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Clinical and Microbiological Differences between *Mycobacterium abscessus* and *Mycobacterium massiliense* Lung Diseases

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In recent years, many novel nontuberculous mycobacterial species have been discovered through genetic analysis. *Mycobacterium massiliense* and *M. bolletii* have recently been identified as species separate from *M. abscessus*. However, little is known regarding their clinical and microbiological differences in Japan. We performed a molecular identification of stored *M. abscessus* clinical isolates for further identification. We compared clinical characteristics, radiological findings, microbiological findings, and treatment outcomes among patients with *M. abscessus* and *M. massiliense* lung diseases. An analysis of 102 previous isolates of *M. abscessus* identified 72 (71%) *M. abscessus*, 27 (26%) *M. massiliense*, and 3 (3%) *M. bolletii* isolates. Clinical and radiological findings were indistinguishable between the *M. abscessus* and *M. massiliense* groups. Forty-two (58%) patients with *M. abscessus* and 20 (74%) patients with *M. massiliense* infections received antimicrobial treatment. Both the *M. abscessus* and *M. massiliense* groups showed a high level of resistance to all antimicrobials, except for clarithromycin, kanamycin, and amikacin. However, resistance to clarithromycin was more frequently observed in the *M. abscessus* than in the *M. massiliense* group (16% and 4%, respectively; $P = 0.145$). Moreover, the level of resistance to imipenem was significantly lower in *M. abscessus* isolates than in *M. massiliense* isolates (19% and 48%, respectively; $P = 0.007$). The proportions of radiological improvement, sputum smear conversion to negativity, and negative culture conversion during the follow-up period were higher in patients with *M. massiliense* infections than in those with *M. abscessus* infections. Patients with *M. massiliense* infections responded more favorably to antimicrobial therapy than those with *M. abscessus* infections.

Mycobacterium species are common causes of pulmonary infections in both humans and animals (14). Although members of the *Mycobacterium tuberculosis* complex cause the majority of pulmonary infections worldwide, many nontuberculous mycobacteria (NTM) can cause similar infections (13, 20). In recent years, many novel NTM species have been discovered through the increased application of genetic investigation tools; detailed genetic characterizations have helped define new taxonomic groupings (17, 29). Recently, two new *M. abscessus*-related species, *M. massiliense* and *M. bolletii*, were identified, which were previously grouped with *M. abscessus* (1, 3). The rate of isolation of these two species has been increasing in Japan. However, very little is known about the natural epidemiology and pathogenicity of *M. massiliense* and *M. bolletii* outside outbreak situations. One report found that the ratio of *M. abscessus* to all NTM is much higher in South Korea (19) than in other countries, including Japan.

Here, we aimed to evaluate the epidemiology, clinical and radiological spectrum, treatments, drug susceptibility, and outcome of *M. abscessus* and *M. massiliense* lung diseases during therapy in Japan.

MATERIALS AND METHODS

Study population. We retrospectively reviewed the medical records of patients initially diagnosed with *M. abscessus* lung disease according to the 2007 American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) guidelines (16) between January 1990 and December 2010 at 12 hospitals or institutions in Japan. These *M. abscessus* species were thereafter identified as *M. abscessus*, *M. massiliense*, and *M. bolletii*. Clinical, radiological, microbiological, management, and outcome data were collected from medical files. Permission was obtained from the institutional review board committee of Hokkaido Social Insurance Hospital (approval number 2011-11). Informed consent was waived because of the retrospective nature of the study.

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Microbiological examination. Sputum or bronchoalveolar lavage fluid was used for smears and mycobacterial cultures according to standard methods (5). Any processed specimens that remained were stored at 2°C to 8°C for the duration of culturing in the study to allow the retesting of the specimens that showed a discrepancy in results between culture growth and preliminary identification by a DNA-DNA hybridization (DDH) mycobacterium kit (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan). Samples were cultured by using the Bactec MGIT 960 system or Ogawa solid medium, and the isolates were stored at −30°C to −80°C. *M. abscessus* species were preliminarily identified by microplate DDH technology using the DDH mycobacterium kit (21) at each hospital or institution. We collected all frozen isolates for multisequencing and susceptibility testing. Frozen isolates were recultured by using the MGIT 960 system and Ogawa solid medium and checked for contamination by growth on *p*-nitrobenzoic acid agar medium.

Further differentiation among *M. abscessus* species was performed at the Department of Mycobacteriology, Leprosy Research Centre, National Institute of Infectious Disease, and the Kobe Institute of Health. Sequences of clinical isolates which were previously identified as *M. abscessus* by DDH were compared with the reference *M. abscessus* (JCM 15300^T), *M. massiliense* (JCM 13569^T), and *M. bolletii* (JCM 15297^T) strains. The majority of the 16S rRNA gene, partial aspects of the *hsp65* and *rpoB* genes, and the 16S-23S rRNA internal transcribed spacer (ITS) region were amplified by PCR using AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA) and primers described previously (22). The PCR products were sequenced with the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) on an ABI Prism 310 genetic analyzer (Applied Biosystems). Sequences were analyzed for their similarity to sequences in the GenBank database by using BLAST (http://www.ncbi.nlm.nih.gov/BLAST).

Antimycobacterial susceptibility testing was performed at the National Hospital Organization Kinki-Chuo Chest Medical Center (Osaka, Japan). Susceptibility was determined by MIC methods using the Etest and broth microdilution.

Etest. Isolates of rapidly growing mycobacteria (RGM) were mailed on Ogawa slant medium and subcultured on 5% sheep blood agar at 35°C in ambient air for 72 h. Bacterial suspensions were prepared in cation-adjusted Mueller-Hinton broth (Eiken Chemical, Tokyo, Japan) to a 1.0 McFarland standard and plated onto cation-adjusted Mueller-Hinton agar. Etest strips (Sysmex bioMérieux, Tokyo, Japan) were placed onto Mueller-Hinton agar (Becton Dickinson, Fukushima, Japan) according to the manufacturer's instructions, and the results were read after 72 h of incubation. Final concentration ranges were 0.016 to 256 μg/ml for isoniazid (INH) and ethambutol (EB); 0.002 to 32 μg/ml for rifampin (RFP), ciprofloxacin (CPFX), and moxifloxacin (MFLX); and 0.064 to 1,024 μg/ml for streptomycin (SM). Susceptibility was evaluated according to Clinical and Laboratory Standards Institute (CLSI) breakpoint recommendations (8, 37) and those proposed previously by Woods et al. (36), Wallace et al. (33), Yang et al. (38), and Swenson et al. (27, 28).

Broth microdilution. Serial double dilutions of clarithromycin (CAM), kanamycin (KM), amikacin (AMK), and imipenem (IPM) were prepared at a concentration range of 0.015 to 64 μg/ml according to CLSI recommendations (37). Briefly, pure colonies were cultured in 7H9 broth with 0.2% glycerol in a tube for 3 to 5 days, vigorously vortexed, and then adjusted to a density equivalent of 0.5 on the McFarland scale. Bacterial suspensions in cation-adjusted Mueller-Hinton broth were transferred into the wells of dry microdilution plates containing the antimicrobial agents (Eiken Chemical). The inoculated plates were placed into plastic bags, incubated at 35°C in ambient air, and read after 72 h. The MIC was defined as the lowest concentration of drug that inhibited visible growth. Susceptibility was evaluated according to CLSI breakpoint recommendations (8, 37) and those proposed previously by Shen et al. (24).

Data analysis. The results are expressed as ranges and means or as numbers of patients. Categorical variables were analyzed by using the χ^2 or Fisher's exact test. Continuous variables were analyzed by using the Mann-Whitney U test or *t* test. All *P* values are two sided; a *P* value of <0.05 indicates statistical significance. Statistical analyses were performed by using Excel 2011 (Microsoft) with the add-in software Statcel 3 (OMS Publishing Inc., Saitama, Japan).

RESULTS

Identification of clinical isolates. Clinical isolates from 102 patients previously diagnosed with *M. abscessus* lung disease by DDH were identified by sequence analysis of the 16S rRNA, *hsp65*, *rpoB*, and ITS genes. Seventy-two (71%) isolates were identified as *M. abscessus*, 27 (26%) were identified as *M. massiliense*, and 3 (3%) were identified as *M. bolletii*.

Patient characteristics. We compared clinical characteristics and treatment outcomes among the 72 patients with *M. abscessus* and 27 patients with *M. massiliense* infections. Patients with *M. bolletii* infection were not included for further study because of their small number.

Table 1 summarizes the baseline characteristics of the patients. No significant differences were found between the *M. abscessus* and *M. massiliense* groups in any of the baseline characteristics, including demographic data, underlying conditions, and respiratory symptoms. The distributions of radiographic disease types were similar in both groups, except for bronchiectasis. Bronchiectasis was significantly more frequent in the *M. abscessus* group (73%; 40 of 55) than in the *M. massiliense* group (43%; 10 of 23) ($P = 0.014$). None of these patients tested positive for HIV.

Drug susceptibility. Table 2 shows the drug susceptibility results for 63 *M. abscessus* and 23 *M. massiliense* isolates, and Table S1 in the supplemental material shows the MIC distributions of the strains. The drug was determined to be effective against an isolate if the MIC of the antimicrobial agent was less than the susceptible concentration, as shown in Table 2. Of the parenteral antibiotics, KM and AMK were effective against most *M. abscessus* isolates (95% and 94%, respectively) and *M. massiliense* isolates (100% for both), with no difference between the species ($P = 0.388$ and 0.509, respectively). However, SM was ineffective against both *M. abscessus* (67%) and *M. massiliense* (61%) isolates ($P = 0.617$). Moreover, the rates of IPM drug resistance were significantly higher in *M. massiliense* than in *M. abscessus* isolates (48% and 19%, respectively; $P = 0.007$).

Of the oral antibiotics, CAM was effective against most *M. abscessus* and *M. massiliense* isolates (84% and 96%, respectively; $P = 0.145$). In contrast, MFLX, CPFX, and RFP were ineffective against most *M. abscessus* (92%, 95%, and 97%, respectively) and *M. massiliense* (96%, 91%, and 100%, respectively) isolates, with no difference between the species ($P = 0.488$, 0.883, and 0.534, respectively). We could not interpret the INH and EB susceptibilities because no breakpoints have been established for these drugs.

Antimicrobial treatment and response. Of the 102 patients, 42 (58%) with *M. abscessus*, 20 (74%) with *M. massiliense*, and 2 (67%) with *M. bolletii* infections received antimicrobial treatment for 3 to 178 months (mean = 33 months), 1 to 122 months (mean = 36 months), and 4 to 68 months (mean = 36 months), respectively. Forty-two patients with *M. abscessus* lung disease and 20 patients with *M. massiliense* lung disease were analyzed for treatment response (Table 3). Radiographic improvement rates were lower for patients with *M. abscessus* infection than for those with *M. massiliense* infection (29% and 48%, respectively; $P = 0.101$).

Microbiological responses also differed between the two

TABLE 1 Clinical characteristics of patients with *Mycobacterium abscessus* and *M. massiliense* lung disease

Characteristic[a]	Value for group		*P* value
	M. abscessus ($n = 72$)	*M. massiliense* ($n = 27$)	
Age (yr) [range (mean)]	27–94 (68)	44–84 (67)	0.637
No. of males/no. of females	26/45	13/13	0.233
Mean body mass index (kg/m^2)	19.5	18.8	0.699
No. of patients with symptom			
Cough	25	9	0.931
Sputum	22	9	0.759
Hemoptysis	14	8	0.262
Fever	8	5	0.905
Dyspnea	4	5	0.057
No. of patients with underlying disease			
Previous pulmonary tuberculosis	12	7	0.297
NTM (MAC)	15	4	0.516
Mycosis	4	2	0.798
Interstitial pneumonia	4	2	0.803
COPD	3	2	0.879
Diabetes mellitus	3	3	0.196
Steroid use	3	3	0.196
Malignancy	2	1	0.823
No. of smokers/no. of nonsmokers	33/11	12/8	0.223
No. of patients who consumed alcohol/no. of patients who did not consume alcohol	30/6	11/3	0.490
No. of patients with/no. of patients without radiological finding of:			
Cavitation	28/28	14/9	0.379
Centrilobular lesion	38/19	14/11	0.355
Infiltration	35/16	12/11	0.173
Bronchiectasis	40/15	10/13	0.014
Pleural effusion	2/49	2/22	0.383
No. of patients with positive AFB smear	50	21	0.363

[a] Abbreviations: NTM, nontuberculous mycobacteria; MAC, *Mycobacterium avium* complex; COPD, chronic obstructive pulmonary disease; AFB, acid-fast bacillus.

groups. The initial sputum conversion rates were lower in patients with *M. abscessus* infection than in those with *M. massiliense* infection (31% and 50%, respectively; $P = 0.115$). The sputum acid-fast bacillus (AFB)-positive relapse rate after the initial conversion to a negative result was higher for patients with *M. abscessus* infection than for those with *M. massiliense* infection (65% and 30%, respectively; $P = 0.077$). Thus, the proportion of patients whose sputum converted and remained culture negative during the follow-up period was lower for patients with *M. abscessus* infection than for those with *M. massiliense* infection.

DISCUSSION

The *M. abscessus* group comprises ubiquitous environmental organisms frequently associated with nosocomial outbreaks and pseudo-outbreaks (18, 32, 34). The increasing availability of gene sequencing has tremendously influenced the taxonomy of bacteria, particularly mycobacteria, with many new species being described every year (30).

A new species related to *M. abscessus*, *M. massiliense*, was recently described (3). The species *M. massiliense* was proposed in 2004 based upon nonconventional phenotypic characterization and genotypic studies of 2 isolates recovered from the sputum and bronchoalveolar fluid of a patient in France (3). Since *M. massiliense* is closely related to *M. abscessus*, it is possible that *M. massiliense* infections have overlapped with *M. abscessus* infections in previous reports (3). Although several unique phenotypes that differentiate *M. massiliense* from *M. abscessus* have been identified, they are difficult to characterize using conventional clinical techniques because of the inability to discriminate between RGM due to their overlapping phenotypic patterns (9, 26). The molecular, biological, and clinical characteristics of these strains will help us to better understand and treat severe infections due to RGM (39). The proper identification of members of the *M. abscessus* complex has proven beneficial in both therapeutic management and epidemiological studies. *M. massiliense* is very closely related to *M. abscessus* but showed different susceptibilities to CAM, and their pathogenic potentials have been demonstrated by infections of immunocompetent and immunocompromised hosts (15, 18).

The proportions of *M. massiliense* strains among *M. abscessus* species vary according to geographical distribution. The prevalences of *M. massiliense* were 28% of 40 patients at the National Institutes of Health in the United States (39), 21% of 39 clinical isolates in the Netherlands (31), 22% of 50 patients with cystic fibrosis in France (23), 55% of 150 patients in South Korea (19), and 26% of 102 patients in Japan. There is currently no explanation for the large difference in the prevalences of *M. massiliense* between South Korea and Japan, as they are in the same Asian region.

No significant differences were found between the baseline clinical characteristics in the *M. abscessus* and *M. massiliense* groups, except for bronchiectasis in the radiological findings. Bronchiectasis was found significantly more frequently in the *M. abscessus* group than in the *M. massiliense* group (73% and 43%, respectively; $P = 0.014$). This result is almost consistent with the results of a Korean study (19).

Importantly, favorable microbiological response rates with similar combinations of antibiotic therapy were much higher for *M. massiliense* than for *M. abscessus* lung disease. This could be explained by the differences in CAM resistance. This study demonstrated a high level of resistance to CAM in *M. abscessus* isolates but not in *M. massiliense* isolates, indicating that treatment of *M. abscessus* lung disease may be more difficult. In fact, *M. abscessus* lung disease has been regarded as a chronic, incurable infection for most patients, given the current antibiotic options (4). The low MIC and absence of CAM resistance (except for one isolate) suggest that *M. massiliense* lung disease may be treated more effectively with a CAM-based antibiotic regimen. Recent studies showed that some RGM, such as *M. abscessus* and *M. fortuitum*, have an *erm* gene that induces macrolide resistance (4, 12). It is

TABLE 2 *In vitro* susceptibilities of 63 *Mycobacterium abscessus* and 23 *M. massiliense* isolates to different antimicrobials[a]

Drug	Species	MIC (μg/ml) for categorization of susceptibility of:			Reference(s)	% resistant isolates (no. of resistant isolates)	*P* value
		Susceptible	Intermediate	Resistant			
Clarithromycin	*M. abscessus*	≤2	4	≥8	15	16 (10)	0.145
	M. massiliense					4 (1)	
Kanamycin[b]	*M. abscessus*	≤16	32	≥64	14, 20	5 (3)	0.388
	M. massiliense					0 (0)	
Amikacin	*M. abscessus*	≤16	32	≥64	15	6 (4)	0.509
	M. massiliense					0 (0)	
Imipenem	*M. abscessus*	≤4	8–16	≥32	15	19 (12)	0.007
	M. massiliense					48 (11)	
Moxifloxacin	*M. abscessus*	≤1	2	≥4	15	92 (58)	0.488
	M. massiliense					96 (22)	
Ciprofloxacin	*M. abscessus*	≤1	2	≥4	15	95 (60)	0.883
	M. massiliense					91 (21)	
Isoniazid[c]	*M. abscessus*	NA	NA	NA		NA	
	M. massiliense					NA	
Rifampin[b]	*M. abscessus*	≤1	2	≥4	14, 18	97 (61)	0.534
	M. massiliense					100 (23)	
Ethambutol[c]	*M. abscessus*	NA	NA	NA		NA	
	M. massiliense					NA	
Streptomycin[b]	*M. abscessus*	≤32	NA	≥64	14, 20	67 (42)	0.617
	M. massiliense					61 (14)	

[a] Drug susceptibility results are shown for 63 patients with *M. abscessus* and 23 patients with *M. massiliense* infections. NA, not available.
[b] The CLSI breakpoints (8) for *Staphylococcus* species have been substituted as the breakpoints of these drugs against *M. abscessus* and *M. massiliense* isolates.
[c] The breakpoints for *M. abscessus* and *M. massiliense* isolates have not yet been established.

unknown whether other RGM such as *M. massiliense* have an *erm* gene. Thus, species-level identification is important because antibiotic susceptibilities and therapies differ significantly depending on the RGM species (4, 16). Since the KM and AMK resistance rates were less than 10% for both the *M. abscessus* and *M. massiliense* groups in the present study, KM and AMK could be two key drugs for the treatment of *M. abscessus* and *M. massiliense* lung diseases.

The IPM resistance rate was much lower in the present study than in the Korean study (19) (19% and 44% for *M. abscessus* and 48% and 67% for *M. massiliense*, respectively). The MFLX, CPFX, and RFP resistance rates were >90% for both the *M. abscessus* and *M. massiliense* groups in the present study. These findings are compatible with the fact that the *M. abscessus* complex has been regarded as being fluoroquinolone resistant (7). However, moderate *in vitro* activities of some fluoroquinolones against members of the *M. abscessus* complex have been demonstrated (2, 6, 19, 25). The use of fluoroquinolones as alternative oral agents during combination antibiotic therapy for *M. abscessus* and *M. massiliense* infections should be studied further.

The present study showed poor activities of INH and EB in both the *M. abscessus* and *M. massiliense* groups (MIC_{90} for INH of ≥512 μg/ml; MIC_{90} for EB of ≥32 μg/ml), which are identical to findings reported previously (27). Thus, INH and EB seem to be ineffective against both the *M. abscessus* and *M. massiliense* groups. Another study found that MFLX was active against *M. abscessus* and that a combination of CAM and MFLX was effective against *M. abscessus* strains in *in vitro* models (10). Moreover, some *M. abscessus* isolates are susceptible to the oral drug linezolid (35, 38). However, linezolid was rarely used in our survey to treat *M. abscessus* species lung disease because of the high cost and moderate to severe side effects in Japan. Thus, further studies are required to evaluate active combinations of oral antibiotics and determine their clinical significance.

The present study has several limitations. First, the retrospective study design necessitates the use of medical records for data collection, leading to variations in each factor. Second, the number of sputum specimens collected over time was relatively small. Had samples been collected more frequently, more conversions to negativity and relapses after conversion may have been found. Third, treatment regimens cannot be optimized based solely on retrospective studies with limited follow-up data. Moreover, the use of IPM, cefoxitin, fluoroquinolone, and linezolid is not permitted for the treatment of NTM diseases under the Japanese social health insurance system. Thus, the combination therapy recommended by the ATS/IDSA (16) has not been applied in most cases. Treatment regimens were decided in practice by physicians. Therefore, it is difficult to evaluate the true treatment response in this study.

In conclusion, we found clinically significant differences be-

TABLE 3 Antimicrobial treatments and treatment responses

Characteristic[a]	Value for group		*P* value
	M. abscessus ($n = 72$)	*M. massiliense* ($n = 27$)	
Treatment			
No. of patients with/no. of patients without operation	3/69	3/24	0.201
No. of patients receiving/no. of patients not receiving chemotherapy	42/30	20/7	0.149
No. of patients on chemotherapy regimen of:			
M, R, E	8	5	
M, C, A	6	5	
M	8	0	
M, F	6	0	
H, R, E	2	2	
Other	12	8	
Duration of treatment (mo) [range (mean)]	3–178 (33)	1–122 (36)	0.723
Result			
No. of patients with/no. of patients without radiological improvement	17/41	12/13	0.101
No. of patients with/no. of patients without sputum smear conversion to negativity	17/38	11/11	0.115
No. of patients with relapse/no. of patients without relapse after sputum smear conversion to negativity	13/7	3/7	0.077
Duration of positive sputum results (mo) [range (mean)]	1–120 (25)	1–62 (18)	0.776

[a] Abbreviations: M, macrolides (clarithromycin, erythromycin, and azithromycin); A, aminoglycosides (streptomycin, amikacin, and kanamycin); F, fluoroquinolones (levofloxacin, moxifloxacin, garenoxacin, and gatifloxacin); C, carbapenems (imipenem and meropenem); H, isoniazid; R, rifampin; E, ethambutol.

tween *M. abscessus* and *M. massiliense* lung infections in Japan. Treatment responses rates with CAM-based antibiotic therapy were higher for *M. massiliense* than in *M. abscessus* lung disease. This difference in treatment responses may be explained by the difference in CAM susceptibilities between the two groups. Prospective clinical trials are needed to clarify these aspects.

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Data analysis of reviewed medical records was performed at the Hokkaido Social Insurance Hospital. The identification of *M. abscessus* species by microplate DDH technology was performed at each hospital or institution. Further differentiation among *M. abscessus* species was performed at the Leprosy Research Center, National Institute of Infectious Diseases, and the Kobe Institute of Health. Antimycobacterial susceptibility testing was performed at the National Hospital Organization Kinki-Chuo Chest Medical Center.

We have no potential conflicts of interest to report. All authors have submitted a International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest.

REFERENCES

1. **Adékambi T, Berger P, Raoult D, Drancourt M.** 2006. *rpoB* gene sequence-based characterization of emerging non-tuberculous mycobacteria with descriptions of *Mycobacterium bolletii* sp. nov., *Mycobacterium phocaicum* sp. nov. and *Mycobacterium aubagnense* sp. nov. Int. J. Syst. Evol. Microbiol. **56**:133–143.
2. **Adékambi T, Colson P, Drancourt M.** 2003. *rpoB*-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. J. Clin. Microbiol. **41**:5699–5708.
3. **Adékambi T, et al.** 2004. Amoebal coculture of "*Mycobacterium massiliense*" sp. nov. from the sputum of a patient with hemoptoic pneumonia. J. Clin. Microbiol. **42**:5493–5501.
4. **American Thoracic Society.** 1997. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. Am. J. Respir. Crit. Care Med. **156**(2 Pt 2):S1–S25.
5. **American Thoracic Society.** 2000. Diagnostic standards and classification of tuberculosis in adults and children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. Am. J. Respir. Crit. Care Med. **161**(4 Pt 1):1376–1395.
6. **Blackwood KS, et al.** 2000. Evaluation of *recA* sequences for identification of *Mycobacterium* species. J. Clin. Microbiol. **38**:2846–2852.
7. **Chadha R, Grover M, Sharma A.** 1998. An outbreak of post-surgical wound infections due to *Mycobacterium abscessus*. Pediatr. Surg. Int. **13**:406–410.
8. **Cockerill FR, III, et al.** 2011. Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement, 1st ed. M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA.
9. **Conville PS, Witebsky FG.** 1998. Variables affecting results of sodium chloride tolerance test for identification of rapidly growing mycobacteria. J. Clin. Microbiol. **36**:1555–1559.
10. **Cremades R, et al.** 2009. Screening for sterilizing activity of antibiotic combinations in an acid model of rapidly growing mycobacteria during the stationary phase of growth. Chemotherapy **55**:114–118.
11. **Dauendorffer JN, et al.** 2003. Identification of mycobacterial species by PCR sequencing of quinolone resistance-determining regions of DNA gyrase genes. J. Clin. Microbiol. **41**:1311–1315.
12. **De Groote MA, Huitt G.** 2006. Infections due to rapidly growing mycobacteria. Clin. Infect. Dis. **42**:1756–1763.
13. **El-Solh AA, et al.** 1998. Clinical and radiographic manifestations of uncommon pulmonary nontuberculous mycobacterial disease in AIDS patients. Chest **114**:138–145.
14. **Falkinham JO, III.** 1996. Epidemiology of infection by nontuberculous mycobacteria. Clin. Microbiol. Rev. **9**:177–215.
15. **Freudenberger RS, Simafranca SM.** 2006. Cutaneous infection with rapidly-growing mycobacterial infection following heart transplant: a case report and review of the literature. Transplant. Proc. **38**:1526–1529.
16. **Griffith DE, et al.** 2007. An official ATS/IDSA statement. Diagnosis, treatment, and prevention of nontuberculous mycobacterial disease: Joint Tuberculosis Committee guidelines. Am. J. Respir. Crit. Care Med. **175**:367–416.
17. **Hanna BA, et al.** 1999. Multicenter evaluation of the BACTEC MGIT 960 system for recovery of mycobacteria. J. Clin. Microbiol. **37**:748–752.
18. **Kim HY, et al.** 2007. Outbreak of *Mycobacterium massiliense* infection

associated with intramuscular injections. J. Clin. Microbiol. **45**:3127–3130.
19. **Koh WJ, et al.** 2011. Clinical significance of differentiation of *Mycobacterium massiliense* from *Mycobacterium abscessus*. Am. J. Respir. Crit. Care Med. **183**:405–410.
20. **Koh WJ, Kwon OJ, Lee KS.** 2002. Nontuberculous mycobacterial pulmonary diseases in immunocompetent patients. Kor. J. Radiol. **3**:145–157.
21. **Kusunoki S, et al.** 1991. Application of colorimetric microdilution plate hybridization for rapid genetic identification of 22 *Mycobacterium* species. J. Clin. Microbiol. **29**:1596–1603.
22. **Nakanaga K, et al.** 2011. Multiple cases of cutaneous *Mycobacterium massiliense* infection in a "hot spa" in Japan. J. Clin. Microbiol. **49**:613–617.
23. **Roux AL, et al.** 2009. Multicenter study of prevalence of nontuberculous mycobacteria in patients with cystic fibrosis in France. J. Clin. Microbiol. **47**:4124–4128.
24. **Shen GH, Wu BD, Wu KM, Chen JH.** 2007. In vitro activities of isepamicin, other aminoglycosides, and capreomycin against clinical isolates of rapidly growing mycobacteria in Taiwan. Antimicrob. Agents Chemother. **51**:1849–1851.
25. **Simmon KE, et al.** 2007. Identification of an emerging pathogen, *Mycobacterium massiliense*, by *rpoB* sequencing of clinical isolates collected in the United States. J. Clin. Microbiol. **45**:1978–1980.
26. **Springer B, Stockman L, Teschner K, Roberts GD, Böttger EC.** 1996. Two-laboratory collaborative study on identification of mycobacteria: molecular versus phenotypic methods. J. Clin. Microbiol. **34**:296–303.
27. **Swenson JM, Thornsberry C, Silcox VA.** 1982. Rapidly growing mycobacteria: testing of susceptibility to 34 antimicrobial agents by broth microdilution. Antimicrob. Agents Chemother. **22**:186–192.
28. **Swenson JM, Wallace RJ, Jr, Silcox VA, Thornsberry C.** 1985. Antimicrobial susceptibility of five subgroups of *Mycobacterium fortuitum* and *Mycobacterium chelonae*. Antimicrob. Agents Chemother. **28**:807–811.
29. **Tortoli E.** 2003. Impact of genetic studies on mycobacterial taxonomy: the new mycobacteria of the 1990s. Clin. Microbiol. Rev. **16**:319–354.
30. **Tortoli E.** 2006. The new mycobacteria: an update. FEMS Immunol. Med. Microbiol. **48**:159–178.
31. **van Ingen J, de Zwaan R, Dekhuijzen RP, Boeree MJ, van Soolingen D.** 2009. Clinical relevance of *mycobacterium chelonae-abscessus* group isolation in 95 patients. J. Infect. **59**:324–331.
32. **Viana-Niero C, et al.** 2008. Molecular characterization of *Mycobacterium massiliense* and *Mycobacterium bolletii* in outbreaks of infections after laparoscopic surgeries and cosmetic procedures. J. Clin. Microbiol. **46**:850–855.
33. **Wallace RJ, Jr, Brown-Elliott BA, Crist CJ, Mann L, Wilson RW.** 2002. Comparison of the in vitro activity of the glycylcycline tigecycline (formerly GAR-936) with those of tetracycline, minocycline, and doxycycline against isolates of nontuberculous mycobacteria. Antimicrob. Agents Chemother. **46**:3164–3167.
34. **Wallace RJ, Jr, Brown BA, Griffith DE.** 1998. Nosocomial outbreaks/pseudo-outbreaks caused by nontuberculous mycobacteria. Annu. Rev. Microbiol. **52**:453–490.
35. **Wallace RJ, Jr, et al.** 2001. Activities of linezolid against rapidly growing mycobacteria. Antimicrob. Agents Chemother. **45**:764–767.
36. **Woods GL, et al.** 2000. Multisite reproducibility of Etest for susceptibility testing of *Mycobacterium abscessus*, *Mycobacterium chelonae*, and *Mycobacterium fortuitum*. J. Clin. Microbiol. **38**:656–661.
37. **Woods GL, et al.** 2011. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes. Approved standard, 2nd ed. M24-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
38. **Yang SC, et al.** 2003. High prevalence of antimicrobial resistance in rapidly growing mycobacteria in Taiwan. Antimicrob. Agents Chemother. **47**:1958–1962.
39. **Zelanzy AM, et al.** 2009. Cohort study of molecular identification and typing of *Mycobacterium abscessus*, *Mycobacterium massiliense*, and *Mycobacterium bolletii*. J. Clin. Microbiol. **47**:1985–1995.