Short Communication

First Report on Isolation and Molecular Characterization of Clinical Mycobacterium setense Isolates in Asia

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SUMMARY: We herein present the third set of documented clinical Mycobacterium setense cases. Three clinically unrelated isolates were identified and characterized using various key conventional and molecular diagnostic tests. Phenotypic and molecular data analysis, particularly 16S rDNA, hsp65, and rpoB sequencing, provided evidence of M. setense involvement in clinical infections in Iranian patients. Our findings may shed light on the capability of this rare Mycobacterium sp. to cause infection in both healthy and immunocompromised patients.

We conducted a worldwide literature search and found that, apart from the original article in 2008 that described Mycobacterium setense as a novel species (1), only one additional report has described the isolation of M. setense from clinical specimens (2). The present study describes three unrelated clinical infection cases that were attributed to M. setense on the basis of a number of phenotypic and molecular tests, and it may provide supporting evidence of the clinical relevance of this newly characterized species.

Case 1: A 60-year-old man presented with mild fever (37.6°C), inflammation, joint tenderness, and erythema during the months following left knee joint replacement surgery. Routine laboratory testing on admission revealed elevated C-reactive protein (CRP) level (18 mg/l) and an erythrocyte sedimentation rate (ESR) of 55 mm/h. Sheep blood agar was inoculated with a joint biopsy specimen from the patient: a few colonies appeared after 2 days of incubation at 37°C. Microscopic evaluation of the colonies revealed weak acid-fast bacilli (AFB), which were reproduced in two additional biopsy specimens. We treated the patient with amikacin on the basis of the initial laboratory findings; the patient recovered in less than 2 months.

Case 2: An 18-year-old woman with vertical HIV infection was hospitalized for a persistent subcutaneous abscess in her left hand. There was a history of needle insertion. The condition apparently began as a small nodule that developed because of an accidental injury 8 months earlier, which was ignored at the time and left untreated. The abscess ruptured due to the patient’s actions, and pus was released. She was administered an empirical therapy regimen by her general practitioner, but she showed no obvious improvement. Her major laboratory findings indicated elevated ESR (255 mm/h), elevated CRP level, CD4 counts of 5,000/mm3, and an HIV-DNA virus count of 50 copies/ml. Preliminary examinations of abscess aspirates for common pathogenic bacteria or fungi were negative; however, microscopic examination revealed the presence of AFB, which was confirmed by positive culture on Löwenstein-Jensen (LJ) medium. The abscess was drained successfully, and amikacin was administered. She fully recovered in less than 2 months.

Case 3: A 58-year-old woman was hospitalized for chronic bronchitis. Her medical history was significant: she had undergone renal transplantation and subsequently received steroid and immunosuppressive therapies. Her hepatic and renal functions were within normal range, but her ESR and CRP levels were high (80 mm/h and 45 mg/l, respectively). The patient developed nonproductive cough and fever (39 ± 2°C) with minimal physical findings. The patient’s tuberculin skin test was negative. She was empirically treated with antibiotics, but she continued to have a low-grade fever, and did not show signs of improvement. AFB were detected on direct microscopic examination of the patient’s bronchoalveolar lavage fluid and sputum specimens, which was performed 6 times. The subsequently collected colonies from LJ media culture were suggestive of rapidly growing mycobacteria (RGM). On the basis of the preliminary laboratory findings, the patient was administered trimethoprim-sulfamethoxazole and amikacin, but the eventual state of the patient is unclear since the patient was lost to follow-up.

The isolates, namely, HNTM46, HNTM49, and HNTM91, were subjected to preliminary identification and common RGM antimycobacterial agent susceptibility testing according to the standard procedures described previously (3).

The isolates were also subjected to molecular identification, which included direct sequence analysis of 16S rDNA, hsp65, and rpoB genes, as described in previous studies (4–6). A concatenated phylogenetic tree was con-
Fig. 1. Alignment of selected stretches of 16S rDNA, hsp65, and rpoB genes of Iranian strains of *M. setense* with those of reference strains. Dots indicate that the base pair was identical to that of type strain of *M. tuberculosis.* * *, (16S rDNA positions) according to *E. coli* numbering system; **, (hsp65 positions) according to *M. colombiense* CIP 108962 (GenBank, EU239785); ***, (rpoB positions) according to *M. abscessus* ATCC23003 (GenBank, AF262741).

Fig. 2. Neighbor-joining tree with Kimura’s two-parameter (K2P) distance correction, inferred using MEGA 4, based on the concatenated sequences of 16S rDNA, *rpoB*, and *hsp65* genes of closely related rapidly growing mycobacteria showing the phylogenetic position of the Iranian isolates of *M. setense*. The tree was rooted using *M. tuberculosis* H37Rv as the outgroup. The support of each branch, as determined from 1000 bootstrap samples, is indicated by percentages at each node. The scale bar indicates 0.01 substitution per nucleotide position.

The GenBank accession number for the HNTM46 gene sequences as a representative isolate determined in this work are HQ229607–9.

The isolates recovered on LJ medium were obtained from a rapidly growing, nonpigmented mycobacterial strain that was capable of growing at 25°C or 37°C, but not at 42°C. The isolates were capable of growth on MacConkey agar without crystal violet or on LJ medium that contained 5% NaCl, and were negative for niacin production. The isolates were positive for semi-quantitative and heat-stable (68°C) catalase and arylsulfatase activities (3 days), nitrate reduction, urease activity, tellurite and Tween hydrolysis, and iron uptake. The isolates were susceptible to amikacin, clarithromycin, doxycycline, sulfamethoxazole, streptomycin, imipenem, and ciprofloxacin.

The nearly complete 16S rDNA gene sequence (1,476 bp) and partial *hsp65* and *rpoB* gene sequences from the isolates showed high similarity (100, 99.46, and 99.11%, respectively) with *M. setense* reference strain sequences, corresponding to 0, 6, and 6 nucleotide differences for each gene. The first and second hypervariable signatures of the isolate 16S rDNA sequences were identical to *M. setense* sequences (Fig. 1). The relationship between our isolates and *M. setense* was supported by the concatenated phylogenetic tree of 16S rDNA, *hsp65*, and *rpoB* genes (2,414 bp in total), and by the high bootstrap value obtained using the neighbor-joining method (Fig. 2).

There was renewed interest in so-called opportunistic mycobacteria and increase in the discovery of new mycobacterial species in the 1980s, with the advent of the AIDS epidemic and the unusual and unexpected detection of nontuberculous mycobacteria (NTM) in the panorama of emerging infectious diseases (9).

There have been improvements in the techniques employed for identifying mycobacteria. Computer-assisted identification methods have been developed to analyze phenotypic data (10), and nucleic acid probes with different specificities have been used to identify isolates (11). Similarly, chromatographic analyses of fatty acids, including mycolic acids, provided valuable diagnostic data (12).

Concurrent to these events was the development of technologies that allowed rapid characterization of mycobacterial isolates by either the application of highly specific DNA probes tailored according to the hypervariable signature gene regions of 16S rDNA or by the amplification of key genetic markers, such as 16S rDNA, *hsp65*, *rpoB*, or ITS genes, by PCR, automated product sequencing, and computerized comparison with known species sequences (4–6,13).

To date, the genus *Mycobacterium* comprises over 140 species (http://www.bacterio.cict.fr/), several of which (other than *Mycobacterium tuberculosis*) are increasingly being recognized as significant human pathogens (9,14).

Clinical presentation caused by NTM is often hard to
differentiate from *M. tuberculosis* complex (MTBC). Therefore, it is important to accurately identify NTM since patient management and epidemiological control of infection depend on the mycobacterial species (9,14,15).

Isolation and identification of NTM species in specimens require specific preparatory procedures, including decontamination steps and selective incubation methods (e.g., the use of a carbon dioxide-enriched atmosphere or temperature modifications for optimal growth) (16). In developing countries like Iran, most clinical laboratories do not use these techniques, thus, infections caused by these organisms are likely to be wrongly diagnosed. On the other hand, extensive identification schemes comprising a minimum of 10 phenotypic tests have been inconclusive for identifying the entire spectrum of mycobacterial species. These schemes require experienced interpretation and are limited by subjectivity and low specificity (16,17).

In low-income countries, identification of mycobacteria by employing conventional phenotypic tests alone may result in erroneous or incomplete identification because of the nonavailability of a sufficient number of discriminative tests to identify isolates. However, molecular testing in conjunction with key phenotypic tests has shown significant promise in response to the need for more rapid and accurate identification of clinically relevant NTM (18).

The importance of molecular data for accurate identification of NTM cannot be overemphasized; since in our study PCR-restriction fragment length polymorphism analysis used (5) preliminarily helped identify the unknown organism as a rare NTM species. This may be considered as valuable information that suggests that certain cases should not be included in the tuberculosis register and wrongly treated. When an NTM is involved, the application of the more complicated molecular test—16S rDNA sequencing as the first-line identification tool—and at least one additional genetic marker, i.e., *hsp65* and/or *rpoB* genes sequencing as a complementary test, are necessary for conclusive identification.

The current report describes three nonrelevant clinical cases attributed to *M. setense*, which may serve as supporting evidence that highlights the clinical relevance of this organism. Regarding phenotypic features, the highest 16S rDNA, *hsp65*, and *rpoB* sequence similarities with genes from a typical strain of *M. setense*, provide evidence that the Iranian isolates belong to the *M. setense* sp.

The etiologic attribution was based on microbiological evidence that AFB were microscopically observed in all clinical specimens and were subsequently recovered from pure cultures. Furthermore, no similar isolates were previously detected in our laboratory and during the same period. On the other hand, the isolates were obtained from more than one specimen in all affected patients.

Our findings showed that *M. setense*, despite being rare, is capable of causing infection in healthy and immunocompromised patients. Furthermore, in laborato-

## References


