

***Mycobacterium iranicum* Infection in HIV-infected Patient, Iran**

To the Editor: The species *Mycobacterium iranicum* was described in 2013 (1) on the basis of 8 clinical strains isolated in various countries (Iran, Italy, Greece, the Netherlands, Sweden, and the United States). Recently, the isolation of *M. iranicum* from the sputum of a woman also was reported (2). We report the isolation of this newly recognized species from an HIV-positive patient.

A scotochromogenic, rapidly growing strain was isolated in 2012 from respiratory specimens of an HIV-positive 44-year-old Iranian man with chronic pulmonary disease. The patient had been found to be HIV seropositive (viral load $\geq 1,000$ copies/mL, CD4 lymphocyte count 120/ μ L) in 2004 when he was hospitalized because of fever, weight loss, and oral candidiasis. Treatment with antiretroviral drugs, including stavudine, lamivudine, and nevirapine, was begun. The patient rapidly improved; the fever disappeared, he gained weight, and he was discharged from the hospital. At a 6-month follow-up visit, viral load was 1,000 copies/mL and CD4 lymphocyte count was 420/ μ L. He continued to receive antiretroviral treatment until 2010 when treatment was discontinued because of its high cost.

The man was hospitalized again in 2012 with mild fever, weight loss, chronic chest pain, and nonproductive cough. At that time, the viral load was $\geq 100,000$ copies/mL, and CD4 count was 5 lymphocytes/ μ L. Tuberculin skin test results were negative, radiograph of the chest showed no abnormalities, and routine cultures of sputum and blood were negative for common bacteria. Lactate dehydrogenase level (98 U/L [reference <600 U/L]) was within normal limits,

whereas liver function was abnormal (alanine aminotransferase level 95 U/L [reference <36 U/L], L-aspartate aminotransferase level 85 U/L [reference <29 U/L], alkaline phosphatase 180 U/L [reference 44–147 U/L], and total bilirubin 1.4 mg/dL [reference 0.3–1mg/dL]). Antiretroviral therapy was resumed, which led to an increase in CD4 cells (205 lymphocytes/ μ L after 1 month). The examination by microscopy (Ziehl-Neelsen staining) of 3 sputum samples did not reveal acid-fast bacilli; culture for mycobacteria was not done. Oral treatment with tetracycline was started, but the patient's fever and chest pain remained unchanged.

After bronchoscopy, 2 of 3 bronchial lavage (BAL) samples were found to be positive for acid-fast coccobacilli by microscopy, and rapidly growing, deep orange mycobacteria grew in all 3 cultures. Giemsa stain did not show *Pneumocystis jirovecii* in BAL samples. A standard antituberculosis regimen was undertaken but did not result in substantial improvement. At 1 month follow-up, 1 sputum sample was negative for acid-fast organisms, and 1 BAL specimen was positive by microscopy and in culture. When the isolate was identified as *M. iranicum*, therapy was replaced with a combination of amikacin and ciprofloxacin for 3 months (standard treatment used in Iran for infections caused by rapidly growing mycobacteria), and the patient improved rapidly. Mycobacteria were neither observed nor grew in culture in a BAL specimen obtained 1 month after the change in therapeutic regimen.

Identification of the isolates was initially attempted with biochemical tests, and they were negative for niacin production, nitrate reduction, Tween 80 hydrolysis, and semiquantitative catalase. The tests were positive for urease activity, iron uptake, tellurite reduction, arylsulfatase (3 days after the start of the test), 5% NaCl tolerance, and heat-stable (68°C)

catalase. The genetic sequencing of almost-complete (1,450 bp) 16S rRNA gene (3), a 710-bp fragment of the β -subunit of the RNA polymerase gene (4), and the hypervariable region (402 bp) of the 65-kDa heat-shock protein (5) revealed 99.8%, 99.4%, and 100% identity, respectively, with sequences of the type strain found in GenBank and definitively confirmed the identification.

The clinical criteria required by the American Thoracic Society and Infectious Disease Society of America (3) to assess the importance of the isolation of a nontuberculous mycobacterium from pulmonary specimens include, in adjunct to a specific symptomatology, the presence of nodular or cavitory lung lesions and the exclusion of any other possible cause of the disease. The normal thoracic radiograph findings for the case-patient described here cannot, however, be considered a definitive exclusion criterion: in highly immunocompromised patients, a chest radiograph may show no abnormalities, even when substantial pathologic features of infection are present (6). The microbiological criteria were clearly fulfilled by isolating the organism from multiple sputum specimens and the BAL specimens. The patient's response to the treatment and the disappearance of thoracic symptoms further support the assertion.

Our report confirms the potential pathogenicity of *M. iranicum*. In addition to the case described here, 9 isolations of this species have been reported so far. Among them, the clinical relevance has been demonstrated for 2 strains grown from respiratory specimens of patients with pulmonary disease and for 1 strain isolated from a cutaneous lesion (1,2). The role of an accurate identification, in conjunction with symptoms and radiographic findings, is central to understanding the clinical significance of mycobacteria isolated from pathological specimens.

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Close Relative of Human Middle East Respiratory Coronavirus in Bat, South Africa

To the Editor: The severe acute respiratory syndrome (SARS) outbreak of 2002–03 and the subsequent implication of bats as reservoir hosts of the causative agent, a coronavirus (CoV), prompted numerous studies of bats and the viruses they harbor. A novel clade 2c betacoronavirus, termed Middle East respiratory syndrome (MERS)—CoV, was recently identified as the causative agent of a severe respiratory disease that is mainly affecting humans on the Arabian Peninsula (1). Extending on previous work (2), we described European *Pipistrellus* bat-derived CoVs that are closely related to MERS-CoV (3). We now report the identification of a South Africa bat derived CoV that has an even closer phylogenetic relationship with MERS-CoV.

During 2011–2012, fecal pellets were collected from 62 bats representing 13 different species in the KwaZulu-Natal and Western Cape Provinces of South Africa and stored in RNALater solution (Life Technologies, Carlsbad, CA, USA). Details about the bat sample are available in the online Technical Appendix Table (wwwnc.cdc.gov/EID/article/19/10/13-0946-Techapp1.pdf). RNA was extracted by using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). Screening for CoVs was done by nested reverse transcription PCR using broadly reactive oligonucleotide primers targeting a conserved region in the *RNA-dependent RNA polymerase (RdRp)* gene (online Technical Appendix). PCR results were positive for 5 (8%) of the 62 specimens. PCR amplicons for 4 positive specimens yielded alphacoronavirus sequences related to

recently described bat alphacoronaviruses from South Africa (4). The other positive specimen, termed PML/2011, was from an adult female *Neoromicia cf. zuluensis* bat sampled in 2011; the specimen yielded a novel betacoronavirus (GenBank accession no. KC869678). Online Technical Appendix Figure 1 shows the distribution of this bat species.

To obtain better phylogenetic resolution, we extended the 398-nt *RdRp* fragment generated by the screening PCR to 816 nt, as described (5). PML/2011 differed from MERS-CoV by only 1 aa exchange (0.3%) in the translated 816-nt *RdRp* gene fragment. Thus, PML/2011 was much more related to MERS-CoV than any other known virus. The amino acid sequence of the next closest known relatives of MERS-CoV, from European *Pipistrellus* bats (3), differed from MERS-CoV by 1.8%. The amino acid sequences of viruses from *Nycteris* bats in Ghana (3) and the 2c prototype bat CoVs, HKU4 and HKU5, from China (6) differed by 5.5%–7.7% from MERS-CoV. The smaller 152- to 396-nt *RdRp* fragments of 2c bat CoVs from a *Hypsugo savii* bat in Spain (7), bat guano in Thailand (8), and a *Nyctinomops* bat in Mexico (9) showed no or only partial overlap with the 816-nt fragment generated in this study; thus, a direct comparison could not be done. However, in their respective *RdRp* fragments, these CoVs yielded amino acid sequence distances of 3.5%–8.0% and were thus probably more distant from MERS-CoV than the virus described here.

A Bayesian phylogenetic analysis of the 816-nt *RdRp* sequence confirmed the close relationship between PML/2011 and MERS-CoV (Figure). Their phylogenetic relatedness was as close as that of SARS-CoV and the most closely related bat coronavirus known, Rs672 from a *Rhinolophus sinicus* bat (Figure). Like PML/2011 and MERS-CoV, Rs672 and SARS-CoV showed only 1 aa exchange in