

Isolation of a Novel Strain of *Mycobacterium iranicum* from a Woman in the United States

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A novel strain of *Mycobacterium iranicum*, a recently described nontuberculous *Mycobacterium* species, was isolated from the sputum of a woman. The source of infection was not determined; however, fomite transmission of inhaled aerosolized secretions from her husband's sleep apnea equipment was historically possible.

CASE REPORT

In May 2010, a 58-year-old female, previously diagnosed with viral encephalitis, secondary variegated porphyria, allergies, multiple herniated discs, and recurrent respiratory infections, including pharyngitis and bronchitis, sought medical care for recent-onset fever, chills, congestion, a productive cough, and chest pain. Her symptoms developed within a few days after her husband returned from a trip to Israel, during which he had visited a sick splenectomized elderly relative who died at home of pneumonia, for which a microbiological cause was not investigated. Shortly after returning to the United States, the husband developed fever, chills, a productive cough, chest pain, and nasal congestion. During the use of continuous positive airway pressure (CPAP) equipment for sleep apnea, the husband's secretions from the CPAP machine were accidentally expelled onto the wife's face. Historically, there was no evidence of bronchiectasis in her pulmonary computerized tomography (CT) scan performed in January 2010. Her physical examination findings included a body temperature of 37.8°C (her normal body temperature was reportedly 36.4°C), periorbital edema, diffuse pharyngeal erythema, sinus congestion, and increased bronchial sounds. There was no radiographic evidence of pneumonia; however, because of her symptoms and overall deteriorating health status, she was empirically treated with azithromycin (500 mg on day 1 and 250 mg on days 2 and 3) for 3 days, followed by treatment with clarithromycin (500 mg orally [p.o.], twice a day [b.i.d.]) for 4 weeks, for presumed community-acquired pneumonia. Despite antibiotic therapy, a productive cough persisted. She then developed a persistent abscess (unknown etiology) on the right cheek, near the nasal area, that did not respond to hot packing and topical antibiotics. The abscess was treated with minocycline (100 mg daily) for 2 months, during which time the abscess resolved and her respiratory symptoms improved dramatically. Upon discontinuation of minocycline treatment, her respiratory symptoms worsened. Pulmonary function tests in November 2010 documented respiratory impairment (40% forced vital capacity [FVC] and also 40% forced expiratory volume [FEV1] in the supine position). Three sputum samples, collected between 3 May and 1 June 2011, contained mononuclear and polymorphonuclear cells with Gram-positive cocci in clusters and pairs, Gram-positive bacilli, and scanty epithelial cells. Each sample generated a heavy growth of normal flora.

Acid-fast bacillus stains were negative. Based on rapid growth and characteristic yellowish-orange smooth colonies, a nontuberculous mycobacterium (NTM) isolate was collected on a Lowenstein-Jensen (LJ) slant. Initial phenotypic tests (Illinois Department of Public Health, Chicago, IL) and subsequent testing with high-performance liquid chromatography (HPLC) and a line probe assay (National Jewish Health, Denver, CO) failed to definitively identify the NTM species. Drug MICs were determined by the broth microdilution method per Clinical Laboratory and Standards Institute (CLSI) guidelines (Advanced Diagnostic Laboratories, Denver, CO) (1). The NTM was susceptible to amikacin, kanamycin, tobramycin, cefoxitin, imipenem, ciprofloxacin, doxycycline, moxifloxacin, tigecycline, clarithromycin, azithromycin, augmentin, trimethoprim-sulfamethoxazole, and linezolid. Serology results for *Chlamydomyces pneumoniae* and *Mycoplasma pneumoniae* were negative. The woman was treated with oral doxycycline (100 mg p.o., b.i.d.); however, after 7 days she developed a drug-induced allergic reaction, after which she was treated with minocycline (100 mg p.o., b.i.d., for 3 days). Her respiratory symptoms improved, but because of severe vertigo, the antibiotics were stopped. A chest CT scan in December 2011 revealed bilateral bronchiectasis involving her lower lobes (Fig. 1).

A Lowenstein-Jensen (LJ) slant of the NTM isolate was transported to the Intracellular Pathogens Research Laboratory (IPRL) for molecular identification. Colonies from the LJ slant were emulsified in physiological saline solution. DNA was extracted using an automated QIASymphony instrument according to the manufacturer's instructions. Sequencing of the 16S rRNA gene and 65-kDa heat shock protein gene (*hsp65*) was performed as described previously (2, 3). The partial 16S rRNA gene sequence shared 100% (477/477 bp) homology with *Mycobacterium iranicum* (type strain; GenBank accession number [HQ009482](https://www.ncbi.nlm.nih.gov/nuccore/HQ009482)), whereas the partial *hsp65* sequence

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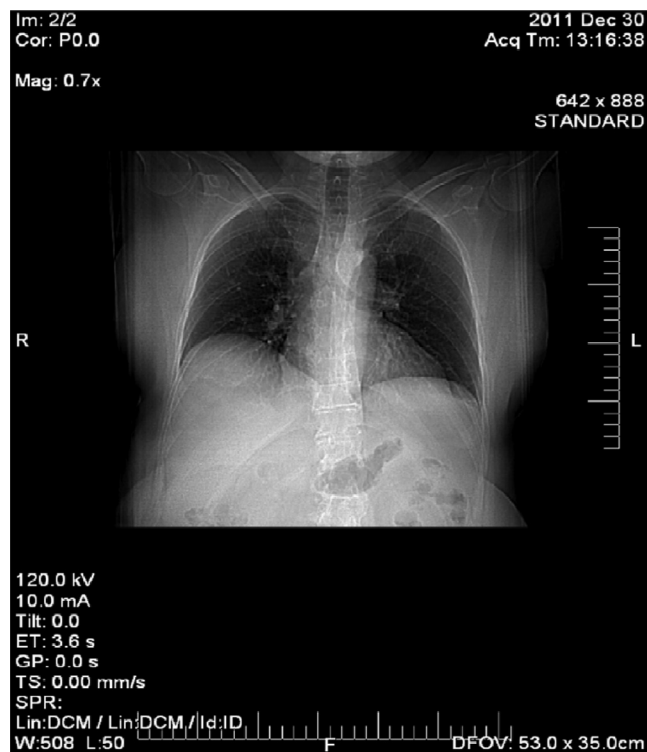


FIG 1 Patient CT scan obtained in December 2011, demonstrating bilateral bronchiectasis.

shared 99% (360/362 bp) homology with *M. iranicum* (GenBank accession number [JQ898289](#)).

NTM species cause a variety of human infections, including chronic debilitating pulmonary disease among elderly persons. NTM infections are environmentally acquired, as these bacteria are often present in soil and various water sources (4). NTM disease prevalence is reportedly increasing, leading some authors to suggest that in the United States, NTM infections may be of greater microbiological importance than tuberculosis (TB) (5, 6).

Species differentiation of NTM has for many years been based on cultural and biochemical tests; however, these tests are time-consuming and phenotypic traits can vary with growth conditions, ultimately leading to inaccurate species identification. Diagnosis based upon broad-range bacterial 16S rRNA gene PCR, followed by direct sequencing, has been used to confirm infections in culture-negative patients (7). However, further differentiation of NTM species may require analysis of genes other than the 16S rRNA gene (3, 8). An increasing prevalence of NTM pulmonary illness is being reported among elderly women in United States (5, 9). It is speculated that NTM disease is more common in the southeastern United States, where increased environmental exposure may contribute to the increasing incidence of NTM pulmonary disease. Defects in pulmonary architecture which may have occurred in this patient due to prior respiratory infections could have impaired her immune responsiveness and thereby promoted trapping of environmental organisms, including NTM, potentially increasing the risk of infection (10, 11). Using a laboratory-based case definition, the burden of NTM infections in Oregon

was predominantly pulmonary, with higher rates in women over 50 years of age. Chronic obstructive pulmonary disease (COPD) was the most common underlying lung disease in males, whereas bronchiectasis was more frequent in females (6).

If the *M. iranicum* infection was acquired in May 2010 and persisted until isolation from sputum in June 2011, it is possible that the organism was a cofactor in the development of the patient's bronchiectasis. Regardless, NTM species now appear to represent an environmental health threat for patients with underlying pulmonary conditions.

M. iranicum is a recently described NTM species that has been isolated from eight patients in six countries (Greece, Italy, Iran, Netherlands, Sweden, and the United States) worldwide. Major phenotypic features include rapid growth of bacteria that produce a yellow-orange pigment. Genotypically, major conserved genetic regions allow differentiation of multiple sequvars. Of the eight reported *M. iranicum* strains, five were isolated from sputum of patients with various pulmonary disorders; clinical significance, according to the criteria of the American Thoracic Society (ATS), was attributed to at least one isolate from Iran (12, 13). The strain obtained from this patient's sputum was identified as *M. iranicum* on the basis of genotypic characteristics. The first third of the 16S rRNA gene was identical to that of the type strain and all the other strains of *M. iranicum* reported to date (12), while this patient's isolate differed from the type strain by two *hsp65* gene nucleotides.

M. iranicum isolates have historically originated from patients residing in Mediterranean countries (12). Although several reports suggest a point source exposure for patients with newly diagnosed NTM infections (5, 14), the source of infection in this patient was not determined. During the husband's respiratory illness in 2010, sputum was not tested. Even though NTM infection in her husband was not confirmed, the patient's exposure to aerosolized secretions from her husband's CPAP machine, in conjunction with the geographical origins of the *M. iranicum* strains isolated to date, suggests that her NTM infection may have been acquired approximately 1 year prior to isolation. Based upon the historical sequence of events, it appears that a novel strain of *M. iranicum* was mechanically transmitted to a woman with chronic illness, after which she developed worsening pulmonary symptomatology and bronchiectasis, thereby supporting the inclusion of *M. iranicum* among the growing numbers of NTM species with pathogenic potential.

Nucleotide sequence accession number. The 16S sequence of the patient's isolate has been submitted to GenBank under accession no. [JX492963](#).

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