AMERICAN SOCIETY FOR MICROBIOLOGY | **Antimicrobial Agents and Chemotherapy**®

# Clinical Characteristics and Treatment Outcomes of Patients with Acquired Macrolide-Resistant *Mycobacterium abscessus* Lung Disease

Hayoung Choi,[a] Su-Young Kim,[a] Dae Hun Kim,[a] Hee Jae Huh,[b] Chang-Seok Ki,[b] Nam Yong Lee,[b] Seung-Heon Lee,[c] Soyoun Shin,[c] Sung Jae Shin,[d] Charles L. Daley,[e] Won-Jung Koh[a]

Division of Pulmonary and Critical Care Medicine, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea[a]; Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea[b]; Korean Institute of Tuberculosis, Cheongju, South Korea[c]; Department of Microbiology and Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, South Korea[d]; Division of Mycobacterial and Respiratory Infections, Department of Medicine, National Jewish Health, Denver, Colorado, USA[e]

**ABSTRACT** Macrolide antibiotics are mainstays in the treatment of lung disease due to the *Mycobacterium abscessus* complex. Although previous studies have reported development of acquired macrolide resistance in this species, limited data are available on the outcomes of lung disease due to macrolide-resistant *Mycobacterium abscessus* subsp. *abscessus*. This study evaluated the clinical features, treatment outcomes, and molecular characteristics of macrolide-resistant isolates of *M. abscessus* subsp. *abscessus*. We performed a retrospective review of medical records and genetic analysis of clinical isolates from 13 patients who had acquired macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease between November 2006 and March 2016. Eleven (85%) patients had the nodular bronchiectatic form of the disease, and two (15%) patients had the fibrocavitary form. When acquired macrolide resistance was detected, 10 (77%) patients were on antibiotic therapy for *M. abscessus* subsp. *abscessus*, and three (23%) patients were on therapy for lung disease due to other nontuberculous mycobacteria. The median treatment duration after detecting resistance was 24.0 months (interquartile range, 16.0 to 43.0 months). Treatment outcomes were poor, and final sputum culture conversion was achieved in only one (8%) patient, after resectional surgery. All 13 clinical isolates demonstrated point mutations at position 2058 ($n = 10$) or 2059 ($n = 3$) of the 23S rRNA gene, which resulted in acquired macrolide resistance. This study indicates that treatment outcomes are very poor after the development of acquired macrolide resistance in patients with *M. abscessus* subsp. *abscessus* lung disease. Thus, more effective measures are needed to prevent development and effectively treat macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease.

**KEYWORDS** nontuberculous mycobacteria, *Mycobacterium abscessus*, macrolides, drug resistance

The prevalence of pulmonary disease caused by nontuberculous mycobacteria (NTM) is increasing worldwide (1, 2). *Mycobacterium abscessus* complex is the most important cause of pulmonary infections by rapidly growing mycobacteria in patients with chronic lung diseases, such as bronchiectasis and cystic fibrosis (3, 4). Currently, *Mycobacterium abscessus* complex can be divided into three subspecies: *Mycobacterium abscessus* subsp. *abscessus*, *Mycobacterium abscessus* subsp. *massiliense*, and *Mycobac-*

*terium abscessus* subsp. *bolletii* (5, 6). Of the three subspecies, *M. abscessus* subsp. *abscessus* is the most common pathogen, followed by *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *bolletii* (7).

Of the rapidly growing mycobacterial pathogens, *M. abscessus* subsp. *abscessus* is the most difficult to treat because of its intrinsic and acquired multidrug resistance (8), and antibiotic treatment success rates are less than 50%, in contrast to the high treatment success rates (80 to 90%) with *M. abscessus* subsp. *massiliense* infection (9–13). Macrolides, such as clarithromycin and azithromycin, are considered the corner-stone for treatment of *M. abscessus* subsp. *abscessus* infections (3). The poor treatment outcomes in *M. abscessus* subsp. *abscessus* lung disease are attributed to inducible mac-rolide resistance, demonstrated by susceptibility to macrolides at day 3 but resistance at day 14 of drug susceptibility testing (DST); this type of resistance is conferred by the ribosomal methyltransferase gene *erm*(41) (14, 15). In addition to inducible macrolide resistance, acquired macrolide resistance, demonstrated by resistance to macrolides at day 3 of DST, can develop during macrolide antibiotic treatment due to mutations in the drug-binding pocket of the 23S rRNA gene (*rrl*) at nucleotide positions 2058 and 2059 (15, 16).

Although previous studies reported that acquired macrolide resistance developed in some patients with *M. abscessus* subsp. *abscessus* infections during macrolide-containing antibiotic treatment (17–19), no published data are available regarding detailed clinical characteristics and treatment outcomes of patients with acquired macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease. The purposes of this study were to evaluate the clinical features and treatment outcomes of patients with acquired macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease and to examine the molecular characteristics of their isolates.

## RESULTS

**Patient characteristics.** A total of 13 patients were diagnosed with acquired macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease during the study period. The clinical characteristics of the patients are summarized in Table 1. For four of the patients, some clinical data were included in recently published articles (12, 20); data on the remaining patients have not been previously reported. There were eight (62%) female patients, and the median age of all patients was 64 years (interquartile range [IQR], 55 to 78 years). Eight (62%) patients had a history of prior treatment for pulmonary tuberculosis. Six (46%) patients had a history of previous treatment for NTM lung disease: four of these patients had *Mycobacterium avium* complex (MAC) infection, one patient had *M. abscessus* subsp. *massiliense* infection, and one patient had MAC infection followed by *M. abscessus* subsp. *massiliense* infection.

Sputum smears were acid-fast bacillus (AFB) positive for all patients at the time acquired macrolide resistance was detected. Two (15%) patients had the fibrocavitary form of lung disease, and 11 (92%) patients had the nodular bronchiectatic form. Cavitary lesions were found on high-resolution computed tomography (HRCT) for 6 (55%) of 11 patients with the nodular bronchiectatic form.

**Antibiotic therapy before detection of acquired macrolide-resistant *M. abscessus* subsp. *abscessus*.** For one (8%) patient, macrolide resistance was detected upon transfer to our hospital after long-term antibiotic treatment at another hospital. For 12 (92%) patients, macrolide resistance developed during antibiotic treatment at our institution. All patients received macrolide treatment, and the median duration of exposure to a macrolide was 19.0 months (IQR, 10.5 to 42.5 months) before detection of acquired macrolide resistance.

In 10 (77%) patients, macrolide resistance was detected during combined antibiotic therapy for *M. abscessus* subsp. *abscessus* lung disease, which consisted of intravenous (i.v.) amikacin, i.v. cefoxitin (or imipenem), and an oral macrolide, with or without fluoroquinolone, doxycycline, and clofazimine. In three (23%) patients, macrolide re-sistance was detected during antibiotic therapy for other NTM lung diseases: two patients with MAC infection had received an oral macrolide, rifampin, and ethambutol,

**TABLE 1** Clinical characteristics of patients ($n = 13$) with acquired macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease at diagnosis

| Clinical characteristics[a] | Value[b] |
|---|---|
| Female | 8 (62) |
| Age, years | 64 (55–78) |
| BMI, kg/m$^2$ | 19.9 (18.2–22.0) |
| Nonsmoker | 11 (85) |
| Previous treatment for pulmonary tuberculosis | 8 (62) |
| Previous treatment for NTM lung disease | 6 (46) |
| | |
| Comorbidities | |
| COPD | 1 (8) |
| Bronchiectasis | 12 (92) |
| Chronic pulmonary aspergillosis | 1 (8) |
| Chronic heart disease | 1 (8) |
| | |
| Laboratory findings | |
| Positive sputum AFB smear | 13 (100) |
| ESR, mm/h | 39 (13–102) |
| CRP, mg/dl | 0.3 (0.1–1.6) |
| | |
| Radiologic findings | |
| Fibrocavitary form | 2 (15) |
| Nodular bronchiectatic form | 11 (85) |
| With cavity | 6 |
| Without cavity | 5 |
| | |
| Pulmonary function test | |
| FVC, liters | 2.33 (1.94–3.30) |
| FVC, % predicted | 75 (61–91) |
| FEV$_1$, liters | 1.85 (1.31–2.56) |
| FEV$_1$, % predicted | 80 (57–92) |

[a]Abbreviations: BMI, body mass index; NTM, nontuberculous mycobacteria; COPD, chronic obstructive pulmonary disease; AFB, acid-fast bacilli; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; FVC, forced vital capacity; FEV$_1$, forced expiratory volume in 1 s.
[b]Data are presented as number (percentage of total) or as median (interquartile range).

and one patient with *M. abscessus* subsp. *massiliense* infection had received an oral macrolide, i.v. amikacin, and i.v. cefoxitin (Table 2).

**Treatment and outcomes after detection of acquired macrolide-resistant *M. abscessus* subsp. *abscessus*.** The treatment regimens after detection of macrolide resistance and subsequent treatment outcomes are summarized in Table 3. Of 13 patients, two (15%) did not receive antibiotic therapy after detection of macrolide resistance: one patient had been observed at an outpatient clinic after completing

**TABLE 2** Treatment regimens before detection of macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease[a,b]

| Treatment regimen | Value | Duration of macrolide exposure, mo |
|---|---|---|
| Antibiotic therapy for *M. abscessus* subsp. *abscessus* lung disease | | |
| Macrolide + i.v. antibiotics[c] ± FQ ± DOX ± CFZ | 10 (77) | 25.5 (9.3–45.0) |
| | | |
| Antibiotic therapy for other NTM lung disease | | |
| Macrolide + RIF + EMB[d] | 2 (15) | 17.0 (NA) |
| Macrolide + i.v. antibiotics[e] | 1 (8) | 11.0 (NA) |
| | | |
| Total | 13 (100) | 19.0 (10.5–42.5) |

[a]The total number of patients in the study was 13. Abbreviations: i.v., intravenous; FQ, fluoroquinolone; DOX, doxycycline; CFZ, clofazimine; NTM, nontuberculous mycobacteria; RIF, rifampin; EMB, ethambutol; NA, not available.
[b]Data are presented as number (percent) or as median (interquartile range).
[c]Intravenous antibiotics included amikacin and cefoxitin (or imipenem).
[d]Combination antibiotic therapy for *M. avium* complex lung disease.
[e]Combination antibiotic therapy for *M. abscessus* subsp. *massiliense* lung disease.

**TABLE 3** Treatment modalities and outcomes after detection of macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease[a,b]

| Treatment modality | Value |
|---|---|
| Antibiotic therapy | |
|     Amikacin | 6 (46) |
|     Cefoxitin or imipenem | 6 (46) |
|     Macrolide | 11 (85) |
|     Fluoroquinolone | 1 (8) |
|     Clofazimine | 10 (77) |
|     Amikacin inhalation | 7 (54) |
| Surgical resection | 2 (15) |
| Total treatment duration, mo | 24.0 (16.0–43.0) |
| Treatment outcome | |
|     Favorable outcome | 0 |
|     Sputum culture conversion after surgery | 1 (8) |

[a]The total number of patients in the study was 13.
[b]Data are presented as number (percent) or as median (interquartile range).

antibiotic therapy for MAC infection, and another patient stopped receiving antibiotic therapy for *M. abscessus* subsp. *abscessus* lung disease after detection of macrolide resistance due to drug side effects. Another 11 (85%) patients received antibiotic therapy for macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease. After detection of macrolide resistance, macrolides continued to be prescribed for all patients. Intravenous (i.v.) amikacin ($n = 6$ [46%]), i.v. cefoxitin or imipenem ($n = 6$ [46%]), an oral fluoroquinolone ($n = 1$ [8%]), or clofazimine ($n = 10$ [77%]) and amikacin inhalation ($n = 7$ [54%]) were used at the discretion of the attending physicians. The median duration of antibiotic therapy after detection of macrolide resistance was 24.0 months (IQR, 16.0 to 43.0 months). Two (15%) patients underwent surgical resections, consisting of lobectomy and wedge resection of another lobe at 6.9 months for one patient and pneumonectomy at 6.5 months for the second patient, after the detection of macrolide resistance.

Based on the occurrence and timing of sputum culture conversion (see Materials and Methods), no patient achieved a favorable outcome after antibiotic treatment alone. Sputum culture conversion was achieved only by the patient who had undergone surgical resection of the left upper lobe, with the conversion occurring subsequent to surgery. During the median follow-up period of 35.4 months (IQR, 13.8 to 59.9 months), two (15%) patients died, one at 3 months and the other at 8 months after the detection of macrolide resistance.

**Genetic analysis of macrolide-resistant *M. abscessus* subsp. *abscessus* isolates.** *M. abscessus* subsp. *abscessus* isolates were available from all patients for genetic analysis, and all isolates had very high clarithromycin MICs ($\geq$64 $\mu$g/ml) at day 3 of DST. All *M. abscessus* subsp. *abscessus* isolates showed *rrl* mutations at position 2058 (10/13 [77%]) or 2059 (3/13 [23%]), with mutations of adenine to guanine (7/13 [54%]), adenine to cytosine (5/13 [38%]), or adenine to thymine (1/13 [8%]) (Table 4). The *rrl* genotypes were 100% concordant with antibiotic susceptibility testing phenotypes, and the 13 isolates with *rrl* mutations were clarithromycin resistant. Eleven isolates contained the T28 sequevar of the *erm*(41) gene, whereas the other two isolates contained the *erm*(41) C28 sequevar. The characteristics of the *M. abscessus* subsp. *abscessus* isolates from the 13 patients after the development of macrolide resistance are summarized in Table 5.

## DISCUSSION

This is the first study to investigate the detailed clinical characteristics and treatment outcomes of patients with acquired macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease, as well as the molecular characteristics of the disease isolates. Overall, the treatment outcome was very poor, with limited effective treatment options. Of 13

**TABLE 4** Genetic analysis of macrolide-resistant *M. abscessus* clinical isolates

| Mutation or sequevar | No. (%) of patients with mutation[a] |
|---|---|
| Presence of point mutation in *rrl*[b] | 13 (100) |
|   Adenine → guanine | 7 (54) |
|     A2058G | 4 |
|     A2059G | 3 |
|   Adenine → cytosine | 5 (38) |
|     A2058C | 5 |
|     A2059C | 0 |
|   Adenine → thymine | 1 (8) |
|     A2058T | 1 |
|     A2059T | 0 |
| | |
| 28th sequevar of *erm*(41)[c] | |
|   T28 | 11 (85) |
|   C28 | 2 (15) |

[a]The total number of patients in the study was 13.
[b]Nucleotides 2058 and 2059 (*Escherichia coli* numbering) of *rrl*, for which the wild-type sequence is AA. A, adenine; G, guanine; C, cytosine; T, thymine.
[c]Numbering system of *erm*(41), with the GTG start codon as 1.

patients, only one (8%) achieved sputum culture conversion, which occurred after surgical resection. During the median follow-up period of 35.4 months after detection of macrolide resistance, two (15%) patients died.

Macrolide resistance in *M. abscessus* subsp. *abscessus* can be intrinsic or acquired depending on the resistance mechanism. Intrinsic macrolide resistance is associated with inducible resistance, involving the presence of a functional *erm*(41) gene, which encodes a type of methyltransferase known to methylate 23S rRNA (14). Acquired macrolide resistance involves spontaneous mutations in the *rrl* gene, which encodes 23S rRNA, that are selected during macrolide-containing antibiotic therapy (15). Whereas *M. abscessus* subsp. *abscessus* strains with inducible macrolide resistance show low MICs at day 3 and require longer incubation times (up to 14 days) for the induction of resistance, *M. abscessus* subsp. *abscessus* strains with acquired macrolide resistance show high MICs by day 3 (21).

Treatment success rates after antibiotic therapy for *M. abscessus* subsp. *abscessus* lung disease are less than 50% (9–13), and these low rates are attributed to inducible macrolide resistance associated with *erm*(41) or to the acquisition of resistance due to *rrl* mutations. Although many previous studies have reported on inducible macrolide resistance in *M. abscessus* subsp. *abscessus* (9–13), very few studies have evaluated acquired macrolide resistance in *M. abscessus* subsp. *abscessus* (17–19). Clinically acquired mutational resistance in *M. abscessus* subsp. *abscessus* occurs relatively infrequently (17). In our previous study, acquired macrolide resistance associated with *rrl* gene mutations developed in only 13% of patients who had persistently positive sputum cultures after more than 12 months of antibiotic therapy (12). Recently, Maurer et al. reported the acquisition of macrolide resistance in *M. abscessus* subsp. *abscessus* isolates in three of five patients with chronic *M. abscessus* subsp. *abscessus* lung disease who received long-term macrolide antibiotic therapy (18). In addition, Rubio et al. reported that on follow-up, three patients who initially had *M. abscessus* subsp. *abscessus* strains with the wild-type *rrl* sequence eventually developed acquired macrolide resistance (19). In those studies, acquired macrolide resistance was associated with macrolide resistance mutations in the 23S rRNA gene, which occurred regardless of the presence of an inducible *erm*(41) methylase. However, detailed clinical characteristics and long-term prognoses were not available in the previous studies.

In the present study, acquired macrolide resistance was detected during antibiotic therapy for *M. abscessus* subsp. *abscessus* lung disease in 10 patients (10/13 [77%]). After an intensive phase of combination i.v. antibiotic therapy for *M. abscessus* subsp. *abscessus* lung disease, the continuation phase usually consists of oral antibiotics such as macrolides, fluoroquinolones, and doxycycline, as described in our previous studies

**TABLE 5** Characteristics of isolates from the 13 patients studied

| Patient | Isolate | No. of mo since first isolate[a] | DST[b] | MIC, μg/ml (day 3/day 14)[c] | Colony morphotype[d] | erm(41)[e] | rrl[f] |
|---|---|---|---|---|---|---|---|
| 01 | 01-1 | 0 | R | >64 | Mixed (R) | T28 | GA |
| | | | | | Mixed (S) | T28 | AA |
| 02 | 02-1 | 0 | R | >64 | Mixed (R) | T28 | AA |
| | | | | | Mixed (S) | T28 | CA |
| | 02-2 | 16 | R | >64 | S | T28 | CA |
| 03 | 03-1 | 0 | R | >64 | S | T28 | AG |
| 04 | 04-1 | 0 | R | >64 | R | T28 | CA |
| 05 | 05-1 | 0 | R | >64 | R | C28 | GA |
| | 05-2 | 4 | R | >64 | R | C28 | GA |
| | 05-3 | 6 | R | >64 | R | C28 | GA |
| | 05-4 | 8 | R | >64 | R | C28 | GA |
| | 05-5 | 11 | R | >64 | R | C28 | GA |
| | 05-6 | 19 | R | >64 | R | C28 | GA |
| | 05-7 | 25 | R | >64 | R | C28 | GA |
| | 05-8 | 31 | R | >64 | S | C28 | GA |
| 06 | 06-1 | 0 | R | >64 | S | C28 | CA |
| | 06-2 | 6 | R | 64 | S | C28 | CA |
| | 06-3 | 10 | R | 64 | Mixed (R) | C28 | CA |
| | | | | | Mixed (S) | C28 | CA |
| 07 | 07-1 | 0 | R | >64 | R | T28 | TA |
| | 07-2 | 3 | R | >64 | R | T28 | GA |
| | 07-3 | 5 | R | 64 | R | T28 | GA |
| | 07-4 | 7 | R | >64 | R | T28 | GA |
| | 07-5 | 14 | R | >64 | R | T28 | GA |
| 08 | 08-1 | 0 | R | >64 | R | T28 | CA |
| | 08-2 | 8 | R | >64 | R | T28 | CA |
| | 08-3 | 9 | R | >64 | R | T28 | CA |
| | 08-4 | 13 | R | >64 | R | T28 | CA |
| 09 | 09-1 | 0 | R | >64 | Mixed (R) | T28 | CA |
| | | | | | Mixed (S) | T28 | CA |
| | 09-2 | 2 | R | >64 | Mixed (R) | T28 | CA |
| | | | | | Mixed (S) | T28 | CA |
| | 09-3 | 4 | R | >64 | Mixed (R) | T28 | CA |
| | | | | | Mixed (S) | T28 | CA |
| 10 | 10-1 | 0 | R | 64 | R | T28 | AG |
| | 10-2 | 2 | R | 64 | Mixed (R) | T28 | AG |
| | | | | | Mixed (S) | T28 | AG |
| 11 | 11-1 | 0 | R | >64 | R | T28 | GA |
| | 11-2 | 1 | R | 32 | R | T28 | GA |
| 12 | 12-1 | 0 | R | >64 | R | T28 | AG |
| | 12-2 | 1 | R | >64 | S | T28 | CA |
| | 12-3 | 5 | R | >64 | S | T28 | CA |
| | 12-4 | 15 | R | >64 | S | T28 | CA |
| | 12-5 | 18 | R | >64 | S | T28 | CA |
| 13 | 13-1 | 0 | R | >64 | R | T28 | GA |
| | 13-2 | 2 | R | >64 | R | T28 | CA |
| | 13-3 | 7 | R | >64 | R | T28 | CA |

[a]The first isolate was a clinical isolate that was firstly confirmed to be resistant to macrolide on DST.
[b]For DST: R, resistance.
[c]The microdilution method was used to determine the MIC for clarithromycin.
[d]For colony morphotype: S, smooth; R, rough.
[e]Numbering system of erm(41), with the GTG start codon as 1.
[f]Nucleotides 2058 and 2059 (E. coli numbering) of rrl, for which the wild-type sequence is AA. A, adenine; G, guanine; C, cytosine; T, thymine.

(9, 12, 22). Our current study showed that acquired macrolide resistance can occur in *M. abscessus* subsp. *abscessus* lung disease after macrolide monotherapy or weak macrolide-containing antibiotic regimens, consistent with previous findings (17). To prevent mutational macrolide resistance, a stronger regimen should be considered at the outset for treatment of *M. abscessus* subsp. *abscessus* lung disease. Such a regimen might include the recently proposed clofazimine or inhaled amikacin (4, 20, 23, 24), which we administered to some of our patients following the identification of acquired macrolide resistance.

In our study, acquired macrolide resistance was detected during antibiotic therapy for other NTM lung diseases in three patients (3/13 [23%]), with two cases of MAC and one case of *M. abscessus* subsp. *massiliense* lung disease. It is not uncommon to isolate *M. abscessus* subsp. *abscessus* during treatment of MAC lung disease, and microbiologic and clinical follow-up is important to determine the significance of *M. abscessus* subsp. *abscessus* isolation in such cases (25). As MAC treatment usually consists of macrolide, rifampin, and ethambutol, *M. abscessus* subsp. *abscessus* infection during MAC treatment could result in exposure to macrolide monotherapy for *M. abscessus* subsp. *abscessus* because neither rifampin nor ethambutol is effective in *M. abscessus* subsp. *abscessus* infection (26, 27). Macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease also developed during antibiotic treatment of *M. abscessus* subsp. *massiliense* lung disease in one of our patients. An oral macrolide can be effective in the continuation phase of treatment of *M. abscessus* subsp. *massiliense* lung disease (28), but this raises the possibility that new infections with *M. abscessus* subsp. *abscessus* may be exposed to this monotherapy and acquire macrolide resistance.

In *M. abscessus* subsp. *abscessus* lung disease, the rates of achieving negative sputum culture conversion were reported as only 25 to 42% (9–13). In this study, no patients with acquired macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease showed negative sputum culture conversion after antibiotic therapy alone, even after use of oral clofazimine and/or inhaled amikacin, which was reported to have clinical efficacy in patients with refractory *M. abscessus* subsp. *abscessus* lung disease (20, 23, 24). This implies that *M. abscessus* subsp. *abscessus* lung disease might be much more difficult to treat after acquisition of acquired macrolide resistance, regardless of intrinsic inducible macrolide resistance. Further research is needed to establish optimal treatment regimens for macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease.

During macrolide-based antibiotic treatment, *M. abscessus* subsp. *abscessus* can develop acquired macrolide resistance through point mutations at position 2058 or 2059 in *rrl* (15, 16, 18). Mutations at these positions were found in the *M. abscessus* subsp. *abscessus* isolates of all patients in the present study, with the isolates resistant to clarithromycin at a high level (MICs of ≥64 μg/ml at day 3). The frequencies of mutations at position 2058 or 2059 differ between studies. Some studies showed that the majority of mutational changes involved A2059 (17, 29), whereas other studies found that most mutations involved A2058 (18, 30). In our study, 23% involved A2059, with the remaining 77% involving A2058, and there was no difference in clarithromycin MICs based on mutation position, although only small numbers of isolates were studied.

Acquired resistance occurs not only in *M. abscessus* subsp. *abscessus* with the macrolide-susceptible *erm*(41) C28 sequevar but also in *M. abscessus* subsp. *abscessus* with the intrinsically macrolide-resistant *erm*(41) T28 sequevar (15, 18, 19, 30, 31). In our study, of the 13 isolates with *rrl* mutations, 85% had the T28 sequevar and 15% had the C28 sequevar of *erm*(41). A recent study of mutants selected *in vitro* for acquired macrolide resistance found that only 19% of mutants derived from the T28 sequevar had *rrl* mutations, whereas 100% of mutants derived from the C28 sequevar had *rrl* mutations (29). In clinical isolates, *rrl* mutations were observed more frequently in isolates of the T28 sequevar than in isolates of the C28 sequevar (18, 30). If macrolide treatment pressure continues, *M. abscessus* subsp. *abscessus* is likely to develop a stable resistant lineage. Notably, none of the resistance-conferring mutations in *rrl* was found to affect bacterial fitness to a major degree; although the A2059G mutation demon-

strated a small but significant fitness cost (2.4 to 2.6% per generation), the A2058G mutation had a nonsignificant cost (0.5 to 1.4% per generation) (32). Nevertheless, the existence of the inducible *erm*(41) gene suggests that acquisition of an *rrl* mutation confers some biofitness disadvantage in the absence of macrolide antibiotics.

Our study has several limitations. First, it was conducted at a single referral center and included a small number of patients. Second, treatment regimens were chosen at the discretion of attending physicians, without an established institutional protocol. Further studies with a larger number of patients are needed to evaluate the efficacy of antibiotic therapy in treating macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease.

In conclusion, we found that acquired macrolide resistance can develop in patients with *M. abscessus* subsp. *abscessus* lung disease after macrolide monotherapy or with weak antibiotic regimens in the continuation phase after initiation phases that include multiple intravenous antibiotics. Treatment outcomes are poor after the development of macrolide resistance, and therefore, prevention of macrolide resistance is important, with more effective therapies needed to treat *M. abscessus* subsp. *abscessus* lung disease both before and after the development of macrolide resistance.

## MATERIALS AND METHODS

**Study population.** We reviewed the medical records for all patients who had macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease between November 2006 and March 2016, as identified from the NTM Registry of Samsung Medical Center (a 1,979-bed referral hospital in Seoul, South Korea) (12). All patients fulfilled the diagnostic criteria of NTM lung disease (3). This retrospective study was approved by the institutional review board (IRB) of Samsung Medical Center (IRB application no. 2016-07-137). Patient information was anonymized and deidentified prior to analysis; therefore, requirements for informed consent were waived.

**Radiographic and microbiologic examination.** Chest radiography and high-resolution computed tomography (HRCT) were available at the time of detection of acquired macrolide-resistant *M. abscessus* subsp. *abscessus* in all patients. The fibrocavitary form of the disease (previously called the upper lobe cavitary form) was defined by the presence of cavitary opacities mainly in the upper lobes. The nodular bronchiectatic form was defined by the presence of bronchiectasis and multiple nodules on chest HRCT, regardless of the presence of small cavities in the lungs (12, 33).

Sputum acid-fast bacillius(AFB) smears and cultures were obtained using standard methods (34). *M. abscessus* subsp. *abscessus* was identified and differentiated from *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *bolletii* by a PCR and restriction fragment length polymorphism method based on the *rpoB* gene or by reverse blot hybridization assay of the *rpoB* gene (9, 12, 28), followed by multilocus sequencing analysis of *rrs*, *hsp65*, and *rpoB* (35). Each isolate was also genotyped with regard to the presence of a T-to-C mutation at position 28 of the *erm*(41) gene, as the C28 polymorphism abrogates inducible resistance (15).

DST was performed at the Korean Institute of Tuberculosis using a broth microdilution method (21). The MIC of clarithromycin was determined at days 3 and 14 after incubation; *M. abscessus* subsp. *abscessus* isolates were considered susceptible (MIC $\leq 2$ $\mu$g/ml at days 3 and 14), resistant (MIC $\geq 8$ $\mu$g/ml at day 3), or inducibly resistant (susceptible at day 3 but resistant at day 14) to clarithromycin (21). MICs for azithromycin were not determined, as clarithromycin is the class drug for macrolides (21).

Once macrolide resistance was detected, *M. abscessus* subsp. *abscessus* isolates were stored at $-80°C$ for further analysis. To detect point mutations at position 2058 or 2059 (*Escherichia coli* numbering) in the 23S rRNA gene, PCR was performed to amplify the region corresponding to domain V of the 23S rRNA gene, according to a method described previously (36). Primers 23SF1 and 23SRIII were used for PCR and sequencing (36).

**Antibiotic therapy and treatment outcomes.** Although the initial treatment regimens for *M. abscessus* subsp. *abscessus* lung disease are standardized at our institution (9, 12, 22), treatment regimens for macrolide-resistant *M. abscessus* subsp. *abscessus* lung disease were not standardized during the study period. Patients with mild symptoms at the time macrolide resistance was detected received oral antibiotics at the outpatient clinic. Patients with severe symptoms were hospitalized and received intravenous (i.v.) amikacin (15 mg/kg [of body weight]/day in two divided doses or 15 mg/kg/day with adjustment of a once-daily dose to target peak serum drug level of 55 to 65 $\mu$g/ml), plus cefoxitin (200 mg/kg/day; maximum of 12 g/day in three divided doses) or imipenem (750 mg three times per day) for 2 to 4 weeks (9, 12, 22), along with oral antibiotics. For oral antibiotics, treatment with a macrolide (clarithromycin at 1,000 mg/day or azithromycin at 250 mg/day) was continued, and additional drugs, such as a fluoroquinolone (ciprofloxacin at 1,000 mg/day or moxifloxacin at 400 mg/day), clofazimine (100 mg/day), or inhaled amikacin (250 to 500 mg/day), were used at the discretion of the attending physicians.

Treatment outcomes were assessed by sputum culture conversion after the detection of macrolide-resistant *M. abscessus* subsp.*abscessus* lung disease; conversion was defined as three consecutive negative cultures, with the time of conversion defined as the date of the first negative culture (9, 12). A

favorable outcome was defined as sputum culture conversion within 12 months after treatment initiation and maintenance for ≥12 months with treatment.

**Statistical analysis.** Data are presented as the median and interquartile range (IQR) for continuous variables and as frequency (percentage) for categorical variables. All statistical analyses were performed using SPSS Statistics for Windows, version 23.0 (IBM, Armonk, NY).

## REFERENCES

1. Prevots DR, Marras TK. 2015. Epidemiology of human pulmonary infection with nontuberculous mycobacteria: a review. Clin Chest Med 36: 13–34. https://doi.org/10.1016/j.ccm.2014.10.002.

2. Stout JE, Koh WJ, Yew WW. 2016. Update on pulmonary disease due to non-tuberculous mycobacteria. Int J Infect Dis 45:123–134. https://doi .org/10.1016/j.ijid.2016.03.006.

3. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ, Jr, Winthrop K. 2007. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 175:367–416. https://doi.org/10.1164/rccm.200604-571ST.

4. Floto RA, Olivier KN, Saiman L, Daley CL, Herrmann JL, Nick JA, Noone PG, Bilton D, Corris P, Gibson RL, Hempstead SE, Koetz K, Sabadosa KA, Sermet-Gaudelus I, Smyth AR, van Ingen J, Wallace RJ, Winthrop KL, Marshall BC, Haworth CS. 2016. US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis. Thorax 71(Suppl 1):i1–i22. https://doi.org/10.1136/thoraxjnl -2015-207360.

5. Griffith DE, Brown-Elliott BA, Benwill JL, Wallace RJ, Jr. 2015. *Mycobacterium abscessus*. "Pleased to meet you, hope you guess my name. . . ." Ann Am Thorac Soc 12:436–439. https://doi.org/10.1513/AnnalsATS .201501-015Ol.

6. Tortoli E, Kohl TA, Brown-Elliott BA, Trovato A, Leão SC, Garcia MJ, Vasireddy S, Turenne CY, Griffith DE, Philley JV, Baldan R, Campana S, Cariani L, Colombo C, Taccetti G, Teri A, Niemann S, Wallace RJ, Jr, Cirillo DM. 2016. Emended description of *Mycobacterium abscessus*, *Mycobacterium abscessus* subsp. *abscessus* and *Mycobacterium abscessus* subsp. *bolletii* and designation of *Mycobacterium abscessus* subsp. *massiliense* comb. nov. Int J Syst Evol Microbiol 66:4471–4479.

7. Koh WJ, Stout JE, Yew WW. 2014. Advances in the management of pulmonary disease due to *Mycobacterium abscessus* complex. Int J Tuberc Lung Dis 18:1141–1148. https://doi.org/10.5588/ijtld.14.0134.

8. Nessar R, Cambau E, Reyrat JM, Murray A, Gicquel B. 2012. *Mycobacterium abscessus*: a new antibiotic nightmare. J Antimicrob Chemother 67:810–818. https://doi.org/10.1093/jac/dkr578.

9. Koh WJ, Jeon K, Lee NY, Kim BJ, Kook YH, Lee SH, Park YK, Kim CK, Shin SJ, Huitt GA, Daley CL, Kwon OJ. 2011. Clinical significance of differentiation of *Mycobacterium massiliense* from *Mycobacterium abscessus*. Am J Respir Crit Care Med 183:405–410. https://doi.org/10.1164/rccm .201003-0395OC.

10. Lyu J, Kim BJ, Kim BJ, Song JW, Choi CM, Oh YM, Lee SD, Kim WS, Kim DS, Shim TS. 2014. A shorter treatment duration may be sufficient for patients with *Mycobacterium massiliense* lung disease than with *Mycobacterium abscessus* lung disease. Respir Med 108:1706–1712. https:// doi.org/10.1016/j.rmed.2014.09.002.

11. Park J, Cho J, Lee CH, Han SK, Yim JJ. 2017. Progression and treatment outcomes of lung disease caused by *Mycobacterium abscessus* and *Mycobacterium massiliense*. Clin Infect Dis 64:301–308. https://doi.org/ 10.1093/cid/ciw723.

12. Koh WJ, Jeong BH, Kim SY, Jeon K, Park KU, Jhun BW, Lee H, Park HY, Kim

DH, Huh HJ, Ki CS, Lee NY, Kim HK, Choi YS, Kim J, Lee SH, Kim CK, Shin SJ, Daley CL, Kim H, Kwon OJ. 2017. Mycobacterial characteristics and treatment outcomes in *Mycobacterium abscessus* lung disease. Clin Infect Dis 64:309–316. https://doi.org/10.1093/cid/ciw724.

13. Diel R, Ringshausen F, Richter E, Welker L, Schmitz J, Nienhaus A. 2017. Microbiological and clinical outcomes of treating non-*Mycobacterium avium* complex nontuberculous mycobacterial pulmonary disease: a systematic review and meta-analysis. Chest 152:120–142. https://doi .org/10.1016/j.chest.2017.04.166.

14. Nash KA, Brown-Elliott BA, Wallace RJ, Jr. 2009. A novel gene, *erm*(41), confers inducible macrolide resistance to clinical isolates of *Mycobacterium abscessus* but is absent from *Mycobacterium chelonae*. Antimicrob Agents Chemother 53:1367–1376. https://doi.org/10.1128/AAC.01275-08.

15. Bastian S, Veziris N, Roux AL, Brossier F, Gaillard JL, Jarlier V, Cambau E. 2011. Assessment of clarithromycin susceptibility in strains belonging to the *Mycobacterium abscessus* group by *erm*(41) and *rrl* sequencing. Antimicrob Agents Chemother 55:775–781. https://doi.org/10.1128/AAC .00861-10.

16. Shallom SJ, Moura NS, Olivier KN, Sampaio EP, Holland SM, Zelazny AM. 2015. New real-time PCR assays for detection of inducible and acquired clarithromycin resistance in the *Mycobacterium abscessus* group. J Clin Microbiol 53:3430–3437. https://doi.org/10.1128/JCM.01714-15.

17. Wallace RJ, Jr, Meier A, Brown BA, Zhang Y, Sander P, Onyi GO, Bottger EC. 1996. Genetic basis for clarithromycin resistance among isolates of *Mycobacterium chelonae* and *Mycobacterium abscessus*. Antimicrob Agents Chemother 40:1676–1681.

18. Maurer FP, Ruegger V, Ritter C, Bloemberg GV, Bottger EC. 2012. Acquisition of clarithromycin resistance mutations in the 23S rRNA gene of *Mycobacterium abscessus* in the presence of inducible *erm*(41). J Antimicrob Chemother 67:2606–2611. https://doi.org/10.1093/jac/dks279.

19. Rubio M, March F, Garrigo M, Moreno C, Espanol M, Coll P. 2015. Inducible and acquired clarithromycin resistance in the *Mycobacterium abscessus* complex. PLoS One 10:e0140166. https://doi.org/10.1371/ journal.pone.0140166.

20. Yang B, Jhun BW, Moon SM, Lee H, Park HY, Jeon K, Kim DH, Kim SY, Shin SJ, Daley CL, Koh WJ. 2017. A clofazimine-containing regimen for the treatment of *Mycobacterium abscessus* lung disease. Antimicrob Agents Chemother 61:e02052-16. https://doi.org/10.1128/AAC.02052-16.

21. Clinical and Laboratory Standards Institute. 2011. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; approved standard, 2nd ed. CLSI document M24-A2. Clinical and Laboratory Standards Institute, Wayne, PA.

22. Jeon K, Kwon OJ, Lee NY, Kim BJ, Kook YH, Lee SH, Park YK, Kim CK, Koh WJ. 2009. Antibiotic treatment of *Mycobacterium abscessus* lung disease: a retrospective analysis of 65 patients. Am J Respir Crit Care Med 180:896–902. https://doi.org/10.1164/rccm.200905-0704OC.

23. Martiniano SL, Wagner BD, Levin A, Nick JA, Sagel SD, Daley CL. 5 May 2017. Safety and effectiveness of clofazimine for primary and refractory nontuberculous mycobacterial infection. Chest https://doi.org/10.1016/ j.chest.2017.04.175.

24. Olivier KN, Shaw PA, Glaser TS, Bhattacharyya D, Fleshner M, Brewer CC, Zalewski CK, Folio LR, Siegelman JR, Shallom S, Park IK, Sampaio EP, Zelazny

AM, Holland SM, Prevots DR. 2014. Inhaled amikacin for treatment of refractory pulmonary nontuberculous mycobacterial disease. Ann Am Thorac Soc 11:30–35. https://doi.org/10.1513/AnnalsATS.201307-231OC.

25. Griffith DE, Philley JV, Brown-Elliott BA, Benwill JL, Shepherd S, York D, Wallace RJ, Jr. 2015. The significance of *Mycobacterium abscessus* subspecies *abscessus* isolation during *Mycobacterium avium* complex lung disease therapy. Chest 147:1369–1375. https://doi.org/10.1378/chest.14-1297.

26. Kang YA, Koh WJ. 2016. Antibiotic treatment for nontuberculous mycobacterial lung disease. Expert Rev Respir Med 10:557–568. https://doi.org/10.1586/17476348.2016.1165611.

27. Ryu YJ, Koh WJ, Daley CL. 2016. Diagnosis and treatment of nontuberculous mycobacterial lung disease: clinicians' perspectives. Tuberc Respir Dis (Seoul) 79:74–84. https://doi.org/10.4046/trd.2016.79.2.74.

28. Koh WJ, Jeong BH, Jeon K, Kim SY, Park KU, Park HY, Huh HJ, Ki CS, Lee NY, Lee SH, Kim CK, Daley CL, Shin SJ, Kim H, Kwon OJ. 2016. Oral macrolide therapy following short-term combination antibiotic treatment for *Mycobacterium massiliense* lung disease. Chest 150:1211–1221. https://doi.org/10.1016/j.chest.2016.05.003.

29. Mougari F, Bouziane F, Crockett F, Nessar R, Chau F, Veziris N, Sapriel G, Raskine L, Cambau E. 2017. Selection of resistance to clarithromycin in *Mycobacterium abscessus* subspecies. Antimicrob Agents Chemother 61:e00943-16. https://doi.org/10.1128/AAC.00943-16.

30. Mougari F, Amarsy R, Veziris N, Bastian S, Brossier F, Bercot B, Raskine L, Cambau E. 2016. Standardized interpretation of antibiotic susceptibility testing and resistance genotyping for *Mycobacterium abscessus* with regard to subspecies and *erm41* sequevar. J Antimicrob Chemother 71:2208–2212. https://doi.org/10.1093/jac/dkw130.

31. Lee SH, Yoo HK, Kim SH, Koh WJ, Kim CK, Park YK, Kim HJ. 2014. Detection and assessment of clarithromycin inducible resistant strains among Korean *Mycobacterium abscessus* clinical strains: PCR methods. J Clin Lab Anal 28:409–414. https://doi.org/10.1002/jcla.21702.

32. Sander P, Springer B, Prammananan T, Sturmfels A, Kappler M, Pletschette M, Böttger EC. 2002. Fitness cost of chromosomal drug resistance-conferring mutations. Antimicrob Agents Chemother 46:1204–1211. https://doi.org/10.1128/AAC.46.5.1204-1211.2002.

33. Kim HS, Lee KS, Koh WJ, Jeon K, Lee EJ, Kang H, Ahn J. 2012. Serial CT findings of *Mycobacterium massiliense* pulmonary disease compared with *Mycobacterium abscessus* disease after treatment with antibiotic therapy. Radiology 263:260–270. https://doi.org/10.1148/radiol.12111374.

34. American Thoracic Society. 2000. Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Care Med 161:1376–1395. https://doi.org/10.1164/ajrccm.161.4.16141.

35. Kim SY, Shin SJ, Jeong BH, Koh WJ. 2016. Successful antibiotic treatment of pulmonary disease caused by *Mycobacterium abscessus* subsp. *abscessus* with C-to-T mutation at position 19 in erm(41) gene: case report. BMC Infect Dis 16:207. https://doi.org/10.1186/s12879-016-1554-7.

36. Jamal MA, Maeda S, Nakata N, Kai M, Fukuchi K, Kashiwabara Y. 2000. Molecular basis of clarithromycin-resistance in *Mycobacterium avium-intracellulare* complex. Tuber Lung Dis 80:1–4. https://doi.org/10.1054/tuld.1999.0227.