KEYWORDS
- Nontuberculous mycobacteria • NTM • Mycobacterium • Identification
- Antimicrobial susceptibility testing

KEY POINTS
- Among adults 65 years or older, from 1997 to 2007, the annual prevalence of pulmonary NTM disease significantly increased from 20 to 47 cases per 100,000 persons, or 8.2% per year. Women were 1.4 times more likely to be a pulmonary NTM case than men. Relative to white individuals, Asian/Pacific Islander individuals were twice as likely to be a case, whereas black individuals were half as likely.
- For optimal recovery of mycobacteria, clinical specimens from nonsterile body sites must be subjected to digestion, decontamination, and concentration. This procedure aims to eradicate more rapidly growing contaminants, such as normal flora (other bacteria and fungi), while not seriously affecting the viability of the mycobacteria.
- One of the most urgent questions that needs to be addressed rapidly by the mycobacteriology laboratory is whether *Mycobacterium tuberculosis* complex or NTM is involved. NAA assays are excellent tools for the purpose, and can be used directly on the clinical specimens of patients suspected of having mycobacterial disease, allowing same-day reporting of results. However, these tests are usually evaluated primarily with respiratory specimens and adequate information of their performance on nonrespiratory specimens stratified to different body compartments is often lacking.
- The Centers for Disease Control and Prevention recommends the use of both liquid and solid media for the growth detection of mycobacteria to decrease the time to detection and to increase the yield of growth detection.
- With the recent advances in chemistry and automation of instrumentation, DNA sequencing of variable genomic regions offers a rapid, accurate, and relatively inexpensive method for the identification of mycobacteria. The most routinely used and reliable method of this kind is the amplification and sequence analysis of hypervariable regions of the gene encoding 16S rRNA.
MICROBIOLOGY

Together with the genera Corynebacterium and Nocardia, the genus Mycobacterium forms a monophyletic taxon, the so-called CMN group, within the phylum Actinobacteria. The genus Mycobacterium is highly diverse, thanks to its ancient origin and years of evolution in multiple habitats. Historically, the species within the genus Mycobacterium have been classified based on their growth rate in a subculture as rapid (visible growth in <7 days) and slow growers (growth detection >7 days), and on their pigment production as scotochromogenic (pigment production in dark), photochromogenic (pigment production after exposure to light), or nonchromogenic.1–5

Previously, the identification of mycobacteria used a panel of cultural characteristics and biochemical tests; however, these assays are not only unacceptably time consuming, but also often inaccurate, laborious, or not capable of identifying the mycobacterium species at all. In addition, some fastidious species (eg, Mycobacterium haemophilum or Mycobacterium genavense) require special growth conditions (hemin source or unusually acidic pH), necessitating rarely used and special media, as well as an exquisite collaboration between the clinician requesting the test and the laboratory professional performing the test.1–5

The plethora of newly described species seen in the past decades (Table 1) is in part the consequence of the availability and increased reliability of new DNA-sequencing methods that are capable of differentiating even closely related species and an increased frequency of isolation of mycobacteria. The latter may be the result of newly emerging manmade reservoirs for certain species. From 41 valid species in 1980, currently this genus encompasses 169 recognized species and 13 subspecies (Fig. 1) (http://www.bacterio.net/mycobacterium.html).1–5

The Mycobacterium genus includes strict pathogens, potentially or opportunistic pathogens, and nonpathogenic saprophytic species. According to the presently prevailing terminology, the mycobacteria species that earlier were referred to as atypical mycobacteria or mycobacteria other than tuberculosis are now called nontuberculous mycobacteria (NTM). Gene sequence similarities within the genus sequences (>94.3% for 16S rRNA gene) and robust phylogenetic reconstructions using concatenated sequences of housekeeping genes have confirmed the natural division among slow-grower and rapid-grower mycobacteria, and also have demonstrated that all slow growers belong to a single evolutionary branch that emerged from the rapidly growing mycobacteria.2–5 This feature is intrinsically linked to their pathogenic ability to infect humans and, therefore, all obligatory pathogens and most opportunistic pathogens belong the slow-growing evolutionary branch.5

EPIDEMIOLOGY

NTM are ubiquitous environmental microorganisms that can be recovered from soil and fresh water and seawater (natural and treated).1–5 Until recently there was no evidence of human-to-human or animal-to-human transmission of NTM. However, 2 recent findings investigating outbreaks in patients with cystic fibrosis using thorough conventional epidemiologic and state-of-the-art molecular typing investigations, such as whole-genome sequencing, have challenged the dogma of person-to-person transmission indicating potential transmission of Mycobacterium abscessus subspecies massiliense and M abscessus between these patients.6,7 Because NTM may be found in both natural and manmade reservoirs, human infections are suspected of being acquired from these environmental sources. However, the identification of the specific source of infection is usually not possible. NTM diseases are usually not
Table 1  
List of novel nontuberculous mycobacteria species since 2004 causing pulmonary disease or were isolated from respiratory specimens

<table>
<thead>
<tr>
<th>Novel Species</th>
<th>Year</th>
<th>Growth</th>
<th>Closely Related to</th>
</tr>
</thead>
<tbody>
<tr>
<td>M abscessus ssp abscessus</td>
<td>2011</td>
<td>Rapid</td>
<td>M abscessus</td>
</tr>
<tr>
<td>M abscessus ssp bolletii</td>
<td>2011</td>
<td>Rapid</td>
<td>M abscessus</td>
</tr>
<tr>
<td>M alsiense*</td>
<td>2007</td>
<td>Slow</td>
<td>M szulgai/M malmoense</td>
</tr>
<tr>
<td>M arosiense</td>
<td>2008</td>
<td>Slow</td>
<td>M avium/M intracellulare</td>
</tr>
<tr>
<td>M arupense</td>
<td>2006</td>
<td>Slow</td>
<td>M nonchromogenicum</td>
</tr>
<tr>
<td>M aubagnense</td>
<td>2006</td>
<td>Rapid</td>
<td>M mucogenicum</td>
</tr>
<tr>
<td>M barrassiae*</td>
<td>2006</td>
<td>Rapid</td>
<td>M moriokaense</td>
</tr>
<tr>
<td>M boenickei</td>
<td>2004</td>
<td>Rapid</td>
<td>M porcinum</td>
</tr>
<tr>
<td>M bouchudurhonense</td>
<td>2009</td>
<td>Slow</td>
<td>M avium</td>
</tr>
<tr>
<td>M chimaera</td>
<td>2004</td>
<td>Slow</td>
<td>M avium/M intracellulare</td>
</tr>
<tr>
<td>M colomobiense</td>
<td>2006</td>
<td>Slow</td>
<td>M avium</td>
</tr>
<tr>
<td>M conceptionense</td>
<td>2006</td>
<td>Rapid</td>
<td>M fortuitum</td>
</tr>
<tr>
<td>M cosmeticum</td>
<td>2004</td>
<td>Rapid</td>
<td>M smegmatis</td>
</tr>
<tr>
<td>M europaeum</td>
<td>2011</td>
<td>Slow</td>
<td>M simiae</td>
</tr>
<tr>
<td>M florentinum</td>
<td>2005</td>
<td>Slow</td>
<td>M lentiflavum/M triplex</td>
</tr>
<tr>
<td>M fragae</td>
<td>2013</td>
<td>Slow</td>
<td>M celatum</td>
</tr>
<tr>
<td>M fukienense**</td>
<td>2013</td>
<td>Rapid</td>
<td>M chelonae/M abscessus</td>
</tr>
<tr>
<td>M houstonense</td>
<td>2004</td>
<td>Rapid</td>
<td>M fortuitum</td>
</tr>
<tr>
<td>M insubricum</td>
<td>2009</td>
<td>Rapid</td>
<td>M farcinogenes/M houstonense/M senegalense</td>
</tr>
<tr>
<td>M iranicum</td>
<td>2013</td>
<td>Rapid</td>
<td>M gilvum</td>
</tr>
<tr>
<td>M koreense</td>
<td>2012</td>
<td>Slow</td>
<td>M triviale</td>
</tr>
<tr>
<td>M kyorinense</td>
<td>2009</td>
<td>Slow</td>
<td>M celatum</td>
</tr>
<tr>
<td>M mantenii</td>
<td>2009</td>
<td>Slow</td>
<td>M scrofulaceum</td>
</tr>
<tr>
<td>M marseillense</td>
<td>2009</td>
<td>Slow</td>
<td>M avium</td>
</tr>
<tr>
<td>M massiliense</td>
<td>2004</td>
<td>Rapid</td>
<td>M abscessus ssp bolletii</td>
</tr>
<tr>
<td>M monacense</td>
<td>2006</td>
<td>Rapid</td>
<td>M doricum</td>
</tr>
<tr>
<td>M nebraskense</td>
<td>2004</td>
<td>Slow</td>
<td>M scrofulaceum</td>
</tr>
<tr>
<td>M noviomagense</td>
<td>2009</td>
<td>Slow</td>
<td>M xeopi</td>
</tr>
<tr>
<td>M paragordonae</td>
<td>2014</td>
<td>Slow</td>
<td>M gordonae</td>
</tr>
<tr>
<td>M parakoreense</td>
<td>2013</td>
<td>Slow</td>
<td>M koreense</td>
</tr>
<tr>
<td>M paraseoulense</td>
<td>2010</td>
<td>Slow</td>
<td>M seoulense</td>
</tr>
<tr>
<td>M paraterrae*</td>
<td>2010</td>
<td>Slow</td>
<td>M terrae</td>
</tr>
<tr>
<td>M parascrofulaceum</td>
<td>2004</td>
<td>Slow</td>
<td>M scrofulaceum</td>
</tr>
<tr>
<td>M phocaicum</td>
<td>2006</td>
<td>Rapid</td>
<td>M mucogenicum</td>
</tr>
<tr>
<td>M riyadhense</td>
<td>2009</td>
<td>Slow</td>
<td>M szulgai</td>
</tr>
<tr>
<td>M saskatchewanense</td>
<td>2004</td>
<td>Slow</td>
<td>M interjectum</td>
</tr>
<tr>
<td>M senuense</td>
<td>2008</td>
<td>Slow</td>
<td>M terrae</td>
</tr>
<tr>
<td>M seoulense</td>
<td>2007</td>
<td>Slow</td>
<td>M scrofulaceum</td>
</tr>
<tr>
<td>M sherrisii</td>
<td>2004</td>
<td>Slow</td>
<td>M simiae</td>
</tr>
<tr>
<td>M shinjukuense</td>
<td>2011</td>
<td>Slow</td>
<td>M tuberculosis H37Rv/M marinum/M ulcerans</td>
</tr>
<tr>
<td>M timonense</td>
<td>2009</td>
<td>Slow</td>
<td>M avium</td>
</tr>
<tr>
<td>M yongonense</td>
<td>2013</td>
<td>Slow</td>
<td>M intracellulare</td>
</tr>
</tbody>
</table>

* List of prokaryotic names without standing in nomenclature.

mandatory to report, as they are not communicable, and surveillance data are not only limited but also unreliable.\textsuperscript{1,8}

The average annual (2004–2006) site-specific prevalence among 4 integrated health care delivery systems ranged from 1.4 to 6.6 per 100,000. Prevalence was 1.1-fold to 1.6-fold higher among women relative to men across sites. Among persons aged 60 years or older, annual prevalence increased from 19.6 per 100,000 from 1994 to 1996, to 26.7 per 100,000 from 2004 to 2006.\textsuperscript{9}

Marras and colleagues\textsuperscript{10} reported an increase in the number of pulmonary NTM isolates in Ontario, Canada, from 9.1 per 100,000 in 1997 to 14.1 per 100,000 in 2003. In a follow-up study, the same research group measured the prevalence and temporal trends of pulmonary NTM disease among residents of Ontario from 1998 to 2010. Five-year prevalence increased from 29.3 cases per 100,000 persons from 1998 to 2002, to 41.3 per 100,000 in 2006 to 2010.\textsuperscript{11}

The prevalence and trends of pulmonary NTM-associated hospitalizations in the United States were estimated using national hospital discharge data from 11 states with continuous data available from 1998 through 2005. Pulmonary NTM hospitalizations increased significantly with age among both sexes: relative prevalence for persons 70 to 79 years of age compared with those 40 to 49 years of age was 15 per 100,000 for women (9.4 vs 0.6) and 9 per 100,000 for men (7.6 vs 0.83). Annual prevalence increased significantly among men and women in Florida (3.2%/year and 6.5%/year, respectively) and among women in New York (4.6%/year), with no significant changes in California.\textsuperscript{12}

Adjemian and colleagues\textsuperscript{13} described the prevalence and trends of pulmonary NTM disease among adults aged 65 years or older throughout the United States in a nationally representative 5% sample of Medicare Part B beneficiaries from 1997 to 2007. From 1997 to 2007, the annual prevalence significantly increased from 20 to 47 cases per 100,000 persons, or 8.2% per year. Women were 1.4 times more likely to be a
pulmonary NTM case than men. Relative to white individuals, Asian/Pacific Islander individuals were twice as likely to be a case, whereas black individuals were half as likely (Figs. 2 and 3).

PATHOGENESIS AND CLINICAL SIGNIFICANCE

NTM may result in colonization, infection, and disease. NTM that are also called opportunistic mycobacteria may become pathogenic in certain conditions (Box 1), whereas other so-called saprophytic NTM never or very rarely cause diseases. Colonization can be defined by the absence of host immune reaction, whereas in the event of infection, the host may respond with skin test reaction or antibody production but without disease manifestation. Colonization and infection can be transient, intermittent, and prolonged. Because humans are in regular contact with NTM in the environment, NTM can be detected in the respiratory and gastrointestinal tract or on the skin in healthy individuals.

NTM may occur in natural and manmade environments, such as treated urban water and sewage systems, swimming pools, hot tubs, pedicure foot baths and showers, tattoo inks, fish tanks, or medical devices, such as endoscopes and their washing machines, ice machines used to refrigerate surgical solutions, or inadequately sterilized surgical equipment or solutions. Certain NTM, such as *Mycobacterium avium*, *Mycobacterium chelonae*, or *Mycobacterium marinum*, are more commonly recoverable in artificial sources, whereas the natural reservoir of *Mycobacterium kansasii* and *Mycobacterium xenopi* is unknown. Other species, such as *Mycobacterium gordonae*, are common in both natural and artificial sources. NTM can form a biofilm on a wide range of organic (plastic, silicone, rubber, PVC) and inorganic material (glass and

![NTM Cases/100,000 Persons](image)

metals, metallic fluids of machines) due to their hydrophobic cell wall, and their resistance to disinfectants, antibiotics, or heavy metals. Both in natural and manmade environments, biofilms may have an important role in protecting NTM against aggressive external factors and in promoting their colonization. This colonization of NTM in biofilms may lead to contaminations that can be source of pseudoinfections or NTM diseases. Pseudoinfections may be the result of contamination during collection of specimens (eg, biofilms in improperly cleaned endoscopes) or contamination during laboratory testing (contaminated water source for reagent preparation). Although pseudoinfections do not necessarily cause disease, they may create difficult diagnostic confusion.\textsuperscript{1,2,8}


Box 1
Underlying conditions predispose to nontuberculous mycobacteria (NTM) lung disease

- Bronchiectasis
- Chronic obstructive pulmonary disease
- Alpha-1-antitrypsin anomalies
- Pneumoconiosis
- Pulmonary alveolar proteinosis
- Immunosuppressive states (ie, use of anti–tumor necrosis factor-alpha biologics, posttransplantation immunosuppression, HIV infection)

Therefore, when NTM are suspected as the etiologic agent of disease (called a mycobacteriosis), the definitive diagnosis should always be supported by repeated isolation of the NTM from several specimens of the patient or a single specimen if collected aseptically from a closed lesion (Box 2). However, laboratory identification of potentially pathogenic or saprophytic NTM alone are not enough to dictate patient care, and laboratory results should always be correlated with the individual’s clinical presentation and radiologic and histologic findings (Table 2) to determine the clinical significance of the specimen and make the correct diagnosis. This requires a partnership and ongoing consultation between the laboratory and clinicians.

DIAGNOSIS

Accurate, rapid microbiological diagnosis of tuberculosis (TB) and other mycobacterial infections begins with proper specimen collection and rapid transport to the laboratory. To ensure collection of the best possible specimen, the health care worker has to be properly trained and the patient provided with clearly presented and fully understood instructions for sputum and other specimen collection to obtain a quality specimen with adequate volume and to avoid contamination with NTM. Improper specimen

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**Box 2**

Clinical and microbiological criteria for diagnosing NTM

**Clinical (both required)**

1. Pulmonary symptoms, nodular or cavitary opacities on chest radiograph, or a high-resolution computed tomography scan that shows multifocal bronchiectasis with multiple small nodules and

2. Appropriate exclusion of other diagnoses

**Microbiologic**

1. Positive culture results from at least 2 separate expectorated sputum samples. If the results from (1) are nondiagnostic, consider repeat sputum acid-fast bacilli (AFB) smears and cultures

or

2. Positive culture result from at least 1 bronchial wash or lavage

or

3. Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture-positive for NTM

4. Expert consultation should be obtained when NTM are recovered that are either infrequently encountered or that usually represent environmental contamination

5. Patients who are suspected of having NTM lung disease but do not meet the diagnostic criteria should be followed until the diagnosis is firmly established or excluded

6. Making the diagnosis of NTM lung disease does not, per se, necessitate the institution of therapy, which is a decision based on potential risks and benefits of therapy for individual patients

collection and contamination of specimens with NTM, especially in tap water, can seri-
ously hamper the determination of clinical significance of specimens. The best example
of this significant problem is the use of nonsterile bronchoscopes (rinsed with nonsterile
or nonfiltered water that contained NTM).14–16 To avoid false-positive culture or nucleic
acid amplification (NAA)-related pseudo-outbreaks due to “mycobacterium-contami-
nated” bronchoscopes, these researchers recommend rinsing the instrument with ster-
ile or filtered water, and for amplification tests, a sterile prewash of the bronchoscopes
be performed and analyzed along with the actual clinical specimen.15,16 However, the
use of routine environmental microbiological testing of bronchoscopes for quality
assurance has not yet been established, although the implementation of an effective
pathogen surveillance program is recommended by the American College of Chest
Physicians and American Association for Bronchology.17

To provide the best results, collect multiple specimens (especially respiratory), if
possible, and the volume of a sputum specimen should exceed 5 mL.18 Histologic pa-
rameters also can provide useful information regarding specimen sampling. There is
evidence that biopsy specimens that show necrotizing granulomas, non-necrotizing
granulomas, poorly formed granulomas, or acute inflammation are optimal for myco-
bacterial growth detection. However, biopsy specimens showing only fibrotic or hyali-
nized granulomas, nonspecific chronic inflammation, reactive or reparative changes,
malignancy, or no significant abnormalities are less appropriate for mycobacterial
culture and staining.19

Most respiratory specimens will contain microorganisms other than mycobacteria.
Therefore, the specimen should be refrigerated if transportation is delayed more than
1 hour, or otherwise overgrowth of more rapidly growing contaminants may occur.20

### SPECIMEN PROCESSING

For optimal recovery of mycobacteria, clinical specimens from nonsterile body sites
must be subjected to digestion, decontamination, and concentration. This procedure
aims to eradicate more rapidly growing contaminants, such as normal flora (other bac-
teria and fungi), while not seriously affecting the viability of the mycobacteria.18 Biopsy
samples or body fluids from normally sterile sites do not require pretreatment and can
be directly inoculated onto culture media. The efficacy of decontamination procedures
is highly influenced by the time of exposure to the reagent used for decontamination,
the toxicity of that reagent, the efficiency of centrifugation, and the killing effect of heat
buildup during centrifugation.18 NTM, in particular rapidly growing mycobacteria
(RGM), are more vulnerable to decontamination than *Mycobacterium tuberculosis*.
Therefore, it is important to keep in mind that even the mildest decontamination
methods, such as the widely used N-acetyl-L-cysteine/NaOH method, can kill approx-
imately 33% of the mycobacteria in a clinical specimen, whereas more overzealous

<table>
<thead>
<tr>
<th>Signs and Symptoms</th>
<th>Radiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough (chronic)</td>
<td>Fibrocavitary: <em>M. avium</em> complex</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Nodular and interstitial nodular infiltrates: <em>M. avium</em> complex</td>
</tr>
<tr>
<td>Weight loss</td>
<td>Fibrocavitary: <em>M. kansasii</em></td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>Multilobar, reticulonodular, or mixed reticulonodular-alveolar</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>Opacities: <em>M. abscessus</em> complex</td>
</tr>
</tbody>
</table>

methods can kill up to 70%. In addition, samples from particular patient populations might need special attention regarding the homogenization and decontamination method to be used. This is especially true for respiratory specimens from patients with bronchiectasis or cystic fibrosis, as NTM are being recovered from these patients with increasing frequency. Because oftentimes specimens of these patients may also contain Gram-negative rods, such as *Pseudomonas aeruginosa*, which can overgrow the culture medium and thus prevent the isolation of NTM, it was recommended that the N-acetyl-L-cysteine/NaOH decontamination method should be followed by a 5% oxalic acid treatment. This extra decontamination step can sufficiently reduce the potential overgrowth by *P aeruginosa* and may well improve the recovery rate of clinically significant NTM. However, certain NTM may also be susceptible to oxalic acid, resulting in a reduced growth detection yield; therefore, double-processing by oxalic acid may be restricted to only those specimens from patients who show contamination either by prescreening on a nutrient agar or on cultures for mycobacteria.

A recent study has shown that decontamination by chlorhexidine yielded the isolation of more NTM than the N-acetyl-L-cysteine/NaOH with oxalic acid on solid culture. However, chlorhexidine cannot be used with broth-based culture systems and its improved recovery rate on solid culture was balanced by the higher yield of liquid culture when that was used with N-acetyl-L-cysteine/NaOH and oxalic acid.

**ACID-FAST MICROSCOPY**

Acid-fast microscopy is the fastest, easiest, and least-expensive tool for the rapid identification of patients with mycobacterial infections, and semiquantitative results of smear examinations may be an important aid in determining the clinical significance of specimens with NTM isolates. However, microscopy is unable to distinguish within the *Mycobacterium* genus and between viable and nonviable mycobacteria. The sensitivity of microscopy is influenced by numerous factors, such as the prevalence and severity of tuberculosis or NTM disease, the type of specimen, the quality of specimen collection, the number of mycobacteria present in the specimen, the method of processing (direct or concentrated), the method of centrifugation, and, most importantly, by the staining technique and the quality of the examination. To facilitate proper patient management, all results should be reported to the physician within 24 hours of specimen collection or, if an off-site laboratory is used, within 24 hours after receipt of the specimen.

It is generally accepted that, owing to an average of 10% higher sensitivity, the fluorescent method should be given preference over the carbol fuchsin–based (Ziehl-Neelsen [ZN]) or Kinyoun staining methods. However, it is often forgotten that fluorochrome stains may stain other bacteria damaged by antituberculous drugs at a higher rate than carbol fuchsir, and lead to a false-positive result. This possibility should be considered when the specimen is from a patient on therapy. Recently, it has been shown that vital staining with fluorescent diacetate may serve as a reliable method to microscopically visualize only viable mycobacteria and to rapidly confirm therapeutic failure before culture results are available. It also has been shown that with the application of simple small-membrane filters directly on clinical specimens, the sensitivity of smear microscopy could be well increased above the level of that with centrifugation-based sample preparation methods in paucibacillary HIV-infected individuals as well. Because extrapulmonary specimens of NTM diseases may often contain low amounts of detectable mycobacteria, this approach may warrant further validation on specimens from different body compartments as well.
It is noteworthy that NTM, especially RGM, may be more sensitive to the decolorization procedure with acid alcohol during staining. Indeed, several studies have indicated a clear trend toward less detection of NTM compared with *M tuberculosis* complex by fluorescent microscopy regardless of the source of light (traditional fluorescent lamp or light-emitting diode light source). Because of a tendency toward false-positivity with fluorochrome staining, good laboratory practice requires that any doubtful and smear-positive results should be confirmed. This can be accomplished by a second observer, restaining of the slide using ZN or Kinyoun stain, or by initially preparing 2 smears, one for the fluorescent stain and the other for ZN or Kinyoun in the event of a positive with fluorochrome staining.

**DIRECT NAA ASSAYS**

One of the most urgent questions that needs to be addressed rapidly by the mycobacteriology laboratory is whether *M tuberculosis* complex or NTM is involved. NAA assays are excellent tools for the purpose, and can be used directly on the clinical specimens of patients suspected of having mycobacterial disease, allowing same-day reporting of results. However, these tests are usually evaluated primarily with respiratory specimens and adequate information of their performance on nonrespiratory specimens stratified to different body compartments is often lacking.

The LightCycler Mycobacterium Detection Kit (Roche Products Ltd, Randburg, South Africa) that was developed for use on respiratory specimens was reported to be an accurate tool for the direct detection of *M tuberculosis*, *M avium*, and *M kansasii* within 90 minutes. The assay targets the 16S rRNA gene and uses fluorogenic hybridization probes for species identification by melting curve analysis on the LightCycler 2.0 platform. An internal control also has been integrated in the assay and the platform enables a high-throughput testing capacity.

The Genotype Mycobacteria Direct test (Hain Lifescience GmbH, Nehren, Germany) is based on the amplification of the 23S rRNA gene in an isothermal reaction. Subsequently biotinylated amplicons are hybridized to oligonucleotide probes anchored on a strip. The assay detects the *M tuberculosis* complex, *M avium*, *Mycobacterium intracellulare*, *M kansasii*, and *Mycobacterium malmoense* directly from processed respiratory specimens; however, further studies are needed to determine assay performance for NTM. Recently, a new polymerase chain reaction (PCR) and line probe assay–based test was developed by Nipro Co (Osaka, Japan) for the rapid detection of *M tuberculosis* complex and rifampin and isoniazid resistance-associated mutations along with the rapid detection and identification of 3 NTM (*M avium*, *M intracellulare*, and *M kansasii*) directly in clinical specimens. The assay was evaluated directly on 163 processed sputum samples and showed a sensitivity of 90.2% for the *M tuberculosis* complex, 84.6% for *M avium*, 54.5% for *M intracellulare*, and 80.0% for *M kansasii*.

**GROWTH DETECTION**

The Centers for Disease Control and Prevention recommends the use of both liquid and solid media for the growth detection of mycobacteria to decrease the time to detection and to increase the yield of growth detection. Growth detection is still indispensable for the following reasons: (1) culture is more sensitive for the detection of mycobacteria than acid–fast microscopy or NAA (especially in paucibacillary disease or in certain extrapulmonary specimens); (2) semiquantitative results of NTM colony counts on solid media may be useful to determine clinical significance or assess response to therapy; (3) growth is necessary for precise identification
(eg, *M chelonae* and *M abscessus* complex, *M marinum* and *Mycobacterium ulcerans*, *M kansasii*, and *M gastri*); (4) phenotypic antimicrobial susceptibility tests (AST) require viable organisms, whereas detection of particular resistance-associated molecular mechanisms require pure culture and large biomass; and (5) genotyping of particular cultured NTM (ie, *M avium*, *M abscessus*, *M chelonae*) can be used for epidemiologic purposes.

Broth-based culture systems have the potential to significantly decrease turnaround time for growth detection of mycobacteria. These systems include the manual Mycobacteria Growth Indicator Tube (MGIT; Becton-Dickinson Diagnostic Instrument Systems, Sparks, MD) and fully automated systems, such as the BACTEC MGIT 960 (Becton-Dickinson Diagnostic Instrument Systems), the BACTEC 9000 MB (Becton-Dickinson Diagnostic Instrument Systems), the MB/BacT 3D (bioMerieux, Durham, NC), and the ESP-II (Trek Diagnostic Systems, Oakwood Village, Ohio).

With the exception of the BACTEC 9000 MB systems, these growth detection systems cannot be used for direct inoculation of blood. Blood samples can be inoculated into these systems only after lysis and centrifugation steps. The paucibacillary nature of NTM infections of certain organs or body sites or the difficulty of adequate specimen collection from particular extrapulmonary compartments often time hamper the growth detection and laboratory confirmation of NTM diseases. Recently, it has been shown that both the mycobacterial yield and time to detection of growth detection in the MGIT 960 system could significantly be improved with the addition of a simple nutrient broth (modified Dubos liquid medium) in paucibacillary pediatric samples.

Although the broth-based systems have decreased the time to detection to 1 to 3 weeks, a solid medium should be used for those strains that may not grow well in liquid media. In particular, this holds true for *M haemophilum*, which will grow better on solid media (supplemented with hemin or hemoglobin as an iron source). It is noteworthy that *M avium* subsp *paratuberculosis* also requires additional nutrients (egg yolk and the siderophore mycobactin J) in both liquid and solid media for optimal growth, whereas *M ulcerans* may be optimally recovered with egg yolk supplementation.

However, other species like *M genavense* show a better recovery rate in liquid media especially at an acidic pH (pH 5.5). Similar to liquid media, the pH of solid media also can significantly influence the growth of mycobacteria. It has been shown that for slowly growing mycobacteria, based on the testing of 16 different species, the optimal pH in Löwenstein-Jensen (LJ) medium was between 5.8 and 6.5. As for RGM, the optimal pH was between 7.0 and 7.4, with the exception of *M chelonae*, which preferred an acidic pH. These findings indicate that the routinely applied LJ medium with pH 7.0 is not optimal for the isolation of all mycobacteria. Therefore, in areas endemic for lung or other diseases caused by NTM, inoculation to an additional LJ slant with an acidic pH, or to Ogawa medium (pH 6), is recommended.

A further advantage for culturing mycobacteria on solid media is that growth can be quantified to better determine clinical significance of the isolate or assess therapeutic response, and colony morphology (Figs. 4 and 5) and pigmentation can be examined to identify mixed infections with more than one mycobacterium species (Fig. 6).

In addition, growth detection of *M xenopi*, *M ulcerans*, or *M genavense* often requires further incubation of cultures up to 12 weeks or incubation. Although most of the clinically significant slowly growing NTM can be isolated by incubating cultures between 35 and 37°C, *M haemophilum* and *M marinum* grow only at 28 to 30°C, *M ulcerans* at 25 to 33°C, and some strains of *M chelonae* grow only at 28 to 33°C.
Therefore, cultures of all skin, joint fluid, and bone specimens should be incubated at both 28 to 30°C and 35 to 37°C.¹,²

IDENTIFICATION

*Nucleic Acid Hybridization Methods*

The commercially available AccuProbe (Hologic Gen-Probe Inc, San Diego, CA) nucleic acid hybridization assay allows the rapid identification of the *M tuberculosis* complex, the *M avium* complex, *M avium*, *M intracellulare*, *M gordonae*, and *M kansasii* within 2 hours following growth detection in culture, as they do not include an

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**Fig. 4.** *M avium* (smooth [A] and translucent [B]).

**Fig. 5.** *M abscessus* (rough).
amplification step. Cross-reaction has been reported with AccuProbe MTB complex with isolates of either *Mycobacterium celatum* types 1 and 3 or *Mycobacterium terrae* when the test is not performed at the proper hybridization temperature (between 60 and 61°C rather than 60 ± 1°C). It has been reported that, in rare circumstances, some strains of *M gordoneae* and *M kansasii* may be falsely negative with AccuProbe. This caveat should be considered when other assays lead to the identification of an isolate, such as *M gordoneae* or *M kansasii*. DNA/RNA probes are usually capable of identifying mycobacteria in contaminated liquid cultures depending on the extent of the contamination because they have a sensitivity and specificity of nearly 100% when at least 10⁵ organisms are present.

Wallace and colleagues pointed out the potential inadequacies of nonsequencing identification methods, such as hybridization DNA probe assays, 16S rRNA gene multiplex PCR, or PCR restriction fragment length polymorphism analysis (PRA), for closely related NTM species, such as *M intracellulare* and *M chimaera*. Their study supports the notion that lumping mycobacteria into groups or complexes will obscure unique characteristics, such as their ecology, epidemiology, virulence, and even susceptibilities to antimicrobial agents.

The Invader assay (Third Wave Technologies, Madison, WI) also was shown to be a reliable hybridization method to rapidly identify mycobacteria isolates. This assay can accurately discriminate single-base differences and can measure directly on genomic DNA without prior amplification by using isothermal conditions. The assay has correctly identified a total of 888 clinical and reference strains and was able to identify 116 (95.1%) of 122 positive liquid cultures of the MGIT 960 system within 4 hours.

**PCR and Restriction Fragment Length Polymorphism Analysis**

The most widely adopted PRA method is based on a 441-bp sequence of the *hsp65* gene. Later, Brunello and colleagues developed a modified PRA of the *hsp65* gene with a new algorithm describing 54 species, including 22 species that were not described previously. Recently, Sajduda and colleagues showed that *hsp65*
PRA analysis aided with automated fluorescence capillary electrophoresis offers the advantage of higher accuracy and rapidity. Alternative diagnostic algorithms also were developed based on the PRA of the dnaJ gene, the 16S-23S DNA spacer region, and on the rpoB gene of mycobacteria. The first method identified 48 species, 40 subspecies, and 4 subtypes, whereas the second method identified 50 species and 13 subtypes. A drawback of PRA is misidentification due to intraspecies genetic variability (if the PRA pattern is not distinct).

**Line Probe Assays**

Three commercially available (except in the United States) line probe assay–based (LPA) tests, the Inno-LiPA Mycobacteria assay (Innogenetics N.V., Ghent, Belgium) and the Genotype Mycobacterium CM and AS assays (Hain Lifescience GmbH, Nehren, Germany) target the 16S-23S rDNA spacer region and the 23S rDNA for identification of mycobacteria. These LPAs are based on the solid phase reverse hybridization of biotinylated PCR amplicons of the target region to oligonucleotide probes arranged on a membrane strip. The Inno-LiPA test is capable of detecting and identifying the \( M \) \( tuberculosis \) complex and an additional 15 NTM, the GenoType Mycobacterium CM test is capable of identifying the \( M \) \( tuberculosis \) complex and an additional 24 NTM, and the GenoType Mycobacterium AS test is capable of identifying of an additional 19 NTM from solid and liquid media. LPAs also may enable the simultaneous detection of species in mixed cultures. The Inno-Lipa Mycobacteria assay also was used for the direct detection of NTM in respiratory samples, with an overall clinical sensitivity and specificity of 79.5% and 84.6%, respectively. The recently introduced Speed-oligo Mycobacteria test (Vircell, Cordoba, Spain) is also a DNA strip–based assay to rapidly differentiate the most frequently isolated mycobacteria in clinical isolates. It is a PCR-based test targeting the 16S rRNA (for genus detection) and 16S-23S rRNA (for species identification) regions. The kit consists of a user-friendly lyophilized PCR mix with a noncompetitive internal amplification control. PCR products are detected by a dipstick device and the entire test can be completed within 2 hours. The assay is intended for the rapid detection of \( M \) \( tuberculosis \) sp, \( M \) \( fortuitum \), \( M \) \( avium-intracellulare \), \( M \) \( tuberculosis \) complex, \( M \) \( kansasii \), \( M \) \( gordonae \), and \( M \) \( abscessus-chelonae \) complex. The test provided concordant results for these species in 177 of 182 isolates (including 61 \( M \) \( tuberculosis \) complex isolates). Following a simple purification test of the PCR amplicon, direct 16S DNA sequencing of a \( M \) \( mycobacterium \) genus–positive specimen also may be performed in 24 hours for rapid identification of additional NTMs.

**DNA Sequencing**

With the recent advances in chemistry and automation of instrumentation, DNA sequencing of variable genomic regions offers a rapid, accurate, and relatively inexpensive method for the identification of mycobacteria. The most routinely used and reliable method of this kind is the amplification and sequence analysis of hypervariable regions of the gene encoding 16S rRNA. For taxonomic and phylogenetic purposes, 2 hypervariable sequences, regions A and B, of the 16S rDNA are usually investigated.

However, identification of new species or some species that cannot be well differentiated by the 16S rDNA (\( M \) \( ulcerans \) and \( M \) \( marinum \), \( M \) \( kansasii \) and \( M \) \( gastri \), \( M \) \( che- lonae \) and \( M \) \( abscessus \) complex [\( M \) \( abscessus \) subsp \( abscessus \) and \( M \) \( abscessus \) subsp \( bolletii \)]) may require the use of alternative DNA-sequencing methods, such as amplification and DNA sequencing of the rpoB, gyrB, hsp65, recA, sodA, dnaJ, 32-kDa protein genes or the 16S-23S rRNA gene spacer. It has been shown
that clear-cut results with DNA sequencing are not the rule, as public or commercial databases may be inaccurate or may not include all established species.\textsuperscript{83}

Accurate and unambiguous identification at the species and even subspecies level may be clinically important because of differences in susceptibilities against antimicrobial agents, virulence, epidemiology, and ecology.\textsuperscript{55,84,85} However, the use of a single DNA-sequencing target may not always provide this necessary answer, as it was shown in the case of identification of different members of the \textit{M abscessus} complex. The taxonomic definition of the \textit{M abscessus} complex is controversial. Based on their \textit{rpoB} sequences earlier, 2 new species, \textit{Mycobacterium massiliense} and \textit{Mycobacterium bolletii}, were proposed. However, recent studies have shown that these new species cannot be distinguished by biochemical and mycolic acid pattern testing and indicated less genomic divergence expected for distinct species using \textit{rpoB} sequencing alone, suggesting an intergroup lateral transfer of the gene.\textsuperscript{76,86,87} Therefore, it has been proposed to unite \textit{M massiliense} and \textit{M bolletii} as \textit{M abscessus} subsp \textit{bolletii} and identify a new taxon \textit{M abscessus} subsp \textit{abscessus}.\textsuperscript{86} However, a recent whole-genome sequencing–based phylogenetic investigation gave additional support to subgroup the \textit{M abscessus} complex into 3 subspecies.\textsuperscript{7} More recently, a multilocus DNA sequence analysis approach involving 8 housekeeping genes, a multspacer sequence typing, and simple and robust PCR-based typing scheme targeting 4 discriminatory locations identified from array-based comparative genomic hybridization was reported to be an effective tool to identify members of the \textit{M abscessus} group.\textsuperscript{76,80,87,88}

The advantage of simultaneous amplification, sequencing, and analysis of 16S rRNA and \textit{rpoB} genes also was highlighted by a recent study that applied the RipSeq dual locus identification system (iSentio, Bergen, Norway).\textsuperscript{89}

\textbf{MALDI-TOF MS}

Whole-cell matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) can be used in the identification of mycobacteria. This technology is designed to provide a protein “fingerprint” based on the desorbed ions from the cell surfaces. Bacterial cells are applied to a target plate after inactivation and extraction, and overlaid with a matrix solution. After analysis, the instrument software automatically acquires and analyzes the data and generates a profile for comparison to a database of reference spectra composed of previously well-characterized isolates with an algorithm that can analyze mixed electropherograms obtained by sequencing of 2 different gene targets in one step.\textsuperscript{90} The integration of the 16S rRNA gene and \textit{rpoB} sequencing allowed the identification of 50 additional organisms to the species level compared with identification using the 16S rRNA gene alone. In addition, this approach allowed the same step distinction of members of the \textit{M abscessus-chelonae} complex or \textit{M kansasii} from \textit{M gastri}. The dual-locus algorithm was able to identify closely related 16S rRNA gene reference sequences for 138 of 139 samples and closely related \textit{rpoB} gene reference sequences for 134 of 139 samples without manual inspection of the mixed electropherograms or database scrutiny.

In a recent study from the University of Washington, rapidly growing mycobacteria were extracted after 3 to 7 days of growth, and slowly growing mycobacteria were extracted after 14 to 21 days.\textsuperscript{90} The MALDI-TOF MS results were compared with results obtained from DNA sequencing of the 16S rRNA, \textit{rpoB}, and/or \textit{hsp65} genes. A total of 198 clinical strains, representing only 18 \textit{Mycobacterium} species, were correctly identified to the species level 94.9\% of the time using an extraction developed by the investigators, and compared with an augmented database. Both the Bruker MALDI Biotyper (Bruker, Fremont, CA) and the bioMerieux Vitek MS system
(bioMerieux, Durham, NC) resulted in correct species-level identifications for 94.4% of these strains. The investigators noted that neither misidentification was clinically relevant. Furthermore, their data suggest that the age of the colonies used for testing should closely match the age of those colonies used for database creation.

GENOTYPING

Genotyping methods have been successfully applied to assess strain relatedness and recognize nosocomial outbreaks, pseudo-outbreaks, mixed infections, or laboratory contamination also with NTM.\(^2\) A recent review by Jagielski and colleagues\(^91\) provides a thorough overview on current methods of molecular typing of mycobacteria. The choice of molecular typing method highly depends on the species, the sample, the setting under investigation, and the expected outcome. The most commonly used methods are pulsed-field gel electrophoresis, insertion sequence (IS)-based typing, methods based on minisatellite sequences (e.g., variable number of tandem repeat, major polymorphic tandem repeat), repetitive sequence-based PCR, random amplified polymorphic DNA analysis, amplified fragment length polymorphism analysis, or multilocus sequence typing.\(^91\) With the advance of genotyping technologies, extensive intraspecies genetic divergence could be revealed in NTM, such as the *M avium* complex, *M kansasii*, or the *M abscessus* complex. These findings also suggested that particular subgroups of an NTM species may be associated with different degree of pathogenicity in humans, distinct clinical manifestation, disease progression, and susceptibility to certain antibiotics.\(^6,92–95\) More recently, a whole-genome sequencing-based study, which provides higher resolution than more conventional typing methods, has identified less diversity of *M abscessus* subsp *massiliense* in clustered patients with cystic fibrosis than that observed within serial isolates from a single individual. This finding strongly indicates transmission between patients during an outbreak investigation. In addition the clusters of *M abscessus* subsp *massiliense* also showed evidence of transmission from a patient with mutations associated with macrolide and amikacin resistance to other patients.\(^7\) Newer molecular typing methods, such as whole-genome sequencing, will likely be able to help identify more reliable and meaningful markers associated with the adaption of particular NTM in specific hosts and environmental habitats, or the evolutionary development of clinically significant NTM and their clinically significant subtypes. This new information also should help to better understand why certain species or certain intraspecies subtypes become clinically more significant while others rarely do. In addition, it is also important to collect genetic typing information on patients with different NTM infections so that we not only can understand what is the link between NTM infections and associated diseases (such as cystic fibrosis, different stages of chronic obstructive bronchitis, or bronchiectasis), but can better identify individuals at risk for these infections or determine differences in prognosis.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

The presently accepted recommendations and guidelines for *in vitro* AST of clinically significant NTM isolates are summarized in the recent recommendation of the American Thoracic Society (ATS) and Infectious Disease Society of America (IDSA) from 2007 and the revised guidelines of the Clinical and Laboratory Standards Institute (CLSI) from 2011.\(^1,96\) A more recent review by Brown-Elliott and colleagues\(^8\) provides additional important recommendations and updates.

The role and relevance of *in vitro* AST of NTM to guide the treatment and clinical management of patients with NTM disease is under continuous debate. The basis
of this debate is that in contrast to *M. tuberculosis*, clinical response to antituberculosis drugs or antibiotics has been shown to correlate only with some compounds and only in some NTM (eg, *M. avium* complex and macrolides or *M. kansasii*, *M. marinum*, or *M. fortuitum*), whereas similar correlation for several other clinically significant NTM (eg, *M. abscessus*) were not or could not be established. On the other hand, in contrast to *M. tuberculosis*, most clinically significant initial NTM isolates already show natural resistance or high *in vitro* breakpoints for several antibiotics and, therefore, AST on NTM seems to be logical, and also to accumulate evidence on their *in vitro* AST patterns. However, it is important to keep in mind that performing AST on clinically nonsignificant NTM isolates is a waste of time and resources, and results may generate confusion in patient management. Because most NTM are ubiquitous in soil and water, determination of the clinical significance of an isolate is warranted before initiating any AST. A further problem is that determination of AST in NTM can be method and species dependent. 

Therefore, establishing valid, representative and reproducible AST breakpoints for therapeutic compounds is an important first step to reliably distinguish susceptible and resistant populations and to derive epidemiologic cut-offs (ECOFFs), as it was also indicated by a recent study by Hombach and colleagues on slowly growing NTM. Determining such ECOFFs on a meaningful number of clinically significant isolates using wild-type drug susceptibility distributions, comparing these ECOFFs with pharmacokinetics and pharmacodynamics data and subsequent selection of clinical antibiotic susceptibility breakpoints (CBP) in clinical studies has been suggested to determine the clinical value of *in vitro* AST findings and setting of CBPs. 

The gold standard method for AST of rapidly growing NTM is a broth microdilution assay. AST testing of rapidly growing NTM should be guarded by quality control by using CLSI-recommended reference strains to ensure not only quality testing but also reproducibility of minimal inhibitory concentration (MICs) within the recommended and acceptable ranges of antimicrobials tested. It is noteworthy that incubation due to acidification of broth pH in CO₂ should be avoided, as this can negatively influence testing for macrolides. In addition, MIC results for imipenem and meropenem, and tetracycline, may be invalid after more than 5 days of incubation because of stability-related problems. Isolates that are susceptible to clarithromycin should be further incubated for 14 days to rule out inducible macrolide resistance, which is a common phenomenon due to the presence of an rRNA methylase *erm* gene present in most clinically significant rapidly growing NTM. To decrease turnaround time and save resources on prolonged incubation, routine DNA sequencing of particular *erm*, such as *erm* (41) in *M. abscessus*, may facilitate both rapid macrolide inducibility detection and species identification within the *M. abscessus* complex. Additional methods, such as the agar disk diffusion method and Etest (bioMerieux), have not been standardized by CLSI regarding validation of end points or showed problems with reproducibility and therefore are not recommended for AST of rapidly growing NTM at this point. 

For slowly growing NTM, no single AST method is recommended. Both broth (microdilution or macrodilution) and solid AST (eg, agar disk elution for *M. haemophilum* that requires more prolonged incubation) methods may be used following proper species-specific intralaboratory validation, and under adequate quality control measures. Recommendations of the ATS, IDSA, and CLSI for slowly growing NTM AST are summarized in **Box 3**. 

Macrolides are playing a central role in treatment of *M. avium* complex diseases, and *in vitro* AST for clarithromycin and clinical response has shown a good correlation.
Therefore, rapid molecular detection, predicting macrolide resistance in clinically significant isolates, may be beneficial to shorten turnaround times to identify patients with macrolide resistance and potential treatment failure. Previous studies have found that mutations in 80% to 100% of high-level macrolide-resistant *M. avium* complex isolates could be detected at nucleotides 2058 and 2059 in the peptidyl transferase loop of the 23S rRNA gene. However, the same mutations could be detected only between 10% and 20% of *M. avium* complex strains with lower level of macrolide resistance. Recently, Maurer and colleagues investigated the role of clarithromycin resistance–associated mutations in the 23S rRNA (*rrl*) gene in patients with chronic *M. abscessus* infection undergoing clarithromycin therapy. Follow-up isolates demonstrated acquisition of resistance mutations in the *rrl* gene in addition to the presence of an inducible Erm methylase, indicating that routine 23S rRNA sequencing in *M. abscessus* can be a valuable aid to rapidly detect high-level macrolide resistance in these patients.

IDENTIFIED FOCUS AREAS IN NTM RESEARCH

In a recent editorial, Daley and Glassroth described difficulties finding answers to important NTM questions: (1) Robust multicenter trials, although more costly to conduct, would provide the patient numbers needed to conduct rigorous and adequately powered clinical trials in patients with NTM infections. (2) Studies that analyze trial results stratified by factors that are known to affect outcomes are important, as treatment outcomes are worse in patients who have acid-fast bacilli smear-positive disease and those with cavitary disease. (3) Patients with NTM have differing

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**Box 3**

Recommendations of the American Thoracic Society and Infectious Disease Society of America for drug susceptibility testing of slowly growing mycobacteria

1. Clarithromycin susceptibility testing is recommended for new and previously untreated *M. avium* complex isolates. No other drugs are recommended for susceptibility testing of new and previously untreated *M. avium* complex isolates. There is no recognized value for testing of first-line antituberculosis agents with *M. avium* complex.

2. Clarithromycin susceptibility testing should be performed for *M. avium* complex isolates from patients who fail macrolide therapy or prophylaxis.

3. Previously untreated *M. kansasii* isolates should be tested *in vitro* only to rifampin. Isolates of *M. kansasii* that show susceptibility to rifampin will also be susceptible to rifabutin.

4. *M. kansasii* isolates resistant to rifampin should be tested against a panel of secondary agents, including rifabutin, ethambutol, isoniazid, clarithromycin, fluoroquinolones, amikacin, sulfonamides, and linezolid.

5. *M. marinum* isolates do not require susceptibility testing unless patient fails treatment after several months.

6. There are no current recommendations for one specific method of *in vitro* susceptibility testing for fastidious NTM species and some less commonly isolated NTM species.

7. Validation and quality control should be in place for susceptibility testing of antimicrobial agents with all species of NTM.

extent of disease, different clinical presentations, and various comorbidities. With limited resources, focus must be put on clinically relevant species, such as *M. avium* complex and *M. abscessus*, as there are more than 160 species of NTM, and the treatment of these infections varies. Precise speciation is very important because outcomes of treatment may vary by species and even subspecies. (4) *M. avium* complex organisms are typically considered similar in the clinical response to therapy and clinical presentations, there are worse outcomes with treatment of *M. intracellulare* compared with *M. avium*; similarly, patients with *M. abscessus* subsp. *massiliense* have better treatment outcomes than those with *M. abscessus* subsp. *abscessus*. (5) Determination of the best measure of treatment success is still to be determined. Long-term follow-up is lacking, and the rate of reoccurrence needs to be evaluated versus reinfection.

REFERENCES


