Efficient Differentiation of *Mycobacterium abscessus* Complex Isolates to the Species Level by a Novel PCR-Based Variable-Number Tandem-Repeat Assay

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A novel duplex PCR method based on variable-number tandem-repeat targets to discriminate among *Mycobacterium abscessus* complex isolates was developed and evaluated in 85 clinical isolates. The assay accuracy was confirmed by a multiple-target sequence analysis. The duplex PCR assay is a one-step, reliable, and accurate assay for discriminating *M. abscessus* species.

*Mycobacterium abscessus* belongs to a group of rapidly growing mycobacteria (RGM) that cause a wide spectrum of infections in humans (6, 17). Recently, *M. abscessus* complex was divided into three closely related *Mycobacterium* species: *M. abscessus* (sensu stricto) (hereafter referred to as *M. abscessus*), *M. massiliense*, and *M. bolletii* (1, 4).

*M. abscessus* is the most drug-resistant mycobacterial species known and exhibits unsatisfactory treatment response rates, especially for patients with pulmonary disease (6, 17). Inducible resistance of *M. abscessus* to clarithromycin has been suggested as an explanation for the lack of efficacy of clarithromycin-based treatments against the bacterium (14). In contrast, *M. massiliense* shows marked susceptibility to clarithromycin due to the absence of inducible resistance to macrolides (8). Therefore, treatment response rates to clarithromycin-based antibiotic therapy are higher in patients with *M. massiliense* than in those with *M. abscessus* lung disease (10). *M. bolletii*, a rare pathogen at present, has also been shown to be highly resistant to clarithromycin (3, 9, 11). Since recent studies suggest that antibiotic susceptibility and treatment outcomes differ significantly among these species, the species-level identification of *M. abscessus* complex isolates has been strongly recommended in order to determine the clinical significance and to assist in managing patients (8, 10).

Despite progress in our ability to identify mycobacterial species using molecular methods, *M. abscessus* complex isolates cannot be reliably identified at the species level by sequencing a single genetic locus such as *rpoB* or *hsp65* (13). Currently, the species-level identification of *M. abscessus* complex solely depends on multiple sequencing analysis targeting several genes, including *rpoB*, *hsp65*, *gnd*, *glpK*, *secA*, and *sodA* (2, 13, 15). This process requires a number of additional, laborious steps (e.g., cloning) to obtain the sequences of the products, leading to expensive and complicated procedures that must be done in reference laboratories.

Increasingly, variable-number tandem-repeat (VNTR) analyses have been used for the molecular typing of isolated mycobacterial strains as well as for mycobacterial species identification (7, 16). In this study, a novel duplex PCR method based on variable-number tandem-repeat targets to discriminate among *Mycobacterium abscessus* complex isolates was developed and evaluated in 85 clinical isolates.

A total of 85 clinical isolates were collected between January 2001 and December 2006 in the Samsung Medical Center, Seoul, South Korea, according to the diagnostic criteria published by the American Thoracic Society (17). The 85 strains were initially identified as *M. abscessus* by PCR-restriction fragment length polymorphism (PRA) analysis of *rpoB* (12).

To obtain our VNTR targets, the entire genome of *M. abscessus* ATCC 19977 (GenBank accession no. CU458896.1) was examined using a tandem repeat finder program (http://tandem.bu.edu/trf/trf.html). In target loci from the 1,496 repeats initially selected by the program, the 16 loci were selected according to the following criteria; repeat number of at least twice, sequence match of at least 85%, and a longer period size than 20 bp. Screening of the 16 selected loci was done against three reference strains, *M. abscessus* ATCC 19977, *M. massiliense* CIP108297, and *M. bolletii* CIP108541. During screening, two loci were identified that could discriminate among the members of the *M. abscessus* complex. The two pairs of primers, VNTR11 and Mab2, amplified the following regions of the *M. abscessus* ATCC 19977 genome: 2951442 to 2952583 (designated VNTR11) and 4058506 to 4058576 (designated VNTR23) (Table 1). The DNA template was amplified in a 20-µl volume using the two pairs of primers (1 µM each primer). The amplification profile consisted of 10
min at 95°C, followed by 30 cycles, with 1 cycle consisting of 30 s at 95°C, 30 s at 63°C, and 45 s at 72°C. We extended the established method to 85 clinical strains independently identified by a multiple-target sequence analysis of \( rpoB \), \( hsp65 \), \( gnd \), and \( glpK \) as previously described (Table 1) (2, 5, 12). Complete identity agreement was found between the two assays, and both methods revealed that the 85 clinical strains were subdivided into 42 \( M.\) \textit{abscessus} strains, 39 \( M.\) \textit{massiliense} strains, and 4 \( M.\) \textit{bolletii} strains. In further characterization of the 85 strains, VNTR11 showed a length polymorphism of two to four copies (179 to 278 bp) in \( M.\) \textit{abscessus} with only one copy in \( M.\) \textit{massiliense} and \( M.\) \textit{bolletii}. \( M.\) \textit{massiliense} showed no tandem repeats (non). In comparison, \( M.\) \textit{bolletii} showed one pattern with two copies of VNTR23. Interestingly, none of the \( M.\) \textit{massiliense} strains were positive for VNTR23 (Fig. 1).

For the easy and precise differentiation of \( M.\) \textit{abscessus} species, duplex PCR with redesigned primers, Mab1 and Mab2, was developed to avoid amplification overlaps between the two loci (Fig. 2). Capillary electrophoresis (Agilent Technologies, Waldbronn, Germany) was applied to ensure accurate differentiation of amplicon size. The primer specificity of the two VNTR targets was evaluated by performing PCR under the same conditions using six clinically important mycobacteria (\( M.\) \textit{terrei} ATCC 15755, \( M.\) \textit{chelonae} ATCC 35752, \( M.\) \textit{avium} complex ATCC 25291, \( M.\) \textit{peregrinum} ATCC 14467, \( M.\) \textit{fortuitum} complex ATCC 25291, \( M.\) \textit{pseudotuberculosis} ATCC 19977, \( M.\) \textit{tuberculosis} subsp. \( M.\) \textit{-bovis} ATCC 27294), and \( M.\) \textit{glycosidolyticum} ATCC 19977, and \( M.\) \textit{bolletii} showed one pattern with two copies of VNTR23. Interestingly, none of the \( M.\) \textit{massiliense} strains were positive for VNTR23 (Fig. 1).

![FIG. 1. Amplified products obtained by PCR using the VNTR11 and VNTR23 primer sets. (A) VNTR11 consists of one to four copy numbers (179 to 278 bp) with a 33-bp tandem repeat. (B) VNTR23 consists of one to three copy numbers (196 to 238 bp) with a 21-bp tandem repeat. In 85 clinical isolates, \( M.\) \textit{abscessus} showed two to four copies of VNTR11 and one or two copies of VNTR23. In comparison, \( M.\) \textit{bolletii} showed one copy of VNTR11 and two copies of VNTR23; \( M.\) \textit{massiliense} showed one copy of VNTR11. \( M.\) \textit{massiliense} showed no tandem repeats (non). Lanes M, molecular size markers (in base pairs); lanes 1, 2, 3, and 4, \( M.\) \textit{abscessus} SM56, 25, 14, and 50, respectively; lanes 5 and 6, \( M.\) \textit{massiliense} SM04 and 38, respectively; lanes 7 and 8, \( M.\) \textit{bolletii} SM19 and 61, respectively.](http://jcm.asm.org/Downloaded from http://jcm.asm.org/ on March 22, 2020 by guest)
tion of M. abscessus. No amplification was seen in any of the mycobacterial species tested (data not shown).

The duplex PCR of M. abscessus complex provides a simple criterion for interpretation: if there is one band on the gel (one amplification product with primer Mab1 only), the result is positive for M. massiliense; when two amplification bands (Mab1 and Mab2 primers) are displayed, they are either M. abscessus (>393 bp) or M. bolletii (393 bp); and if there is a band of >393 bp in length, the result is interpreted as M. abscessus.

This is the first study to demonstrate the precise identification of M. abscessus complex to the species level using VNTR targets. The assay not only accurately distinguished M. abscessus complex isolates from other mycobacterial species, including M. chelonae and M. fortuitum, but also discriminated among M. abscessus complex isolates to the species level.

An effective discrimination among closely related yet pathogenetically diverse members of M. abscessus complex will support a better diagnosis and treatment regimen as well as further our understanding of the epidemiology of these pathogens.

To extend these results, studies of the association between clinical outcomes, distinct pathogenesis, drug resistance, and VNTR profile using a greater number of VNTR targets are under way.

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