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Rapid Diagnosis of *Mycobacterium abscessus* Bacteremia Using Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

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Mycobacterium abscessus was isolated from cultures of seven blood samples from a 64-year-old diabetic female who was admitted due to steroid-unresponsive adrenal insufficiency. The isolates were difficult to identify using the conventional commercial systems, VITEK 2 (bioMérieux, France) or MicroScan (Siemens Healthcare Diagnostics, USA), but were rapidly identified as *M. abscessus* by a matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS)-based Bruker Biotyper system (Bruker Daltonics, USA). Identification of *M. abscessus* was confirmed by a reverse hybridization-based assay (Genotype Mycobacterium CM/AS 12, Hain Lifescience) and direct sequencing of a heat-shock protein gene. After removal of her central venous catheter, the patient was successfully treated with a combination therapy comprising clarithromycin, amikacin, cefoxitin, and imipenem. Our findings demonstrate that MALDI-TOF MS can facilitate rapid and accurate identification of *M. abscessus* from blood cultures, which enables prompt administration of appropriate therapy following catheter removal. **(Ann Clin Microbiol 2016;19:77-81)**

Key Words: Bacteremia, MALDI-TOF, *Mycobacterium abscessus*

INTRODUCTION

Mycobacterium abscessus comprises a group of rapidly growing (within 7 days on solid media), multidrug-resistant, nontuberculous mycobacteria (NTM) species that are ubiquitous in soil and water [1]. *M. abscessus* causes pulmonary and skin and soft-tissue infections [2,3]. Although *M. abscessus* bacteremia is rare in Korea, the number of *M. abscessus* bacteremia cases reported worldwide has increased [3-5]. Correct identification of *M. abscessus* has clinical relevance, especially because there are closely related species, *M. massiliense* and *M. bolletii*, which showed different susceptibility to clarithromycin, and their pathogenic potentials [6]. However, diagnosis of *M. abscessus* bacteremia can be challenging, because identification of this rapidly growing NTM using conventional identification systems is problematic [5]. Recently, the introduction of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) provides a rapid identification for clinical isolates of NTM. The identification ability of MALDI-TOF MS for NTM has been assessed and improved by several studies in various setting of the composition of database, or sample pretreatment [7,8]. Here, we report a rare case of *M. abscessus* bacteremia in which blood isolates were identified by MALDI-TOF MS. The findings demonstrate that MALDI-TOF MS can facilitate diagnosis of *M. abscessus* bacteremia.

CASE REPORT

A 64-year-old female suffering from adrenal insufficiency was transferred to our hospital because of non-responsiveness to steroid replacement therapy. She had a history of hypertension, diabetes mellitus, and hypoglycemic encephalopathy. On admis-

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sion, she was febrile and, 3 months prior, had undergone insertion of a peripheral central venous catheter (CVC), which remained *in situ*. A chest X-ray showed no evidence of active lung lesions. Seven cultures blood of samples obtained on admission days 1 (one of two blood sets), 2 (two of two blood sets), and 6 (four of four blood sets) yielded a gram-positive bacillus (Fig. 1). Antibiotic therapy with cefazolin was started on the day of admission; on day 6, this therapy was changed to ertapenem and teicoplanin, and, on day 10, it was changed to a combination of clarithromycin, amikacin, cefoxitin, and imipenem. The CVC was removed on day 6, and a culture of the catheter tip yielded no growth. The patient suffered from intermittent fever (temperature, 37.4-39.3°C) from the day of admission, but this gradually subsided and blood cultures became negative on day 10. The patient's condition improved and she was discharged from the hospital on day 20.

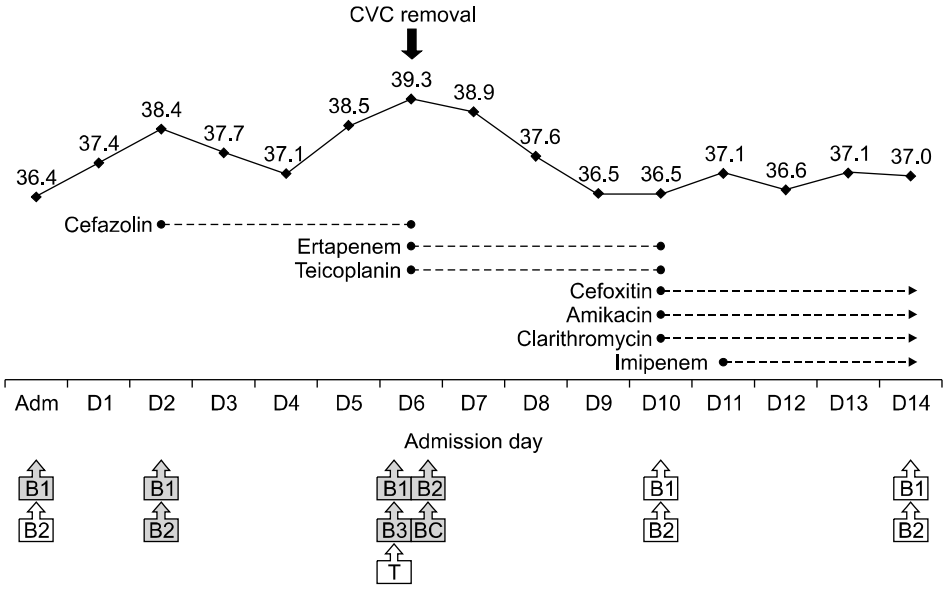

Fig. 1. Hospital course of patient with *Mycobacterium abscessus* bacteremia. Solid lines with diamonds indicate body temperature, and dotted lines with circles indicate the period of antimicrobial agent usage. Empty boxes denote negative cultures, and gray-shaded boxes denote positive cultures. Abbreviations: CVC, peripheral central venous catheter; B, peripheral blood culture; BC, catheter blood culture; T, catheter tip culture. Numbers in boxed indicate number of cultures performed on that day.

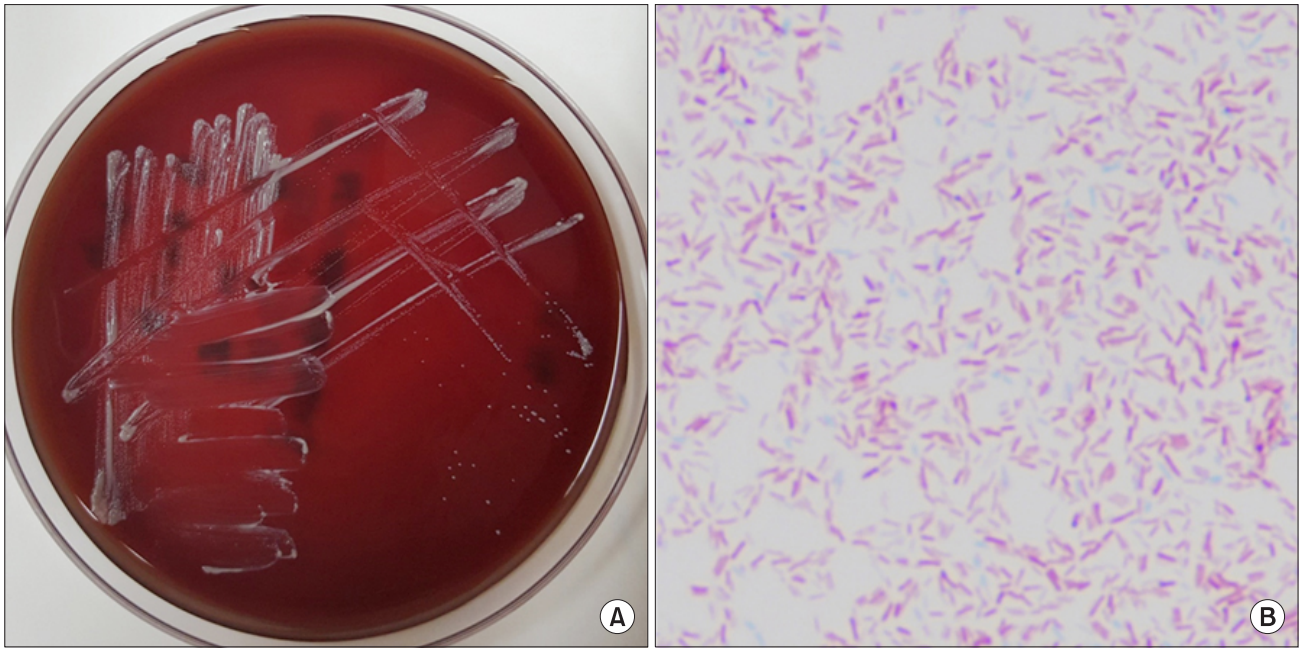

Fig. 2. (A) Macroscopic and microscopic appearance of the isolate. Growth on sheep blood agar as non-hemolytic, white pinpoint colonies after 72 h of incubation at 35°C in an aerobic environment. (B) The isolates yielded positive results of acid-fast staining (×1,000).

The isolates grew on sheep blood agar as non-hemolytic, white pinpoint colonies after 72 h of incubation at 35°C in an aerobic environment (Fig. 2). The bacteria produced catalase. The isolates were identified as *Kocuria rosea* (94% probability) using VITEK 2 with a GP card (bioMérieux, Marcy-l'Étoile, France) and as *Micrococcus* and related species using MicroScan (Siemens Healthcare Diagnostics, West Sacramento, CA, USA) systems. Using MALDI Biotyper (software version 3.1, reference database version 4.0.0.1, Bruker Daltonics, Billerica, MA, USA) with direct colony extraction method by which the microbes are directly applied as a thin film on a spot of the target slide (a reusable 96 wells stainless steel target slide, Bruker Daltonics), allowed to dry at room temperature, followed by the addition of 1 μL of HCCA (α-cyano-4-hydroxycinnamic acid) matrix onto each sample spot dried at room temperature. The isolates were identified as follows: *Mycobacterium abscessus* (score: 1.731), *Tissierella praeacuta* (1.333), *Rhodococcus ruber* (1.325), and other species (scores lower than 1.3). When the isolates were retested with protein extraction method using ethanol/formic acid, they were identified as *Mycobacterium abscessus* (score: 2.055). The isolate was found to be acid-fast. The identification as *M. abscessus* was confirmed by reverse hybridization-based assay (Genotype Mycobacterium CM/AS 12, Hain Lifescience), and direct DNA sequencing of the *hsp65* region [9]. A BLAST search of the sequence revealed 100% sequence homology with a *Mycobacterium abscessus* strain (HM454214.1), *Mycobacterium abscessus* subsp. *abscessus* strain (KT185518.1), and *Mycobacterium abscessus* subsp. *bolletii* strain (KT185533.1), whereas the similarity with *Nocardia farcinica* strain HN11062 (KF432771.1) was 99.77% (440/441 bp) (Fig. 3). Antimicrobial susceptibility testing at the Korean Institute of Tuberculosis [10] indicated that the isolate exhibited inducible resistance to clarithromycin (MIC, 2 μg/mL and >64 μg/mL); resistance to ciprofloxacin (>16 μg/mL), doxycycline (>32 μg/mL), and moxifloxacin (8 μg/mL); and intermediate resistance to amikacin (>32 μg/mL), cefoxitin (>32 μg/mL), and imipenem (8 μg/mL).

DISCUSSION

To date, about 20 species of rapidly growing NTM that are capable of causing bloodstream infections have been identified [5]. However, reports of cases of bloodstream infections by rapidly growing NTM have been limited [4,5] which may reflect, in part, the difficulty in identifying this species from blood cultures. *M. abscessus* isolates recovered from blood cultures could be regarded as skin contaminants or misidentified as *Corynebacterium* spp., because they are Gram-positive, non-motile, and catalase-positive bacilli. In the present study, the isolates were misidentified by VITEK 2 with a GP card and

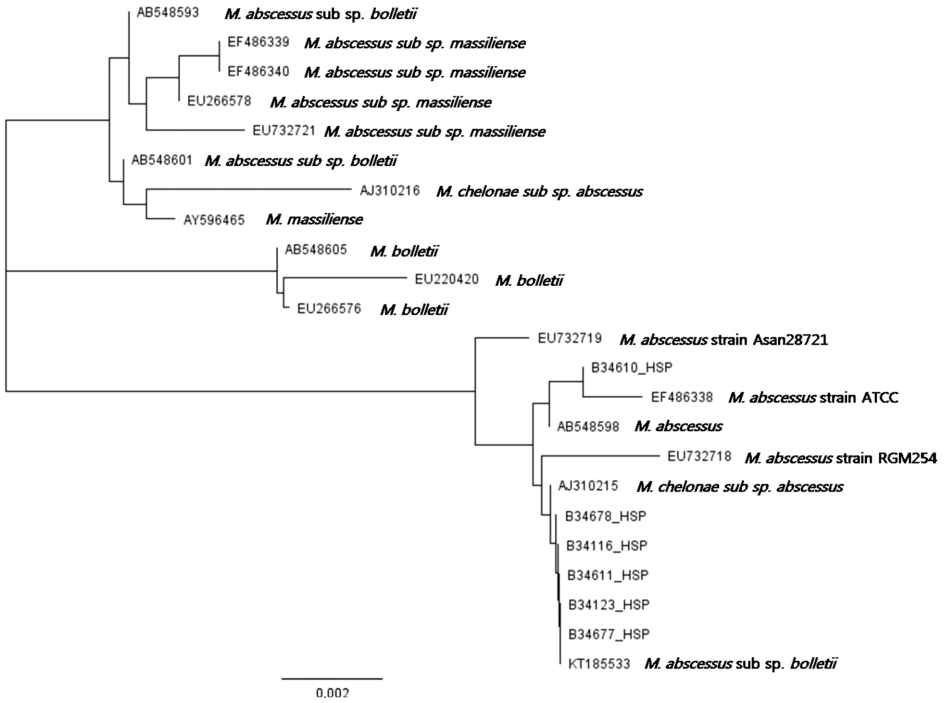

Fig. 3. Phylogenic trees of *hsp65* sequences of the six isolates (B34610_HSP, B34678_HSP, B34116_HSP, B34611_HSP, B34123_HSP, B34677_HSP) obtained from the patient in this study.

Microscan, but they were correctly identified as *M. abscessus* by MALDI-TOF MS. The identification of *M. abscessus* was supported by a positive AFB stain result. Our case highlighted that MALDI-TOF MS can be considered as a rapid diagnostic tool of bacteremia caused by rapid growing NTM including *M. abscessus*.

Recent studies have shown that *M. abscessus* and other mycobacterial species can be identified by MALDI-TOF MS [7,8,11,12]. In study which compares MALDI-TOF MS and reverse hybridization-based assay for the identification of NTM, the concordance analysis between the two methods showed agreement in 96.9% [7]. However, these studies utilized colonies grown on Lowenstein medium or Middlebrook 7H11 agar, which are used for mycobacterial culture, because most isolates were from respiratory specimens. The present study showed that *M. abscessus* blood isolates grown on blood agar plates could be easily identified by MALDI-TOF MS. Compared with >24 h for sequencing, the short turnaround time of the MALDI-TOF MS method (15 min) enables rapid diagnosis of *M. abscessus* bacteremia.

Our patient exhibited multiple risk factors for *M. abscessus* infection, including immunosuppression, diabetes mellitus, and the long-term presence of a CVC [3,13]. The isolates were resistant to various antibiotics, including inducible resistance to clarithromycin, but CVC removal and combination therapy yielded a successful clinical outcome. Although the CVC tip culture yielded no growth, we postulate a catheter-related bloodstream infection based on the following: first, this isolate was present continuously in seven blood cultures from the day of admission until that of CVC removal. Second, the fever subsided after CVC removal, which was prior to initiation of treatment of *M. abscessus*. Third, no typical radiological findings of *M. abscessus* pulmonary infection were evident, and sputum cultures and AFB stains were negative. In conclusion, this case shows that *M. abscessus* bacteremia case can be rapidly diagnosed by MALDI-TOF MS, albeit the automated bacterial identification systems fail to identify them.

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=국문초록=

말디토프 질량분석법으로 신속 진단한 *Mycobacterium abscessus*에 의한 혈류 감염 1예

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*Mycobacterium abscessus*는 신속 발육하는 비결핵성 마이코박테리움에 속하며, 주로 폐감염과 창상 감염 등을 유발한다. 저자들은 국내에서 말디토프 질량분석법에 의해 최초로 진단된 *M. abscessus* 균혈증을 보고하고자 한다. 본 증례에서는 스테로이드 치료에 반응하지 않는 부신기능저하증으로 입원한 64세 여자, 당뇨 환자에서 7회에 걸친 혈액 배양 결과에서 *M. abscessus*가 분리되었다. 분리된 균주는 전통적인 동정 시스템인 VITEK 2 (bioMérieux, France)나 MicroScan (Siemens Healthcare Diagnostics, USA)으로는 잘못 동정되었으나, 말디토프 질량분석법에 의해 *M. abscessus*로 정확하게 동정되었으며, 이는 heat shock protein 유전자 염기서열 분석으로 확인되었다. 환자는 중심정맥관 제거와 함께 clarithromycin, amikacin, cefoxitin, imipenem 병합요법으로 치료되었다. 본 증례는 혈액배양에서 분리되는 *M. abscessus*의 동정이 말디토프 질량분석법을 통해 신속하고 정확하게 이뤄질 수 있으며, 이러한 동정이 중심정맥관 제거와 함께 시기적절한 치료로 이어져 임상적으로 유용할 수 있음을 시사하였다. [Ann Clin Microbiol 2016;19:77-81]

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