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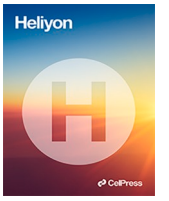

Research article

A rare family outbreak of *Mycobacterium abscessus* infection in immunocompetent fraternal triplets

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A R T I C L E I N F O

Keywords:
Non-tuberculous mycobacterium
Mycobacterium abscessus pulmonary disease
Fraternal triplets
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A B S T R A C T

Background: Mycobacterium abscessus (*M. abscessus*) infection is rare in children who were previously healthy, particularly in infants. We present the first report of a family outbreak of *M. abscessus* infection among immunocompetent infant triplets.
Methods: We reviewed triplets' demographic data, laboratory tests and imaging examinations to describe their clinical features. We performed whole-exome sequencing to rule out primary immunodeficiency disorders. We used DNA sequencing for *M. abscessus* subspecies identification.
Results: The fraternal triplets (triples A, B and C) presented with a 10-day history of cough. Triple A also experienced a brief episode of fever, and triple B had tachypnea. Chest CT scans showed pulmonary masses and nodules in triples A and C, and cavities in triple B. Cultures of sputum and bronchoalveolar lavage fluid from all triplets yielded *M. abscessus*. Further subspecies identification showed that isolates from triples A and C were *M. abscessus* subsp. *massiliense*, and isolates from triple B were *M. abscessus* subsp. *abscessus* (MAA). After eight months of combination therapy, the pulmonary lesions of the triplets improved significantly.
Conclusion: Our study confirms that *M. abscessus* pulmonary disease can occur in immunocompetent infants. We hypothesize that the simultaneous infection of the triplets may be associated with their prematurity and extensive environmental exposure. This study highlights the importance to include *M. abscessus* infection in the differential diagnosis of pulmonary masses and/or cavities, regardless of the age of onset or the presence of underlying pathology or susceptible genes.

Mycobacterium abscessus (*M. abscessus*) is a rapidly growing non-tuberculous mycobacterium (NTM) that is widely distributed in

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natural environments such as soil and water [1,2]. *M. abscessus* infection is challenging to diagnose because it resembles other mycobacterial infections clinically and radiologically. Moreover, the organism is resistant to many antibiotics, and has most strains have inducible macrolide resistance, which complicates treatment strategies [3]. *M. abscessus* pulmonary disease generally affects elderly patients [4,5]. However, it is extremely rare in children who were previously healthy, particularly in infants, unless they have immunodeficiency or structural lung abnormalities, such as cystic fibrosis or primary ciliary dyskinesia [6]. Only four sporadic cases [7–10] with *M. abscessus* infection have been reported in previously healthy or immunocompetent children, and no family outbreaks involving infants have been reported. We present a rare case of *M. abscessus* pulmonary disease in fraternal triplets who were 3-month--old infants without any underlying pathology. To the best of our knowledge, this is the first report of a family outbreak of *M. abscessus* infection among immunocompetent infant triplets.

1. Methods

1.1. Subjects

Infants who met the diagnostic criteria of *M. abscessus* pulmonary disease were enrolled in the study after being referred to the Children's Hospital Affiliated to Zhengzhou University. Demographic information was recorded. Inflammatory markers, immune function, and cultures of blood, sputum, and bronchoalveolar lavage fluid (BALF) were obtained. Additionally, chest computed tomography (CT) scans were performed to evaluate pulmonary diseases. *M. abscessus* pulmonary disease was diagnosed based on the 2020 Guidelines from ATS/ERS/ESCMID/IDSA [1], which included clinical, radiographic, and microbiologic criteria. This study was approved by the Ethics Committee of Children's Hospital Affiliated to Zhengzhou University (2023-K-060). Informed written consent was obtained from all parents/legal guardians.

1.2. Whole-exome sequencing (WES)

Genomic DNA was extracted using a QIAamp Blood Midi Kit (QIAGEN). The amplified DNA was captured using GenCap WES capture kit. The whole exons of genomic and 20 bp of the flanking intronic regions were sequenced on DNBSEQ (DNBSEQ-T7) with 150 bp paired-end reads. After sequencing, the clean reads were mapped to the UCSC hg19 human reference genome using BWA software (http://bio-bwa.sourceforge.net/). The variants of SNP and InDel were detected by the parameter driver of Sentieon software. Variants were further annotated by ANNOVAR software (http://annovar.openbioinformatics.org/en/latest/), and associated with multiple databases, such as 1000 genome, ESP6500, dbSNP, EXAC, HGMD, and predicted by SIFT, PolyPhen-2, MutationTaster, GERP++. The pathogenicity of variants was assessed according to the American College of Medical Genetics and Genomics. Potential pathogenic variants were verified for proband and his parents by Sanger sequencing on ABI3730xl sequencer (Applied Biosystems).

1.3. M. abscessus subspecies identification

We performed DNA sequence analysis of the rpoB, hsp65 and erm(41) genes to determine the subspecies of the pathogen. Bacterial DNA extracted by magnetic bead-based method was used as the template for PCR. The primers for PCR were as follows: rpoB primers, 5'-GGCAAGGTCACCCCGAAGGG-3' and 5'-AGCGGCTGCTGGGTGATCATC-3'; hsp65 primers, 5'-ACCAACGATGGTGTGTCCAT-3' and 5'-CTTGTCGAACCGCATACCCT-3'; erm(41) primers, 5'-ACGTTGGATCCGAGCGCCGTCACAAGATGCACA-3' and 5'-GCGAGAAGCTTGACTTCCCCGCACCGATTCCAC-3'. The PCR mixture was prepared as follows: 5 μl DNA template, 2 × PCR Mixture, 0.2 mM of each primer set, and the final volume was then adjusted to 25 μl with distilled water. The PCR conditions were as follows: initial denaturation at 95 °C for 10 min, 30 cycles of denaturation at 94 °C for 30s, 60 °C for 30s, and 72 °C for 1 min, followed by a final extension at 72 °C for 7 min. DNA sequences were aligned with the homologous sequences of *M. abscessus* standard strains by using BioEdit Sequence Alignment Editor 7.1.3 (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Specifically, the PCR products of the erm(41) for *M. abscessus* subsp. *abscessus* (MAA) were 673 bp. However, the erm(41) DNAs amplified for *M. abscessus* subsp. *massiliense* (MAM) isolates were 397 bp.

2. Results

2.1. Demographic data and clinical features

This study involves three 3-month-old infants who are fraternal triplets from the same family, conceived through in vitro fertilization. The triplets were delivered preterm at 34 weeks and 6 days of gestation. They did not experience asphyxia at birth and were fed with formula. They lived in a rural area with poor sanitation, where a cowshed was located in the yard. They presented with a 10-day history of cough. Only triplet A, the first-born, experienced a brief episode of fever, while triplets B and C, the second- and third-born, remained afebrile. Physical examination revealed normal development for all triplets, with triplet B exhibiting tachypnea with a respiratory rate of 42 breaths per minute. No abnormalities were detected for triplets A and C.

Laboratory tests revealed elevated levels of inflammatory markers, including white blood cell count (WBC), procalcitonin, and interleukin-6 for all triplets. Tuberculin skin test, interferon-γ release assay, and HIV antibody testing were negative. Immunoglobulin levels and lymphocyte subsets were within normal range.

Chest CT scans upon admission illustrated the presence of multiple masses and nodules in the middle and lower lobes of the

bilateral lungs, with mediastinal lymph node enlargement for triplets A and C. Additionally, triplet B had cavities in the lower lobe of the left lung (Fig. 1).

2.2. WES

We performed WES for all triplets but did not detect any pathogenic mutations. Based on the negative results from immunological function tests, dihydrorhodamine assays and WES, we excluded primary immunodeficiency disorders. We also ruled out congenital structural pulmonary abnormalities, such as cystic fibrosis or primary ciliary dyskinesia, based on the negative WES results.

2.3. Culture and drug sensitivity test (DST)

Sputum and BALF samples were obtained from the triplets on two non-consecutive days, and Ziehl-Neelsen staining showed positive results (+++). Cultures consistently demonstrated the growth of *M. abscessus*, and DST results were interpreted in accordance with the Clinical and Laboratory Standards Institute guidelines. The results showed that all triplets exhibited sensitivity to cefoxitin (MIC 16 μg/mL), amikacin (MIC 8 μg/mL) and clarithromycin (MIC 0.12 μg/mL); intermediate susceptibility to linezolid (MIC 16 μg/mL), moxifloxacin (MIC 2 μg/mL) and imipenem (MIC 16 μg/mL); and resistance to doxycycline (MIC 16 μg/mL), minocycline (MIC 8 μg/mL), and tobramycin (MIC 16 μg/mL). The isolated strains did not exhibit inducible resistance to clarithromycin, as they remained sensitive to the antibiotic on both day 3 and day 14.

2.4. Abscessus subspecies identification

M. abscessus isolates from the sputum cultures of the triplets underwent subspecies identification, revealing that isolates from triples A and C were MAM, and isolates from triplet B were MAA. Fortunately, triplet B's MAA had the C28 sequevar (the sequevar with a C at position 28 of erm(41) gene), indicating a potential sensitivity to clarithromycin, which was also consistent with the in vitro susceptibility results.

We also performed an investigation of their living environment for microbial identification. We collected samples from drinking water, domestic water, water dispensers, bathtubs, and air conditioning vents. The drinking water came from a centralized supply in rural areas, while the domestic water came from a well in the yard. We did not detect NTM in the water sources. However, we detected MAM and MAA on the inner walls of the outlet and inlet of the water dispenser, respectively, which the family used daily to prepare formula for the bottle-fed triplets.

2.5. Diagnosis, treatment, and follow-up

Based on the medical history, clinical presentations, pulmonary imaging, multiple sputum/BALF cultures, and microbial identification results, *M. abscessus* pulmonary disease was diagnosed in all three triplets. The initial therapeutic approach consisted of a combination of oral clarithromycin, intravenous imipenem, and cefoxitin. Triplets A and C exhibited resolution of clinical symptoms within a week, normalization of inflammatory markers after ten days, and negative sputum cultures after a month. Subsequently, they

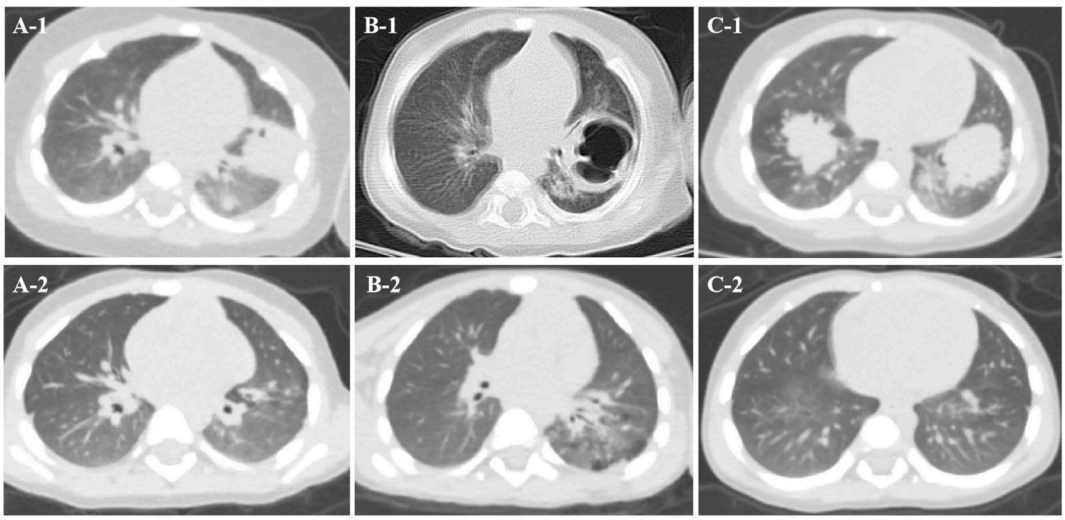

Fig. 1. Chest CT scans of fraternal triplets with pulmonary disease at baseline and after 8 months of treatment. (A-1, C-1) Multiple masses and nodules in both middle and lower lung lobes of triplets A and C at baseline. (B-1) Cavities in the left lower lung lobe of triplet B at baseline. (A-2, C-2) Near-complete resolution of pulmonary lesions in triplets A and C after treatment. (B-2) Disappearance of cavities in triplet B after treatment.

were discharged from the hospital and continued consolidation treatment involving oral clarithromycin, linezolid, and moxifloxacin. For triplet B, cough symptoms subsided after two weeks of the initial treatment, and sputum culture turned negative after a month. However, a follow-up complete blood count indicated an elevation in WBC levels to 20.36×10^9/L, while a subsequent chest CT scan showed no notable decrease in the mass lesion size. As a result, the original treatment regimen was enhanced with the inclusion of tigecycline. One month after the implementation of the modified treatment plan, the patient's WBC normalized, chest X-ray findings improved, and he was discharged from the hospital. Post-discharge, triplet B continued consolidation treatment with oral clarithromycin, linezolid (withdrawal after two weeks due to diarrhea), moxifloxacin, and doxycycline. After eight months of combination therapy, the triplets have experienced a significant improvement in pulmonary lesions as evidenced by the repeated chest CT scans (Fig. 1).

3. Discussion

NTM infection has emerged as a significant public health issue in recent decades, imposing both clinical and economic burdens worldwide. *M. abscessus* is the most prominent group of rapidly growing NTM associated with pulmonary infections and comprises three subspecies: MAA, MAM, and *M. abscessus* subsp. *bolletii* [11] However, *M. abscessus* is an infrequently detected pathogen in children, especially infants. Only four cases have been reported in previously healthy or immunocompetent children [7–10], and only one case was further identified as MAM. In this study, all the triplets met the clinical, radiological, and microbiological diagnostic criteria for NTM lung disease, and excluded immunodeficiency and other chronic lung diseases. Furthermore, they all underwent *M. abscessus* subspecies identification. *M. abscessus* outbreaks usually result from the contamination of medical equipment and inadequate infection control within healthcare settings. To our knowledge, this is the first report of an outbreak of *M. abscessus* infection involving infants within a home environment. We hypothesize that the simultaneous infection of *M. abscessus*in triplets may be associated with premature birth and extensive environmental exposure.

Subspecies identification of *M. abscessus* is of great importance for determining clinical relevance and devising treatment plans. Significant differences exist in cure rates among different subspecies [12]. A recent meta-analysis showed that only 45.6 % of patients with *M. abscessus* pulmonary disease achieved treatment success [12]. MAA is generally considered the most challenging NTM infection to treat, with an average cure rate of only 33.0 % [13]. The difference in treatment outcomes was partly explained by the susceptibility to macrolides [14]. MAA encodes a functional erythromycin ribosomal methylase gene, erm(41), which results in inducible macrolide resistance. Conversely, MAM typically possesses a truncated nonfunctional erm(41), conferring intrinsic susceptibility to macrolides. However, a small number of clinical MAA isolates may exhibit macrolide sensitivity similar to MAM due to a T-to-C substitution at position 28 of the gene (T28C) that inactivates the function of erm(41) [15]. Fortunately, in our report, all the triplets demonstrated good sensitivity to clarithromycin in both in vitro drug sensitivity tests of *M. abscessus* and actual clinical efficacy. This is attributed to the infection of MAM in triples A and C and C28 sequevar of MAA in triplet B.

M. abscessus can present with radiological features such as masses, nodules, bronchiectasis and cavities, with no significant differences between subspecies [16]. However, lower body mass index, bilateral lung involvement, and cavitary-type disease were identified as predictors of disease progression [14]. Cavitary lesion is an independent factor associated with treatment failure in *M. abscessus* pulmonary disease [17], and surgical resection may improve the outcomes. However, in our case triplet B, surgical lung resection was postponed due to age, weight and the extent of the lesions. Fortunately, he achieved complete resolution of cavitary lesions following active standardized combination antibiotic treatment. The optimal treatment regimen and duration for *M. abscessus* pulmonary disease have not yet been established. Multi-drug combination therapy and long-term use are generally recognized as effective [18]. In our study, after eight months of follow-up, the patients remain asymptomatic with improved pulmonary imaging and show satisfactory growth and development. This is consistent with the prognosis of *M. abscessus* pulmonary disease in immunocompetent children reported in the literature. Further follow-up studies are needed to determine the long-term sequelae and immune responses of the triplets with *M. abscessus* pulmonary disease.

The limitations of this study arise from its reliance on metagenomic next-generation sequencing for environmental sampling, which precluded the isolation of *M. abscessus* strains. Consequently, we were unable to explore the molecular epidemiological association between environmental and clinical isolates of *M. abscessus*.

In conclusion, we report an extremely rare case of a familial outbreak of *M. abscessus* infection among immunocompetent infant triplets. We hypothesize that the simultaneous infection of the triplets may be associated with their prematurity and extensive environmental exposure. Identification of subspecies and drug resistance genes can facilitate the treatment. This case highlights the importance to include *M. abscessus* infection in the differential diagnosis of pulmonary masses and/or cavities, regardless of the age of onset or the presence of underlying pathology or susceptible genes.

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Role of funder

The National Regional Medical Center Opening Project had no role in the design and conduct of the study.

Conflict of interest disclosures

The authors have indicated they have no potential conflicts of interest to disclose.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Children's Hospital Affiliated to Zhengzhou University (Approval no. 2023-k-060). In addition, informed consent was obtained from the patients for the publication of all images, clinical data and other data included in the main manuscript.

Data availability statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

CRediT authorship contribution statement

Bingyan Zhou: Writing – original draft, Data curation. **Yibing Cheng:** Supervision, Conceptualization. **Haijun Wang:** Supervision, Formal analysis. **Li Lin:** Writing – review & editing, Methodology, Investigation. **Huiwen Zheng:** Methodology, Formal analysis, Data curation. **Yuelin Shen:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] C.L. Daley, J.M. Iaccarino, C. Lange, E. Cambau, R.J. Wallace Jr., C. Andrejak, E.C. Böttger, J. Brozek, D.E. Griffith, L. Guglielmetti, G.A. Huitt, S.L. Knight, P. Leitman, T.K. Marras, K.N. Olivier, M. Santin, J.E. Stout, E. Tortoli, J. van Ingen, D. Wagner, K.L. Winthrop, Treatment of nontuberculous mycobacterial pulmonary disease: an official ATS/ERS/ESCMID/IDSA clinical practice guideline, Eur. Respir. J. 56 (1) (2020 Jul 7) 2000535, https://doi.org/10.1183/13993003.00535-2020.

[2] F. Mougari, L. Guglielmetti, L. Raskine, I. Sermet-Gaudelus, N. Veziris, E. Cambau, Infections caused by Mycobacterium abscessus: epidemiology, diagnostic tools and treatment, Expert Rev. Anti Infect. Ther. 14 (12) (2016) 1139–1154, https://doi.org/10.1080/14787210.2016.1238304.

[3] S. Cowman, J. van Ingen, D.E. Griffith, M.R. Loebinger, Non-tuberculous mycobacterial pulmonary disease, Eur. Respir. J. 54 (1) (2019) 1900250, https://doi.org/10.1183/13993003.00250-2019.

[4] Y. Tan, B. Su, W. Shu, X. Cai, S. Kuang, H. Kuang, J. Liu, Y. Pang, Epidemiology of pulmonary disease due to nontuberculous mycobacteria in Southern China, 2013-2016, BMC Pulm. Med. 18 (1) (2018 Nov 9) 168, https://doi.org/10.1186/s12890-018-0728-z.

[5] K. Furuuchi, K. Morimoto, T. Yoshiyama, Y. Tanaka, K. Fujiwara, M. Okumura, K. Izumi, Y. Shiraishi, S. Mitarai, H. Ogata, A. Kurashima, K. Yoshimori, K. Ohta, H. Goto, Y. Sasaki, Interrelational changes in the epidemiology and clinical features of nontuberculous mycobacterial pulmonary disease and tuberculosis in a referral hospital in Japan, Respir. Med. 152 (2019 Jun) 74–80, https://doi.org/10.1016/j.rmed.2019.05.001.

[6] G.S. Lamb, J.R. Starke, Mycobacterium abscessus infections in children: a review of current Literature, J Pediatric Infect Dis Soc 7 (3) (2018 Aug 17) e131–e144, https://doi.org/10.1093/jpids/piy047.

[7] H. Liu, F. Dong, J. Liu, J. Liu, Y. Pang, S. Zhao, J. Lu, H. Li, Successful management of Mycobacterium abscessus complex lung disease in an otherwise healthy infant, Infect. Drug Resist. 12 (2019 May 15) 1277–1283, https://doi.org/10.2147/IDR.S198461.

[8] M.M. Alramadhan, J.R. Murphy, M.L. Chang, Extensive Mycobacterium abscessus Pneumonia in an immunocompetent infant with No underlying lung pathology, Case Rep Infect Dis. 2021 (2021) 6615722, https://doi.org/10.1155/2021/6615722.

[9] A. Sands, E. Klepper, M. Bolton, Mycobacterium abscessus pneumonia in an immunonormal infant, Pediatr. Infect. Dis. J. 41 (12) (2022) e537–e539, https://doi.org/10.1097/INF.0000000000003681.

[10] A.F. Freeman, K.N. Olivier, T.T. Rubio, G. Bartlett, J.W. Ochi, R.J. Claypool, L. Ding, D.B. Kuhns, S.M. Holland, Intrathoracic nontuberculous mycobacterial infections in otherwise healthy children, Pediatr. Pulmonol. 44 (11) (2009 Nov) 1051–1056, https://doi.org/10.1002/ppul.21069.

[11] M.D. Johansen, J.L. Herrmann, L. Kremer, Non-tuberculous mycobacteria and the rise of Mycobacterium abscessus, Nat. Rev. Microbiol. 18 (7) (2020) 392–407, https://doi.org/10.1038/s41579-020-0331-1.

[12] N. Kwak, M.P. Dalcolmo, C.L. Daley, G. Eather, R. Gayoso, N. Hasegawa, B.W. Jhun, W.J. Koh, H. Namkoong, J. Park, R. Thomson, J. van Ingen, S.M. H. Zweijpfenning, J.J. Yim, Mycobacterium abscessus pulmonary disease: individual patient data meta-analysis, Eur. Respir. J. 54 (1) (2019 Jul 11) 1801991, https://doi.org/10.1183/13993003.01991-2018.

[13] D.E. Griffith, C.L. Daley, Treatment of Mycobacterium abscessus pulmonary disease, Chest 161 (1) (2022) 64–75, https://doi.org/10.1016/j.chest.2021.07.035.

[14] J. Park, J. Cho, C.H. Lee, S.K. Han, J.J. Yim, Progression and treatment outcomes of lung disease caused by Mycobacterium abscessus and Mycobacterium massiliense, Clin. Infect. Dis. 64 (3) (2017) 301–308, https://doi.org/10.1093/cid/ciw723.

[15] K. Kumar, C.L. Daley, D.E. Griffith, M.R. Loebinger, Management of Mycobacterium avium complex and Mycobacterium abscessus pulmonary disease: therapeutic advances and emerging treatments, Eur. Respir. Rev. 31 (163) (2022) 210212, https://doi.org/10.1183/16000617.0212-2021.

[16] H. Nagano, T. Kinjo, J. Fujita, T. Kishaba, Radiological findings in nontuberculous mycobacterial pulmonary diseases: a comparison between the Mycobacterium avium complex and the Mycobacterium abscessus complex, PLoS One 17 (7) (2022) e0271660, https://doi.org/10.1371/journal.pone.0271660.

[17] W.J. Koh, S.M. Moon, S.Y. Kim, M.A. Woo, S. Kim, B.W. Jhun, H.Y. Park, K. Jeon, H.J. Huh, C.S. Ki, N.Y. Lee, M.J. Chung, K.S. Lee, S.J. Shin, C.L. Daley, H. Kim, O.J. Kwon, Outcomes of Mycobacterium avium complex lung disease based on clinical phenotype, Eur. Respir. J. 50 (3) (2017 Sep 27) 1602503, https://doi.org/10.1183/13993003.02503-2016.

[18] K. Jeon, O.J. Kwon, N.Y. Lee, B.J. Kim, Y.H. Kook, S.H. Lee, Y.K. Park, C.K. Kim, W.J. Koh, Antibiotic treatment of Mycobacterium abscessus lung disease: a retrospective analysis of 65 patients, Am. J. Respir. Crit. Care Med. 180 (9) (2009 Nov 1) 896–902, https://doi.org/10.1164/rccm.200905-0704OC.