommends AST for *Mycobacterium avium* complex (MAC) isolates to be performed in broth by either the microdilution or macrodilution method (451, 452). For both methods, care should be taken to select transparent colonies for testing (451). The optimal pH for test media has not been established, but it is accepted that macrolides are more active *in vitro* under mildly alkaline conditions (pH 7.3 to 7.4) than in acidic media at pH 6.8, which is the pH of the soon-to-be-unavailable radiometric Bactec 460 medium (Becton Dickinson, Sparks, MD). Laboratory studies have shown broth microdilution results to be 90% in concordance with the results of the Bactec 460 medium (pH adjusted to 7.3 to 7.4) and the VersaTREK broth (Trek Diagnostics, Cleveland, OH) macrodilution methods (16, 361). To date, the CLSI has made no preference regarding the pH except to recommend different breakpoints for each pH range. For example, at pH 6.8 (Middlebrook 7H9 medium), the intermediate breakpoint for clarithromycin should be 32 μg/ml, whereas with testing at pH 7.3 to 7.4 (cation-adjusted Mueller-Hinton broth [CAMHB]), the clarithromycin intermediate breakpoint is 8 to 16 μg/ml (451, 452).

Generally, all wild-type (untreated) strains of the MAC are susceptible to macrolides (172, 264). Resistance to macrolides has been defined as clarithromycin MICs of ≥64 μg/ml at pH 6.8 and ≥32 μg/ml at pH 7.3 to 7.4. Intermediate MICs (16 to 32 μg/ml depending upon the pH of the media), suggesting a mixed population of MAC organisms, are rare and should be confirmed. Patients with confirmed intermediate clarithromycin MICs should be carefully monitored with repeat cultures for the possibility of emerging macrolide resistance (451).

***Mycobacterium kansasii*.** The treatment regimens for *M. kansasii* include rifampin, ethambutol, and isoniazid (INH) or an alternative regimen with the substitution of clarithromycin for INH. Rifabutin should be used as a substitute for rifampin for HIV patients on protease inhibitors (132). Isolates susceptible to rifampin are also susceptible to rifabutin, so no additional testing is required. AST is not usually required for untreated (wild) isolates of *M. kansasii*, since MICs fall into a narrow range, with the exception of those of INH. The INH MICs for *M. kansasii* range from 0.5 to 5 μg/ml, so the standard MTBC critical concentration of 0.2 μg/ml usually shows resistance, and the 1.0-μg/ml concentration yields variable results, making testing and the interpretation of INH results technically difficult. Despite the laboratory issues with INH, treatment with the above-described regimens is usually successful (132, 451). However, treatment failure can occur (418). When treatment failure occurs, the isolate is almost always resistant *in vitro* to rifampin and occasionally one or more of the companion drugs as well. Therefore, the CLSI and ATS/IDSA recommend AST of isolates from patients who fail or have a poor response to therapy (132, 451).

Because treatment failure is always associated with rifampin and/or clarithromycin resistance, the current CLSI recommendation is to test all initial isolates of *M. kansasii* against rifampin and clarithromycin only (140, 418, 451, 452). For isolates found to be rifampin resistant (MIC > 1 μg/ml), secondary antimicrobials, including amikacin, ciprofloxacin, ethambutol, linezolid, moxi-

floxacin, rifabutin, and trimethoprim-sulfamethoxazole, should be tested (451). INH and streptomycin may be useful, but breakpoints for susceptibility and resistance for NTM have not been established (451).

The methods used for the testing of isolates of *M. kansasii* include the method of proportion, similar to that for MTBC, and either macrodilution or microdilution broth methods. However, the CLSI strongly recommends broth microdilution in cation-adjusted Mueller-Hinton broth plus 5% OADC (451).

***Mycobacterium marinum.*** *M. marinum* is the cause of "swimming pool" or "fish tank" granuloma and is most often acquired from a soft tissue injury to the hand or arm in freshwater or salt water (132, 214). Currently, routine AST of isolates of *M. marinum* is not required due to its consistent susceptibility to antimicrobial agents most often used for treatment, including rifampin, ethambutol, doxycycline-minocycline, TMP-SMX, and clarithromycin. Furthermore, there is a minimal risk of acquired resistance to these agents and disease because the species is usually localized, with small numbers of organisms being present (132, 451).

For patients with *M. marinum* who fail to respond to treatment after several months and continue with positive cultures, however, AST may be recommended (179, 451, 452).

There have been few comparative AST studies with *M. marinum*. However, the broth microdilution method using CAMHB supplemented with OADC has been recommended by the CLSI. Test antimicrobials include rifampin, ethambutol, clarithromycin, doxycycline-minocycline, and amikacin (451).

The optimal temperature for strains of *M. marinum* is 30°C to 33°C, and thus, *in vitro* AST is best performed at 30°C rather than 35°C to 37°C, as used for most other slowly growing NTM (451).

**Miscellaneous slowly growing nontuberculous mycobacteria.** Among the slowly growing NTM, as stated above, the ATS/IDSA and CLSI recommendations apply best to MAC, *M. kansasii*, and *M. marinum* isolates (132, 451). However, other species of NTM may cause human disease, and AST of such isolates should be considered in regard to their clinical significance. For example, AST should be performed on isolates from sterile body fluids, tissues, or multiple sputum samples (114).

Species such as the *M. terrae-M. nonchromogenicum* group (318, 357), *M. simiae* (101, 391), *M. szulgai* (160, 389, 399), *M. celatum* (290, 392), *M. xenopi*, and *M. malmoense* should be tested by using the criteria recommended for isolates of rifampin-resistant *M. kansasii*, although the latter two species may require temperature adjustment (42°C to 45°C) and pH adjustment (pH 6.0), respectively, for optimal growth (451). Both *M. xenopi* (351) and *M. malmoense* (58, 149, 456) may also require extended incubation.

**Fastidious slowly growing nontuberculous mycobacteria.** Several slowly growing NTM associated with clinical disease exist, and susceptibility testing data are limited. These species include *M. haemophilum*, which requires hemin or ferric ammonium citrate and lower temperatures (28°C to 30°C) for growth (338); *M. genavense* (43, 45), which requires extended incubation for more than 6 weeks using a macrobroth system; and *M. ulcerans*, which requires a lengthy incubation (4 to 6 weeks) and grows best at 30°C (451). Because of the lack of experience in susceptibility testing of these species, no standard method for susceptibility testing of these species has been recommended by the CLSI. Laboratories are advised to establish their own in-house validation for susceptibility testing of these species or to send these isolates to a qualified mycobacterial reference laboratory (451).

## ANTIMICROBIAL ACTIVITY AND MECHANISMS OF DRUG RESISTANCE

The antimycobacterial agents with broad-spectrum activity are believed to target the same proteins and organelles as other bacteria, although specific data for mycobacteria are often lacking. That said, the mechanisms of resistance to these agents are consistent with this view. Much of our understanding of the mechanisms of clinically acquired resistance in pathogenic mycobacteria has come from studies of *M. tuberculosis* and has been the subject of several recent reviews (316, 462). The purpose of this review is to focus on studies of NTM where possible. Table 2 provides a summary of drug targets and known or possible mechanisms of resistance in NTM.

### Inhibition of Mycobacterial Cell Wall Synthesis

**Carbapenems and cephalosporins.** Carbapenems (e.g., imipenem and meropenem) and cephalosporins (e.g., cefoxitin and ceftriaxone), like other β-lactam antimicrobials, are inhibitors of peptidoglycan synthesis. The β-lactams bind to, and irreversibly inhibit, penicillin-binding proteins (PBP) or D,D-transpeptidases. The PBP are responsible for the intermolecular peptide bridges and are required for the formation of mature peptidoglycan from nascent peptidoglycan strands; these bridges are critical to the structural integrity of peptidoglycan. More specifically, the PBP catalyze the bridging between molecules from the fourth amino acid (D-alanine) of one peptide side chain to the third amino acid (*meso*-diaminopimelic acid) of an adjacent intermolecular peptide chain, forming a 4-3 cross-link (85, 204). Although the blocking of the formation of the 4-3 cross-link leads to a disruption of the cell wall, a downstream effect of the buildup of peptidoglycan fragments and precursors is the activation of the hydrolase autodigestion of mature peptidoglycan (206, 346), further disrupting the cell wall.

In addition to the 4-3 cross-links, mycobacteria, like other bacteria, can also form 3-3 cross-links between the peptide side chains of the peptidoglycan (85, 204, 205). Such cross-links are catalyzed by D,L-transpeptidases and reflect a remodeling of mature peptidoglycan, and they may represent an adaptive response (204). As well as targeting the PBP, there is evidence that carbapenems bind to (and probably inhibit) the mycobacterial D,L-transpeptidases (204).

Clinically acquired resistance to β-lactams has not been characterized for mycobacteria, although this may change in the near future with the renewed interest in this class of agents as antituberculosis agents (in combination with clavulanic acid) (165, 166). The intrinsic resistance of mycobacteria to β-lactams is likely to result from the combined effects of cell wall permeability, the affinity of the penicillin-binding proteins for these agents, and the presence of β-lactamases (106, 177, 252). Evidence suggests that β-lactams enter mycobacteria via the MspA porin (or its ortholog), the loss of which increases resistance to these agents (90, 363). However, there is no evidence that changes in porins underlie differences in intrinsic resistance (or acquired resistance) to carbapenems and/or cephalosporins. Porin loss has a significant fitness cost (362), which may outweigh the resistance benefit to the organism. Despite this, there is indirect evidence that the permeability barrier of the cell wall may play a critical role in the

**TABLE 2** Summary of drug targets and known or possible mechanisms of resistance in NTM

| Agent | System or process inhibited | Molecular target(s)[a] | Mechanism(s) of acquired resistance[b] | Mechanism(s) of intrinsic resistance[b] |
|---|---|---|---|---|
| β-Lactams (carbapenems and cephalosporins) | Peptidoglycan synthesis | PBP, D,L-transpeptidases | Mutation in PBP | D,L-Transpeptidases (cephalosporins), β-lactamases |
| Ethambutol | Arabinogalactan/arabinomannan synthesis | EmbB | Mutations in *embB*, *embR*, and other genes in the *emb* operon | Polymorphisms in *embB*, *lfrA*, efflux pump |
| Isoniazid | Mycolic acid synthesis | InhA | Mutations in *katG* or *inhA* | Lack of prodrug activation |
| Glycopeptides | Peptidoglycan synthesis | D-Alanine–D-alanine terminal amino acids | Unknown | Unknown |
| Aminoglycoside | Protein synthesis | Ribosome | Mutations in 16S rRNA gene *rpsL*, aminoglycoside phosphotransferase | Aminoglycoside acetyltransferases and phosphotransferases |
| Tetracycline | Protein synthesis | Ribosome | Mutations in 16S rRNA gene, ribosome protection [*otr*(A) and *tet*(M)], efflux [*tet*(K), *tet*(L), *tet*(V), *otr*(B), and *tap*] | Ribosome protection [*otr*(A) and *tet*(M)], efflux [*tet*(K), *tet*(L), *tet*(V), *otr*(B), and *tap*] |
| Glycylcycline | Protein synthesis | Ribosome | Unknown | Unknown |
| Macrolide-ketolide | Protein synthesis | Ribosome | 23S rRNA gene mutations | *erm* genes |
| Oxazolidinones | Protein synthesis | Ribosome | 23S rRNA gene mutations | Unknown |
| Fluoroquinolone | DNA replication/gene expression | DNA gyrase | Mutations in *gyrA* | *lfrA* efflux pump |
| Rifamycin | RNA synthesis | RNA polymerase | Mutations in *rpoB* | ADP-ribosylation |
| Trimethoprim | Folate metabolism | DHFR | Mutations in DHFR | Polymorphisms in DHFR |
| Sulfonamides | Folate metabolism | DHPS | Mutations in DHPS | Unknown |

[a] PBP, penicillin-binding protein (D,D-transpeptidases); DHFR, dihydrofolate reductase; DHPS, dihydropteroate synthetase.
[b] The role of the cell wall in resistance is omitted as it is assumed to be significant in all intrinsic resistances.

intrinsic resistance of mycobacteria to β-lactams (107, 177, 252, 303).

The affinities of purified mycobacterial PBP for β-lactams appear to be in the same range as those of the PBP of *Escherichia coli* (177), despite the higher level of mycobacterial intrinsic resistance to these agents. This suggested that, at least for some mycobacteria, the PBP might not contribute to intrinsic β-lactam resistance. A possible role in acquired resistance was indicated by several studies that reported PBP with reduced drug binding in laboratory-isolated mutants with increased resistance to β-lactams (106, 252). Another mechanism that may affect β-lactam susceptibility is a change in the predominant transpeptidase involved in peptidoglycan synthesis, that is, a shift from PBP (D,L-transpeptidases) to D,D-transpeptidases. Such an adaptive response, leading to the acquisition of β-lactam resistance, has been reported for *Enterococcus faecium* (229). It is intriguing that the peptidoglycan of *M. abscessus* may have predominantly 3-3 intermolecular cross-links (205), formed by D,D-transpeptidases rather than the classic PBP, which may explain the resistance of this organism to some β-lactams that are active against other mycobacteria.

Perhaps the most extensively studied aspect of mycobacterial susceptibility to β-lactams is the production of degradative enzymes, or β-lactamases. The destruction of penicillin by a mycobacterium (*M. tuberculosis*) was first reported in 1945 by Woodruff and Foster (448), and, largely from the work of Kasik et al. several decades later, it became clear that penicillinase/β-lactamase activity was present in both slowly growing and rapidly growing mycobacteria and that each organism appeared to have several proteins with penicillinase/β-lactamase activity (182–185). Since then, it has become clear that β-lactamases are ubiquitous in mycobacteria, and the genomic data are consistent with previous observations that each organism may have multiple enzymes with β-lactamase activity. For instance, *M. abscessus* has nine (GenBank accession number CU458896) and *M. avium* has six (GenBank accession number CP000479) β-lactamase or β-lactamase-like genes. In contrast, *M. tuberculosis* has only two β-lactamase or β-lactamase-like genes, with *blaC* being considered the most significant (113).

That mycobacteria have β-lactamases affecting the activity of β-lactams in clinical use is not in doubt, and overall, these enzymes have a broad substrate specificity (260, 303). However, it is less clear whether they play a significant role in either intrinsic resistance or acquired resistance to β-lactams in NTM. Several studies of the β-lactamases of *M. fortuitum* found that the level of these enzymes did correlate with susceptibility to some β-lactams, such as amoxicillin, ampicillin, and benzylpenicillin (107, 260, 303). Similar results were found with *M. smegmatis* (113). In contrast, another study did not find a clear association between susceptibility and β-lactamase activity (106). An explanation for such discrepancies may come from the finding that at least some mycobacterial β-lactamases are inducible (260). That said, a common finding has been that β-lactamases of NTM appear to have little, if any, effect on susceptibility to imipenem and cefoxitin (106, 107, 113, 303), the β-lactams with perhaps the most significant clinical utility against NTM.

The possibility that the antimycobacterial activity of β-lactams may be limited by β-lactamases has led to studies of enzyme inhibitors as potentiators. The most studied β-lactamase inhibitor

of mycobacteria is clavulanic acid, and this inhibitor has been shown to increase the susceptibility of NTM to β-lactamase substrates such as amoxicillin (27, 106, 107).

**Ethambutol.** Although ethambutol is primarily an antituberculosis agent, it is a component of some regimens for infections with slowly growing mycobacteria. It is of limited use for rapidly growing mycobacteria and is considered inactive against other microorganisms.

The direct effect of ethambutol on mycobacteria is a disruption of cell wall synthesis, in particular the inhibition of the synthesis of arabinogalactan (AG) and, and to a lesser extent, lipoarabinomannan (LAM) (92, 207, 246, 371). A likely candidate for the actual molecular target of ethambutol is the arabinosyl transferase encoded by the *embB* gene; this has come largely from analyses of mutations in the *emb* operon that confer resistance to ethambutol (8, 210). Although the disruption of the synthesis of AG and LAM is believed to be the primary direct effect of ethambutol, evidence suggests that a secondary target of this agent may be spermine metabolism. Studies from more than 2 decades ago demonstrated that the growth inhibition caused by ethambutol could be reversed by the addition of spermidine, and the mycobacterial spermidine synthase enzyme is inhibited by ethambutol (283, 298). The disruption of the synthesis of AG and LAM, and possibly spermine, explains the direct antimycobacterial activity of ethambutol; however, the key utility of this agent for anti-NTM treatment regimens is the changes in the permeability of the cell wall to other antimycobacterial agents.

As indicated above, the primary mechanism of acquired resistance to ethambutol is a mutation in the *embB* gene (8, 210). The *embB* gene is part of an operon comprising either two genes, *embA-embB*, or three genes, *embC-embA-embB* (32, 379), depending on the mycobacterial species. The *emb* operon is regulated by the product of the *embR* gene (32). The majority of resistance-associated *emb* mutations are in the *embB* gene, although mutations in other parts of the *emb* operon have been described (306). However, not all missense mutations in this operon confer resistance, and mutations in the codon 306 region have been reported for multidrug-resistant *M. tuberculosis* strains that are still susceptible to ethambutol (249). A role for mutations in spermidine synthase has not been explored for such organisms. A further complication of the acquisition of ethambutol resistance is that it appears to be a multistep process involving an increase in the expression level of the *emb* operon and the selection of mutations in, primarily, the *embB* gene (379).

The mechanisms of intrinsic resistance to ethambutol are largely uncharacterized, although polymorphisms in the *embB* gene are associated with intrinsic resistance to ethambutol in some mycobacteria (8). There is evidence to suggest that the mycobacterial efflux pump LfrA may affect susceptibility to ethambutol (323); however, the effect is small and unlikely to fully explain the high-level intrinsic resistance to this agent expressed by some mycobacteria. As for many other antimicrobial agents, the mycobacterial cell wall likely represents an important factor in limiting the activity of ethambutol.

**Isoniazid.** Isoniazid is considered primarily an antituberculosis agent, but it does have activity against several NTM species, including *M. kansasii* and *M. xenopi*. Isoniazid, or isonicotinic acid hydrazide (INH), is a prodrug that the target organism must modify to its active form. The active derivatives of isoniazid are not fully characterized, but the main form is believed to be an isoniazid-NAD adduct (93, 270, 445). Isoniazid-NAD forms from the interaction of INH and $NAD^+$ in the presence of $Mn^{2+}$ and $O_2$, which is catalyzed by the catalase-peroxidase KatG (445). Indeed, the lack of a suitable catalase-peroxidase to catalyze this process is the primary reason why most mycobacteria are not susceptible to isoniazid.

The first insights into the mechanism of action of isoniazid were made over 3 decades ago with the demonstration that this agent inhibited mycolic acid synthesis (370, 372). More recently, the primary target of activated isoniazid was identified as one of the fatty acid synthesis II (FAS II) enzymes, enoyl-acyl carrier protein (ACP) reductase, or InhA (20, 197, 203). The inhibition of InhA explains the primary effects of isoniazid on mycobacteria, and the crystal structure of the isoniazid-NAD adduct bound to InhA has been determined (93). There is evidence that activated isoniazid may target other proteins (12), including dihydrofolate reductase (DHFR) (13) and the FAS II enzyme KasA (236, 355, 356). The role of these alternative targets in the antimycobacterial activity of activated isoniazid is still in question (197, 203, 433).

As mentioned above, most species of bacteria, including most mycobacteria, are intrinsically resistant to isoniazid, which is largely a consequence of a lack of intracellular activation of this prodrug. There is also evidence that mycobacteria may have an isoniazid efflux pump (75, 403), although it is not clear whether it has a role in intrinsic resistance. In *M. tuberculosis*, clinically acquired resistance to isoniazid is associated primarily with mutations in the *katG* and *inhA* genes (245, 256). To date, there have been no publications describing the mechanisms of clinically acquired isoniazid resistance in *M. kansasii*, although it is assumed that they would be equivalent to those in *M. tuberculosis*.

**Glycopeptides.** The glycopeptide agents in clinical use are vancomycin and teicoplanin. Although there have been some reported successes with vancomycin as an antimycobacterial agent in humans and animals (150, 176, 208), mycobacteria are usually considered to be resistant to this agent. Although direct studies of the action of vancomycin in mycobacteria are lacking, indirect evidence for the peptidoglycan precursors present in mycobacteria following exposure to these agents suggests that the mechanism of action of vancomycin is the same as that in other bacteria (228). Vancomycin binds to the D-alanine–D-alanine terminal amino acids of the peptide side chains of peptidoglycan and in doing so prevents the 4-3 cross-linking by the PBP D,L-transpeptidase and other processes in the final assembly of mature peptidoglycan (83).

Most pathogenic mycobacteria are intrinsically resistant to glycopeptide antimicrobial agents. Since the peptide side chains of the mycobacterial peptidoglycan have the canonical binding site for vancomycin, it seems most likely that resistance to vancomycin relates to the permeability barrier of the cell wall. Vancomycin is not lipophilic and thus is unlikely to permeate the outer lipid-rich layer of the cell wall. Furthermore, this drug is a large molecule (for an antimicrobial agent) and is believed to be too large to pass through mycobacterial porins (90). The presence of VanA or VanB (or homologs), the primary mechanism of clinically significant vancomycin resistance in enterococci (83), has not been described for mycobacteria.

### Inhibition of Protein Synthesis

The protein synthesis-inhibiting antimycobacterial agents all target the ribosome and interfere with the nascent peptide chain

formation. With the exception of the macrolides, ketolides, lincosamides, and streptogramins, the mechanisms of action and resistance of the different classes of agents are distinct.

**Aminoglycosides.** The aminoglycosides inhibit protein synthesis by binding to the ribosome near the A site (for a review, see reference 444). This changes the state of the tRNA binding to the A site and also interferes with mRNA decoding, although some aminoglycosides can also inhibit the ribosomal translocation of tRNA. As a consequence of the interference of tRNA in the ribosome, aminoglycosides also disrupt the proofreading function of the A site, which can lead to frameshift errors and the readthrough of stop codons, with an increase in the frequency of mistranslated proteins (232, 343). Although there have been limited direct studies establishing these findings with mycobacteria, the activities of different aminoglycosides on specific mutant ribosomes in *M. smegmatis* are consistent with this view (156).

The primary mechanism of acquired resistance to aminoglycosides in mycobacteria is based on the modification of the 30S subunit of the ribosome, i.e., the drug target. The modifications are caused by mutations in either the 16S rRNA gene or the *rpsL* gene, which encodes the S12 ribosomal protein (44, 81, 110, 162, 186, 238, 257, 258, 358, 360). Although 16S rRNA mutations can confer high-level resistance to aminoglycosides (e.g., MIC $>$ 1,024 μg/ml for 2-deoxystreptamine aminoglycosides against *M. abscessus* [300]), such mutations tend to confer a lower level of resistance than *rpsL* mutations, and despite the chemical similarity between the different aminoglycosides, resistance to one agent does not necessarily result in resistance to all agents (285). For instance, a 16S rRNA gene mutation at position 1408 in *M. abscessus* confers resistance to 2-deoxystreptamine aminoglycosides (e.g., amikacin and kanamycin) but not resistance to streptomycin (300). For all the pathogenic mycobacteria studied, the 16S rRNA and *rpsL* gene mutations account for up to 90% of acquired aminoglycoside resistance. Thus, another mechanism must account for the other acquired resistances; there is some evidence that it may involve changes in cell wall permeability (240).

In addition to screening for ribosome mutations, a focus of studies on acquired and intrinsic mycobacterial resistance to aminoglycosides has been characterizing the role of acetyltransferase enzymes that inactive these agents. Such enzymes confer aminoglycoside resistance in other bacteria (348).

From studies 2 decades ago, rapidly growing mycobacteria are known to produce aminoglycoside-acetylating enzymes (168), and a substrate profile analysis revealed two patterns of 3-*N*-acetyltransferase with broad and narrow specificities (395). More recently, DNA homology studies identified putative aminoglycoside acetyltransferases in the chromosomes of both rapidly growing and slowly growing mycobacteria, including *M. tuberculosis* (5). The cloning and overexpression of the aminoglycoside acetyltransferase gene *aac(2′)-Id* of *M. smegmatis* conferred a 4- to 16-fold increase in the MIC of a range of aminoglycosides, and the disruption of the gene resulted in a drop in the MIC of an equivalent magnitude (4, 5). In contrast, the expression of the *M. tuberculosis* putative aminoglycoside acetyltransferase in *M. smegmatis* did not increase resistance to this class of agent, despite the demonstration that the enzyme can modify aminoglycosides (24). Regardless of the presence of aminoglycoside acetyltransferase-encoding genes in mycobacteria, the results of several studies indicated that mycobacterial acetyltransferase enzymes do not correlate with susceptibility to these agents (155, 419). Thus, the role of these enzymes in either intrinsic or acquired resistance to aminoglycosides is not clear.

As well as acetyltransferases, the known genomes of both rapidly growing and slowly growing mycobacteria have genes that encode putative aminoglycoside phosphotransferases. For instance, the complete genome sequences for *M. abscessus* (GenBank accession number CU458896) and *M. avium* (GenBank accession number CP000479) were annotated with 5 and 1 putative aminoglycoside phosphotransferase genes, respectively. A study by Ramon-Garcia et al. (308) linked resistance to streptomycin, but not to other tested aminoglycosides, to the expression of the APH(3″)-Ic phosphotransferase identified in a strain of *M. fortuitum*. The gene encoding this enzyme was detected in fewer than 20% of *M. fortuitum* clinical isolates. In contrast, Ho et al. (155) failed to detect aminoglycoside phosphotransferase (or nucleotidyltransferase) activity in lysates of *M. fortuitum*, *M. kansasii*, and MAC cells. Therefore, clinically significant aminoglycoside resistance conferred by phosphotransferases is likely to be unusual for most mycobacterial species.

**Tetracyclines and glycylcyclines.** The tetracyclines, such as tetracycline and doxycycline, and the glycylcyclines, such as tigecycline, bind to the 30S subunit of the ribosome and inhibit protein synthesis. Primarily, tetracyclines impede the access of tRNA to the A site of the ribosome. Although tetracycline appears to be able to interact with the ribosome at multiple points, the highest-affinity binding sites are consistent with the interference of the A site (11, 47, 292). Evidence indicates that the high-affinity binding of glycylcyclines to the 30S ribosomal subunit is the same as, or overlaps, that of tetracycline (28, 34, 278), with additional interactions that may explain the higher affinity of tigecycline for the ribosome (278).

The acquisition of resistance to the tetracyclines in bacteria is associated primarily with ribosome protection and drug efflux (for reviews, see references 74 and 320). Ribosome protection proteins (RPP) have homology to the elongation factors EF-Tu and EF-G, critical factors for normal ribosome function. However, the RPP are believed to confer resistance by displacing tetracycline from ribosomes (320). Evidence suggests that ribosome protection does not confer resistance to tigecycline (301). To date, two ribosome protection protein genes, *otr*(A) and *tet*(M), have been described for tetracycline-resistant mycobacteria (279, 328), although their role in tetracycline resistance in these organisms has yet to be confirmed. Efflux pumps (or putative efflux pumps) that are associated with increased resistance to tetracyclines appear to be common in mycobacteria. These include homologs of genes found in other bacteria, including *tet*(K), *tet*(L), *tet*(V), and *otr*(B) (91, 279). The findings that the *tet* and *otr* RPP and efflux genes found in mycobacteria have sequence homology with the archetypal genes and that the genes are not found in all isolates suggest that these genes were transferred horizontally (279). Thus, these genes may represent mechanisms of clinically acquired resistance.

In addition to the Tet and Otr efflux pumps, mycobacteria also have a genus-specific tetracycline efflux pump, Tap (3, 307). The expression of this efflux pump can increase tetracycline MICs by 8- to 16-fold in experimental systems (3, 307), yet the presence of the *tap* gene in rapidly growing mycobacteria did not correlate with resistance to doxycycline or tigecycline (104). Furthermore, the gene may not be present in all isolates of "*tap*-positive" *Mycobacterium* species (104).

Tetracycline resistance in some bacteria can be manifested by enzymatic drug inactivation (for a review, see references 74 and

320). Homology searches of the genomic sequence data suggest that mycobacteria lack genes encoding tetracycline-inactivating enzymes, and there is no experimental evidence demonstrating such an activity. Although acquired resistance to tigecycline has not yet been described for mycobacteria, it is of interest that the Tet(X) enzyme can inactivate tigecycline (250). The mobility of the *tet* genes raises the possibility that the *tet*(X) gene may be acquired by mycobacteria, resulting in tigecycline resistance. Mutations in the 16S rRNA gene can confer resistance to tetracyclines (327), although these are not common mechanisms of clinically acquired resistance and have not been described for mycobacteria.

**Macrolides and ketolides.** The macrolides and the keto derivatives, the ketolides, act by binding in the peptide exit tunnel of the ribosome (125, 147, 273, 341) and thus preventing the growing peptide chain from vacating the peptidyltransferase center of the ribosome. This is believed to "gum up" the ribosome and prevent the further elongation of the nascent peptide chain. Although the different agents may bind in slightly different places in the exit tunnel, the ultimate effect is the same (286).

Since the inhibitory effect of macrolides and ketolides is largely a passive blocking of the peptide exit channel, mechanisms of acquired resistance to these agents involve modifications of the drug-binding site to reduce the binding of the agents. In mycobacteria, which have only one or two copies of the rRNA (*rrn*) operon, acquired resistance is almost always associated with a mutation in the 23S rRNA gene leading to a base change at either position 2058 or 2059 (*E. coli* numbering); these are critical rRNA residues involved in the binding of macrolides to ribosomes, including in mycobacteria (286). These mutations confer resistance to macrolides and ketolides in mycobacteria as wells as lincosamides and streptogramin B (239, 264, 286). The acquisition of 23S rRNA gene mutations in NTM during therapy with macrolides has been reported for several species, including *M. abscessus*, the MAC, *M. chelonae*, *M. fortuitum*, and *M. kansasii* (59, 237, 239, 264, 421). Although 23S rRNA gene mutations are believed to account for most of the acquired macrolide resistance, there is evidence that other, as-yet-uncharacterized, ribosome mutations may account for some of the acquired macrolide resistance in *M. avium* (97).

The primary mechanism of clinically acquired macrolide resistance in mycobacteria has been known for over a decade, yet only recently have we gained insight into the specific mechanisms of intrinsic resistance in this genus. The mechanism of resistance is conferred by inducible *erm* methylase genes and has been described for 10 species of RGM (189, 261–263, 266), including the *erm*(38) genes of *M. goodii* and *M. smegmatis*; the *erm*(39) genes of *M. boenickei*, *M. fortuitum*, *M. houstonense*, *M. neworleansense*, and *M. porcinum*; the *erm*(40) genes of *M. mageritense* and *M. wolinskyi*; and the *erm*(41) genes of *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *bolletii*. These *erm* genes represent four *erm* gene classes (319), and they fall into two predicted evolutionary groups: (i) *erm*(38), *erm*(39), and *erm*(40) and (ii) *erm*(41). The *erm* genes in the first group are all >70% identical and are located in the same region of the chromosome, i.e., adjacent to the *folD* gene. The *erm* alleles in the second group are genetically unrelated to the first group and are most closely related to *erm*(37), the *erm* gene of *M. tuberculosis* (263).

The *erm* genes of the RGM encode rRNA methylases, which confer resistance to mycobacteria by methylating an adenine in the peptidyltransferase region of the 23S rRNA (226, 227). This is equivalent to the *erm* genes of other bacteria (319, 321) and results in the reduced binding of macrolides (and related agents) to the ribosome. The *erm*(38) methylase (and probably others in the same evolutionary group) leads predominantly to ribosome monomethylation, even though it has the capacity to dimethylate rRNA (226, 227). The monomethylated state accounts for the type II resistance phenotype, i.e., resistance to macrolides, ketolides, and lincosamides but not streptogramins. The similarity of the *erm*(41) methylases of *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *bolletii* to that of *erm*(37) of *M. tuberculosis* suggests that the activities of the enzymes are similar, i.e., the monomethylation of one or more adenines in the peptidyltransferase center (226, 227). Like the other mycobacterial *erm* methylases, *erm*(41) confers a type II resistance phenotype.

A great deal of interest has been focused on the ketolides, partly because they are considered poor or weak inducers of *erm* genes in Gram-positive pathogens such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* (18, 42, 459). Another advantage of ketolides is that ribosome modification by *erm* monomethylases does not confer high-level resistance to these agents in some organisms (220). These properties suggested that the activity of ketolides might be relatively unaffected by mycobacterial *erm* genes, since the genes are inducible (261–263, 266) and tend to result in a predominance of monomethylated ribosomes (226, 227). However, ketolides such as telithromycin and HMR3004 were found to be good inducers of mycobacterial *erm* genes (10, 263), which resulted in high-level resistance to these agents (226, 227, 262, 263).

The phenotypes associated with the expression of the mycobacterial *erm* genes suggest that these genes likely compromise macrolide-based therapies; i.e., the presence of these genes would be expected to give an infecting organism a selective advantage with little fitness cost, as the genes are inducible. It is intriguing, therefore, that isolates of normally *erm* gene-positive organisms have been found with inactive or deleted *erm* genes (26, 189, 263, 266). Such mutant *erm* genes have been described for *M. fortuitum* and *M. abscessus*, and it seems reasonable to assume that similar inactive alleles are present in some isolates of other *erm* gene-positive RGM. These Erm mutant organisms are highly susceptible to macrolides such as clarithromycin. This creates a complication for predicting macrolide resistance by using molecular screens for the mycobacterial *erm* genes, as a significant proportion would be expected to be macrolide susceptible.

The elucidation of the primary mechanisms of high-level acquired and intrinsic resistance to macrolides (i.e., 23S rRNA mutation and the expression of *erm* genes) does not explain why some of these agents are more active against mycobacteria than others. The differential cell wall permeability to macrolides appears to be an important factor: macrolides (and most likely ketolides) are lipophilic; they enter mycobacteria by passive diffusion through the cell wall (96). Clarithromycin was found to enter mycobacteria faster than the less lipophilic erythromycin, and this correlated with the higher level of antimycobacterial activity of the former agent (96). A role for the mycobacterial cell wall in limiting the activity of macrolides is supported by the effect of ethambutol enhancing the activity of these agents (311).

In other bacterial pathogens, macrolide efflux pumps represent significant mechanisms of macrolide resistance, such as MexXY-OprM of *Pseudomonas aeruginosa* (for a review, see reference 297) and MefA of *Streptococcus* species (94). Based on the

effect of efflux inhibitors on susceptibility and cell-associated drug levels in the MAC, it was proposed that mycobacteria have a macrolide efflux pump (324). Whether there is an active macrolide transport system has yet to be determined, and consequently, it is not clear whether macrolide efflux plays a significant role in either intrinsic or acquired macrolide resistance in mycobacteria.

**Oxazolidinones.** The oxazolidinones are a new class of ribosome-targeting antimicrobial agents, of which the only FDA-approved agent is linezolid. Oxazolidinones target the ribosome, binding to the peptidyltransferase center but overlapping the A-site pocket (174, 440). In this position, linezolid interferes with the positioning of tRNA entering the A site and may also prevent the normal functioning of the peptidyltransferase center by changing its conformation (174, 440).

Not surprisingly, bacterial resistance to linezolid is conferred by mutations in the ribosome that reduce the binding of this agent. The primary site for resistance-associated mutations is the 23S rRNA gene in domain V (46); the specific mutated residues are distinct for those that confer resistance to the macrolides and ketolides. Studies of linezolid resistance in mycobacteria, both laboratory-derived mutants and clinical isolates, identified resistance-associated 23S rRNA mutations (154, 224, 335). In addition to the 23S rRNA gene, oxazolidinone resistance has been associated with mutations in the L3 and L4 ribosomal protein genes *rplC* and *rplD* (161, 222, 223, 447); such mutations have not yet been described for mycobacteria. Despite the fact that oxazolidinone resistance can be conferred by ribosome mutations, they incur a significant fitness cost to the organism (35). In fact, there have been reports of linezolid-resistant organisms, with 23S rRNA gene mutations, reverting to the wild type in the absence of drug, with a return to a susceptible phenotype (241, 242).

There have been few studies of acquired resistance to linezolid in mycobacteria. A study of 4 linezolid-resistant *M. tuberculosis* isolates did not identify any mutations in the 23S rRNA or *rplD* gene (317); *rplC* was not screened in that study. In studies of *in vitro*-selected linezolid-resistant mycobacteria, a significant proportion lacked 23S rRNA mutations (154, 335). Mutations in the *rplC* or *rplD* genes in the *in vitro*-selected mutants were not screened in those studies, although the resistance phenotypes were not consistent with ribosomal protein gene mutations (154, 335). Thus, there must be at least one other currently uncharacterized mechanism of oxazolidinone resistance in mycobacteria.

In *Staphylococcus* spp., oxazolidinone resistance is conferred by the Cfr rRNA methylase, encoded by the *cfr* gene (14, 221, 223, 382). This enzyme methylates the 23S rRNA at position 2503 (382), and the gene is associated with mobile elements (41, 243, 382). Intriguingly, the presence of *cfr* in clinical isolates of *Staphylococcus aureus* with *rplC* ribosomal protein gene mutations has been reported (223). The presence of *cfr* in linezolid-resistant mycobacteria has not been reported, and the genome sequence of *M. abscessus*, which is considered intrinsically resistant to linezolid, lacks DNA homologous to this gene.

### Inhibition of Nucleic Acid Synthesis

**Fluoroquinolones.** The primary target for the fluoroquinolones is the DNA gyrase, which relaxes the supercoiling of the DNA ahead of the DNA helicase and DNA replication complex. By inhibiting the DNA gyrase, the strain of the supercoiled DNA will prevent DNA replication. As with other bacteria, quinolones bind to the mycobacterial DNA gyrase and inhibit this enzyme, even in species considered intrinsically resistant to these agents (143). The DNA gyrase is encoded by the *gyrA* and *gyrB* genes. As well as the DNA gyrase, the other bacterial type II topoisomerase, topoisomerase IV, is also a target of quinolones. The role of topoisomerase IV is to unlink the newly synthesized DNA strands so that the chromosomes can segregate during cell division, although this enzyme also has a function equivalent to that of the DNA gyrase. In other bacteria, the genes encoding topoisomerase IV have various names, such as *grlB* and *grlA* in *Staphylococcus aureus* and *parC* and *parE*. Intriguingly, pathogenic mycobacteria such as *M. abscessus*, *M. avium*, and *M. tuberculosis* appear to lack a topoisomerase IV ortholog, although the genome data for other species such as *M. smegmatis* and *Mycobacterium vanbaalenii* suggest that they may have genes that encode this enzyme.

In other pathogenic bacteria, acquired high-level resistance to fluoroquinolones involves a stepwise accumulation of mutations in the genes encoding the DNA gyrase and topoisomerase IV (40). However, since pathogenic mycobacteria lack the latter enzyme, high-level resistance is associated primarily with mutations in the DNA gyrase or *gyrA* gene (293). In addition, as for other bacteria, drug efflux is also believed to be involved in resistance to fluoroquinolones in mycobacteria. In particular, interest has been focused on the LfrA efflux pump, encoded by the *lfrA* gene (215, 219, 336, 373). Although this pump does not appear to be capable of conferring high-level resistance, it may be part of a stepwise shift in phenotype toward high-level resistance.

The mechanism(s) of intrinsic resistance to fluoroquinolones is unclear, as the binding of quinolones to DNA gyrases from different mycobacteria does not predict resistance (143). The differential permeability of the cell wall is probably involved in intrinsic resistance, and it has been proposed that efflux may also be significant. However, since the LfrA efflux pump is conserved in mycobacteria, it is unclear whether this mechanism is significant (104, 336). The expression of *lfrA* is regulated by LfrR (33), and thus, it is possible that the drug phenotype correlates with the level of *lfrA* expression. There is some evidence that mycobacteria may have another, as-yet-uncharacterized, fluoroquinolone efflux pump (215). Despite the evidence for these processes, it is not clear whether they play a significant role in intrinsic, clinically significant resistance in mycobacteria.

**Rifamycins.** The rifamycins (e.g., rifampin and rifabutin) bind to the prokaryotic DNA-dependent RNA polymerases and are potent inhibitors of these enzymes (212). The RNA polymerase is comprised of five subunits, and the binding site of rifamycins is within the β-subunit, which is the catalytic center of the enzyme. However, evidence suggests that rifampin (and presumably the other rifamycins) binds to the DNA/RNA channel (63, 196) rather than in the catalytic center of the β-subunit. Thus, rifamycins act by blocking the initiation of RNA synthesis; once the enzyme has loaded the DNA template and has begun RNA elongation, rifamycins are blocked from access to their binding site. In this respect, the mechanism of action for rifamycins has some similarity to that of macrolides.

There is a significant variability in the susceptibilities of mycobacteria to the rifamycins, although much of this variability is believed to result from the impermeability of the mycobacterial cell wall, as the purified DNA-dependent RNA polymerases are sensitive to these agents (122, 167).

Acquired rifamycin resistance in *M. tuberculosis* has been well documented (316, 462) and is conferred primarily by mutations

in the *rpoB* gene, which encodes the β-subunit of the RNA polymerase (378). Similar mutations in the *rpoB* genes of *M. kansasii*, *M. leprae*, the MAC, and *M. ulcerans* also confer rifamycin resistance (30, 190, 195, 233, 275), although a significant proportion of resistant MAC strains may lack a missense mutation in this gene (195). The most common mutations associated with clinically acquired resistance in *M. tuberculosis* cluster in an 80-bp region of the *rpoB* gene, with approximately 80% of isolates having a mutation in codon 526 or 531 (305, 461). Evidence suggests that a similar clustering of resistance-associated mutations also occurs in other mycobacteria.

Interestingly, the mutations associated with resistance to different rifamycins do not always match, an observation which appears to explain why rifamycins such as rifapentine, rifabutin, and rifalazil are active *in vitro* against some *M. tuberculosis* strains that are resistant to rifampin (248, 281, 438, 454). However, the most common *rpoB* mutations in rifampin-resistant *M. tuberculosis* (i.e., codons 526 and 531) also confer high-level resistance to the other rifamycins.

Although clinically acquired resistance to rifamycins in mycobacteria results from the selection of organisms with *rpoB* gene mutations, the mechanisms underlying the lack of efficacy of rifamycins or intrinsic resistance are less clear. As with other antimicrobial agents, the cell wall most likely has a significant role in intrinsic resistance to rifamycins. However, unlike most other antimycobacterial agents, evidence suggests that active rifamycin efflux from mycobacteria is minimal at best (287) and, thus, probably not contributory to either acquired or intrinsic resistance. A possible alternative mechanism that affects rifamycin activity is drug inactivation by ADP-ribosylation (29). This process is mediated by ADP-ribosylases encoded by the *arr* gene; homologs of the *arr* genes are found throughout the bacterial kingdom, including mycobacteria (87). The expression of the *arr* gene in *M. smegmatis* was correlated with a reduced activity of rifampin (302), and the enzyme appears to be able to inactivate other rifamycins, including rifabutin (29). The *arr* gene is present in the genome of *M. abscessus* (GenBank accession number YP_001701343), which is considered to be intrinsically resistant to rifamycins. In addition to ADP-ribosylases, rifamycin resistance may be conferred by some flavin adenine dinucleotide (FAD)-monooxygenases, although to date, this has been demonstrated only for *Rhodococcus equi* (9).

**Trimethoprim and sulfonamides.** Trimethoprim and sulfonamides, such as sulfamethoxazole, are inhibitors of microbial folate metabolism (353, 354). The disruption of this process leads directly to a reduction in the synthesis of the building blocks of nucleic acids and some amino acids (194). Although trimethoprim and sulfonamides interfere with the same process, the molecular targets of trimethoprim and sulfamethoxazole are different. Trimethoprim binds to and inhibits the dihydrofolate reductase (DHFR), inhibiting the conversion of dihydrofolate into tetrahydrofolate. The *dfrA* gene encodes the mycobacterial DHFR. Sulfonamides bind to the enzyme dihydropteroate synthetase (DHPS) and inhibit the conversion of 7,8-dihydro-6-hydroxy-methylpterin-pyrophosphate and *para*-aminobenzoic acid (PABA) to 7,8-dihydropteroate. In fact, sulfonamides are competitive antagonists of the binding of PABA to DHPS. Although chemically distinct from sulfonamides, the antileprosy agent dapsone is also an inhibitor of DHPS (198). Depending on the species, either the *folP1* gene or the *sulI* genes encode the mycobacterial type 1 DHPS. Although most of the data on the interaction of trimethoprim and sulfonamides come from nonmycobacterial species, several studies demonstrated that trimethoprim and sulfonamides bind to and inhibit the DHFR and DHPS of NTM (7, 17, 25, 38, 65, 67, 72, 116, 272, 326, 352).

Although resistance to trimethoprim and sulfonamides has been reported for mycobacteria, the underlying mechanisms have not been elucidated in most cases. For *M. avium*, intrinsic trimethoprim resistance appears to correlate with the resistance of the DHFR to inhibition by this agent (366). Furthermore, an *in vitro*-selected trimethoprim-resistant variant of *M. smegmatis* had a mutated DHFR with reduced inhibition by this agent (352). Similarly, dapsone resistance in *M. leprae* is associated with mutations in the *folP1* gene (62, 128, 209, 439), suggesting that sulfonamide resistance is also probably related to the resistance of the DHPS.

In other bacteria, acquired resistance to these agents is also conferred by mutations in the genes encoding DHFR and DHPS (354). High-level resistance to trimethoprim has been associated with mutations in the regulatory region of the DHFR gene leading to an increase in the expression level of the enzyme by 2 orders of magnitude (111). However, the horizontal transfer of resistant alleles accounts for the acquisition of sulfonamide and trimethoprim resistances in many normally susceptible pathogens (169, 353, 354). The acquisition of these exogenous alleles is believed to occur via plasmids, although many are also associated with integrons (169, 353, 354).

## TREATMENT OF NONTUBERCULOUS MYCOBACTERIAL INFECTIONS

### Pulmonary

***Mycobacterium avium* complex.** The *M. avium* complex (MAC) is the mycobacterial pathogen most commonly isolated from respiratory samples. The complex was originally composed of two species, *M. avium* and *M. intracellulare* (132).

Within the *M. avium* species, four subspecies (*M. avium* subsp. *avium*, *M. avium* subsp. *sylvaticum*, *M. avium* subsp. *hominissuis*, and *M. avium* subsp. *paratuberculosis*) have been designated (394).

*M. avium* subsp. *avium*, previously referred to as *M. avium*, is the etiologic agent of avian tuberculosis (TB). Disease in birds is classical and is the source of the type strain, ATCC 25291. It has only rarely been associated with human disease, which is caused by *M. avium* subsp. *hominissuis*. The latter subspecies is a recognized cause of human disease such as disseminated infection in AIDS, cervical lymphadenitis in children, and chronic lung disease in patients with cystic fibrosis (CF) and older adults with bronchiectasis (394).

*M. avium* subsp. *sylvaticum* is also known as the "wood pigeon bacillus" and causes TB-like lesions in these birds. The type strain, ATCC 48898, was isolated from the liver and spleen of a wood pigeon (394).

*M. avium* subsp. *paratuberculosis* is the cause of Johne's disease, a chronic granulomatous enteric infection which was originally identified in cattle and wildlife. There is evidence that this subspecies may also have a role in the pathogenesis of Crohn's disease of the bowel in humans, at least in some patients (394). Human infection is difficult to diagnose due to the pathogen's extremely slow growth and dependence on mycobactin (a mycobacterial growth factor) (394).

Molecular studies have revealed the presence of more than 30 additional taxonomic groups or sequence variants (sequevars) within the MAC. These taxa have been grouped as MAC "X" and are distinct from *M. avium* and *M. intracellulare*. Two members of this group were recently elevated to species status (*M. colombiense* [253] and *M. chimaera*). Five other taxa have been given species status, *M. vulneris* (400), *M. marseillense* (333), *M. timonense* (333), *M. arosiense* (21, 388), and *M. bouchedurhonense* (333), and were proposed for addition to the *M. avium* complex based on their genetic relatedness (386, 387).

Tortoli et al. (386, 387) suggested an unusually high virulence of *M. chimaera* in seven immunocompetent patients. *M. colombiense* (253) and *M. avium* thus far seem to be associated more often with disseminated disease, especially among HIV-positive ($HIV^+$) patients (132). In a 2006 study, 227 clinical MAC isolates from Thailand were differentiated into species and types by using PCR-enzyme analysis of the *hsp65* gene (299). Those investigators characterized three distinct types of *M. avium* (*M. avium* I, II, and III) and two types of *M. intracellulare* (Min I and Min IV) by PCR-restriction enzyme analysis of the 65-kDa heat shock protein. The distributions of *M. avium* and *M. intracellulare* types varied among isolates from different sources. Seventy-seven percent of blood isolates were *M. avium* I isolates, and 10.3% were *M. avium* II isolates. In contrast, 51.9% of the pulmonary specimens were positive for *M. intracellulare* (44.2% were *M. intracellulare* I). *M. intracellulare* IV was isolated from blood more often than *M. intracellulare* I. For other specimens, *M. avium* I was the most common type seen: 80.6% of 67 isolates (mostly blood samples) from $HIV^+$ patients were *M. avium* I isolates, while 11.9% were *M. avium* II and 7.5% were *M. intracellulare* IV isolates (299). Additionally, patients with *M. avium* infection were younger adults (20 to 39 years old), while patients with *M. intracellulare* infection were older adults (≥60 years old). Those authors concluded that *M. avium* may cause a more disseminated, invasive disease than *M. intracellulare* (299). A prior study of data from 1999 to 2003 at a large cancer center in Texas showed that *M. intracellulare* was more pathogenic in older women (aged ≥50 years) regardless of the underlying disease (145).

To complicate the identification and reporting of MAC isolates, the subspecies of *M. avium*, including *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, and *M. avium* subsp. *hominissuis*, have identical 16S rRNA gene sequences and thus are not separated by 16S rRNA gene sequencing. *M. colombiense*, *M. intracellulare*, and *M. chimaera* differ from *M. avium* by only 6 bp, 9 bp, and 10 bp, respectively, and only 1 bp discriminates *M. chimaera* and *M. intracellulare* (66, 394).

Sequencing of the 16S-23S internal transcribed spacer (ITS) region has been useful for studies of the variability within the MAC (274). More than 45 MAC sequevars, including Mav A to Mav H for *M. avium* and Min A to Min D for *M. intracellulare*, have been identified (120, 121). Many other MAC sequevars, designated MAC A to MAC Y (365) and Nov, exist for those novel species of the MAC that are not *M. avium* or *M. intracellulare*, some of which have been recently established as *M. chimaera* (MAC A), *M. marseillense* (MAC D), *M. arosiense* (MAC S), and *M. colombiense* (MAC X). The new MAC species *M. bouchedurhonense* and *M. timonense* contain new ITS sequevars that cluster with MAC Q and MAC L/M, respectively (21, 333, 365). Other subspecies, such as *M. avium* subsp. *avium*, *M. avium* subsp. *silvaticum*, and *M. avium* subsp. *paratuberculosis*, have also been assigned to the Mav A sequevars (66, 394).

Similar to the ITS region, the sequencing of the *hsp65* gene (Telenti fragment) is not adequate for the differentiation of isolates of *M. avium* subsp. *avium*, *M. avium* subsp. *silvaticum*, and *M. avium* subsp. *paratuberculosis* and most *M. avium* subsp. *hominissuis* isolates. However, a clear differentiation of these subspecies is possible by sequencing outside the Telenti fragment (368, 394).

**(i) Presentation of disease.** The treatment considerations for MAC lung disease differ based upon the presentation of disease and findings on chest radiographs and high-resolution computerized tomography (CT) (HRCT) scans (132). Traditionally, MAC apical fibrocavitary disease has been associated with males in their late 40s and early 50s, usually with a history of heavy cigarette smoking and often excessive alcohol use (132). This type of MAC disease behaves much like tuberculosis and is most rapidly progressive, often resulting in extensive lung destruction and mortality if left untreated (132).

The second type of MAC presentation is more commonly seen in nonsmoking, white, postmenopausal (mean age, 60 years) women (188). On chest CT scans, these patients have nodular and interstitial infiltrates frequently involving the right middle lobe or lingula area. This type of MAC presentation is also referred to as "nodular bronchiectasis" or "nodular bronchiectasis disease," and the progression of the disease is generally slower than that of MAC fibrocavitary disease (132). Symptomatically, patients often have chronic cough and debilitating fatigue. Less frequently, patients may present with fever, hemoptysis, and/or weight loss. Follow-up may require months to years to show clinical changes. The HRCT findings for this type of MAC presentation generally show multiple, small (≤5-mm), peripheral nodules ("tree in bud") on the bronchovascular tree and cylindrical bronchiectasis. Of interest, a recent report from Queensland, Australia, indicated that the pattern of pulmonary disease in that area has changed from primarily cavitary disease in middle-aged men to fibronodular disease in women (381).

Patients with nodular MAC disease often have complicating respiratory cultures positive for other pathogens, such as *Pseudomonas aeruginosa* and other bacteria and occasionally other NTM, including *M. abscessus*. Treatment is usually directed at the eradication of the nonmycobacterial pathogen(s) in addition to the MAC strain (132).

Controversy still exists as to whether bronchiectasis is the result of mycobacterial infection or is a risk factor for mycobacterial infection (132, 375). One recent study suggested that granulomatous inflammation was the cause of bronchiectasis. In other presentations, such as that with cystic fibrosis (CF) or prior pulmonary TB, bronchiectasis clearly antedates MAC disease (123, 132). For further discussion, see Risk Factors and Immunodeficiency Defects below.

Even with the introduction of macrolides, MAC lung disease presents challenging treatment issues for the clinician. The optimal treatment regimen for pulmonary MAC infection has not been established. Until the introduction of the macrolides in the 1990s, the management of MAC was dismal. Since that time, the cornerstone of MAC treatment has been a macrolide (clarithromycin or azithromycin) (Table 1).

It is known that most first-line antituberculous agents have poor *in vitro* activity, and *in vitro* MIC testing results do not cor-

relate well with the clinical response. Thus, there is no role for the routine *in vitro* testing of these agents, including rifampin, rifabutin, streptomycin, and ethambutol (191, 192, 314, 453).

Early MAC clinical trials typically incorporated three drugs, including rifampin or rifabutin, ethambutol, and a macrolide (132–137, 407, 408, 431, 432). Gordin et al. showed that rifabutin did not improve efficacy when added to a macrolide and ethambutol but did decrease the development of macrolide resistance in 14% of patients in a series of AIDS patients with disseminated MAC (DMAC) disease who had a bacteriologic response at 16 weeks of therapy. However, there was no difference in the incidence of resistance in the nonresponder group (130, 132, 138).

A subsequent study of more than 300 patients with pulmonary disease on a three-drug macrolide-containing regimen that included ethambutol and rifampin or rifabutin given three times weekly (TIW) showed a macrolide resistance rate of only 4%. This study emphasized the importance of ethambutol and a rifamycin in protection against macrolide resistance in both responders and nonresponders (138).

Multiple studies have shown the importance of ethambutol as a component of the treatment regimen for pulmonary MAC infection (130, 132, 139, 345). The primary and most serious adverse effect associated with ethambutol is ocular toxicity. This is clearly a dose-related toxicity and not a hypersensitivity reaction. Patients on a high dose of drug (usually 25 mg/kg of body weight daily) present with blurred vision; decreased acuity; central scotomas; and, often, a loss of red-green color discrimination, and optic or retrobulbar neuritis may occur in one or both eyes. Sometimes, the central fiber of the optic nerve is affected, but more often, peripheral constriction of the visual fields occurs (139).

Patients receiving ethambutol should be monitored for visual acuity and red-green color discrimination. In the majority of cases, ethambutol ocular toxicity is reversible upon the cessation of the drug. In rare cases, no improvement is seen (139).

The macrolide remains the major therapeutic agent for the treatment of MAC lung disease. Clarithromycin, as the class agent for the two newer macrolides (clarithromycin and azithromycin), has shown excellent *in vitro* activity against isolates of the MAC, in contrast to the antituberculous agents discussed above. Although azithromycin is chemically considered an azalide rather than a "true" macrolide, the term "macrolide" has been used for both agents because of the comparable activities and close similarity of the two drugs (132, 134, 136). Both azithromycin and clarithromycin have shown increased concentrations in phagocytes and tissues, including the lung (100, 132, 181, 408).

Not only the types (oral and parenteral) of companion drugs used but also the duration and need for daily versus intermittent dosing (three times weekly) must be considered in the management of MAC lung disease.

Over the past 2 decades, several studies have shown the efficacy of clarithromycin- and azithromycin-containing regimens with companion drugs (ethambutol, rifampin, or rifabutin with or without streptomycin) (132–137, 432). All wild-type (untreated) MAC isolates have susceptible MICs ($\leq$8 μg/ml) of clarithromycin compared to resistant isolates, with MICs of $\geq$32 μg/ml, which are associated with microbiologic and clinical relapse (132, 138, 432). The latter isolates also have a point mutation in the macrolide-binding peptidyl transferase region of the 23S rRNA gene that is not present in susceptible strains of the MAC (138, 237, 239).

Multiple studies of daily treatment regimens containing macrolides with companion drugs have shown these regimens to be safe and effective for the treatment of MAC lung disease. However, they are not well tolerated, especially in older women (132–134, 136, 407, 408). Subsequent studies have also concluded that intermittent treatment using macrolide-containing regimens appears to be comparable to daily regimens in pulmonary MAC disease but better tolerated (132–134, 136, 432a).

Thus, the therapeutic regimens discussed above emphasize the need for multiple-drug therapy in the treatment of MAC lung disease. The ATS has recently stated that a three-drug regimen (macrolide, ethambutol, and a rifamycin) is preferred to a two-drug regimen because of concern for the development of macrolide resistance. The potential role of other antimicrobials such as fluoroquinolones, clofazimine, and inhaled amikacin has not yet been established. Macrolides should never be used as a single-agent therapy (132, 407, 408).

Some experts believe that microbiological cure may not be possible in some populations, including the elderly, frail individuals for whom drug intolerance and drug interactions are not uncommon. More aggressive regimens may be more suitable for patients with rapidly progressive, usually cavitary disease, and less aggressive treatment may be appropriate for patients with drug intolerance and/or indolent disease. The addition of a fourth agent, such as injectable streptomycin or amikacin, is based on the age, weight, and clinical condition of the patient (132).

**(ii) Cystic fibrosis.** In recent studies, patients with underlying lung disease such as CF were more likely to show progressive changes in HRCT scans (365). The largest multicenter prevalence study of pulmonary NTM involving 1,582 patients with CF (mean age, 18.9 years) was performed in France recently (329). This prospective study showed an NTM prevalence of 6.6%, with 3.6% of patients meeting the current ATS criteria for clinical significance, compared to a previous U.S. multicenter study with an NTM prevalence of 13%, with MAC isolates recovered in 72% of patients in 21 U.S. centers (132, 276, 277, 329). In the American study, an older age of CF patients (mean age, 26 $\pm$ 11 years) ($P$ = 0.0002) was the most significant predictor of NTM disease (276). Those investigators concluded that the association of NTM with milder lung disease (higher forced expiratory volume [FEV] and lower frequency of concomitant *P. aeruginosa* infections) suggested that patients with more severe CF disease die prior to having enough exposure to acquire the NTM or that some factor related to the ability to attain older age with mild CF disease may predispose these patients to NTM (276, 277). Those authors also found that cultures of *M. intracellulare* were often smear positive (46% versus 18%) and found in multiple samples (39% versus 23%) compared to those of *M. avium* (276, 277). Abnormal HRCT findings were also predictive of the progression of disease in patients with MAC infection, emphasizing the need for close monitoring of CF patients with NTM. CF patients with MAC infection tended to meet the ATS criteria for clinical significance more often than did CF patients with other NTM. Although most CF patients with cultures positive for NTM did not meet the ATS criteria for disease at the time of the first positive culture, the ATS recommends the careful monitoring of these patients with cultures, especially for macrolide-resistant NTM, which may develop due to the low-dose and long-term azithromycin therapy administered for its immunomodulatory properties (132, 331, 332). Other clinical measures, such as CT chest scans, lung function

studies, and weight assessment, before the beginning of specific treatment for NTM may aid in the interpretation of the response to treatment (376). CF patients with pancreatic insufficiency and possible concomitant drug interactions may also have an inconsistent absorption of oral drugs. Thus, an assessment of drug levels may be needed (129, 132).

Finally, the diagnostic and treatment regimens for NTM lung disease in patients with CF are currently the same as those for patients without CF. However, because of underlying disease, multiple concomitant infections, and various medical therapies for other associated conditions, the application of the diagnostic criteria and treatment options may be more limited or difficult (132, 276).

For treatment purposes, given the overlapping coverage of some drugs (e.g., aminoglycosides) used to treat NTM with bacterial CF pathogens such as *P. aeruginosa*, Olivier et al. (276) recommended that airway clearance measures should be intensified and that bacterial airway pathogens should be treated aggressively before the initiation of treatment for MAC disease or other NTM in order to be better able to assess the efficacy of treatment for NTM infection (374).

**(iii) MAC hypersensitivity pneumonitis.** Recently, a syndrome known as "hot tub lung," associated with the MAC and exposure to indoor spas, aeration systems, and at least one household shower, has been described (6, 102, 132, 231). Because of the uncertainty of this syndrome as an inflammatory or an infectious process or a combination of both processes, there are no standardized guidelines on which to base treatment recommendations. In contrast to typical MAC lung disease, corticosteroids may encourage recovery. Antimycobacterial therapy, if deemed necessary, is usually given for shorter time periods (i.e., 3 to 6 months), provided that symptoms resolve, chest radiographs improve, and sputum cultures are negative. The consensus among mycobacterial infectious disease experts is that a complete avoidance of the use of indoor hot tubs is necessary for patients with documented hypersensitivity pneumonitis (132).

***Mycobacterium abscessus.*** *M. abscessus* is the causative agent of more than 80% of pulmonary disease due to RGM (132, 141). Multiple cultures of *M. abscessus* from respiratory sites are usually diagnostic of disease. *M. abscessus* can present similarly to MAC disease, most often as a chronic lung infection in older, nonsmoking women with bronchiectasis (132).

Preliminary studies have shown that *M. abscessus* is second only to the MAC in prevalence among patients with CF and the third most common NTM respiratory pathogen in the United States after the MAC and *M. kansasii* (132). A 2010 report also showed that *M. abscessus* was the predominant species among patients with cancer and NTM pulmonary disease (312).

The treatment of *M. abscessus* has been hampered by its resistance to standard antituberculous agents and other antimicrobials. Acquired mutational resistance to amikacin and other aminoglycosides is often seen in patients with CF. Although wild-type (untreated) isolates have susceptible or intermediate MICs of macrolides at 3 days, isolates of *M. abscessus* subsp. *abscessus* have resistant MICs after extended incubation, which is the result of an inducible methylase gene [*erm*(41)] which confers macrolide resistance (263). The presence of a functional *erm* gene in *M. abscessus* subsp. *abscessus* (formerly *M. abscessus*), in contrast to its absence in *M. abscessus* subsp. *bolletii* (formerly *M. massiliense*), has recently been studied. Patients with *M. abscessus* subsp. *bolletii* infection had a higher response rate (approximately 90%) than patients with *M. abscessus* subsp. *abscessus* infection (approximately 25%) (1, 193).

The macrolides clarithromycin and azithromycin are the major therapeutic agents for *M. abscessus* subsp. *bolletii*, which lacks a functional *erm* gene, but the importance of macrolides is much less certain for isolates of *M. abscessus* subsp. *abscessus*, which has a functional *erm* gene (193). Doses of 500 mg of clarithromycin twice daily and 250 to 500 mg azithromycin have been previously described for the treatment of infections caused by the *M. abscessus* group.

Untreated isolates of *M. abscessus* also have low or intermediate MICs (≤32 to 64 μg/ml) of cefoxitin, and approximately half of the isolates show resistance to linezolid (≥32 μg/ml). When linezolid is used for the treatment of NTM lung infection, some investigators recommend the concomitant administration of pyridoxine to reduce the effects of peripheral neuropathy. Most isolates are resistant to doxycycline, ciprofloxacin, and moxifloxacin. Some isolates show susceptibility or intermediate susceptibility to imipenem, but the percentage is difficult to assess, since *in vitro* testing yields inconsistent results, and most laboratories do not report MICs for this drug, as per previous CLSI recommendations (452).

Tigecycline is a newer parenteral minocycline derivative that has potent *in vitro* activity against isolates of *M. abscessus*. Tigecycline MICs for most isolates of *M. abscessus* is ≤1 μg/ml, but there are no established breakpoints of this agent for mycobacteria (132, 451, 452).

The most active agent against *M. abscessus* is amikacin. It is administered daily or three times weekly depending upon clinical circumstances. This dose should provide peak drug levels in the low 20-μg/ml range (132). For difficult situations, such as macrolide-resistant *M. abscessus* strains, most experts recommend a combination of parenteral agents based on *in vitro* MICs. Amikacin combined with cefoxitin (12 g in divided doses) or imipenem (1 g twice daily) for a minimum of 8 weeks is recommended for the initial treatment of adults with *M. abscessus* subsp. *bolletii* infection, while adult patients with *M. abscessus* subsp. *abscessus* infection generally remain on both drugs for the duration of therapy (132, 193). In less serious cases or for patients who cannot tolerate some of the treatment regimens, some symptoms can be managed with intermittent treatment using a macrolide in combination with one or more parenteral agents or an even less intensive regimen of periodic parenteral antibiotics or oral macrolide therapy. A major caveat is that patients who have difficulty with antibiotic regimens should consult an expert in *M. abscessus* lung disease to explore available regimen options (132).

Most experts recommend a goal of 6 to 12 months of sputum cultures negative for *M. abscessus* subsp. *bolletii* as a reasonable endpoint for treatment, but currently, there are no antimicrobial regimens sufficient to achieve this endpoint for patients with *M. abscessus* subsp. *abscessus* infection. Six months of culture negativity is the usual endpoint for the latter subspecies. Alternatively, some experts recommend other endpoints, such as symptomatic and/or radiographic improvement or decreased numbers of organisms and/or positive respiratory cultures. Multidrug regimens including amikacin and cefoxitin or amikacin plus imipenem for 2 to 4 months may result in clinical and microbiological improvement (132) (Table 1).

***Mycobacterium chelonae.*** Pulmonary infection with *M. che-*

*lonae* occurs but is less commonly seen than infection with *M. abscessus*. The successful regimen for the treatment of *M. chelonae* lung infection probably includes a macrolide, since isolates of *M. chelonae* do not possess an *erm* gene that would induce macrolide resistance, combined with one or more antimicrobials selected on the basis of results of *in vitro* susceptibility testing. Unlike *M. abscessus* lung disease, tobramycin is the preferred aminoglycoside rather than amikacin based on *in vitro* MICs. Approximately 25% of isolates of *M. chelonae* are susceptible to ciprofloxacin and doxycycline. Linezolid is also an option, as more than half of the isolates have susceptible MICs of this agent. Although an optimal regimen has not been established for the treatment of *M. chelonae* infection, most experts recommend a treatment duration that would include 12 months of negative sputum cultures, just as for most other lung diseases caused by NTM (132) (Table 1).

***Mycobacterium fortuitum.*** *M. fortuitum* lung disease is rare and most often associated with patients who have gastroesophageal disorders with chronic vomiting, including achalasia and lipoid pneumonia (422).

Because isolates of *M. fortuitum* are typically susceptible to several oral and parenteral agents, including the quinolones, sulfonamides, imipenem, doxycycline (50%), cefoxitin (50%), and the aminoglycosides, most experts recommend that therapy with at least two antimicrobials with *in vitro* activity should be given until the patient has at least 12 months of negative sputum cultures (132) (Table 1).

Recent studies have indicated that isolates of *M. fortuitum* and the related species *M. boenickei*, *M. houstonense*, and *M. neworleansense* contain an inducible *erm*(39) gene that confers macrolide resistance. This resistance is not always detected with a routine 3-day incubation and may require additional incubation for up to 14 days to observe this resistance (261, 262, 266). With this fact in mind, macrolides should be used judiciously for the treatment of *M. fortuitum-M. smegmatis* group (including *M. goodii*) lung disease.

A rare case of pulmonary infection due to *M. fortuitum* associated with an antigen-selective defect in cell-mediated immunity to the organism was also described (244). A defect in cellular immunity to purified protein derivative (PPD) from *M. fortuitum* was shown to be antigen selective, evidenced by a normal response to PPD from *M. tuberculosis* and several other antigens accompanying extended *M. fortuitum* infection (244).

***Mycobacterium immunogenum* and hypersensitivity pneumonitis.** As previously described, *M. immunogenum* has been recovered from metalworking fluids (MWF) of metal grinders with hypersensitivity pneumonitis (441). The main therapy for these patients is removal from MWF and corticosteroids. Only rarely is antimicrobial therapy given (132).

***Mycobacterium mucogenicum.*** *M. mucogenicum* is rarely associated with pulmonary infection. When found in respiratory samples, the species most often represents contamination from an environmental source, especially tap water (423).

***Mycobacterium malmoense.*** Although rarely recovered in the United States, most isolates of *M. malmoense* have been found in northern Europe, Zaire, and Japan (124, 132, 151, 458). Worldwide, the prevalence of *M. malmoense* is second only to the MAC in isolates from sputum samples and lymph nodes of children. A recent study from the Netherlands showed that 80% of 40 patients with pulmonary disease associated with *M. malmoense* had cavitary disease and were males with preexisting chronic obstructive disease (157).

*In vitro* susceptibility testing of *M. malmoense* may be difficult due to its often poor growth. The original isolates of *M. malmoense* (recovered prior to the advent of macrolides and quinolones) were reported to be susceptible to ethambutol, kanamycin, ethionamide, and cycloserine but resistant to multiple agents including isoniazid, streptomycin, rifampin, and capreomycin (132, 342). Hoefsloot and colleagues (157) reported susceptibility to rifampin (72%), rifabutin (96%), cycloserine (98%), and macrolides (100%), in contrast to resistance to amikacin and streptomycin (both 70%), ciprofloxacin (61%), and isoniazid (100%), in 46 isolates of *M. malmoense* cultured from 2002 to 2006. The lack of consistency in susceptibility and, like the MAC, a lack of correlation between *in vitro* susceptibilities and clinical responses may account for some of the difficulties in treatment. Combination therapy with isoniazid, rifampin, and ethambutol with and without quinolones and macrolides has resulted in microbiological improvement (58, 61, 132, 313, 315) in some cases. The British Thoracic Society has advised a combination regimen of rifampin and ethambutol with or without isoniazid, a quinolone, and a macrolide for a total of 2 years for the treatment of *M. malmoense* (254).

***Mycobacterium simiae.*** Patients with *M. simiae* infection are usually older nonsmoking females with bronchiectasis, the same patient population at risk for MAC and *M. abscessus* infections.

Treatment for *M. simiae* is difficult due to the extreme resistance of this species to most antimicrobials, including MICs of >4 μg/ml for rifabutin. Although the optimal therapeutic regimen has not been established, most experts recommend a macrolide-based regimen along with a quinolones (preferably moxifloxacin rather than ciprofloxacin) and a sulfonamide for the successful treatment of *M. simiae* infection (101, 132) (Table 1).

***Mycobacterium szulgai.*** Pulmonary disease due to *M. szulgai* is very similar to that caused by *M. tuberculosis*. Most patients are males over 50 years of age with risk factors similar to those for cavitary MAC disease (alcohol abuse, smoking, chronic obstructive pulmonary disease [COPD], and a history of pulmonary TB). Because *M. szulgai* is rarely found in the environment, the diagnosis of *M. szulgai* may often be made with one positive culture in the appropriate clinical setting (132, 389, 399).

Unlike MAC isolates, *M. szulgai* isolates are often susceptible *in vitro* to most antituberculous agents. Most experts recommend combinations of two or more susceptible antimicrobials. Again, although an optimal regimen has not been established, a macrolide-containing regimen combined with two or more antimicrobials and 12 months of sputum negativity are probably sufficient for successful treatment (132).

***Mycobacterium terrae* complex.** The *M. terrae* complex is common in tap water, so only rarely is it considered clinically significant when recovered from sputum. A few cases of *M. terrae* complex (*M. terrae*, *M. nonchromogenicum*, *M. triviale*, and *M. arupense*) lung disease have been described, including a case of localized cavitary lung disease and another patient on high-dose chemotherapy who had undergone an autologous bone marrow transplant (132, 357).

Although the optimum treatment for *M. terrae* complex pulmonary disease has not been established, the use of a macrolide plus ethambutol or other agents, such as trimethoprim-sulfame-

thoxazole and a quinolone, based on *in vitro* susceptibility, is likely to be successful (132).

Recently, isolates of a new species, *M. arupense*, have been included in this complex. Currently, the numbers of isolates tested are inadequate to establish an optimal regimen (79).

***Mycobacterium xenopi.*** *M. xenopi* differs from most of the other pulmonary NTM in its preference for higher temperatures (i.e., 42°C to 45°C) (225).

In Canada, the United Kingdom, and other areas of Europe, *M. xenopi* is second to the MAC as a cause of NTM lung disease. *M. xenopi* is recovered less often in the United States, although it has been reported from the northeastern and southeastern areas of the United States (132, 313).

The response of *M. xenopi* to treatment has been variable and does not always correlate with *in vitro* susceptibility. Susceptibility testing of *M. xenopi* can be difficult due to its poor growth in broth and its thermophilic requirement, and this may also account for the lack of quality MIC data. Some reports indicated a susceptibility of the species to most first-line antituberculous drugs, whereas other reports asserted resistance to rifampin, ethambutol, and isoniazid (Table 1). Most experts, however, recommend a macrolide-containing regimen similar to those for MAC infections and continuation of the regimen until at least 12 months of negative sputum cultures have been achieved (331). Some investigators advocate 15 to 18 months of negative cultures to prevent a possible relapse of *M. xenopi* infection. One observation indicated that while conversion may occur within 12 months, relapse rates are high, even with macrolide-containing regimens. Some investigators prefer to add a quinolone with or without an initial regimen of streptomycin (132).

***Mycobacterium kansasii.*** *In vitro* susceptibility testing of *M. kansasii* should be restricted to rifampin, and testing against other agents is not warranted due to the consistent susceptibility pattern of rifampin-susceptible *M. kansasii*. Rates of conversion of sputum cultures with rifampin are almost 100%, and treatment failure is almost always associated with rifampin resistance (2, 216, 418, 451). The ATS treatment recommendations include a daily regimen of rifampin, ethambutol, and isoniazid with pyridoxine until the sputum cultures are negative for 12 months. Because of the critical importance of the rifamycins to the regimen for treatment of *M. kansasii* infection, patients on antiretroviral regimens may substitute a macrolide or moxifloxacin for a rifamycin (132).

Griffith et al. (140) also showed the efficacy of a three-times-weekly macrolide-containing regimen combined with ethambutol and rifampin until cultures are negative for 12 months for a small group of 14 patients with *M. kansasii* lung disease. Many physicians currently utilize this regimen, although it has not been studied in larger clinical trials.

For patients with rifampin-resistant *M. kansasii* infection, a three-drug regimen based on *in vitro* susceptibilities, including a macrolide, ethambutol, moxifloxacin, sulfonamide, or streptomycin, has been recommended by the ATS (132) (Table 1). As with MAC lung disease, 12 months of sputum negativity while on treatment seems to be a reasonable duration of treatment (132, 140) for both rifampin-susceptible and rifampin-resistant isolates for either daily or intermittent treatment regimens.

**Miscellaneous species.** Other NTM, including *M. mageritense* (170, 414), *M. haemophilum* (347, 436), *M. goodii* (48), *M. triplex*, *M. lentiflavum* (330), *M. celatum* (290, 291), *M. interjectum*, *M. intermedium*, *M. branderi*, *M. heidelbergense*, and *M. heckeshornense*, have been recovered from respiratory sites and may be associated with disease (54, 367, 385, 436). Additionally, *M. brumae*, *M. confluentis*, *M. kubicae*, and *M. holsaticum* have been recovered from respiratory samples; however, their pathogenicity has not been confirmed (385, 386). *M. haemophilum* may be an underrecognized cause of pulmonary disease and should be considered especially for AFB smear-positive, culture-negative patients (436). Currently, there is a paucity of data concerning treatment for these species, and no specific treatment has been established. However, as for many NTM, most experts recommend *in vitro* susceptibility testing and a macrolide-containing regimen with companion drugs such as rifampin and ethambutol for slowly growing species. For rapidly growing species (*M. brumae*, *M. confluentis*, and *M. goodii*), if found to be clinically significant, a macrolide along with one or two companion drugs based on *in vitro* susceptibility test results is probably warranted (132). Isolates of *M. goodii* carry the *erm*(38) gene and, thus, may be inducibly resistant; consequently, infections may be intractable to macrolide-based treatments. Isolates of *M. mageritense*, however, are uniformly resistant to macrolides, owing to the presence of the *erm*(40) gene, necessitating an alternate regimen of perhaps a quinolone with a sulfonamide and/or doxycycline (170, 414).

## Surgery

In general, surgical resection is advocated for patients in specific circumstances who have localized lung disease in one lung and who can tolerate the surgery. Patients who fail drug therapy, develop macrolide-resistant mycobacteria, or have severe complications such as hemoptysis may be candidates for surgery (132, 175). One caveat is that lung surgeries should be performed by surgeons experienced in this type of surgery to avoid unnecessary complications.

Results of surgery vary; however, in the macrolide era, morbidity and mortality have generally decreased compared to those associated with premacrolide surgeries (349, 350). In the premacrolide era, Corpe (82) reported a 7% perioperative mortality rate with a rate of significant postoperative complications of 18% for the surviving patients from a series of 131 patients before macrolides were available. Another study of 37 patients showed improved long-term outcomes and only two relapses, with less morbidity and no mortality (251).

Later, Pomerantz et al. (296) reported that 13 patients with infected middle lobe, lingula, or both (12 with MAC infection and 1 presumably with *M. abscessus* infection, but details of the species identification were not given) had successful surgeries with no deaths and only 2 patients who required additional antibiotic treatment. Shiraishi et al. (349, 350) also indicated that 94% of 33 patients who underwent pulmonary resection in Japan were sputum culture negative following surgery. Those authors did, however, note two late deaths: one patient died of unknown causes 12 years after the surgery, and another died of respiratory failure 2 years postoperatively. Those authors also stated that the high rate of conversion to a sputum-negative status prior to surgery is crucial to the low rate of postoperative complications, although this is not possible for all patients. Thus, those authors advocated surgical resection as early as possible before the disease becomes too extensive (349). Another study of 28 patients in a Texas hospital also showed that >90% of patients achieved a culture-negative status and remained in control of the disease permanently following surgical resection (268).

Recently, Yu and colleagues (455) described a cohort of 134 patients with MAC infection who were treated with antimicrobials several months prior to thoracoscopic lung resection. This was the largest reported study of patients undergoing thoracoscopic anatomic lung resection due to pulmonary NTM disease or bronchiectasis. There was no operative mortality, and complications were reported for only 12 cases (7%).

A previous study by Zhang et al. (460) reported less successful outcomes for a cohort of 790 patients. Success rates were 70 to 98%, compared the success rate of 97% reported by Yu et al. (455).

For other mycobacterial lung infections, such as that caused by *M. kansasii*, antimicrobial treatment without surgery is usually adequate. In contrast, patients with *M. abscessus* (178), *M. simiae*, and *M. xenopi* (202) infections may require surgical resection for the permanent control of their disease (269). There was also a report of a successful pneumonectomy in an infant with severe necrotic lung infection due to *M. fortuitum* (280).

### Disseminated Disease

***Mycobacterium avium* complex.** In the early 1980s and 1990s, disseminated disease was often associated with MAC infection in patients with AIDS. Prior to the discovery of AIDS, there were fewer than 100 cases of disseminated nontuberculous mycobacterial (DNTM) infection reported (163, 164, 446). Due to the development of better antiretroviral therapy, the rate of DNTM disease due to disseminated MAC (DMAC) infection in the setting of advanced HIV disease has decreased, although children with AIDS often have DMAC infection. A recent study showed that approximately 8% of patients with cancer had disseminated DNTM infection involving at least one end organ (312).

Treatment recommendations for DMAC follow the ATS guidelines for pulmonary MAC infection (132) (Table 1). In the setting of HIV, at least two but preferably three drugs, one of which is either clarithromycin or azithromycin, are administered. Typically, ethambutol and either rifampin or rifabutin are given with an injectable drug such as amikacin for the first 1 to 2 months of therapy. Treatment with clofazimine has been associated with increased mortality; thus, it is not recommended. Treatment for DMAC infection should be continued for life in patients who respond poorly or have consistently low CD4 cell counts. Treatment may be discontinued for patients who respond well and have a consistent increase in their CD4 cell counts (>100) (132, 405, 457).

The successful treatment of DMAC disease involves the treatment of HIV infection and also the treatment of the MAC infection. Treatment of HIV is intended to improve the patient's immune status, although this is often difficult, as most adults have high viral loads and CD4 cell counts of <10. Contraindications and various interactions of treatment agents can be problematic.

Like pulmonary MAC disease, a macrolide is essential for the treatment of DMAC disease (132). Clarithromycin has been shown to clear bacteremia more rapidly than azithromycin, although both macrolides have been used (434). Azithromycin may provide an advantage in that fewer other drugs have interactions with azithromycin than with clarithromycin. For the selection of rifamycins, rifabutin is usually recommended because it can more easily combine with antiretroviral agents. The usual drug regimen is a macrolide, ethambutol, rifabutin, and often amikacin (initially), with all drugs given daily (132).

***Mycobacterium chelonae.*** Although rare, most disseminated disease due to RGM is associated with *M. chelonae* (377, 401, 410, 428). Disseminated *M. chelonae* typically presents as multiple subcutaneous nodules with a predilection for the extremities, presumably as a consequence of hematogenous spread (377). In a series of 100 skin, soft tissue, and bone isolates over a 10-year period, 53% of the cases were disseminated cutaneous disease (410). The majority of these infections were associated with corticosteroids, chemotherapy, organ transplantation (25%), rheumatoid arthritis (25%), or other immune disorders (26%) (410). Of the 53 patients with disseminated infection, 92% were receiving low-dose corticosteroids with or without other immunosuppressive agents. Only one patient was HIV positive, and only one had cancer (410).

In the 1980s, hemodialysis was a major health care-associated procedure with reported cases of disseminated disease, most of which were due to *M. abscessus* (428). Such hemodialysis-associated NTM infections are rare in 2011.

The treatment of infections due to *M. chelonae* was problematic prior to the introduction of the macrolides. *In vitro* susceptibility studies have shown all pretreatment isolates to be inhibited by the macrolides and tobramycin. One caveat for disseminated disease due to *M. chelonae* is that a rapid development of macrolide resistance may occur with macrolide monotherapy. Thus, a second agent is recommended (377). Unlike *M. abscessus* and the *M. fortuitum* group, isolates of *M. chelonae* do not contain an *erm* gene to induce macrolide resistance. Mutational macrolide resistance is due to a single point mutation in the gene coding for the 23S rRNA involving adenine at position 2058 or 2059 (421). Although only approximately 40% of the isolates of *M. chelonae* have MICs of imipenem of ≤8 μg/ml, imipenem is still considered one of the best parenteral agents for the treatment of disease. This is especially important since none of the isolates of *M. chelonae* have susceptible MICs of cefoxitin (132). Nonmacrolide oral agents with susceptibility include doxycycline (20%), quinolones (~15%), and sulfonamides (≤7%) (401). Linezolid has also been demonstrated to be a useful agent for the treatment of disseminated disease caused by *M. chelonae*, although the risk of toxicity requires the careful monitoring of long-term therapy (i.e., 3 to 6 months) (55). Approximately 94% of isolates of *M. chelonae* have linezolid MICs in the susceptible range (53, 415). Because isolates vary in their susceptibilities to these oral agents, *in vitro* susceptibility testing to determine the optimal drug treatment is warranted (410, 426).

The current recommendation is treatment with parenteral agents in combination with a macrolide in the initial phase of the disease. The duration of therapy for disseminated disease should be at least 6 months to avoid relapse (377, 401). Many patients are not acutely ill and are not hospitalized; however, patients and physicians often desire an exclusively oral treatment regimen. Fewer than 40% of the strains of *M. chelonae* have been inhibited by doxycycline and/or ciprofloxacin, but this should be confirmed by the *in vitro* testing of individual isolates (54, 369, 410). Moxifloxacin may be a plausible alternate drug if *in vitro* MICs are considered to be susceptible. Additionally, in cases of disseminated *M. chelonae* infection, linezolid may be a useful agent in combination with pyridoxide (to decrease the probability or severity of peripheral neuropathy often associated with linezolid) (55).

***Mycobacterium fortuitum.*** Disseminated disease caused by other NTM such as *M. fortuitum* is rare, even in immunocompro-

mised patients. Choueiry et al. (76) described a patient with a desmoid tumor and an infected catheter site who developed disseminated *M. fortuitum* infection. Another case was reported in which a 76-year-old male with chronic lung disease developed disseminated disease apparently originating in the lungs and spreading to bone and skin. He was treated successfully with amikacin and ciprofloxacin with a sulfonamide (60).

Underlying disease with disseminated *M. fortuitum* has reportedly been associated with meningitis, pneumonia following pacemaker implantation, sterile pyuria with caliectasis, vertebral osteomyelitis, liver and spleen granulomas following aortic aneurysm repair, esophageal ulcers, and chest wall infection following median sternotomy (173). The recommended treatment for disseminated *M. fortuitum* infection has involved an initial 2- to 6-week course of antimicrobials including two parenteral agents (i.e., amikacin and cefoxitin or imipenem) followed by an additional 2- to 6-month regimen of one or two oral agents (macrolides, sulfonamide, doxycycline, and quinolones). Whenever possible, surgical excision is recommended (60). However, quinolones should not be administered as single agents due to the increased risk of resistance. Furthermore, macrolides should be used with caution due to the probability of the presence of the *erm* gene, which induces macrolide resistance (262, 266). *In vitro* susceptibility testing should be performed in order to determine optimal agents, since some species within the *M. fortuitum* group have variable susceptibilities to macrolides and tetracyclines.

**Miscellaneous disseminated infections.** Disseminated NTM infection has also been seen with other species, including *M. genavense* (39, 45, 126, 380), *M. conspicuum* (359), *M. simiae* (259), *M. celatum* (290, 392), *M. triplex* (78, 158), *M. lentiflavum* (271), *M. haemophilum* (338), and *M. malmoense* (73, 456), especially in the setting of HIV or other immunosuppressive disorders (386).

Treatment for disseminated disease due to these other NTM generally involves a multidrug regimen including clarithromycin. Treatment for disease associated with species such as *M. celatum*, *M. lentiflavum*, and *M. malmoense* is controversial, as only a few reports of clinical outcomes are available. Some reports stated variable susceptibilities for some agents, such as ethambutol, rifampin, and rifabutin. Reports of susceptibility to quinolones and other agents such as aminoglycosides have also been discrepant. Because the susceptibility patterns of these species have not been established, the CLSI and the ATS recommend that *in vitro* susceptibility testing be performed on individual isolates (132, 451).

### Other Nontuberculous Mycobacteria

Disseminated cutaneous *M. abscessus* infection is unusual but, like disseminated infections due to *M. chelonae*, occurs most often in patients receiving corticosteroids and commonly involves the lower extremities (132, 409). Disseminated cutaneous disease has also been associated with newly described or less frequently encountered species, including *M. bohemicum*, *M. genavense*, *M. mageritense*, the *M. terrae* complex, and *M. wolinskyi* (132). The *M. terrae* complex is often associated with indolent chronic disease following blunt trauma, especially to the hand or wrist (68, 132, 318, 357). Treatment regimens for infection with the latter species (except for *M. genavense*, which is difficult to grow for susceptibility testing) are based primarily on results of *in vitro* susceptibility testing.

**Disease from Thailand.** Chetchotisakd et al. (71) described 16 cases of unusual disseminated chronic RGM infection from Thailand (9 isolates were identified as *M. abscessus*, 1 was identified as *M. fortuitum* followed by an episode of *M. abscessus*, and the remainder were identified only as *M. chelonae-M. abscessus* group isolates) in adults who presented with bilateral cervical lymphadenitis and the involvement of other organs. Sweet's syndrome, a reactive skin disease that is generally associated with various infections, inflammatory or lymphoproliferative disease, or solid organ transplantation, was the most common presentation, seen in nine patients in the series. Twelve patients had disseminated disease involving other organs. None of the patients had AIDS or a known immunodeficiency, but almost one-half had other infections associated with cell-mediated immunity, tuberculosis, cryptococcosis, or multiple pathogens (71).

Patients received an aminoglycoside (amikacin or kanamycin) in combination with a macrolide (clarithromycin or azithromycin). Some patients received initial therapy with imipenem or cefoxitin for 1 month, depending upon the results of the *in vitro* susceptibility testing of individual isolates. Two patients died, and two patients were lost to follow-up. However, the remaining 12 patients showed a regression in the sizes and numbers of lymph nodes, although lymph nodes and other organs remained culture positive during treatment. Five patients responded with no early subsequent positive cultures, but three of them relapsed after up to 18 months of treatment (71).

### Ophthalmic Infections

Increasing numbers ophthalmic infections due to NTM have been reported over the past 2 decades. The majority of mycobacterial ocular infections are caused by the RGM, specifically *M. abscessus* and *M. chelonae*, although occasional sporadic infections and at least one outbreak have been caused by *M. szulgai*, a slowly growing NTM (52, 115, 160).

Risk factors for ocular NTM infections include the use of corticosteroids, immunosuppression (including AIDS), and laser-assisted *in situ* keratomileusis (LASIK) and other ophthalmic procedures (69, 118, 160, 334, 443).

The treatment of NTM ocular infections is often performed by the local instillation of aminoglycosides or quinolones. Topical treatments with antimicrobials such as clarithromycin, amikacin, and tobramycin have been used successfully, often in combination with ophthalmic solutions with quinolones (69, 77, 84, 115, 118, 150, 171). Additionally, broad-spectrum topical antimicrobials, including quinolones or aminoglycosides, are often administered to surgical patients with the goal of minimizing infection with bacterial or NTM pathogens. The use of antibiotics fortified in concentrations higher than those available commercially may be an option, especially for serious ophthalmic infections. However, ophthalmic NTM infections are often refractory to antimicrobials, and lamellar keratectomy or penetrating keratoplasty is often the last alternative.

Generally, the susceptibility testing of ocular isolates is similar to the testing for the same species from other sites. Based on *in vitro* susceptibilities, the most active agents against *M. abscessus* are usually amikacin and macrolides. In contrast, for isolates of *M. chelonae*, the lowest MICs generally are those of tobramycin, macrolides, and quinolones (52).

A major problem in the interpretation of *in vitro* susceptibility testing in ocular infections is that the evaluation of MICs involves the use of breakpoints for systemic agents and may not be appli-

cable for topical agents. Physicians should be aware that because antimicrobial susceptibility to agents recommended for the treatment of mycobacterial infections is based upon systemic breakpoints, susceptibility testing of agents used topically for ocular infections may not show a correlation. In these cases, consultation with physicians experienced in the treatment of mycobacterial ocular infections should be considered. Nevertheless, until this issue is addressed by the CLSI and studies are performed to establish breakpoints for topical agents, MICs for some ocular pathogens, including NTM, should be interpreted with caution (52).

### Infection of Bones and Joints

***Mycobacterium terrae-Mycobacterium nonchromogenicum-Mycobacterium arupense.*** Infections of bones and joints may involve almost any NTM. *M. marinum* and *M. terrae-M. nonchromogenicum* may cause chronic tenosynovitis, especially in the hand. Infection with the *M. terrae* complex may be slow to resolve with antimicrobial treatment alone and often requires the surgical debridement of infected tissues (318).

As discussed above, the *M. terrae* complex includes *M. terrae*, *M. nonchromogenicum*, *M. triviale*, and the recently described species *M. arupense*. However, most case reports were published prior to the molecular era of identification of species, and thus, the organisms reported may be of uncertain identification and/or significance. As noted by Smith et al. (357), generally, *M. terrae* and *M. triviale* have been considered nonpathogenic, while infections with *M. nonchromogenicum* more often represent true disease.

Smith and colleagues (357) reviewed over 60 reports from the literature on *M. terrae* complex (not including *M. arupense*) infections. They noted that 44% of the patients had no underlying or reported clinical conditions. Only one-half of patients with tenosynovitis who were monitored for 6 months were noted to have clinical improvement or cure. The other half required extensive debridement, tendon extirpation, or amputation (357). Again, it should be noted that the majority of these cases were reported before laboratories performed molecular identification methods. The clinical significance of *M. arupense* remains unclear. The first 65 isolates of this species were mostly (48 of 65) from respiratory specimens, and only 1 (the type strain) was recovered from a tendon (79). Since the original report, an additional isolate of *M. arupense*, from a diabetic patient with tenosynovitis in Taiwan, was reported (393).

The optimal antimicrobial regimen for the treatment of infections due to the *M. terrae* complex is unknown; however, most experts agree that a macrolide-containing regimen similar to the MAC regimen is probably effective (357).

In contrast, most infections with *M. marinum* are more rapidly resolved. Drug therapy is relatively easy, and the standard treatment consists of two or more agents such as a macrolide, sulfonamide, rifampin, rifabutin, and ethambutol for a period of approximately 3 to 4 months (1 to 2 months following the resolution of symptoms). Treatment failure has been associated with deep-structure involvement but is not usually due to the failure of the antimicrobial regimen. Susceptibility testing is not routinely recommended and should be reserved for cases of treatment failure. Surgical debridement as an adjunct to antimicrobial therapy may be indicated, especially in cases involving closed spaces of the hand or in cases of treatment failures (132, 451).

**Miscellaneous nontuberculous mycobacteria in bone and joint infections.** Other species that have been associated with bone or joint infection include *M. wolinskyi* (48); *M. haemophilum* (295, 347); the *M. fortuitum* group (153), including *M. setense* (201); *M. goodii* (48); *M. abscessus* (234); and *M. chelonae* (98, 410). Recently, 13/20 cases of *M. goodii* and *M. wolinskyi* infections were associated with osteomyelitis, one following postsurgical (hardware) replacement (48). Of 42 cases of *M. fortuitum* group infections, Wallace et al. (411) reported 12 (29%) cases with osteomyelitis, 9 of which were subsequent to open fracture and 3 of which followed a penetrating traumatic injury. Patients with osteomyelitis often have positive cultures for ≥12 weeks, so a minimum of 6 months of antimicrobial treatment is necessary (426).

Newly described mycobacterial species in patients with bone and joint infection have also been described. These species include *M. hodleri*, *M. branderi*, *M. lacus*, *M. lentiflavum*, *M. immunogenum*, *M. setense*, *M. houstonense*, *M. boenickei*, *M. mageritense*, and *M. austroafricanum* (132, 340, 385, 386, 398).

Synovitis with *M. haemophilum* has also been reported. Septic arthritis and osteomyelitis are less common in immunocompromised patients (347). Prolonged courses of multidrug therapy, most often including a macrolide, rifampin, amikacin, and/or quinolones, may be necessary. Most isolates of *M. haemophilum* are resistant to ethambutol. Among a series of 17 immunocompromised patients, the median duration of therapy was 10 months (347).

### Prosthetic Joint Infections

Eid et al. (99) recently reported a review of 18 cases of prosthetic joint infections from the past 4 decades at the Mayo Clinic involving 10 cases of *M. fortuitum* infection, 6 cases of *M. chelonae* infection, and 1 case each of *M. abscessus* and *M. smegmatis* infection. All patients were >60 years old, and the time from implantation to the onset of symptoms varied from 3.5 to 312 weeks. Those authors stated that the disparity in times might be related to improvements in surgical and aseptic techniques.

Currently, recommendations for treatment include the removal of the prosthesis, a combination of appropriate antimycobacterial therapy for a duration of at least 6 months, and resection arthroplasty (99, 132).

Subsequently, Tompkins et al. (383) reported a case of nosocomial total-knee-arthroplasty-associated *M. goodii* infection in a 63-year-old woman with type 2 diabetes and morbid obesity. Previous cases of *M. goodii* bursitis likely introduced during intrabursal injections or during a subsequent surgery and an *M. goodii* infection associated with an infected prosthetic inguinal hernia repair mesh have also been reported (119, 383).

### Central Nervous System Infection

In an early pediatric review of NTM disease in 1972, Lincoln and Gilbert reported only 12 cases of disseminated disease, including 6 patients with definitive central nervous system (CNS) disease and 1 case that was "highly probable." Three patients had *M. kansasii* infection (one a dual infection with *M. tuberculosis*), two patients had MAC infection, and two patients had organisms listed simply as "scotochromogens" (217).

A second report in 1979 by Wolinsky (446) (considered a landmark study at the time) described one case each of meningitis involving *M. kansasii*, the MAC, and *M. gordonae*. It should be emphasized that both of those studies occurred prior to the mo-

lecular era of identification to the species level, and treatment was not detailed.

Currently, among non-HIV- and HIV-infected patients, the majority of CNS infections due to NTM involve the MAC. A review in 2004 noted that at least 53 of 63 cases (84%) of NTM CNS infection in HIV-infected patients involved the MAC. HIV-infected patients with CNS infections may also be infected with other species, including *M. kansasii*, *M. gordonae*, and *M. fortuitum* (164). Only six cases were due to *M. kansasii*, two involved *M. fortuitum*, and one case each was due to *M. genavense* and *M. triplex*. Among HIV-negative patients, at least 32 cases of CNS infection associated with NTM have been reported, including 11 due to the MAC, three involving *M. kansasii*, 12 due to *M. fortuitum*, 2 each associated with *M. gordonae* and *M. chelonae-M. abscessus*, and 1 each due to *M. malmoense* and the *M. terrae* complex (112, 405).

Murray et al. (255) reported a case of brain abscess involving the MAC in a patient with AIDS whose treatment for MAC infection had been discontinued after immune reconstitution. The patient responded favorably following surgical excision and the reinstitution of his MAC antimicrobials (255). Flor et al. (112) stated that the overall mortality rate for patients with CNS MAC infection was 77%, although incomplete documentation has made estimates of mortality due to meningitis difficult.

Clarithromycin, rifampin, and ethambutol penetrate the CNS well. However, with inflamed meninges, the newer quinolones and amikacin may not be adequate. Intrathecal therapy with aminoglycosides has been proposed, but no clinical studies have been performed (132).

**Rapidly growing mycobacteria.** Fifteen cases of RGM CNS infections have been described, with 13 (87%) being associated with *M. fortuitum* and 2 (13%) involving the *M. chelonae-M. abscessus* group. The presence of catheters and local trauma, including prior surgery, were the most significant risk factors. Two patients had AIDS, 1 patient had chronic otitis media, and 13 patients had meningitis (405).

Most investigators recommend aggressive surgical debridement and treatment with antimicrobials based on species identification and *in vitro* susceptibility testing for the treatment of CNS infection due to RGM. Treatment should include parenteral therapy with amikacin and cefoxitin or imipenem in combination with at least one oral agent for a total duration of at least 6 months. Initial parenteral regimens may be discontinued if the patient responds favorably after 4 to 6 weeks. No single regimen has been established for the treatment of CNS infection due to RGM, but preferably, three to four drugs should be given, at least initially (132, 405).

A case of posttraumatic meningitis caused by *M. abscessus* has also been described (230). The patient had sustained a knife wound to the neck 6 months prior to admission. The injury had resulted in quadriparesis and left facial droop. Subsequent magnetic resonance imaging (MRI) of the brain revealed an abnormal meningeal enhancement, and MRI of the spine showed an abnormal enhancement of the cervical and thoracic spine. Cerebrospinal fluid (CSF) cultures yielded *M. abscessus*. Initial treatment for *M. tuberculosis* was stopped after the patient developed hepatotoxicity, and treatment with amikacin and clarithromycin was initiated. An Ommaya reservoir was implanted, and intrathecal amikacin was added to the regimen. The patient refused the removal of the reservoir after it was suspected to be colonized with *M. abscessus* and died a month later with overwhelming meningitis but had no evidence of disseminated disease (negative blood cultures). The treatment regimen failed despite the achievement of therapeutic levels of clarithromycin in CSF and the continued *in vitro* susceptibility of the infecting organism (230).

Although not proven, those authors postulated that other than the possible colonization of the Ommaya reservoir with *M. abscessus*, another possible explanation for the failure of the treatment regimen might be that the minimum bactericidal concentration (MBC) of clarithromycin was increased in acidic media such as the acidic CSF in meningitis (230). Further studies are necessary to assess this hypothesis.

***Mycobacterium kansasii.*** Only nine cases of CNS disease due to *M. kansasii* have been reported; six of these cases involved HIV-positive patients.

Treatment for CNS disease due to *M. kansasii* follows the ATS guidelines for pulmonary *M. kansasii* infection (rifampin, ethambutol, isoniazid, or clarithromycin). For patients with HIV infection, rifabutin may be substituted for rifampin (132).

Flor et al. (112) reviewed 62 cases of meningitis and found that 6 cases were due to *M. kansasii*. Those authors stated that the rate of morbidity related to *M. kansasii* meningitis was high despite appropriate antimicrobial therapy.

***Mycobacterium gordonae.*** Although controversial as a pulmonary pathogen, rare cases of CNS infection associated with *M. gordonae* have been described. One case was a child with hydrocephalus who had two ventriculoatrial shunts placed at 18 days and 7 months of age. The child was treated empirically with streptomycin and isoniazid but continued to grow *M. gordonae* in CSF and ascites specimens. The patient improved after the shunt was removed and antimicrobials were reinstituted. The investigators of that study hypothesized that the organism was implanted onto the valve of the second shunt and that a third shunt (ventriculoperitoneal) spread the infection to the peritoneum and meninges (112, 405).

The second case of CNS disease due to *M. gordonae* was a 23-year-old woman with a history of a ventriculoperitoneal shunt during the excision of a cerebellar medulloblastoma 9 years earlier. The patient was treated with steroids for presumed hypersensitivity pneumonitis and hepatitis 9 months prior to the second admission. Again, the shunt was proposed by those authors to be the route of infection, and the patient was successfully treated with rifampin, ethambutol, and kanamycin based on prior *in vitro* susceptibility test results (405).

***Mycobacterium genavense.*** Treatment of CNS infection with *M. genavense* may be difficult due to its inability to grow on standard susceptibility media. However, previous cases have been successfully treated with multiple antimicrobials, including clarithromycin, quinolones, and ethambutol (43).

**Miscellaneous.** Treatments for other CNS infections, including *M. terrae* complex, *M. malmoense*, and *M. triplex* infections, should follow ATS guidelines for the treatment of pulmonary disease and current clinical experience with infections from other sites (132, 405).

### Otitis Media

The majority of NTM otitis media infections have been associated with *M. abscessus*. Although rare, otologic infections with *M. fortuitum* in adults, associated with the use of topical steroid otic drops, have been reported (15, 267). The largest single study in-

volved 21 cases and occurred in the era prior to the era of routine testing and treatment with macrolides (117).

Franklin and colleagues (117) tested 21 RGM isolates (20 *M. abscessus* isolates and 1 *M. chelonae* isolate) from sporadic infections following the placement of tympanostomy tubes. Isolates were resistant to sulfonamides, doxycycline, and ciprofloxacin. Seventy-six percent and 95% of the isolates of *M. abscessus* were susceptible to imipenem and cefoxitin, respectively (117). Often, infections have been refractory to treatment with antimicrobials alone and have required extensive debridement with prolonged antimicrobials.

Risk factors for otitis media include the use of topical steroid-containing drops and localized trauma with pressure equalization (PE) tube insertion. High-level mutational cross-resistance with all aminoglycosides, including neomycin, gentamicin, tobramycin, and amikacin, has been seen for isolates of *M. abscessus*, presumably related to previous treatment with aminoglycosides that did not clear the infection but which selected for mutational antimicrobial resistance (117).

Thus, *in vitro* susceptibility testing is recommended and repeat testing may be necessary for patients refractory to treatment, because resistance to antimicrobials may develop during therapy. Therapy involves long-term antimicrobials, including a macrolide and amikacin plus cefoxitin or imipenem for 3 to 6 weeks, followed by long-term oral treatment for at least 6 months. Although the 1994 study was performed prior to the widespread use of clarithromycin, as noted above, those authors suggested that topical erythromycin drops might enhance oral therapy, and thus, the newer macrolide clarithromycin might also have potential therapeutic benefit (117). Surgical debridement and the removal of all foreign bodies, including PE tubes, are also necessary.

Three other case reports of successful treatment regimens prior to the study by Franklin et al. were reported by Moerman and colleagues (247). One child who had no tympanostomy tubes had recurrent otitis at 4 months of age. A polypoid mass was found in the external ear canal, but cultures were negative. Skin tests were done, and therapy consisting of rifampin and isoniazid was begun and ended after a symptom-free period of 2 months. The patient remained free of infection. Another child reportedly had *M. chelonae* ("*M. chelonei*" prior to the taxonomic change) infection following the insertion of PE tubes and surgery 2 years earlier. He was treated with a sulfonamide, and following a radical mastoidectomy, he healed rapidly (247).

### Lymphadenitis

Cervical adenitis due to the MAC is the most commonly seen NTM disease in children between 1 and 5 years of age in the United States (132). No risk factors predisposing children to cervical adenitis have been found, but children who have undergone BCG vaccination have a decreased risk of MAC cervical lymphadenitis. In Scandinavia and other northern areas of Europe, *M. malmoense* and *M. haemophilum* are also common causes of lymphadenitis. Recently, a case of *M. lentiflavum* cervical lymphadenitis following oral surgery in an adult was described in Italy (289). The incidence of *M. scrofulaceum*, which was previously a common cause of lymphadenitis, has been decreasing (132). NTM lymphadenitis is rarely seen in adults who are HIV negative.

In adults with mycobacterial lymphadenitis, once *M. tuberculosis*, which is found in >90% of cases, is ruled out of the diagnosis, the MAC is the most likely causative agent. The discrimination of tuberculous lymphadenitis from NTM lymphadenitis is essential, because tuberculous lymphadenitis requires drug treatment in addition to public health reporting. For lymphadenitis in an immunocompetent adult, complete surgical excision is required. Antimicrobial treatment may not be necessary. For patients with extensive MAC lymphadenitis or a poor response to surgery, a macrolide-based regimen should be considered (132).

Rarely, newly described species, including *M. palustre*, *M. parmense*, and *M. tusciae*, have been implicated in lymphadenitis in children. Despite antimicrobial treatments, the patients in each case were cured only after surgical excision (105, 384, 386, 390).

Recently, *M. colombiense*, a newly described member of the MAC and most commonly recovered from HIV-infected patients, was isolated in a case of lymphadenopathy in a 25-month-old child in France and in another child in Spain. Neither child was known to be immunocompromised. The reports did not include a description of the therapy used, but those authors emphasized the identification of the species in order to determine appropriate drug treatment and clinical management (404).

An unusual presentation of chronic bilateral cervical lymphadenopathy was also reported and is discussed above in "Disease from Thailand."

### Catheter Infections and Bacteremia

Generally, all NTM may be associated with catheter infections and bacteremia, and treatment is based on guidelines for disseminated disease for individual species (132). Catheter-related infections are typically associated with the use of long-term central venous catheters. Other than central venous catheters, hemodialysis shunts, peritoneal catheters, and ventriculoperitoneal shunts may also become infected with mycobacteria (19, 54, 57).

Catheter-associated infections and/or sepsis is the most common health care-associated type of infection due to the RGM in both immunosuppressed and immunocompetent patients (54, 57, 339). The organisms not only may cause mycobacteremia but also may present as local wound drainage from an exit site or tunnel infection. The most commonly recovered RGM species or groups include *M. fortuitum*, *M. abscessus*, and the *M. mucogenicum* group. Central venous catheter infections are the most important clinical infections caused by the latter group. In a study by Wallace et al. (423), 9 out of 20 clinical cases (45%) of infection caused by *M. mucogenicum* were due to blood and central venous catheters. *M. mucogenicum* has also been associated with catheter-related peritonitis in patients on dialysis (19). Additionally, the newly described species in the *M. mucogenicum* group, *M. phocaicum*, was isolated in an outbreak of mycobacteremia in five patients with central venous catheters in a hospital in Texas (80).

The treatment of *M. mucogenicum* group infection is based on the results of the *in vitro* susceptibility testing of the individual strain. Typically, the species is susceptible to multiple antimicrobials, including aminoglycosides, cefoxitin, clarithromycin, minocycline, doxycycline, quinolones, and imipenem (132, 423).

Like *M. mucogenicum*, recent studies (in 2010) have shown that catheter-related sepsis is the major clinical illness caused by the pigmented RGM species *M. neoaurum* and a newly described species, *M. bacteremicum* (57). Another newly described species, *M. brumae*, has also been the cause of catheter-related sepsis (208).

Initial treatment for catheter-related infections involves the removal of the infected catheter followed by antimicrobial treatment appropriate for the species involved in the infection (54).

The duration of treatment for NTM catheter sepsis varies but is usually at least 6 to 12 weeks to prevent a relapse of infection (54, 132, 304). However, recent studies have indicated that although systemic infections with mycobacteria in leukemic children are rare, they may require up to 2 years of therapy, even with the removal of the catheter (211).

### Cutaneous Infections

Skin and soft tissue infections can be divided into localized types of infection resulting from trauma, including surgery, and disseminated cutaneous infections, which originate from a cutaneous site but spread throughout the body.

**Disseminated cutaneous infection.** For a description of disseminated cutaneous infection, see "Disseminated Disease" above.

**Localized cutaneous infection.** Traumatic wound infections, especially following open fractures, puncture wounds, or surgery, often involve RGM, especially the *M. fortuitum* group, *M. chelonae*, and *M. abscessus*. Diagnosis is made by a culture of the organism from a wound drainage or tissue biopsy specimen (54, 132, 424, 426, 428). The majority of infections typically occur 4 to 6 weeks following the traumatic event, and approximately 40% of the injuries in a series of 85 isolates from the United States and Queensland, Australia, involved the lower extremities (54). Stepping on a nail was the most frequent preceding event leading to infection (54, 340, 411). Clinically, the infection ranges from localized cellulitis or abscesses to osteomyelitis. The latter type of infection often involves *M. fortuitum*, whereas the former is frequently associated with *M. abscessus* or *M. chelonae* (54, 410).

A recent study showed that patients with cutaneous infections with *M. chelonae* and *M. abscessus* tended to be older and often on some type of immunosuppressive therapy (396). Interestingly, AIDS is not considered a significant risk factor for the development of cutaneous infections due to *M. chelonae* (54, 410).

Cutaneous infections associated with *M. abscessus*, *M. chelonae*, or the *M. fortuitum* group should be treated according to guidelines for treatment for the specific species involved. Monotherapy with a macrolide for localized infections with *M. chelonae* may be sufficient, whereas multiple antimicrobials are essential if the infection is disseminated or if other species are involved. The risk of the development of macrolide resistance was emphasized previously (132, 401). For example, in localized infections involving isolates of the *M. fortuitum* group, other agents may be necessary due to the presence of the *erm* gene, which may induce resistance to macrolides (266). Again, *in vitro* susceptibility testing is crucial in order to assess alternative therapeutic regimens.

The debridement of infected sites may also be critical adjunctive therapy depending upon the extent of the disease. Some investigators previously advocated the aggressive debridement of all infected subcutaneous tissues and skin, often followed by closure or grafting, if necessary, along with appropriate antimicrobial treatment (284, 294, 310). Currently, incision and drainage may be important for some NTM infections, but aggressive debridement is no longer necessary given the available antimicrobials, unless the infection is extensive or is a relapse infection (132).

## RISK FACTORS AND IMMUNODEFICIENCY DEFECTS

### Underlying Bronchiectasis

Bronchiectasis is defined as irreversible dilation of the peripheral airways associated with excessive mucus production related to damaged airways and is thought to be a common cause of infection, especially chronic types such as mycobacterial or fungal infections. During acute inflammatory stages, the walls of the bronchi thicken with edema and inflammatory cells. The cell-mediated immune response produces an infiltration of lymphocytes, macrophages, and antigen-presenting cells. In turn, chronic inflammation leads to the destruction of the elastin layer in the bronchial walls (123).

Although NTM pulmonary disease and bronchiectasis are most certainly associated with each other, the pathophysiological link between the risk factors of patients (primarily older women) and the development of bronchiectasis has not yet been elucidated (131). Bronchiectasis and pulmonary NTM disease are thought to be a common final manifestation of several conditions, including recurrent infections which result in a granulomatous inflammatory response (37, 123, 132, 435). A variety of primary diseases (e.g., cystic fibrosis) is associated with bronchiectasis. The MAC and *M. abscessus* are the predominant NTM associated with pulmonary disease in the United States, although other NTM, such as *M. malmoense*, *M. kansasii*, and *M. simiae*, etc., may be more common in other geographic areas (213, 218). Continued environmental exposure makes NTM reinfections, especially MAC reinfections, likely for patients with nodular bronchiectasis (432). Recent studies from the United States have shown that most patients with nodular bronchiectasis are infected with multiple genotypes. Reinfection with MAC isolates after successful treatment occurs primarily (approximately 90%) in patients with nodular bronchiectasis. In that same study, only three patients (13%) with cavitary MAC disease showed reinfections (432). The mean time to a new MAC infection after the stopping of therapy was found to be only 24 months (432).

Controversy exists as to whether MAC disease is the primary disease in patients with nodular bronchiectasis or, rather, the immunocompromising condition of bronchiectasis predisposes these patients to MAC disease (123). A retrospective study by Fujita and colleagues in 2003 (123) proposed that the destruction of the bronchial structure due to granuloma formation throughout the airways was probably the principal cause of bronchiectasis in MAC infection in nine patients with MAC lung disease. Those authors noted cartilage destruction, ulceration of the bronchial mucosa, and narrowing of the airways along with an extensive formation of MAC cells in granulomas. These findings suggest that bronchiectasis was caused by MAC infection due to the destruction of the bronchial structure (123).

Patients with nodular bronchiectasis and MAC infection often have additional cultures positive for bacterial pathogens such as *Pseudomonas aeruginosa* and/or other NTM, including *M. abscessus* and *M. chelonae*, which may complicate the assessment and management of the primary NTM disease. Thus, treatment strategies for managing bronchiectasis, such as airway clearance measures and the suppression or prevention of concomitant bacterial infections, may improve the patient's symptoms and, ultimately, the outcome of the pulmonary infection (132, 435).

A U.S. study from 1 January 1999 to 31 December 2001 of patients with a diagnosis of bronchiectasis (excluding cystic fibrosis) estimated the prevalence of bronchiectasis to be approximately 272/100,000 persons over 75 years of age, with age and sex distributions (primarily women) similar to those for pulmonary NTM (435).

In a recent survey, 15% of patients for whom pulmonary NTM

was the primary diagnosis had bronchiectasis as a secondary diagnosis (37). The authors of that study stated that the true percentage of patients with bronchiectasis was probably higher due to the fact that the definition of NTM pulmonary disease included bronchiectasis. Those authors also hypothesized that bronchiectasis may have been underreported, since diagnosis without a computed tomography (CT) scan was difficult, and not all patients had CT scans done. Furthermore, because that study included only the hospitalized population, and many cases of NTM disease are diagnosed and managed in an outpatient clinic, the number of patients with bronchiectasis may have been underestimated (37).

Lam et al. (200) showed that a culture response with intermittent (three times weekly [TIW]) therapy occurred in 21% of patients with cavitary MAC disease without bronchiectasis and in a similar percentage (20%) of patients with cavitary MAC and bronchiectasis. The culture responses were 72% for patients with bronchiectasis and no cavitary disease and 67% for patients without cavitary disease or bronchiectasis. The presentations and outcomes in that study varied depending upon whether the patient had cavitary or noncavitary disease without regard to the presence of bronchiectasis (200). Overall, those authors concluded that on the basis of culture, high-resolution CT scans, and symptoms, the TIW treatment appeared to be less effective in patients with cavitary disease, especially those with a history of COPD, bronchiectasis, or previous treatment for MAC infection (200).

Recent studies from Japan have shown that a higher dose of clarithromycin (800 mg versus 400 mg) per day is associated with more rapid culture conversion in patients of similar genders, ages, and body masses and with HRCT scans with bronchiectatic nodular lung disease and MAC infection (148). Both of these doses were less than the 1,000 mg per day (500 mg twice daily) recommended by the ATS/IDSA for these patients (132). More research is needed to detect a possible genetic defect in patients with nodular bronchiectasis and NTM lung disease.

### Genetic Mutations

Recently, a rare new form of severe immunodeficiency syndrome correlated with CXC chemokine receptor 4 (CXCR4) dysfunction was implicated in the cases of two young female patients with severe disseminated MAC infections, one with a fatal outcome. Both patients' immune defect was marked by a severe B-cell lymphopenia, a severe defect in natural killer cells, and systemic granulomatosis of an unknown significance. Although steroid treatment failed in both cases, gamma interferon 2α (IFN-γ2α) was effective in one patient (95).

### Tumor Necrosis Factor

Tumor necrosis factor alpha (TNF-α), a proinflammatory cytokine, has a variety of functions, including an involvement in apoptosis, granuloma formation, increasing the number of bactericidal proteins, enhancing superoxide anion production, and preventing the replication of mycobacterial phagosomes (89). A recent study showed that the levels of TNF-α in MAC infection are inversely related to the virulence of the mycobacterial serovar (322).

Patients receiving anti-TNF-α therapy are known to be at risk for tuberculosis and other granulomatous diseases (442). Patel and colleagues cited cytokine (IFN-γ) activation of infected macrophages and TNF-α as the mechanisms responsible for the development of immunity against pathogens such as mycobacteria (282).

A recent survey of 239 U.S. Food and Drug Administration (FDA) Med Watch System (442) reports of patients receiving anti-TNF-α therapy suggested that cases of NTM disease associated with anti-TNF-α therapy were twice as frequent as cases of MTBC associated with anti-TNF-α therapy in the United States. Seventy-five percent of patients with NTM (174 cases) were receiving infliximab, 41 patients (17%) were receiving etanercept, and 19 (8%) were receiving adalimumab. Only 105 cases (44%) met the NTM disease criteria according to the ATS/IDSA case definition (132). The majority (65%) of the 105 cases were women (median age, 63 years), 73 (69%) of the confirmed or probable cases were receiving infliximab, 25 (24%) were receiving etanercept, and 7 (7%) were receiving adalimumab. Fifty-two (49%) of the patients had MAC infection, 20 (19%) had RGM infection, and 8 (8%) had *M. marinum* infection (442).

Seventy-five percent of the patients surveyed were receiving anti-TNF-α therapy for rheumatoid arthritis. Sixty-eight (65%) of these patients also received prednisone, and 58 (55%) received methotrexate. Twenty-five (24%) of all patients reported the presence of at least one of the following conditions: bronchiectasis (5%), chronic obstructive pulmonary disease (10%), diabetes mellitus (5%), and rheumatoid lung (4%) (442).

Not surprisingly, since TNF-α inhibition is known to increase the risk of extrapulmonary and disseminated disease, nearly one-half of the patients surveyed had extrapulmonary disease. Although no study has directly compared the risk of NTM disease for users of infliximab to that for users of other anti-TNF-α agents, Winthrop et al. (442) advised that the use of infliximab may pose a higher risk for NTM disease because of either the drug itself or the characteristics of the patients taking the drug. For example, those authors noted that infliximab users were more likely to also be using methotrexate at the time of diagnosis. It remains to be seen when or if anti-TNF-α therapy can be safely reinstituted or continued in patients being treated for mycobacterial infections (442).

### Serovar-Specific Glycopeptidolipids

Although the virulence and multidrug resistance of *M. avium* are due partly to the resilient cell wall found in all mycobacteria, the MAC contains an additional electron-dense layer composed partially of nonspecific glycopeptolipids (nsGPLs) and serovar-specific highly antigenic glycopeptolipids. The latter serovar-specific glycopeptidolipids (ssGPLs) are found only in this group of mycobacteria. Of the 31 known serovars of *M. avium*, only a few cause disease in humans. It is thought that the variations in the ssGPL determine the pathogenicity of the serovar and may also be a factor in differences in host/organism immunity. Recent studies have shown that *M. avium* can control various host signaling pathways, such as the mitogen-activated protein kinase and nuclear factor pathways (322). This modulation of specific intramacrophage signaling cascades can result in pro- and anti-inflammatory cytokine production and phagolysosome fusion, resulting in host disease or mycobacterial death (322).

### Gamma Interferon and Interleukin-12 Receptor Defects

Gamma interferon (IFN-γ) is a cytokine produced by T lymphocytes and natural killer (NK) cells that is critical for the containment and killing of intracellular organisms (325). IFN-γ stimulates macrophages to produce interleukin-12 (IL-12) and TNF-α, which in turn stimulate helper T cells and NK cells. Mycobacterial infection also begins the stimulation of the macrophage production of IL-12. Subsequently, IFN-γ binds to its receptor on mac-

rophages, resulting in activation with reactive oxygen species, chemokines, and cytokine production (144). The IFN-γ receptor complex is composed of IFN-γ receptor 1 (R1) (ligand biding) and IFN-γ R2 (signal transducing). These two receptors function together to upregulate multiple genes involved in immunity, including TNF-α and IL-12 (309).

Previous studies have shown that the IFN-γ and IL-12 pathways are inextricably involved in the pathogenesis of autoimmune diseases as well as tumor development, control, and containment and the cell death of intracellular organisms such as mycobacteria (325). Several reports have shown that patients who had defects in these pathways developed serious and sometimes fatal infections with MTBC and NTM (142, 144, 309).

Recent studies indicated that IFN-γ is released by activated NK cells in the early stages of *M. avium* infection. Furthermore, as the infection progresses, both T-helper and Tc cells also secrete IFN-γ. The exact function of IFN-γ is still being debated, although it appears that the destruction and removal of *M. avium* are major functions (322).

IFN-γ R1 is a class II cytokine receptor devoid of intrinsic kinase or phosphatase activities. The receptor functions both genetically and functionally like a housekeeping gene. Mutations with both recessive and dominant inheritance patterns result in an IFN-γ R1 deficiency (325). Dominant IFN-γ R1 and recessive IFN-γ R1 deficiencies are most often associated with BCG and NTM infections.

Patel et al. (282) recently defined mutations in seven different genes involved in the IL-12-dependent, high-output IFN-γ pathway. Those authors showed that patients with a rare X-linked recessive mutation of the gene encoding the NF-κB essential modulator may also develop a wide array of infections, including mycobacterial infections, in the absence of any recognized primary or secondary immune deficiency. Additional studies have also described a familial autosomal dominant IFN-γ receptor mutation in which three members of the same family developed disseminated mycobacterial infections as children (146).

Patients with a complete IFN-γ R1 deficiency have been shown to develop incurable, usually fatal, mycobacterial infections, including BCG (if immunized with this vaccine) and NTM including the MAC, *M. chelonae*, *M. fortuitum*, and *M. smegmatis*, prior to the age of 3 years despite continuous antimycobacterial treatment. The organisms involved in infections tend to be more refractory to treatment, and the alteration of the cytokine pathways is not an option. Aggressive extensive antimicrobial treatment is critical for these patients. Histologically, patients with MAC or BCG may develop lesions similar to those of lepromatous leprosy, which contain mainly macrophages with acid-fast organisms and only a small amount of T lymphocytes. In contrast, patients with *M. chelonae*, *M. fortuitum*, and *M. smegmatis* infections may have lesions with only a small number of acid-fast organisms. These children usually develop fever and lose weight and may develop lymphadenopathy and hepatosplenomegaly with a probable dissemination of disease. Bone marrow transplantation appears to be the only possible potential cure for this immune problem (282).

A partially dominant IFN-γ R1 deficiency is usually associated with more limited NTM disease or BCG infection, often with bone involvement in children but not adults (325). The clinical phenotype is milder than that of the partially recessive and completely recessive phenotypes and in most cases can be controlled with IFN-γ therapy in combination with antimycobacterial agents (282).

Genotypic-phenotypic correlations have been elucidated for specific genes, some of which have therapeutic implications (325). In the absence of HIV infection or other cell-mediated immune defects, Patel and colleagues (282) proposed several conditions that should be investigated for defects in the type 1 cytokine cascade, including patients with disseminated or recurrent NTM infections.

IFN-γ R2 is another member of the class II cytokine family. Mutations in this receptor are less frequent than those in IFN-γ R1. There are likely intracellular pathways and complex molecular mechanisms involved with this receptor that remain to be characterized (325). IFN-γ R2 is the signal-transducing chain of the IFN-γ receptor. The surface expression of the IFN-γ R2 chain is more tightly regulated than that of IFN-γ R1 and, as such, is the most important factor related to the cellular IFN-γ response (282). A complete deficiency of IFN-γ R2 is the least frequent cause of genetic susceptibility to NTM disease but is no less severe than a complete IFN-γ R1 deficiency.

Rapkiewicz et al. (309) reported a 9-year-old child with an autosomal recessive IFN-γ R2 deficiency who developed hepatoportal venopathy due to disseminated MAC infection. After 8 months of antimycobacterial treatment along with IFN-α and granulocyte-macrophage colony-stimulating factor (GM-CSF), the patient continued to have lymphadenopathy. IFN-α treatment was discontinued, and IFN-γ treatment was begun. A year later, the patient had painful generalized lymphadenopathy with multiple liver lesions and ascites. Despite aggressive treatment, the patient died. Those authors noted that a partial resolution of disease and decreases in the organism burden and granulomatous inflammation seemed to correlate with the duration of IFN-α and antibiotic treatment, thus emphasizing the pivotal role of the cytokine pathway in the control of NTM infection (309).

A partial IFN-γ R2 deficiency was also reported for a 20-year-old patient with a history of BCG and *M. abscessus* infection (282).

Patients with IL-12 mutations also retain IFN-γ responsiveness and can also benefit from the addition of IFN-γ to their antimicrobial regimen. The need for antibiotic maintenance has not yet been clarified for patients who initially clear their first mycobacterial infection. Generally, new episodes of infection do not occur, although the patients remain genetically and functionally deficient in IL-12 (325).

Patients with a complete IL-12-p40 deficiency are rare, and cases are usually familial. These patients also show increased genetic susceptibility to BCG and NTM infections. This condition appears to be more severe than an IL-12 receptor β1 (IL-12Rβ1) deficiency and is fatal in 38% of those affected (282).

A complete IL-12Rβ1 deficiency in the most frequent cause of genetic susceptibility to mycobacterial disease and has been diagnosed in 40% of patients with a known inherited disorder of the IL-IFN system (144). These patients have a complete absence of responsiveness to IL-12 and IL-23. Antigenic stimulation (BCG or MAC) of patient peripheral blood lymphocytes has resulted in decreased levels of IFN-γ production. Patients with IL-12Rβ1 defects have a milder phenotype than those with complete IFN-γ R1 or R2 deficiencies, likely due to residual IL-12-independent IFN-γ-mediated immunity. The clinical course of disease is usually milder, and BCG vaccination or disease may provide some protection against subsequent NTM infections. Patients are respon-

sive to antimycobacterial drugs. The overall genetic susceptibility has been reported to be 45%, with only 11% mortality (282). Haerynck et al. (144) described a 9-year-old girl with relapsing bilateral cervical adenitis and increased markers of inflammation, decreased lymphocyte counts, and intestinal malabsorption. The MAC was cultured from bone marrow, urine, stool, sputum, and an abdominal lymph node. Immunologic investigation showed a defect in the IL-12/23–IFN-γ axis (specifically, IL-12Rβ1). After treatment with subcutaneous IFN-γ, amikacin, rifabutin, clofazimine, and clarithromycin, her lymphadenopathy decreased, and she gained weight. A few months later, inflammation of the intestines and lymph nodes increased severely, and although IFN-γ injections were increased, the patient developed lung edema and died (144).

Gruenberg and colleagues (142) described a second child who, at 19 months of age, was diagnosed with MAC and *Streptococcus pneumoniae* infection due to an IL-12Rβ1 mutation (142). The case represented the first documented case of sepsis due to *S. pneumoniae* in a patient with an IL-12Rβ1 deficiency. The child remained on multiple antimicrobials, including ciprofloxacin, clarithromycin, rifampin, and ethambutol along with IFN-γ at the time the article was written.

Other deficiencies such as STAT1 (signal transducer and activator transcription required for signal transduction for both type I and type II interferons), TyK2 (signal transduction induction of tyrosine phosphorylation), and NEMO (nuclear factor critical and nonredundant components of the NF-κB signaling pathway) deficiencies have also been associated with genetic susceptibility to NTM infections (282).

Because of the difficulty in the treatment of NTM infections resulting from defects in the IFN-γ/IL-12 pathway, most experts recommend a stepwise approach, which probably ultimately involves referral to a research laboratory that specializes in these cases. The general workup includes diagnosis by flow cytometric analysis. Evaluation for mutations at the sequence level should be performed.

### HIV/AIDS

Recently, Chastellier et al. (70) confirmed that strains of *M. avium-M. intracellulare* used different pathways for survival and the maintenance of virulence. One intriguing pathway involves the actual rescue of the mycobacteria from the phagolysosomal environment by returning the phagolysosome to an immature state by the inhibition of nuclear factors and phagolysosome fusion. Thus, the mycobacteria remain alive and appear to be capable of replication. Although the fusion of phagosomes with lysosomes is intended to kill the mycobacteria, Chastellier and coworkers (70) showed that the mycobacteria are not always destroyed and that some appear to replicate normally.

*M. avium* can also activate anti-inflammatory cytokines and may even survive in phagolysosomes. Thus, in a patient with severe immunosuppression, such as that due to HIV/AIDS, these intracellular survival mechanisms can cause major treatment problems (70, 322).

### Cystic Fibrosis

Cystic fibrosis (CF) is a serious autosomal recessive illness that occurs in the Caucasian population as a result of mutations in the CF transmembrane conductance regulator (CFTR) gene (64). Recent studies have reported a 36 to 50% prevalence of CFTR mutations in patients with NTM compared to the prevalence in the general population (188, 463). Kim and colleagues (188) advised that half of the patients in a 2008 study and in previous studies did not have mutations identified and that almost all cases were lacking features of classic CF disease. Thus, pulmonary NTM infections in these patients could not be ascribed solely to mutations in CFTR.

Although the IFN-γ/IL-12 pathways were shown to be important for the development of disseminated infection with NTM, there are currently no data to confirm their role in CF disease (188). Immunity in patients with CF is generally normal, and the number of pulmonary NTM infections has been shown to increase with age in CF and be common in CF patients with less severe disease (276, 277). Previously, other investigators suggested that patients with CF are more vulnerable to NTM lung disease because of obstructive airway defects, poor nutritional status, and frequently associated diabetes mellitus and steroid treatments at an older age (108). Cullen et al. (86) indicated that because the signs and symptoms of NTM are often difficult to distinguish from those of the chronic bacterial lung infections seen in advanced stages of CF, there may be an underestimation of the role of NTM in the decline in lung function among these patients.

Patients with CF are predisposed to airway infections with unusual organisms, including NTM, although the clinical impact of NTM infections in these patients remains uncertain, since some investigators reported no adverse events for patients with NTM (22, 23, 86, 108, 180, 187, 188, 218, 276, 277, 288, 329, 332, 337, 344, 437). The clinical significance of NTM in CF has not been completely elucidated, but older age (mean age, 21 ± 9 years) was the most significant predictor of the isolation of NTM from patients with CF in a multicenter U.S. study in 2003 (276, 277). That same study showed that 72% of the patients had MAC infection and that 16% had *M. abscessus* infection. Molecular typing showed that all patients within each center had unique NTM strains, thus affirming an earlier study by Bange and colleagues (22) which showed that person-to-person transmission and the nosocomial acquisition of NTM among CF patients are rare or do not occur (180, 276, 277, 344).

Subsequent studies of 385 CF patients under the age of 24 years (mean age, 12 years) in pediatric centers in Paris, France, showed a prevalence of 8% for NTM infections. In contrast to the U.S. study, *M. abscessus* (39%) was the most common NTM cultured, followed by the MAC (21%) and *M. gordonae* (18%). Notably, *M. abscessus* was recovered from patients of all ages, but MAC isolates were not seen in patients under 15 years of age (288). Interestingly, in a multicenter study in Israel in 2008, the overall prevalence of NTM was 22% among 186 CF patients (mean age, 24 years); the most frequently recovered species was *M. simiae* (also the most common NTM in non-CF infections in Israel), followed by 31% *M. abscessus* and 14% MAC isolates (213, 218). Another study from Sweden revealed *M. abscessus* (6%) to be the predominant NTM species in CF patients, followed by MAC (3%) and *M. lentiflavum* (1%) isolates (180). Additionally, Jönsson and colleagues (180) showed that a rough phenotype was associated with persistent airway disease in 81% of the patients studied, compared to only 10% of patients with a smooth phenotype. Those investigators further asserted that the rough phenotype might increase the biofilm-producing ability of the organism.

A larger multicenter nationwide prevalence study among 1,582 CF patients (mean age, 18.9 years) in France also showed that *M. abscessus* (3%) and the MAC (1.5%) were the most commonly isolated species and the only species which met the ATS bacterio-

logical criteria for NTM lung disease (329). Of interest, 40% of the *M. abscessus* isolates belonged to the newly described species *M. abscessus* subsp. *bolletii* (329).

Various treatment modalities have been recommended for CF patients with NTM infection and concomitant infection with other pathogens (127). Typical bacterial species such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* are usually treated with antimicrobials such as aminoglycosides, which may also be effective against NTM. Thus, most investigators believe that airway clearance treatment of the bacterial pathogens should be intensified prior to the initiation of antimycobacterial treatment in order to allow a better assessment of how bacterial treatment may affect NTM disease (276, 277).

Previous studies have shown that high doses of ibuprofen inhibit the migration, adherence, swelling, and aggregation of neutrophils as well as the release of liposomal enzymes (331). The use of ibuprofen has also been shown in laboratory studies to inhibit monocyte chemotaxis. Some investigators speculate that this nonsteroidal anti-inflammatory drug may also change the immune system and allow the progression of diseases such as NTM. Moreover, ibuprofen has also been shown to inhibit chloride secretion and thus have a direct effect on CFTR function, which may genetically predispose the patient to NTM (86). Further uncovering of the relationship regarding the use of ibuprofen in these patients remains to be studied.

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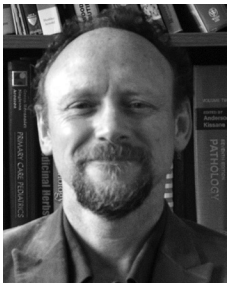

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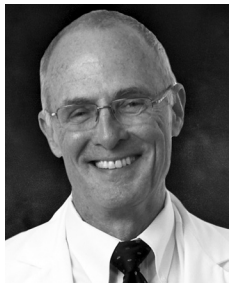

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