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Clinical Characteristics, Treatment Outcomes, and Resistance Mutations Associated with Macrolide-Resistant *Mycobacterium avium* Complex Lung Disease

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Macrolide antibiotics are key components of the multidrug treatment regimen for treating lung disease (LD) due to *Mycobacterium avium* complex (MAC). Despite the emergence of macrolide resistance, limited data are available on macrolide-resistant MAC-LD. This study evaluated the clinical features and treatment outcomes of patients with macrolide-resistant MAC-LD and the molecular characteristics of the macrolide-resistant isolates. A retrospective review of the medical records of 34 patients with macrolide-resistant MAC-LD who were diagnosed between January 2002 and December 2014 was performed, along with genetic analysis of 28 clinical isolates. Nineteen (56%) patients had the fibrocavitary form of MAC-LD, and 15 (44%) had the nodular bronchiectatic form. *M. intracellulare* was the etiologic organism in 21 (62%) patients. Approximately two-thirds (22/34 [65%]) of the patients had been treated with currently recommended multidrug regimens that included macrolide, ethambutol, and rifamycin prior to the emergence of macrolide resistance, and none had been treated with macrolide monotherapy. The median duration of treatment after the detection of macrolide resistance was 23.0 months (interquartile range, 16.8 to 45.3 months). Treatment outcomes were poor after the development of macrolide resistance, with favorable treatment outcomes achieved in only five (15%) patients, including two patients who underwent surgical resection. One-, 3-, and 5-year mortality rates were 9, 24, and 47%, respectively. Molecular analysis of 28 clinical isolates revealed that 96% (27/28) had point mutations at position 2058 or 2059 of the 23S rRNA gene. Our analyses indicate that more effective therapy is needed to treat macrolide-resistant MAC-LD and prevent its development.

The *Mycobacterium avium* complex (MAC) predominantly consists of *M. avium* and *M. intracellulare* and in many countries has been reported as the most common etiology of lung disease caused by nontuberculous mycobacteria (NTM) (1, 2). Newer macrolides, such as clarithromycin and azithromycin, are cornerstones in the antibiotic treatment of MAC lung disease (MAC-LD) (3–7), and macrolides, and perhaps amikacin, are the only drugs with a consistent correlation between *in vitro* susceptibility results and clinical response in MAC-LD (8–12). Therefore, a macrolide-based multidrug regimen that consists of a macrolide, a rifamycin (rifampin or rifabutin), and ethambutol, with or without the initial use of streptomycin or amikacin, is the currently recommended standard therapy for patients with MAC-LD (13).

Because macrolides are key drugs for the treatment of MAC-LD, the development of macrolide resistance indicates a poor treatment outcome and increased mortality (14, 15). However, there have been only two previous studies evaluating the risk factors, clinical characteristics, and treatment outcomes of macrolide-resistant MAC-LD, and the results are inconsistent, especially regarding the risk factors and optimal treatment modalities (14, 15). The aim of this study was to evaluate the clinical features and treatment outcomes of patients with macrolide-resistant MAC-LD, as well as the molecular characteristics of macrolide-resistant MAC isolates.

MATERIALS AND METHODS

Study populations. The medical records of all patients with macrolide-resistant MAC-LD identified from the NTM Registry of Samsung Medical Center (a 1,979-bed referral hospital in Seoul, South Korea) from January 2002 to December 2014 were reviewed. All the patients fulfilled the diagnostic criteria of NTM lung disease (13). This retrospective study was approved by the Institutional Review Board (IRB) of Samsung Medical Center (IRB no. 2016-02-004). Informed consent was waived for the use of medical data because patient information was anonymized and deidentified prior to analysis.

Radiographic and microbiologic examination. The fibrocavitary form (previously referred to as the upper lobe cavitary form) of MAC-LD was defined by the presence of cavitary opacities and pleural thickening,

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mainly in the upper lobes, regardless of the presence of underlying chronic obstructive lung disease in the rest of the lungs. The nodular bronchiectatic form of MAC-LD was defined by the presence of multifocal bronchiectasis and clusters of small nodules on chest high-resolution computed tomography (HRCT), regardless of the presence of small cavities in the lungs (13, 16). The HRCT images were evaluated by four of us (S.M.M., H.Y.P., B.W.J., and W.-J.K.), and consensus was obtained.

Sputum acid-fast bacillus (AFB) smears and cultures were obtained using standard methods, as described previously (16). During the study period, NTM species were identified by a PCR and restriction fragment length polymorphism method based on the *rpoB* gene or by a reverse blot hybridization assay of the *rpoB* gene (17–20). Drug susceptibility testing for clarithromycin was performed using the broth microdilution method at the Korean Institute of Tuberculosis (21). Isolates with a MIC of 32 μg/ml or greater were considered resistant (21). MICs for azithromycin were not determined, as clarithromycin is the class drug for macrolides (21).

MAC isolates were stored at −80°C for further analyses. For the detection of 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 previously described (22). The primers 23SF1 and 23SRIII were used for PCR and sequencing (22).

Antibiotic therapy and treatment outcomes. Patients with macrolide-resistant MAC-LD received combination antibiotic therapy. For most patients, the following drug dosages were used: clarithromycin, 1,000 mg/day; azithromycin, 250 mg/day; ethambutol, 15 mg/kg of body weight/day; rifampin, 450 mg/day for a body weight of <50 kg or 600 mg/day for a body weight of ≥50 kg; rifabutin, 300 mg/day. Streptomycin (25 mg/kg intramuscularly two or three times a week), moxifloxacin (400 mg/day), or clofazimine (100 mg/day) was also used at the discretion of the attending physicians.

Sputum examinations were performed 1, 3, and 6 months after initiation of antibiotic treatment and then at 2- to 3-month intervals during treatment. Sputum conversion was defined as three consecutive negative cultures, with the time of conversion defined as the date of the first negative culture (17–19). A favorable outcome was defined as sputum culture conversion within 12 months after initiation of treatment and maintenance of a negative culture for 12 months or longer on treatment (17–19). An unfavorable outcome was defined as no sputum culture conversion or death. Death was attributed to MAC-LD when patients had failure of sputum culture conversion and progression of MAC-LD at the time of death. Patients who died after sputum culture conversion to negative, who died of acute respiratory failure regardless of sputum culture conversion, or who were lost to follow-up and died from unknown causes were categorized under "death due to all causes." All dates of patient deaths were ascertained from the medical records or the database of the National Health Insurance Service.

Statistical analysis. All data are presented as medians and interquartile ranges (IQRs) for continuous variables and as numbers and percentage for categorical variables. Data were compared by the Mann-Whitney U test for continuous variables and by the Pearson χ^2 test or Fisher's exact test for categorical variables. A two-sided P value of <0.05 was considered to indicate a statistically significant difference for all analyses. All analyses were performed with SPSS statistical software (SPSS version 23; IBM, Armonk, NY).

RESULTS

Patient characteristics. Macrolide-resistant MAC-LD was diagnosed in 34 patients during the study period. The median age for all patients was 65 years (IQR, 61 to 70 years), and the majority (n = 23 [68%]) of the patients were male. None of the patients were infected with human immunodeficiency virus. Twenty-five (74%) patients had a history of prior treatment of pulmonary tuberculosis, and four (12%) had a history of previous successful treatment of NTM lung disease caused by MAC, *M. abscessus*, or *M. massiliense*. Chronic obstructive pulmonary disease (n = 11 [32%]) and chronic pulmonary aspergillosis (n = 11 [32%]) were common comorbid diseases (Table 1).

The etiologic organisms of MAC-LD were *M. intracellulare* in 21 (62%) patients and *M. avium* in 13 (38%) patients. The sputum AFB smear was positive in 27 (80%) patients at the time of detection of macrolide resistance. Chest radiography and HRCT were available for all patients. Fifteen (44%) patients had the nodular bronchiectatic form, and 19 (56%) had the fibrocavitary form of the disease. Cavitary lesions were found by HRCT in all patients with the fibrocavitary form and in eight patients (53%) with the nodular bronchiectatic form. The median preoperative forced vital capacity (FVC) was 2.25 liters (IQR, 1.84 to 2.98 liters), and the forced expiratory volume in 1 s (FEV_1) was 1.96 liters (IQR, 1.36 to 2.31 liters). The patients with the fibrocavitary form had lower FVC and FEV_1 than those with the nodular bronchiectatic form (Table 1).

Previous antibiotic therapy before the detection of macrolide-resistant MAC. All patients had received macrolide-based combination treatment, and the median duration of exposure to macrolide was 30.4 months (IQR, 20.1 to 38.7 months) before the detection of macrolide resistance. Thirty-two patients (94%) received clarithromycin, and two patients (6%) received azithromycin followed by clarithromycin. The most common previous treatment regimen (n = 22 [65%]) consisted of macrolide, rifamycin, and ethambutol, with (n = 16) or without (n = 6) streptomycin. A combination of macrolide with another drug, excluding ethambutol, was administered to 10 patients (29%). In these 10 patients, ethambutol was discontinued due to optic neuritis (n = 9) or skin rash (n = 1). One patient received the two-drug regimen of macrolide with ethambutol. Macrolide-resistant MAC lung disease developed in one patient after completing treatment with combined antibiotic therapy that included oral clarithromycin for *M. massiliense* lung disease (Table 2) (23, 24).

Treatment and outcomes after the detection of macrolide-resistant MAC. The treatment regimens after the detection of macrolide resistance and the treatment outcomes are summarized in Table 3. After the detection of macrolide resistance, the macrolide was discontinued in 18 (53%) patients and continuously prescribed in 16 (47%) patients (azithromycin in 10 patients and clarithromycin in 6 patients). Rifamycin, as either rifampin (32 patients) or rifabutin (2 patients), was used in all patients (n = 34 [100%]), and ethambutol (n = 25 [74%]) was used in most patients. Moxifloxacin or clofazimine was added to the regimen for 17 (50%) and 4 (12%) patients, respectively. Streptomycin was administered in 13 (38%) patients after the detection of macrolide resistance and was more frequently used in patients with the fibrocavitary form (10/19 [53%]) than those with the nodular bronchiectatic form (3/15 [20%]). The median duration of antibiotic therapy after the detection of macrolide resistance was 23.0 months (IQR, 16.8 to 45.3 months). Two (6%) patients with the fibrocavitary form underwent surgical resection with lobectomy, one at 3.7 months and the other at 7.7 months after the detection of macrolide resistance.

As shown in Table 3, only five (15%) patients had favorable outcomes, and 29 (85%) had unfavorable outcomes. The proportions of patients with favorable outcomes were similar between patients with the nodular bronchiectatic form (2/15 [13%]) and those with the fibrocavitary form (3/19 [16%]). During the me-

TABLE 1 Clinical characteristics at the time of diagnosis of macrolide-resistant MAC-LD

Characteristic[a]	Value[b]		
	Total	Nodular bronchiectatic form	Fibrocavitary form
Patients	34 (100)	15 (44)	19 (56)
Male	23 (68)	8 (53)	15 (79)
Age (yr)	65 (61–70)	65 (56–72)	66 (62–70)
BMI[b] (kg/m^2)	19.7 (17.3–21.2)	19.5 (18.2–21.2)	19.7 (15.8–21.6)
Nonsmokers	20 (59)	10 (67)	10 (53)
Previous treatment of pulmonary TB	25 (74)	11 (73)	14 (74)
Previous treatment of NTM lung disease	4 (12)	3 (20)	1 (5)
Comorbid disease			
COPD	11 (32)	3 (30)	8 (42)
Chronic pulmonary aspergillosis	11 (32)	2 (13)	9 (47)
Interstitial lung disease	2 (6)	1 (7)	1 (5)
Diabetes mellitus	4 (12)	1 (7)	3 (16)
Chronic heart disease	8 (24)	2 (13)	6 (32)
Chronic liver disease	3 (9)	2 (13)	1 (5)
Etiologic organism			
M. intracellulare	21 (62)	9 (60)	12 (63)
M. avium	13 (38)	6 (40)	7 (37)
Positive sputum AFB smear	27 (80)	10 (67)	17 (90)
Cavitary lesions on chest HRCT	27 (80)	8 (53)	19 (100)
Pulmonary function tests[c]			
FVC (liters)	2.25 (1.84–2.98)	2.90 (2.12–3.27)	2.10 (1.84–2.56)
FVC (% predicted)	63 (52–80)	81 (61–101)	56 (51–71)
FEV_1 (liters)	1.96 (1.36–2.31)	2.13 (1.82–2.70)	1.77 (1.17–2.08)
FEV_1 (% predicted)	77 (54–87)	85 (71–104)	65 (45–82)

[a] BMI, body mass index; TB, tuberculosis; COPD, chronic obstructive pulmonary disease.
[b] Data are presented as number (percent) or as median (interquartile range).
[c] Results were available for 31 patients (14 with the nodular bronchiectatic form and 17 with the fibrocavitary form).

dian follow-up of 39.3 months (IQR, 22.9 to 43.4 months) after the detection of macrolide resistance, all-cause mortality was 50% (17/34), including mortality due to MAC-LD in nine (26%) patients. All-cause mortality was more frequent in patients with the fibrocavitary form (13/19 [68%]) than in those with the nodular bronchiectatic form (4/15 [27%]). The overall cumulative mortality rates at 1, 3, and 5 years were 9% ($n = 3$), 24% ($n = 8$), and 47% ($n = 16$), respectively.

Comparison of variables according to treatment outcomes. There were no differences in age, sex, body mass index, etiologic organism, radiographic type, and the presence of cavitary lesions on HRCT according to treatment outcome. Patients with unfa-

TABLE 2 Previous treatment regimen before detection of macrolide-resistant MAC-LD

Parameter	Value[a]		
	Total ($n = 34$)	Nodular bronchiectatic form ($n = 15$)	Fibrocavitary form ($n = 19$)
Treatment regimen			
Macrolide + ethambutol + rifamycin ± streptomycin	22 (65)[b]	8 (53)	14 (74)
Macrolide + rifampin ± streptomycin or moxifloxacin[c]	10 (29)	5 (33)	5 (26)
Macrolide + ethambutol	1 (3)	1 (7)	0 (0)
Macrolide + ciprofloxacin[d]	1 (3)	1 (7)	0 (0)
Duration of exposure to macrolide (mo)	30.4 (20.1–38.7)	30.3 (20.5–36.7)	31.7 (19.2–46.7)

[a] Data are presented as number (percent) or as median (interquartile range).
[b] Sixteen patients received streptomycin injections.
[c] Ethambutol was discontinued because of adverse effects, such as visual disturbance ($n = 9$; 90%) or skin rash ($n = 1$; 10%).
[d] Macrolide-resistant *M. avium* complex lung disease developed after completion of treatment for *M. massiliense* lung disease in a patient who received combined antibiotic therapy, including oral clarithromycin.

TABLE 3 Treatment modalities and outcomes after detection of macrolide-resistant MAC-LD

Parameter	Value[a]		
	Total ($n = 34$)	Nodular bronchiectatic form ($n = 15$)	Fibrocavitary form ($n = 19$)
Antibiotic therapy			
Macrolide	16 (47)	6 (40)	10 (53)
Rifampin or rifabutin	34 (100)	15 (100)	19 (100)
Ethambutol	25 (74)	10 (67)	15 (79)
Moxifloxacin	17 (50)	8 (53)	9 (47)
Clofazimine	4 (12)	3 (20)	1 (5)
Streptomycin	13 (38)	3 (20)	10 (53)
Surgical resection	2 (6)	0 (0)	2 (11)
Total treatment duration (mo)	23.0 (16.8–45.3)	23.9 (20.8–56.5)	22.4 (11.8–43.9)
Treatment outcome			
Favorable outcome	5 (15)	2 (13)	3 (16)
Unfavorable outcome	29 (85)	13 (87)	16 (84)
Mortality			
Time from detection of resistance to death (mo)	39.3 (22.9–43.4)	33.4 (24.9–41.4)	39.3 (16.6–49.8)
1-yr mortality	3 (9)	0 (0)	3 (16)
3-yr mortality	8 (24)	2 (13)	6 (32)
5-yr mortality	16 (47)	4 (27)	12 (63)
Deaths due to MAC lung disease	9 (26)	3 (20)	6 (32)
Deaths due to all causes	17 (50)	4 (27)	13 (68)

[a] Data are presented as number (percent) or as median (interquartile range).

vorable outcomes were more likely to have sputum AFB smear positivity at the time of detection of macrolide resistance than those with favorable outcomes (25/29 [86%] versus 2/5 [40%]; $P = 0.048$). Regarding treatment regimens, continued treatment with a macrolide ($P = 0.648$) or the addition of moxifloxacin ($P = 0.335$), clofazimine ($P = 0.999$), or streptomycin ($P = 0.999$) had no significant effect on treatment outcome. Patients who underwent surgical resection were more likely to have a favorable outcome ($P = 0.018$) (Table 4).

Genetic-mutation analysis of macrolide-resistant MAC isolates. Among the 34 patients with macrolide-resistant MAC-LD, the MAC isolates of 28 (82%) patients were available for genetic-mutation analysis. Point mutations were found at position 2058 ($n = 13$) or 2059 ($n = 14$) of the 23S rRNA gene in all but one of the isolates (Table 5). The most common mutation was a nucleotide change from adenine to guanine (15/28 [53%]), followed by cytosine (10/28 [36%]) and thymine (2/28 [7%]). There was no significant difference in mutation type according to treatment outcome ($P = 0.335$).

DISCUSSION

This study analyzed the clinical characteristics and treatment outcomes of 34 patients with macrolide-resistant MAC-LD, as well as the molecular characteristics of macrolide-resistant MAC isolates. Overall, our analyses indicate a poor prognosis for patients with

TABLE 4 Comparison of variables according to treatment outcomes after diagnosis of macrolide-resistant MAC-LD

Variable	Value[a]			*P* value
	Total ($n = 34$ [100%])	Favorable outcome ($n = 5$ [15%])	Unfavorable outcome ($n = 29$ [85%])	
Male	23 (68)	2 (40)	21 (72)	0.300
Age (yr)	65 (61–70)	65 (60–69)	65 (61–71)	0.841
BMI[b] (kg/m^2)	19.7 (17.3–21.2)	20.4 (17.4–23.6)	19.5 (17.3–21.2)	0.363
M. intracellulare	21 (62)	3 (60)	18 (62)	0.999
Positive sputum AFB smear	27 (80)	2 (40)	25 (86)	0.048
Fibrocavitary form	16 (47)	3 (60)	16 (55)	0.999
Cavitary lesions on chest HRCT	27 (80)	4 (80)	23 (79)	0.999
Use of macrolide	16 (47)	3 (60)	13 (45)	0.648
Use of moxifloxacin	17 (50)	1 (20)	16 (55)	0.335
Use of clofazimine	4 (12)	0 (0)	4 (14)	0.999
Use of streptomycin	13 (38)	2 (40)	11 (38)	0.999
Surgical resection	2 (6)	2 (40)	0 (0)	0.018

[a] Data are presented as number (percent) or as median (interquartile range).
[b] BMI, body mass index.

TABLE 5 Analysis of genetic mutations of adenines at positions 2058 and 2059 in the 23S rRNA gene of macrolide-resistant MAC clinical isolates ($n = 28$)

Point mutation at position 2058 or 2059[a]	No. (%)
Presence of mutation	27 (96)
Adenine→guanine	15 (53)
A2058G	6
A2059G	9
Adenine→cytosine	10 (36)
A2058C	6
A2059C	4
Adenine→thymine	2 (7)
A2058T	1
A2059T	1
Absence of mutation	1 (4)

[a] *E. coli* numbering. A, adenine; G, guanine; C, cytosine; T, thymine.

macrolide-resistant MAC-LD, with limited effective treatment options. Five-year mortality rates, from development of resistance to time of death, approached 50%, with the death of 26% of patients directly attributed to MAC-LD. Of the 34 patients examined, only 5 (15%) showed favorable outcomes, and there was no statistically significant association between antibiotic regimen and treatment outcome, although patients who underwent surgical resection were more likely to have favorable outcomes. An AFB-positive sputum smear at the time of detection of macrolide resistance was more frequently observed in patients with unfavorable outcomes.

During the management of patients with MAC-LD, preventing the emergence of macrolide resistance is critical, because the development of such resistance is strongly associated with treatment failure and increased mortality (14, 15). The first study on macrolide-resistant MAC-LD from the United States was published in 2006 and found that the major reasons (76%) for the development of macrolide resistance were initial macrolide monotherapy and the combination of a macrolide and a fluoroquinolone (14). That study included patients who were previously enrolled in clinical trials with initial macrolide monotherapy in the early 1990s (25–27). However, a recently published study from Japan found that macrolide monotherapy or a two-drug regimen that included a macrolide were previously administered in only 18% and 12%, respectively, of patients with macrolide-resistant MAC-LD (15). The majority (60%) of patients had received adequate long-term combination therapies prior to the emergence of macrolide resistance (15).

In our study, no patient had received macrolide monotherapy before the detection of macrolide resistance. However, approximately one-third of our patients were treated with a regimen that did not include ethambutol because of its adverse effects. Ethambutol is an important companion drug to prevent the emergence of macrolide resistance (28, 29), but the adverse effects of ethambutol, such as ocular toxicity, are a serious concern, especially in the daily treatment for MAC-LD (30). Our study suggests that treatment regimens without ethambutol for MAC-LD may allow the development of macrolide resistance.

The majority (65%) of our patients received the currently recommended three oral drugs (macrolide, rifamycin, and ethambutol) before the development of macrolide resistance, which is consistent with a recently published study showing that 60% of patients received the recommended combination antibiotic therapy before the development of macrolide resistance (15). These results suggest that the development of macrolide resistance can occur even when patients are treated with multidrug treatment regimens. The development of macrolide resistance under these circumstances may be explained by the relatively low concentrations of key drugs, including macrolides, because the concomitant use of rifamycin often leads to reduced levels of macrolides, particularly clarithromycin, in serum (31, 32).

Another factor that may contribute to the emergence of macrolide resistance during recommended treatment is a high bacterial burden. In the present study, the proportion of cases with the fibrocavitary form of MAC-LD was high (56%), and 80% of patients had smear-positive sputum specimens at the time of detection of macrolide resistance. In our institution, the proportion of cases with the fibrocavitary form was only 19% of newly diagnosed macrolide-susceptible MAC-LD cases (18). This high proportion of the fibrocavitary form (53 to 76%) among cases of macrolide-resistant MAC-LD was also reported in previous studies (14, 15). Additionally, 79% of our patients with the fibrocavitary form were male. It is well known that the fibrocavitary form of MAC-LD typically affects elderly men with underlying lung disease, such as a history of tuberculosis (13). The fibrocavitary form of MAC-LD resembles pulmonary tuberculosis, and these patients usually had AFB-positive sputum smears. However, it was unclear whether these patients previously had culture-confirmed pulmonary tuberculosis, because patients with AFB smear-positive sputum or those displaying chest radiographic findings suggestive of active tuberculosis had generally been presumed to have pulmonary tuberculosis and were treated empirically with antituberculous drugs in areas where tuberculosis is endemic, such as South Korea (33).

In addition, *M. intracellulare* was the etiologic organism in a higher proportion of cases in our current study (62%) than in newly diagnosed cases of MAC-LD in our institution (45%) (18). A previous study also found that the majority (77%) of cases of macrolide-resistant MAC-LD were caused by *M. intracellulare* (14). Some studies have found that *M. intracellulare* is more virulent than *M. avium* and that patients with *M. intracellulare* infection are more likely to have the fibrocavitary form of MAC-LD and more treatment failures than patients with *M. avium* infection (18, 34). The high bacterial burden of a more virulent organism could result in a greater possibility of selection for resistant organisms during the treatment of MAC-LD.

With regard to treatment outcomes, the sputum conversion rate (15%) in the present study was lower than those (26 to 36%) reported in previous studies of macrolide-resistant MAC-LD (14, 15). The 1-year mortality rate in our study (9%) was lower than that (25%) found in a previous study of macrolide-resistant MAC-LD (14), although the 5-year mortality rate was significantly higher (47%) in the present study. Moreover, the 5-year mortality rate in patients with macrolide-resistant MAC-LD in our study was much higher than those (13 to 26%) in patients with newly diagnosed MAC-LD in previous studies (35–37). These time-dependent increases in mortality indicate the difficulty of managing patients with macrolide-resistant MAC-LD. Our results show that the continuation of macrolides or the addition of moxifloxacin, clofazimine, or streptomycin does not improve the treatment success rate after the development of macrolide-resistant MAC-LD. These findings were consistent with those of previous studies, al-

though moxifloxacin or clofazimine can be effective for the treatment of macrolide-susceptible MAC-LD (38–40). In contrast, the combination of surgical resection and parenteral aminoglycoside was associated with favorable treatment outcomes in the treatment of macrolide-resistant MAC-LD (14), although surgical treatment may be associated with high complication rates (41, 42). In the present study, two patients with the fibrocavitary form who underwent surgical resection combined with injectable medication achieved sputum-negative conversion. Although surgical resection can be beneficial in some patients with macrolide-resistant MAC-LD, most patients in our study were not considered to be candidates for surgery because of their compromised general condition and destruction of lung function during the long-term progressive course of treatment prior to the detection of macrolide resistance.

Molecular analysis of the 23S rRNA gene at positions 2058 and 2059 found high frequencies (96%) of point mutations in the patient isolates. The mechanisms of macrolide resistance of MAC have been studied at the molecular level, and point mutations are well recognized to be responsible for macrolide resistance in clinical isolates (43, 44). Of these point mutations, the most common in our study was the transition from adenine to guanine, consistent with the findings of a previous study (14). One study suggested that, in resistant isolates, the mutation from adenine to guanine or cytosine was associated with a high MIC, whereas a low level of resistance was related to the mutation of adenine to thymine or to the absence of point mutations at positions 2058 and 2059 (22). However, in our study, there was no difference in treatment outcome according to mutation type. Point mutations at positions 2058 and 2059 were absent from only one (4%) macrolide-resistant MAC isolate in the present study. Our findings are consistent with those of previous studies showing point mutations at these positions in 80 to 100% of macrolide-resistant MAC isolates (14, 45, 46). In isolates without point mutations at these sites, alterations of membrane permeability and efflux pump activation have been proposed as alternate mechanisms underlying MAC resistance (22, 47). Additionally, although other mutations that confer macrolide resistance in MAC have not been reported, it is possible that such mutations do occur, either at other positions in the 23S rRNA gene or at other genomic sites. Further molecular analyses are needed to identify other mechanisms that contribute to the development of macrolide resistance in MAC.

Although several of our findings are consistent with previous reports, there are some limitations to our study. First, the investigation was conducted at a single medical center, and the number of patients was small for the detection of clinically significant findings or responses to modifications in antibiotic therapy. Second, in our study, it was not feasible to evaluate the incidence of macrolide resistance in our patient population or the risk factors for the development of macrolide resistance in patients with MAC-LD who initiated recommended multidrug antibiotic therapy because 12 (35%) patients had initiated antibiotic therapy at other hospitals and macrolide resistance was detected at the time they were transferred to our hospital. In addition, one patient developed macrolide-resistant MAC-LD after antibiotic treatment for *M. massiliense* lung disease. Previous studies from Japan and the United States showed that macrolide resistance did not develop even in patients with persistent positive sputum cultures after more than 12 months of recommended multidrug antibiotic treatment (48, 49). Our previous study found that 17% of patients with an unfavorable microbiologic response after 12 months of multidrug antibiotic therapy developed macrolide resistance (19). Further studies with larger numbers of patients are needed to evaluate the incidence and risk factors for the development of macrolide resistance, as well as the efficacy of antibiotic therapy for the treatment of macrolide-resistant MAC-LD.

In conclusion, this study found that macrolide resistance developed in the majority of patients with MAC-LD despite the administration of recommended long-term combination therapies. Treatment outcomes are poor and mortality is high after the development of macrolide resistance. However, surgical resection can be an important treatment modality in selected patients. Overall, our analyses indicate that more effective therapy is urgently needed to treat macrolide-resistant MAC-LD.

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