Original Article

*Mycobacterium simiae*: a Possible Emerging Pathogen in Iran

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**SUMMARY:** *Mycobacterium simiae* has been reported worldwide, particularly from the Middle East. This organism has been recognized as a causative agent of pulmonary and disseminated infections. In this study, we used molecular methods to detect this organism from patients who were suspected of having tuberculosis. A total of 117 isolates of mycobacteria were evaluated from different regions of Iran. Isolates were identified using phenotypic methods and gene sequencing of 16S rRNA, rpoB, hsp65, and ITS. Of the 117 isolates, 12 *M. simiae* isolates (10.2%) were identified from different clinical samples, including bronchoalveolar lavage and sputum (n = 8), blood (n = 3), and lymph node biopsy (n = 1). Three isolates (3/12, 25%) were recovered from blood samples of HIV cases when the CD4+ cell count was less than 50/μl. There was no significant relationship between infection and age or gender. Infection with nontuberculous mycobacteria (NTM), including *M. simiae*, is the major problem among immunocompromised patients. The results of this study illustrated the importance of molecular methods for accurate and rapid detection of NTM infections in the treatment of nonresponding patients with suspected tuberculosis.

**INTRODUCTION**

*Mycobacterium simiae* was reportedly restricted to several regions of the world such as Cuba, Gaza, and southern United States (1–5). Recently, this organism has been widely reported from other areas, including the Middle East (6–9). Transmission of *M. simiae* to humans and possible environmental sources of this organism remain unclear; however, the routes of entry are probably respiratory and digestive tracts (10).

In the *M. simiae* complex, *M. simiae* is clinically most relevant. *M. simiae* is one of the most common nontuberculous mycobacteria (NTM) causing lung disease. It causes pulmonary disease in the patients with underlying disease and disseminated infection in both immunocompromised and immunocompetent patients (1,4).

There is limited data on the association between the in vitro susceptibility of the organism and the in vivo response to most drugs (1). *M. simiae* usually shows poor in vivo response to therapy, and most isolates are resistant to first line anti-tuberculosis (TB) drugs such as isoniazid and rifampicin (1,7). The therapy regimen for this pathogen completely differs from that for TB, and newer drugs such as 8-methoxy fluoroquinolone (moxifloxacin), clarithromycin, and trimethoprim/sulfamethoxazole are effective on this mycobacterium. Therefore, rapid detection of *M. simiae*, particularly among immunocompromised patients, is necessary to prescribe an appropriate therapy regimen.

Using conventional bacteriology, it is difficult to detect and identify *M. simiae*, particularly when the result of the niacin test is positive (1). Due to the progress in molecular genetic tools, it has now become possible to detect NTM species (11).

*M. simiae* has been previously reported from Iran (8,9). In the present study, we attempted to detect this organism from patients diagnosed with TB using molecular methods. These patients were empirically treated with anti-TB drugs routinely prescribed to acid-fast bacillus (AFB) sputum smear-positive pulmonary patients. To the best of our knowledge, this is the first study regarding *M. simiae* in Ahvaz, Kermanshah, and Tehran.

**MATERIALS AND METHODS**

A total of 117 clinical strains of mycobacteria were isolated from 190 cases with suspected *Mycobacterium*–related complications at the TB Reference Centre of Ahvaz (Khuzestan, Iran), Masoud Laboratory (Tehran, Iran), and Kermanshah (Kermanshah, Iran) between 2009 and 2012 using standard decontamination procedures, acid-fast staining, and culture on Löwenstein–
described previously (15). The primer sequences of the 16S rRNA, rpoB, hsp65, and ITS regions were as described above using 2 specific primers, Tb11 (5'-ACTGGTGCCAAGGCATCCA-3') and Tb12 (5'-CTTGTTCGAACCCGATCACCT-3'), as described previously (13). Genomic DNA of M. tuberculosis H37Rv4 and double-distilled water were used as positive and negative control, respectively, in all PCR analyses. The PCR-amplified 16S rRNA gene products for each isolate were purified using the GeneJET™ Gel Extraction Kit (Fermentas, Ukraine), according to the manufacturer’s instructions. The sequences of the products were determined using the ABI PRISM® 7700 Sequence Detection System (Applied Biosystems, Foster City, Calif., USA) following the standard protocol of the supplier.

(ii) hsp65: A 439-bp amplified PCR product of the hsp65 gene for each isolate was purified and sequenced, as described above using 2 specific primers, pA (5'-AGAGTTTGATCCTGGCTCAG-3') and pH (5'-AAGGAGGTGATCCAGCCGA-3'), as described previously (13). Genomic DNA of M. tuberculosis H37Rv4 and double-distilled water were used as positive and negative control, respectively, in all PCR analyses. The PCR-amplified 16S rRNA gene products for each isolate were purified using the GeneJET™ Gel Extraction Kit (Fermentas, Ukraine), according to the manufacturer’s instructions. The sequences of the products were determined using the ABI PRISM® 7700 Sequence Detection System (Applied Biosystems, Foster City, Calif., USA) following the standard protocol of the supplier.

(ii) rpoB: A 750-bp fragment of the rpoB gene was amplified and sequenced using 2 specific primers, MycoF (5'-GGCAAGGTCAACCCGAAGGG-3') and MycoR (5'-AAGCGGCTGCTGGTGATCATC-3'), as described previously (15).

(iii) ITS: The universal primers, L (5'-GCTGGGATCACTCTTTCT-3') from the conserved sequence at the 3' end of the 16S rRNA (from position 1525 to position 1543 on the Escherichia coli 16S rRNA) and R (5'-CTGTGTCACCCGCTTCA-3'), which was deduced from the conserved 5' sequence of 23S rRNA (position 23 to position 40 on the Escherichia coli 23S rRNA), were used for amplification of the full length of the ITS region (16).

Analysis of sequence data: All existing relevant sequences of mycobacteria were separately retrieved from the GenBank database (www.ncbi.nlm.nih.gov/genbank) and deposited in the jPhydit program as an in-house-prepared database of 16S rRNA, rpoB, hsp65, and ITS sequences (17). The obtained sequences of different genes for each clinical isolate were aligned and compared to determine the percentage of similarity using the jPhydit program (17). Phylogenetic trees were constructed from DNA sequences using the neighbor-joining (NJ) method and Kimura’s 2-parameter (K2P) distance correction model with 1000 boot-strap replications supported by the MEGA 4.1 software (18).

The GenBank accession numbers of the clinical isolate AFP-000175 as a representative strain of M. simiae strain determined in this study are as follows: JX266703 for 16S rRNA, JX294386 for hsp65, JX294404 for rpoB, and JX270663 for ITS.

RESULTS

Of the 190 TB cases, a total of 117 strains of mycobacteria were recovered from various clinical samples (Fig. 1). Based on phenotypic features, the species spectrum consisted of 94 (80.3%) M. tuberculosis, 12 (10.2%) M. simiae-like, 5 (4.3%) M. kansasi, and 6 (5.2%) M. fortuitum. The isolates belonging to M. tuberculosis, M. fortuitum, and M. kansasi were clearly identified at the species level by phenotypic analysis. Of the 12 M. simiae-like strains, optimum growth was observed at 37°C, with colonies appearing after 4 weeks. No growth was observed at 42°C. On Middlebrook 7H10 agar medium, 12 strains were scotochromogenic with yellow pigmentation. Twelve, 10, and 8 isolates were positive for niacin, tellurite reduction, and urease activity, respectively, and all were positive for semi-quantitative catalase > 45 mm and
negative for 14-day arylsulfatase. The results obtained in this study were sufficient to identify the strains as *M. simiae*-like strains, mainly because they produced niacin and were scotochromogenic with yellow pigmentation.

Twelve *M. simiae*-like strains were isolated on pure culture from BAL and sputum (*n* = 8), blood (*n* = 3), and lymph node biopsy (*n* = 1). All cases with pulmonary disease were symptomatic, and 3 independent BAL specimens from each case were AFB-positive and yielded a pure culture of the same strains of mycobacteria. From cases with disseminated disease, 3 different blood samples were cultured, which led to the isolation of few colonies of mycobacteria. AFB staining was negative for all isolates from all 3 cases with disseminated disease. Slow-growing scotochromogenic mycobacteria from specimens that were obtained from 2 independent fine-needle lymph node biopsies of a patient suffering from cervical lymphadenitis and grown on LJ medium exhibited positive AFB staining. The etiological role of the 12 isolates may be inferred from the fact that AFB exhibited positive AFB staining. The etiological role of the 12 isolates may be inferred from the fact that AFB were recovered in pure culture from different clinical samples of each patient according to the American Thoracic Society (ATS) guidelines (1).

The respective 16S rRNA (1426 bp), rpoB (661 bp), hsp65 (413 bp), and ITS (214 bp) genes from each isolate were identical. Comparison of the 16S rRNA, rpoB, hsp65, and ITS sequences from each isolate using our in-house database in the jPhydit program revealed that *M. simiae* strain ATCC 25275T was the best match for the true identity of the causative microorganism in this study. The 16S rRNA (Fig. 2), other gene (rpoB, hsp65, and ITS) sequences (data not shown) also confirmed the identification of the isolates as *M. simiae*. Based on these data, we concluded that the true identity of the causative microorganism was *M. simiae*.

Based on current data, the rates of *M. simiae* infections in Kermanshah, Khuzestan, and Tehran were 16.1% (5/31 cultures positive), 6.3% (4/63 cultures positive), and 13% (3/23 cultures positive), respectively.

Table 1 shows the baseline characteristics and symptoms of the cases. Three strains (3/12, 25%) were recovered from blood samples of HIV cases when the CD4+ count was less than 50/μl. For each case of disseminated *M. simiae*, the most common symptoms were fever, night sweats, anorexia, and weight loss. The mean age of the 6 female and 6 male patients was 57.1 and 48.3 years, respectively.

**DISCUSSION**

*M. simiae* is commonly isolated from respiratory specimens (4). Previously, this organism was presumed to cause disseminated infections only in immunocompromised individuals only. However, recent reports have confirmed that *M. simiae* can also disseminate in immunocompetent patients (10,19–21). The rate of NTM infections varies geographically worldwide. *M. simiae* has been reported from different parts of Asia, including Iran, Japan, and Turkey (8,9,22,23). This organism may have been an infectious agent in Iran. A poor laboratory diagnostic system is assumed to be the reason for failure in detecting this organism from patients infected with *M. simiae*.

The correlation between *M. simiae* infection and age and gender has remained controversial, although infected patients tended to be older than those infected with TB (7). The rate of *M. simiae* infections among females varies from low to high among studies (5,7). The mean age of the patients was 58.2 ± 16.9 years. Our findings regarding age are in agreement with other reports from Iran (8).

The true infection rate for *M. simiae* is reportedly 9–24% (7,18), which is higher than the rate determined in the present study. A previous study reported that the most frequently isolated NTM were *M. simiae* (7). NTM isolated in the present study included 12 *M. simiae*, 5 *M. kansasii*, and 6 *M. fortuitum* isolates, consistent with the ATS diagnostic criteria and were considered clinically relevant. However, due to the small number of cases, the true incidence of NTM infections is likely understi-
Table 1. Baseline characteristics, symptoms, and outcome of the patients infected by *M. simiae*

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Region</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Post medical history</th>
<th>Sample source</th>
<th>Direct microscopy</th>
<th>Symptom</th>
<th>Chest X-ray</th>
<th>Initial diagnosis/treatment</th>
<th>Treatment after correct identification</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>K7</td>
<td>K</td>
<td>80</td>
<td>Male</td>
<td>—</td>
<td>BAL</td>
<td>+</td>
<td>PC, WL, Fev, Dysp</td>
<td>Infiltrates</td>
<td>TB/RIF, INH</td>
<td>CLR, RIF (3 months)</td>
<td>Alive</td>
</tr>
<tr>
<td>K12</td>
<td>K</td>
<td>60</td>
<td>Female</td>
<td>—</td>
<td>BAL</td>
<td>+</td>
<td>PC, WL, Fev, Dysp</td>
<td>Infiltrates</td>
<td>TB/RIF, INH</td>
<td>No available</td>
<td>Alive</td>
</tr>
<tr>
<td>K13</td>
<td>K</td>
<td>15</td>
<td>Female</td>
<td>—</td>
<td>BAL</td>
<td>+</td>
<td>PC, Hp, WL, Fev, Dysp</td>
<td>Infiltrates</td>
<td>TB/RIF, INH</td>
<td>CLR, EMB, CIP (2 months)</td>
<td>Alive</td>
</tr>
<tr>
<td>K18</td>
<td>K</td>
<td>60</td>
<td>Male</td>
<td>—</td>
<td>BAL</td>
<td>+</td>
<td>PC, WL, Fev, Dysp</td>
<td>Pleural effusion</td>
<td>TB/RIF, INH</td>
<td>RIF, INH, CLR (6 months)</td>
<td>Alive</td>
</tr>
<tr>
<td>K27</td>
<td>K</td>
<td>62</td>
<td>Male</td>
<td>Malignancy</td>
<td>BAL</td>
<td>+</td>
<td>PC, Hp, WL, Fev, Dysp</td>
<td>Infiltrates</td>
<td>TB/RIF, INH</td>
<td>CLR, EMB, CIP (3 weeks)</td>
<td>Dead</td>
</tr>
<tr>
<td>Kh4</td>
<td>Kh</td>
<td>31</td>
<td>Male</td>
<td>AIDS</td>
<td>Blood</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>CLR, EMB, CIP (5 months)</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>Kh7</td>
<td>Kh</td>
<td>45</td>
<td>Male</td>
<td>AIDS, DM</td>
<td>Blood</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>CLR, EMB, CIP (1 month)</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>AFP-000175</td>
<td>T</td>
<td>68</td>
<td>Female</td>
<td>ILD</td>
<td>Sputum</td>
<td>+</td>
<td>PC, Hp, WL, Fev, Dysp</td>
<td>Cavity in left lower lob</td>
<td>TB/RIF, INH</td>
<td>CLR, MOX (2 months)</td>
<td>Alive</td>
</tr>
<tr>
<td>T11</td>
<td>T</td>
<td>40</td>
<td>Female</td>
<td>COPD</td>
<td>BAL</td>
<td>+</td>
<td>PC, Hp, WL, Fev, MA</td>
<td>Cavity in right middle lobe</td>
<td>TB/RIF, INH</td>
<td>CLR, RIF (3 months)</td>
<td>Alive</td>
</tr>
<tr>
<td>Kh19</td>
<td>Kh</td>
<td>12</td>
<td>Male</td>
<td>Previous TB, AIDS</td>
<td>Blood</td>
<td>—</td>
<td>PC, Hp, WL</td>
<td>—</td>
<td>CLR, RIF (3 months)</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>Kh29</td>
<td>Kh</td>
<td>89</td>
<td>Female</td>
<td>RA</td>
<td>Lymph node biopsy</td>
<td>+</td>
<td>Fev, WL</td>
<td>—</td>
<td>TB/RIF, INH</td>
<td>—</td>
<td>Dead</td>
</tr>
<tr>
<td>WK12</td>
<td>Kh</td>
<td>71</td>
<td>Female</td>
<td>Bronchiectasis</td>
<td>BAL</td>
<td>—</td>
<td>PC, Hp, WL</td>
<td>Pleural effusion</td>
<td>TB/RIF, INH</td>
<td>CLR, RIF (3 months)</td>
<td>Alive</td>
</tr>
</tbody>
</table>

1): K, Kermanshah; T, Tehran; Kh, Khuzestan.
2): DM, diabetes mellitus type II; ILD, interstitial lung disease; COPD, chronic obstructive pulmonary disease; TB, tuberculosis; RA, rheumatoid arthritis.
3): BAL, bronchoalveolar lavage fluid.
4): PC, productive cough; Hp, hemoptysis; WL, weight loss; MA, malaise/asthenia; Dysp, dyspnea; Fev, fever.
5): RIF, rifampicin; INH, isoniazid.
6): CLR, clarithromycin; EMB, ethambutol; CIP, ciprofloxacin; MOX, moxifloxacin.
mated. In future, a large-scale prospective cohort study is required to explore the incidence rate of NTM infections.

Similar to a previous study (20), productive cough, dyspnea, malaise, fever, sweat, and body weight loss were the most common signs and symptoms of M. simiae infection among the Iranian patients. However, the most frequent imaging finding was nodular lesions (100%) in Iranian patients, which makes our study distinct from those in a previous study (8). The reason for such difference remains unclear. We believe that socioeconomic and geographic differences may be considered; however, to arrive at a definite conclusion, future study is recommended.

Three patients in our study were HIV- and M. simiae-positive; however, the rate of M. simiae infection, particularly in non-HIV patients, remains vague (22). In an earlier study conducted in Iran, all patients, except 1, were HIV-negative (8). Thus, we think that further studies on a larger scale are warranted to clarify this association.

Unlike M. tuberculosis, M. simiae is not an obligate pathogen. Therefore, it normally does not cause disease in immunocompetent individuals (1). It mainly causes soft-tissue infections, lymphadenitis, and lung diseases and occasionally causes disseminated infection in patients with impaired immune defenses, such as AIDS (1). In the present study, one-fourth of M. simiae-infected patients had disseminated disease; all of them were HIV-positive and had low CD4+ counts. This finding is in agreement with those of previous studies (7, 24, 25). The clinical presentation, radiographic findings, and utility of sputum-based diagnostic modalities may differ between HIV-positive and -negative cases and may differ in HIV-infected individuals as immune suppression progresses (1, 26). These factors increase the complexity of management of patients with HIV/M. simiae coinfections.

All patients in the present study were diagnosed with TB and received treatment for several years (repeated courses of anti-TB drugs) without showing any response to anti-TB drugs. These data highlighted the importance of molecular methods for the accurate and rapid detection of NTM infections, particularly those caused by M. simiae, and the importance of choosing an optimal treatment for nonresponding TB patients.

This study, as well as previously published reports, emphasized the fact that NTM, such as M. simiae, are mainly problematic in an immunocompromised state for either HIV-positive or -negative patients.

Conflict of interest None to declare.

REFERENCES


