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

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### Abstract

**Background:** *Mycobacterium abscessus* complex (MABC) is the most drug resistant of the mycobacterial pathogens. *M. abscessus* subsp. *abscessus* encodes a functional erythromycin ribosomal methylase gene, *erm(41)*, causing inducible macrolide resistance. However, some clinical isolates of *M. abscessus* subsp. *abscessus* harboring nonfunctional *erm(41)* were susceptible to macrolide, even after extended incubation of 14 days. Loss of function of the *erm(41)* genes was associated with a T-to-C substitution at position 28 of the gene (T28C), leading to an amino acid change from Trp to Arg at codon 10. Pulmonary disease caused by *M. abscessus* subsp. *abscessus* strains with a nonfunctional *erm(41)* (C28 sequevar) may be responsive to macrolide-containing antibiotic regimens. Therefore, all *M. abscessus* subsp. *abscessus* strains with a functional *erm(41)* (T28 sequevar) were thought to be resistant to macrolide with extended incubation. Here, we report the first case of pulmonary disease caused by a strain of *M. abscessus* subsp. *abscessus* which was susceptible to macrolide due to T19 sequevar of *erm(41)* gene.

**Case presentation:** A 62-year-old Korean female was referred to our hospital due to chronic cough, sputum, and hemoptysis lasting more than 5 months. The patient’s sputum was positive for acid-fast bacilli staining and nontuberculous mycobacteria (NTM) were isolated twice from sputum specimens. The isolate was identified as *M. abscessus* subsp. *abscessus*. The isolate had a point mutation of C → T at position 19 (C19 → T) in the *erm(41)* gene, instead of expected C28 sequevar of *erm(41)*, and had no *rrl* mutation. The isolate displayed a clarithromycin susceptible phenotype with an Arg → Stop codon change in *erm(41)*. The patient was successfully treated with a macrolide-containing regimen.

**Conclusion:** This is the first case of pulmonary disease caused by a strain of *M. abscessus* subsp. *abscessus* showing clarithromycin susceptible phenotype due to T19 sequevar of the *erm(41)* gene. The *erm(41)* gene is clinically important, and non-functional *erm* alleles may be an important issue for the management of MABC lung disease. The presence of a non-functional *erm(41)* allele in *M. abscessus* subsp. *abscessus* isolates may be associated with better outcomes.

**Keywords:** Nontuberculous mycobacteria, *Mycobacterium abscessus*, Lung diseases, Clarithromycin, Drug resistance

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Background
The prevalence of lung diseases caused by nontuberculous mycobacteria (NTM) is increasing worldwide [1, 2]. Mycobacterium abscessus complex (MABC) is a rapidly growing mycobacterium and is the second most common cause of NTM lung disease after M. avium complex in many countries [3–5]. In addition, MABC has emerged as an important pathogen in patients with cystic fibrosis and chronic lung diseases, such as bronchiectasis [6–10].

MABC is the most drug resistant of mycobacterial pathogens, resulting in limited therapeutic options and a high treatment failure rate [11–14]. MABC is comprised of three closely related subspecies: M. abscessus subsp. abscessus, M. abscessus subsp. massiliense and M. abscessus subsp. bolletii [15, 16]. M. abscessus subsp. abscessus encodes a functional erythromycin ribosomal methylase gene, \( \text{erm}(41) \), which modifies the binding site for macrolide antibiotics, causing inducible macrolide resistance [17–19]. However, some clinical isolates of M. abscessus subsp. abscessus were susceptible to macrolide antibiotics, even after extended incubation of 14 days [20]. Loss of function of \( \text{erm}(41) \) genes was associated with a T-to-C substitution at position 28 of the gene (T28C), leading to an amino acid change from Trp to Arg at codon 10 [17, 18]. Pulmonary disease caused by these strains with M. abscessus subsp. abscessus with C28 sequvar may be responsive to macrolide-containing antibiotic regimens [20]. Conversely, all M. abscessus subsp. abscessus strains with a T28 sequvar were thought to be resistant to clarithromycin with extended incubation [18].

Although there were multiple polymorphisms associated with amino acid changes, only this T28C substitution resulted in loss of \( \text{erm}(41) \) gene function and no other nucleotide substitution was known to be associated with macrolide susceptibility [20]. We report the first case of pulmonary disease caused by a strain of M. abscessus subsp. abscessus that was susceptible to clarithromycin due to a T19 sequvar of the \( \text{erm}(41) \) gene. The patient was treated successfully with macrolide-containing antibiotics. This study was approved by the institutional review board of the Samsung Medical Center.

Case presentation
A 62-year-old Korean female was referred to our hospital due to chronic cough, sputum, and hemoptysis lasting more than 5 months. She was a non-smoker and had no history of previous treatment for pulmonary tuberculosis. The patient was 149.7 cm tall and weighed 40.7 kg. The erythrocyte sedimentation rate was 77 mm/h, and C-reactive protein was 2.37 mg/dL. A human immunodeficiency virus antibody test was negative. A chest computed tomography (CT) scan revealed bronchiectasis and bronchiolitis in both lungs, suggesting the nodular bronchiectatic form of NTM lung disease (Fig. 1a).

The patient’s sputum was positive for acid-fast bacilli staining and NTM were isolated twice from sputum specimens in both solid (3 % Ogawa solid media; Shinyang, Seoul, South Korea) and liquid culture system (Bectec MGIT 960 system; BD Diagnostics, Sparks, MD, USA). To identify an etiological agent, bacteria grown in the MGIT 960 culture system were initially propagated in 7H9 broth (Difico Laboratories, Detroit, MI, USA) supplemented with 10 % (vol/vol) oleic acid-albumindextrose-catalase (OADC; BD Diagnostics) for 7 days at 37 °C. They were then sub-cultured in egg-based 3 % Ogawa solid media (Shinyang, Seoul, South Korea), and genomic DNA was extracted from cultured bacteria. M. abscessus was the initial species identified using a reverse line blot hybridization assay (REBA Myco-ID; M&D, Inc., Wonju, South Korea) based on the \( \text{rpoB} \) gene [21].

To confirm the accuracy of this identification, sequencing analyses of \( \text{rpoB} \), \( \text{hsp65} \), and 16S rRNA were performed using GenBank (http://blast.ncbi.nlm.nih.gov/) with the BLAST algorithm [22–24]. The 16S rRNA sequences were 100 % identical to M. abscessus subsp. abscessus (GenBank accession no. NR074427), M. abscessus subsp. massiliense (GenBank accession no. NR074421), M. chelonae (GenBank accession no. AY457082), and M. abscessus subsp. bolletii (GenBank accession no. NR043236). The \( \text{rpoB} \) sequences showed 99.7 % similarity to those of the M. abscessus subsp. abscessus type strain, with only a 2-base mismatch (GenBank accession no. CU458896). The \( \text{hsp65} \) sequences were 100 % identical to those of the M. abscessus subsp. abscessus type strain (GenBank accession no. CU458896). The isolate was identified as M. abscessus subsp. abscessus by sequencing based method.

Drug susceptibility testing was performed using a broth microdilution method and M. peregrinum ATCC 700686 was used for quality control according to the guidelines [25], revealing that the isolate was susceptible to clarithromycin (minimum inhibitory concentration [MIC], ≤0.5 μg/mL), even after extended incubation for 14 days (Table 1). The isolate was genotyped for \( \text{erm}(41) \) polymorphism and \( \text{rrl} \) mutation, which are known as the main mechanisms of macrolide resistance [26]. The isolate had a point mutation of C→T at position 19 (C19→T) in the \( \text{erm}(41) \) gene, instead of the expected C28 sequvar of \( \text{erm}(41) \). It also had no \( \text{rrl} \) mutation. To the best of our knowledge, the C19→T mutation of \( \text{erm}(41) \) in M. abscessus subsp. abscessus is the first description.

The patient received oral clarithromycin (1,000 mg/d) with an initial 4-week hospitalization for intravenous amikacin and cefoxitin [27, 28]. At day 20 of treatment, clarithromycin was switched to azithromycin (250 mg/d) due to gastrointestinal disturbance. After discharge, the
patient received oral azithromycin for a total duration of 15 months. Her sputum cultures converted to and remained negative after 2 months of antibiotic treatment. Chest CT at 12 months of treatment revealed improvement in consolidations and bronchiolitis (Fig. 1b).

**Discussion**

It is important to distinguish the three subspecies of MABC because of their differences in susceptibility to clarithromycin [29–32]. *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii* are commonly inducible resistant, susceptible, and resistant to clarithromycin, respectively [15].

This is the first case of pulmonary disease caused by a strain of *M. abscessus* subsp. *abscessus* showing a clarithromycin susceptible phenotype due to the T19 sequence of *erm* (41) gene. According to a previously reported paper by Nash et al., *M. abscessus* subsp. *abscessus* strains with T28→C had no inducible resistance to clarithromycin and had low MIC. In the present study, the *M. abscessus* subsp. *abscessus* clinical isolate had a C-to-T mutation at position 19 (C19→T) leading to an Arg→Stop codon change at codon 7 of the *erm* (41) gene. This strain was named SMC-Mabs-T19. The SMC-Mabs-T19 strain revealed low MIC, similar to the *M. abscessus* subsp. *abscessus* strain with a C28 sequevar. Therefore, maintenance of low clarithromycin MIC against the SMC-Mabs-T19 strain might have been a result of the production of non-functional Erm(41). The entire *erm* (41) sequence of the susceptible isolate SMC-Mabs-C19 was unique and differed from that of *M. abscessus* subsp. *abscessus* type strain (GenBank accession no. CU458896) by only two bases: a C-to-T mutation at position 19 (C19→T), and a T-to-C mutation at position 159 (T159→C) (Fig. 2a and b). Of these differences, T159→C was also present in the inducible resistant strains MC719 and UC22 (GenBank accession nos. EU177504 and CP012044, respectively). Therefore, the T19 sequevar was the most likely explanation for the lack of function of *erm* (41) alleles from the SMC-Mabs-T19 strain. Until now, *erm* (41) with a T19 sequevar has not been reported in GenBank. However, we found only two *M. abscessus* subsp. *abscessus* clinical isolates harboring *erm* (41) with C19→G or A point mutations, and more information regarding drug susceptibility was not available (GenBank accession nos. FJ358485 and KP702837, respectively; Fig. 2b).

**Conclusions**

The *erm* (41) gene is clinically important, and non-functional *erm* alleles may be an important issue for management of MABC lung disease. The presence of a non-functional *erm* (41) allele in *M. abscessus* subsp. *abscessus* isolates may be associated with better outcomes.

**Ethics and consent to participate**

This study protocol was approved by the institutional review board of the Samsung Medical Center (IRB approval 2008-09-016).
**Fig. 2** Analysis of DNA sequences in the *erm*(41) of *Mycobacterium abscessus* subsp. *abscessus* clinical isolate SMC-Mabs-T19. **a** Phylogenetic position of isolate SMC-Mabs-T19 and other strains belonging to *M. abscessus* complex based on entire *erm*(41) gene sequences. This tree was constructed using the neighbor-joining method. Percentage values at nodes represent the bootstrap levels supported by 1,000 re-sampled datasets. Scale bars indicate evolutionary distance in base substitutions per site. **b** Sequence alignment of *erm*(41) from SMC-Mabs-T19 and other strains belonging to the *M. abscessus* complex. Base numbering is from the first base of *erm*(41). Identical nucleotides are indicated by an asterisk below sequences. Two deletion sites of *M. abscessus* subsp. *massiliense* are indicated by dashes.
Consent to publish
Written informed consent was obtained from the patient for publication of this Case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Availability of data and materials
All the data supporting the findings is contained within the manuscript.

Abbreviations
CT: computed tomography; erm(41): erythromycin ribosomal methylase gene; MABC: Mycobacterium abscessus complex; NTM: nontuberculous mycobacteria.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
WJK designed the study. SYK and BHJ drafted the manuscript. WJK contributed to the diagnosis and treatment. SYK carried out microbiological analysis. SJS reviewed and edited the manuscript. WJK reviewed and supervised the manuscript. All the authors approved the final version of the manuscript.

Funding
This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future Planning (NRF-2015R1A2A1A01039595) and by a grant of the Korea Health technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HI15C2278). The funders had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

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Received: 20 October 2015 Accepted: 10 May 2016
Published online: 17 May 2016

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