Association between sequevar and antibiotic treatment outcome in patients with *Mycobacterium abscessus* complex infections in Japan

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**Abstract**

**Purpose.** Macrolide susceptibility differs between subspecies in the *Mycobacterium abscessus* complex, likely due to differences in *erm*(41) sequevars. Patients with *M. abscessus* complex infection generally show poor clinical outcomes in response to antibiotic treatment. Here, the association between genotype and treatment outcome was investigated.

**Methodology.** We collected 69 isolates from 35 patients with non-cystic fibrosis bronchiectasis; 24 had *M. abscessus* complex lung disease and non-cystic fibrosis bronchiectasis, and 11 were colonized. Outcome analysis was performed in the 24 infected patients. Molecular analyses, including *erm*(41) and *rrl* sequencing, and variable-number tandem-repeat (VNTR) analysis of 69 isolates, from 24 infected and 11 colonized patients, were performed to elucidate the influence of genotype on antibiotic susceptibility.

**Results.** Among the 24 patients, 18 (14 infected with *M. abscessus* subsp. *abscessus* and 4 with *M. abscessus* subsp. *massiliense*) showed unfavourable outcomes; six (three infected with *M. abscessus* subsp. *abscessus* and three with *M. abscessus* subsp. *massiliense*) exhibited favourable outcomes. Patients with unfavourable outcomes showed acquired clarithromycin resistance (33.3 vs 0 %), mixed sequevars (38.9 vs 16.7 %) and differing VNTR patterns between initial and serial isolates (33.3 vs 16.7 %). In contrast, in the 11 colonized patients, *M. abscessus* subsp. *abscessus* C28 (sequevar 02) and *M. abscessus* subsp. *massiliense* were the most prevalent subspecies.

**Conclusion.** Patients infected with multiple sequevars and genotypes were more likely to exhibit treatment failure and/or recurrence. The precise identification of subspecies and analyses of mycobacterial characteristics may help to predict treatment outcomes in patients with *M. abscessus* complex lung disease.

**INTRODUCTION**

*Mycobacterium abscessus* is an emerging pathogen that is responsible for a wide spectrum of diseases, including respiratory infections, particularly in patients with chronic lung disease or skin, soft tissue and bone infections. Further, this bacterium is known to cause rare disseminated disease in patients with severely immunosuppressed non-cystic fibrosis (CF) [1]. The *M. abscessus* complex was recently divided into three subspecies: *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *bolletii* [2, 3]. Macrolides are key drugs in the treatment of diseases caused by *M. abscessus* complex [1, 4, 5]. However, macrolide susceptibility varies among subspecies [6–8] and is primarily mediated by the inducible expression of an erythromycin ribosomal methylase gene, *erm*(41) [9]. Both *M. bolletii* and *M. abscessus* subsp. *abscessus* harbour intact *erm*(41) genes; however, a T/C polymorphism at position 28 in the chromosome of *M. abscessus* subsp. *abscessus* results in clarithromycin-inducible resistance (T28) or phenotypic susceptibility (C28) [10]. *M. abscessus* subsp. *massiliense* harbours a dysfunctional *erm*(41) gene as a result of...
the deletion of nucleotides 64–65 and 159–432, leading to a macrolide-susceptible phenotype [9]. According to recent reports, \textit{erm}(41) sequence variants in \textit{M. abscessus} subsp. \textit{abscessus} are associated with differences in clarithromycin susceptibility [11–13]. In addition, acquired macrolide resistance [minimum inhibitory concentration (MIC) greater than 8 mg l$^{-1}$] results from point mutations at positions 2057–2059 in the 23S rRNA gene (\textit{rrl}) [14, 15]. Based on these observations, testing for genetic mechanisms and susceptibility is required in cases of treatment failure [16, 17]. However, in a recent report, recurrence in patients with lung disease caused by \textit{M. abscessus} subsp. \textit{massiliense} was due to reinfection with a strain that was indistinguishable from that isolated prior to treatment. Thus, although treatment failure is attributed to resistance selection, it may also be related to particular DNA polymorphisms within the complex, or strain variation [18].

In the present study, the molecular mechanisms underlying clarithromycin resistance were characterized in a collection of \textit{M. abscessus} complex isolates, and polyclonal infections were detected. In addition, we analysed \textit{erm}(41) sequence variants and variable-number tandem repeats (VNTR) in the \textit{M. abscessus} complex to assess their individual roles in phenotypic resistance.

**METHODS**

**Ethics**

All study procedures were approved by the Ethics Committee of Kinki-chuo Chest Medical Center (no. 502) and were performed in accordance with the Declaration of Helsinki. Since this retrospective observational study was performed using the opt-out method through our hospital website, informed consent was not obtained from the patients.

**Study population**

According to our medical records, 35 patients with non-CF bronchiectasis visited the National Hospital Organization Kinki-chuo Chest Medical Center (Sakai-shi, Osaka, Japan) between 1 January 2008 and 30 September 2016. Twenty-four patients were diagnosed with \textit{M. abscessus} complex lung disease based on the diagnostic guidelines published by the American Thoracic Society/Infectious Diseases Society of America [1]. The remaining 11 patients, who did not meet the diagnostic criteria, were referred to as colonized patients. Patients co-infected with other nontuberculous mycobacterial (NTM) species during the study were excluded from the study population. During routine laboratory testing, a total of 69 \textit{M. abscessus} complex isolates were collected from 35 patients. The single isolates from 11 colonized patients were included in the phenotypic and genotypic analyses for comparison, but were excluded from the outcome analysis.

**Treatment outcome**

Patients underwent long-term therapy, administered every 3–6 months, in cases of significant radiographic progression or worsening respiratory symptoms. Patients who could not expectorate sputum after three consecutive negative cultures from sputum specimens were classified as having sustained culture conversion. Final treatment outcomes were classified as either sustained culture conversion with improvement of clinical symptoms and radiographic deterioration (favourable) or treatment failure (unfavourable). Patients with chronic disease who were classified as exhibiting culture conversion without antibiotic treatment were regarded as followed-up [8, 19]. Treatment failure included a lack of culture conversion with progressive radiographic abnormalities and clinical deterioration (progressive) or recurrence after the initial culture conversion (recurrence). Microbiological recurrence was defined by two or more positive culture results after the completion of treatment. Twenty-one patients, excluding 3 followed-up and 11 colonized patients, received combination antibiotic therapy consisting of the following oral macrolides: clarithromycin (800 mg day$^{-1}$) or azithromycin (250 mg day$^{-1}$), an oral fluoroquinolone (sitafloxacin; 100 mg day$^{-1}$), and at least two parenteral antibiotics, such as amikacin (400 mg day$^{-1}$) and imipenem (1500 mg day$^{-1}$).

**Microbiological examination**

Acid-fast bacilli (AFB) smears and mycobacterial cultures were performed as recommended in standard guidelines [1]. Sixty-nine isolates from 35 patients were cultured using the Bactec MGIT 960 system (Becton Dickenson, Fukushima, Japan) or Ogawa KY solid medium (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan), and subspecies were identified based on the following oral macrolides: clarithromycin (800 mg day$^{-1}$) or azithromycin (250 mg day$^{-1}$), an oral fluoroquinolone (sitafloxacin; 100 mg day$^{-1}$), and at least two parenteral antibiotics, such as amikacin (400 mg day$^{-1}$) and imipenem (1500 mg day$^{-1}$).

**erm(41) and rrl sequencing**

Extracted DNA was suspended in 0.3 ml of Tris-EDTA buffer and boiled for 10 min. The \textit{erm}(41) gene was amplified using the forward primer \textit{erm}F (5’-GACCGGGGCC TTCTTCTGTAT-3’) and the reverse primer \textit{erm}R1 (5’-GACTTCCCCGACCGAGTTCC-3’) [16, 21]. Gene variants were identified based on sequencing results [11–13]. The determined \textit{erm}(41) sequences of \textit{M. massiliense} (accession nos FJ358487 to FJ358490), \textit{M. boletii} (accession no. FJ358491) and \textit{M. abscessus} (accession nos FJ358483 to FJ358486) were deposited in GenBank. Sequencing of the 23
rRNA gene (rrl) was performed as described by Rubio et al. [15]. The rrl gene was amplified using primers 19F (5'-G TAGCGAAATTCGTTGCGG-3') and 21R (5'-TTCCCCGT TAGATGCCTTCAG-3') [15]. The PCR cycle conditions for both analyses were as follows: 5 min at 95 °C, followed by 35 cycles of 95 °C for 60 s, 62 °C for 60 s and 72 °C for 90 s, with a final extension at 72 °C for 10 min.

**VNTR genotyping and susceptibility analysis**

Mycobacterial genotyping was performed by VNTR analyses of initial and serial isolates [22]. The tandem repeat (TR) 28 locus was excluded from our analysis as a stable size difference between M. abscessus subsp. abscessus and M. abscessus subsp. massiliense isolates was not detected [23]. Between initial and serial isolates, we categorized infections as mixed-strain infections (when more than one allele was identified at more than one locus) and clonal variants-population infections (when more than one allele was identified at a single locus) [24]. To determine susceptibility, DNA sequencing and VNTR analyses were repeated three times to confirm the results; the sequences and VNTRs were subsequently compared with those of M. abscessus JCM13569T, M. massiliense JCM15300T and M. bolletii JCM15297T as reference sequences, using phenotypic and genotypic antibiograms.

**RESULTS**

**Characteristics of patients**

A total of 69 isolates were obtained; these comprised 58 isolates from 24 patients with disease caused by M. abscessus complex and 11 isolates from colonized patients. Of the 58 isolates from the patients with disease caused by M. abscessus complex, subspecies analysis indicated that 40 were infected with M. abscessus subsp. abscessus and 18 with M. abscessus subsp. massiliense. The 24 patients were divided into 2 groups: 17 patients with lung disease caused by M. abscessus subsp. abscessus and 7 with lung disease caused by M. abscessus subsp. massiliense. Among the 17 patients with lung disease caused by M. abscessus subsp. abscessus, 10 were classified as showing progressive disease, 4 with disease recurrence, 1 exhibited a favourable outcome and 2 were undergoing follow-up. Of the seven patients infected with M. abscessus subsp. massiliense, two were classified as showing progressive disease, two as showing disease recurrence, two showed a favourable outcome and one was undergoing follow-up (Table 1).

**Treatment outcomes**

In the 24 patients, the sequences of erm(41) and rrl were compared between 18 patients with unfavourable responses (14 M. abscessus subsp. abscessus and 4 M. abscessus subsp. massiliense, progressive or recurrent disease) and 6 patients with favourable responses (Tables 1 and 2). As expected, isolates from patients with unfavourable outcomes tended to exhibit clarithromycin resistance at day 3 (6/18, 33.3 vs 0/6, 0 %), mixed sequevars (7/18, 38.9 vs 1/6, 16.7 %) and different VNTR profiles (6/18, 33.3 vs 1/6, 16.7 %). In addition, the erm(41) C28 sequevar (5/7, 71.4 %) was more prevalent in M. abscessus subsp. abscessus from the 11 colonized patients than from those showing unfavourable outcomes (2/14, 14.3 %) or favourable outcomes (0/3, 0 %).

M. abscessus subsp. abscessus in patients with unfavourable outcomes appeared more resistant to clarithromycin than pathogens isolated from patients with favourable outcomes, although the distribution of resistance against amikacin, imipenem, or sitafloxacin was similar among the groups, i.e. favourable outcome, unfavourable outcome and colonization groups (Table 2).

**Clarithromycin susceptibility testing**

The phenotypic susceptibility data for the 69 isolates are summarized in Table S1 (available in the online version of this article). Notably, 7 of the 47 M. abscessus subsp. abscessus isolates were clarithromycin-resistant on day 3. Of the 40 isolates that showed initial sensitivity, 9 were susceptible by day 14, whereas the remaining 31 displayed inducible resistance. In contrast, of the 22 M. abscessus subsp. massiliense isolates, 6 were resistant and 16 were susceptible at either day 3 or day 14.

**erm(41) polymorphisms**

The 69 isolates lacked variation in rpoB and hsp65, which is used for species identification. Based on an erm (41) T/C 28 polymorphism analysis of the 47 M. abscessus subsp. abscessus isolates, 38 T28-type strains were identified in patients with complex-related disease and two were identified in colonized patients, whereas four C28 strains were isolated from two patients with complex-related disease and five with colonization. We also identified erm(41) polymorphisms in 22 M. abscessus subsp.

**Table 1. Patient population and subspecies identified within the Mycobacterium abscessus complex: classification according to treatment outcomes and colonization**

<table>
<thead>
<tr>
<th>Subspecies (patients, isolates)</th>
<th>Progressive</th>
<th>Recurrent</th>
<th>Favourable</th>
<th>Follow-up</th>
<th>Colonization*</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. abscessus subsp. abscessus (n=24, 47)</td>
<td>10 (25)</td>
<td>4 (9)</td>
<td>1 (2)</td>
<td>2 (4)</td>
<td>7 (7)</td>
</tr>
<tr>
<td>M. abscessus subsp. massiliense (n=11, 22)</td>
<td>2 (7)</td>
<td>2 (4)</td>
<td>2 (4)</td>
<td>1 (3)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Total (n=35, 69)</td>
<td>12 (32)</td>
<td>6 (13)</td>
<td>3 (6)</td>
<td>3 (7)</td>
<td>11 (11)</td>
</tr>
</tbody>
</table>

*Colonized patients (seven M. abscessus and four M. massiliense) were excluded from comparisons of subspecies with respect to treatment outcomes.
massiliense isolates with a dinucleotide deletion at position 64–65 and a 273bp truncation, including 18 isolates in 7 patients and 4 isolates from 4 colonized patients, respectively. Discrepancies were observed in four isolates from patients and 4 isolates from 4 colonized patients, respectively. Differences were observed in the sequevars between the initial and post-treatment isolates in seven patients (Tables 3 and 4). Four isolates belonged to sequevar 02 (containing a T28C mutation) and exhibited susceptibility at day 14 (Table 3). However, we did not find any isolates harbouring the erm(41) T19 variant.

### Acquired clarithromycin resistance was associated with treatment outcomes

Among the 17 patients with lung disease caused by *M. abscessus* subsp. *abscessus*, all isolates from one patient showing favourable response and two patients undergoing follow-up were susceptible to clarithromycin on day 3. In contrast, among 10 patients showing progressive disease and 4 showing recurrence, 7 isolates from 4 patients (Mab02, Mab03, Mab05 and Mab13) acquired resistance. Initial isolates from two patients (Mab02 and Mab03) showed susceptibility; however, a new resistant isolate harbouring an A2057G point mutation in rrl was identified after treatment. Initial isolates from Mab05 and Mab13 already displayed resistance, and the former showed double-point mutations at positions 2057 and 2058 (Table 3).

Among the seven patients with *M. abscessus* subsp. *massiliense*, six isolates from two patients (Mm01 and Mm02) in the progression and recurrence groups displayed resistance.
Table 3. Treatment outcomes, phenotypic MICs and genotype information for *M. abscessus* subsp. *abscessus* T/C28 isolates

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Specimen no.</th>
<th>Months*</th>
<th>Clarithromycin susceptibility (MIC; mg l&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>*erm(41) genotype(28)</th>
<th><em>rrl</em> genotype†</th>
<th>VNTR‡</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Day 3</td>
<td>Day 14</td>
<td>T28 sequevar</td>
<td>genovar</td>
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<td>Progressive outcome (n=10)</td>
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<td>WT</td>
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<td>Recurrence outcome (n=4)</td>
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<td>Favourable outcome (n=1)</td>
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<td>Follow-up outcome (n=2)</td>
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<td>11070417</td>
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<td>1</td>
<td>32</td>
<td>T28 sequevar</td>
<td>01</td>
<td>WT</td>
</tr>
</tbody>
</table>

*Months since treatment initiation.*
†*Wild-type (WT) is defined as an isolate with nucleotide AAA at codon 2057 through 2059.*
‡The order of VNTR loci is VNTRs of TR45, TR109, TR116, TR150, TR172, TR86, TR200, TR139, TR167, TR179, TR163, TR101, TR149, TR131, TR2, and TR137.
§GTGACGGGGCTAT in SNPs of the *erm(41)* sequevar.

Yoshida et al., *Journal of Medical Microbiology* 2018;67:74–82
Susceptibility testing and *rrl* sequencing were concordant for mutations at positions 2057 or 2059. Furthermore, the initial and serial isolates from Mm02 showed an acquired mutation at these positions during treatment (Table 4).

**Table 4.** Treatment outcomes associated with phenotypic MICs and genotype in *M. abscessus* subsp. *massiliense* isolates

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Specimen no.</th>
<th>Months*</th>
<th>Clarithromycin susceptibility (MIC; mg l(^{-1}))</th>
<th>erm(41) genotype(28)†</th>
<th><em>rrl</em> genotype‡</th>
<th>VNTR cluster</th>
<th>VNTR§</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 3</td>
<td>Day 14</td>
<td><em>M. massiliense</em></td>
<td>WT</td>
<td>cluster B</td>
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<td>Progressive outcome (n=2)</td>
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</tr>
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<td>&gt;64</td>
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<td>A2059G</td>
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</tr>
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<td>15030218</td>
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<td>15080927</td>
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</tr>
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</table>

VNTR clusters refer to the clusters reported by Yoshida et al. [23].

*Months since treatment initiation.
†*M. abscessus* subsp. *massiliense* erm(41) genotype refers to a dysfunctional gene with deletions of nucleotides 64–65 and 159–432.
‡WT (wild-type) is defined as an isolate with AAA at nucleotides 2057 through 2059.
§The order of VNTR loci is VNTRs of TR45, TR109, TR116, TR150, TR155, TR172, TR86, TR200, TR139, TR167, TR179, TR163, TR101, TR149, TR131, TR2, and TR137.

**Differentiation between clinical isolates by VNTR**

The VNTR profiles of the initial and serial isolates were compared in all 24 patients with *M. abscessus* complex infections (17 *M. abscessus* subsp. *abscessus* and 7 *M. abscessus* subsp. *massiliense*). The VNTRs enabled differentiation between the initial and serial isolates from two patients: *M. abscessus* subsp. *abscessus* isolates from a patient with progressive disease (Mab08) and *M. abscessus* subsp. *massiliense* from a patient with recurrence (Mm04). Serial isolates from three *M. abscessus* subsp. *abscessus* patients (Mab01, Mab03 and Mab04) and one *M. abscessus* subsp. *massiliense* patient (Mm05) revealed different VNTR profiles at only one locus. Furthermore, the initial isolates from two patients (Mab04 and Mab06) exhibited double or triple bands at the locus, but transitioned to a single peak for post-treatment isolates. With the exception of these patients, all initial and post-treatment isolates from the remaining 16 patients were indistinguishable by VNTRs.

**DISCUSSION**

Clarithromycin-resistant *M. abscessus* complex isolates were identified, and the mechanisms underlying resistance were examined. The relationship between inducible macrolide resistance and treatment outcomes with macrolide-based regimens has been examined in several recent reports [8, 9, 23]. The culture conversion rate in patients with pulmonary *M. abscessus* subsp. *massiliense* disease was higher than that in patients with *M. abscessus* subsp. *abscessus* [6, 25]. However, acquired mutational resistance may occur post-therapy [14]. In a recent study, progression rates were similar, despite significantly different treatment outcomes, in
patients with lung disease caused by *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense* [26]. We identified *M. abscessus* isolates with *rrl* point mutations and *erm* (41) sequevar variations. The development of clarithromycin resistance from susceptible *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense* isolates was associated with unfavourable treatment outcomes [15, 27]. Interestingly, three patients (16.7%) acquired clarithromycin resistance with persistent or recurrent positive cultures during treatment, and this was consistent with previously reported rates (13%) [8].

Acquired macrolide resistance by NTM, including in the *M. abscessus* complex, is infrequent after multidrug long-term therapy [8, 28]. In the present study, one patient (Mm01), who failed to produce a negative sputum culture, was infected with macrolide-susceptible *M. abscessus* subsp. *massiliense* during long-term treatment; however, subsequent isolates showed high resistance (>64 mg l\(^{-1}\)) and a mutation in *rrl*. We found that the initial and serial isolates were indistinguishable using VNTRs. A previous report demonstrated that patients with *M. abscessus* subsp. *massiliense* treated with a regimen including macrolide-containing antibiotics develop clarithromycin resistance upon recurrence of infection [27]. The results of our study were consistent with these previous findings, suggesting that episodes of infection during or after treatment typically result from reinfection with same strain.

The mechanism by which clarithromycin resistance is acquired during macrolide-containing antibiotic treatment has not been fully elucidated. In our study, one patient (Mab13) was reinfected with a highly resistant strain carrying wild-type *rrl*. Wild-type strains are typically susceptible to conventional antibiotics. However, low-level resistance may be subject to constitutive efflux pump activation early in the treatment period, which is thought to be the first step in acquiring mutational resistance [27, 29]. Although no low-level clarithromycin-resistant isolates (8 mg l\(^{-1}\)) were observed in our study, strains with intermediate resistance (4 mg l\(^{-1}\)) were found to harbour wild-type *rrl*. Thus, the mechanisms underlying acquired macrolide resistance remain unclear. Furthermore, the observation of the coexistence of wild-type and mutant strains in initial isolates (Mab02 and Mab03) is important, as it indicated that acquired resistance during treatment is difficult to detect using susceptibility testing.

A recent report recommended a 7-day incubation procedure to assess antibiotic resistance [12]. In contrast, Brown-Elliot *et al.* [11] suggested that interpretation after extended incubation was difficult for rapidly growing mycobacteria, as poor growth may lead to false-positive susceptibility results. Furthermore, treated isolates tend to exhibit suboptimal growth in CA-MH broth [11]. As poor growth was observed in some serial isolates in the present study, the interpretation of MICs in our study was consistent with the Clinical and Laboratory Standards Institute (CLSI) guidelines [20] at day 14.

Our analyses revealed a discrepancy in isolates from patient Mm02, which were originally identified as *M. abscessus* subsp. *massiliense*, but were later revealed to be mixed sequevars by *erm* (41) gene sequencing. This is the first study to identify an association between mixed sequevars and unfavourable treatment outcomes. Several cases of *M. abscessus* subsp. *massiliense* with a functional *erm* (41) gene have been reported [15, 16]. Thus, our data supported the development of *erm* (41) gene transfer methods and the investigation of fluctuations in recombinant strains during treatment.

Interestingly, we also found two mixed genetic populations: the acquisition of a point mutation at position 2057 and the wild-type genotype from two patients with progressive *M. abscessus* subsp. *abscessus* disease, and double mutations at positions 2057 and 2058 (Mab05), or positions 2057 and 2059 (Mm02). Further, *erm* (41) sequevar analysis indicated that six isolates (24%) comprised sequevars 01 and 06, which were prevalent in patients with persistent disease. An isolate consisting of sequevar 06 and *M. abscessus* subsp. *massiliense* with truncated *erm* (41) was found in patient Mm02 with progressive disease (Fig. S1). Interestingly results were also observed by VNTR analysis, which showed double or triple bands in the initial isolates and an absence of PCR products in serial isolates. The VNTR patterns with multiple bands appeared to minimize the potential for technical errors by mis-scoring; the clonal stability of each of the 17 VNTR loci has been evaluated in previous reports [22, 23]. In patient Mab04, two different TR 2 alleles (four and six repeats) were simultaneously observed in the initial isolate, whereas only six repeats were observed in a sample collected 11 months post-treatment (Table 2). Patient Mab06, with progressive disease, displayed similar findings, with triple peaks in TR86, and double peaks in TR163 for 24 months. Thus, these data suggest that isolates from patients showing unfavourable response contain clonal variants that differ from the parent strain. Furthermore, we found that two patients (Mab08 and Mm04) were infected with polyclonal populations, based on genetic VNTR profiles. Thus, patients infected with NTM species, including the *M. abscessus* complex, who have undergone unsuccessful treatment are at risk of repeated or multiple infections [14]. Indeed, a recent study suggested that the average patient with pulmonary *M. avium* complex or *M. abscessus* subsp. *abscessus* disease exhibited polyclonal infections [8, 27, 29, 30]. In contrast, VNTR profiles and repetitive element sequence-based PCR (rep-PCR) clusters strongly suggested that patients with CF in the UK were persistently infected with a single strain [18]. According to another study, strains from patients in Spain were identical to the initial isolates [15]. However, CF is relatively rare in the Japanese population; therefore, further analyses with samples obtained from different geographical regions are needed to investigate the risk of polyclonal infection in these patients.

In this study, *erm* (41) gene sequencing was used to classify *M. abscessus* subsp. *abscessus* isolates into sequevars based...
on 13 single nucleotide polymorphisms, of which only dys-
functional sequevar 02 (T28C) with full-length erm(41) was
associated with susceptibility. Interestingly, M. abscessus
subsp. massiliense and sequevar 2 had higher frequencies
than the other sequevars in colonized subjects (Table 2). It
is still unclear whether colonization results from M. absces-
sus subsp. massiliense strains with decreased virulence or
non-functional erm(41). Nevertheless, subspecies identifica-
tion and erm(41) sequencing could help to predict treat-
ment outcomes for M. abscessus complex lung disease.
Previous reports have indicated that M. abscessus subsp.
massiliense is highly transmissible between patients with CF
[16, 31]. Accordingly, if M. abscessus subsp. abscessus seque-
var 02 and M. abscessus subsp. massiliense are more trans-
missible, erm(41) sequevar analyses may be an effective
method for controlling infection.

The present study had several limitations. Most impor-
tantly, it was a single-institution study with a limited patient
population. In addition, we were unable to compare a suffi-
cient number of favourable outcomes for M. abscessus
subsp. massiliense lung disease, as the duration of treatment
and follow-up were short, which may have resulted in
underestimation of favourable outcome rates. We were
unable to verify the roles of complex factors contributing to
success or failure in the present patients; these may include
pharmacological factors such as relationships between drug
concentration profiles and pharmacological effects, and
non-pharmacological factors such as treatment by chest
physiotherapy. Finally, the isolates were only analysed
genetically, and no morphological information was col-
clected; thus, we could not determine whether a particular
strain was associated with persistent infection.

In conclusion, our data indicated that several patients were
infected with polyclonal populations based on genetic
VNTR profiles and erm(41) sequevars. Genetic analysis sug-
gested that some cases of persistent or recurrent disease
resulted from acquired resistance or re-infection. The coex-
tistence of DNA polymorphisms involved in macrolide resis-
tance and erm(41) sequevars suggested that individual
patients were persistently infected with different strains har-
bouring key genetic mutations. Therefore, novel methods to
determine the specific strains responsible for repeated infec-
tions and develop effective measures to reduce patient expo-
sure to pathogens that are difficult to treat are urgently
needed.

Ethical statement
All study procedures were approved by the Ethics Committee of Kinki-
chuo Chest Medical Center (No. 502) and were performed in
accordance with the Declaration of Helsinki. Since this retrospective
observational study was performed using the opt-out method through our
hospital website, informed consent was not obtained from the
patients. All data was anonymized for the study.

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Conflicts of interest
The authors declare that there are no conflicts of interest.


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