Mycobacterium novocastrense–associated Pulmonary and Wound Infections

To the Editor: Although the clinical role of nontuberculous mycobacteria has long been appreciated (*1*), the high endemicity of tuberculosis (TB) in developing countries has overshadowed the emergence of these organisms. They are simply dismissed as being contaminants or are misidentified as *Mycobacterium tuberculosis* (*2*).

No report on *M. novocastrense* has been published since its original description in 1997 (*3*), except for a study in France by N'Guessan et al. (*4*). That study initiated speculation about the possible role of this bacterium in the etiology of human infection. We report isolation of *M. novocastrense* in 2 independent clinical cases—1 from tissue biopsy specimen of an apparently healthy adult, the other from bronchoalveolar lavage of an HIV-infected patient—that will cast light on the clinical relevance of this rare species.

Case-patient 1, a 60-year-old woman, was referred to a hospital because of high fever, productive cough, thoracic pain, and noticeable weight loss. After her husband's AIDS-related death in a state prison, HIV infection had been diagnosed in this case-patient. At admission, laboratory testing showed negative tuberculin skin test result, lymphopenia, an elevated C-reactive protein level of 76 mg/L, an erythrocyte sedimentation rate of 73 mm/h, a viral load of 500 copies/mL, and negative blood culture for bacterial growth. Her outpatient records indicated that she empirically was given numerous courses of antimicrobial drugs because of a provisional diagnosis of bacterial pneumonia, but her condition did not improve. Subsequent hospital referral was prompted by worsening of her symptoms. With a diagnosis of suspected TB, bronchoalveolar lavage fluid was collected. Direct microscopic examination of the specimen showed acid-fast bacilli with subsequent formation of typical colonies of a relatively rapidly growing photochromogenic *Mycobacterium* spp. on Löwenstein-Jensen (LJ) medium. The patient was treated with amikacin, and her condition markedly improved.

Case-patient 2, a 23-year-old woman, sought care for a 6-month history of soft tissue swelling in her left leg resulting from an accidental injury in a paddy field. The lesion had not been treated and subsequently increased in size. The large nodule self-ruptured and excreted yellow-pale pus. She was prescribed minocycline by her general practitioner, but the lesion did not resolve. When the patient arrived at the hospital, her clinical and laboratory assessments were normal. She had negative test results for HIV, hepatitis C, and hepatitis B infections. Erythrocyte sedimentation rate was 255 mm/h. Culture of a biopsy specimen from the cutaneous lesion on ordinary culture media was negative, although microscopic observation of the drainage fluid showed acid-fast bacilli. This finding was confirmed by a positive culture on LJ medium. A repeat specimen resulted in isolation of the same organism in pure culture. The patient was given amikacin and fully recovered in <1 month.

The isolates, i.e., HNTM1 and HNTM10, were subjected to preliminary identification and susceptibility to common antimycobacterial agents for rapidly growing mycobacteria according to standard procedures (*5*,*6*). The isolates were then subjected to molecular identification, which included PCR amplification of a genus-specific region of the 65-kDa heat shock protein (*hsp*) gene (*7*) and the direct sequence analysis of 16S rDNA, *hsp*65, and *rpo*B genes as described (*3*,*8*,*9*). GenBank accession numbers for the gene sequences of HNTM1 as a representative isolate determined in this study are HM807280–HM807282.

The isolates we observed by acid-fast staining of the specimens and recovered on LJ medium were a yellow pigmented photochromogenic species that grew rapidly at 25°C, 37°C, and 42°C. They grew on MacConkey agar without crystal violet and LJ medium containing 5% NaCl; were positive for semiquantitative catalase, arylsulfatase activity in 14 days, and nitrate reduction; and were negative for urease activity, niacin production, tellurite and Tween hydrolysis, heat-stable (68°C) catalase, and iron uptake. They were susceptible to amikacin, clarithromycin, doxycycline, sulfamethoxazole, streptomycin, imipenem, ciprofloxacin, isoniazid, and ethambutol but resistant to rifampin.

The PCR amplification of a 228-bp genus-specific fragment of the *hsp65* gene reliably confirmed that the isolates belonged to the genus *Mycobacterium*. The almost complete 16S rDNA gene sequence (1,476 bp) and partial sequences of *hsp65* and *rpoB* genes of the isolates showed the highest similarity of 99.86%, 99.45%, and 99.7% with that of *M. novocastrense* reference strain. These values correspond to 2-nt differences for each gene.

Our report of 2 independent clinical cases might support evidence the clinical relevance of *M. novocastrense*. Our findings show that *M. novocastrense*, however rare its incidence might be, can cause infection in healthy and immunocompromised patients. However, because of the complexity of identifying nontuberculous mycobacteria, emphasis should be placed on the quality of regional laboratories for TB in developing countries to differentiate isolates to the species level.

This study was supported by the office of vice-chancellor for research, Isfahan University of Medical Sciences.

Hasan Shojaei, Abodolrazagh Hashemi, Parvin Heidarieh, and Abass Daei Naser

Author affiliations: Isfahan University of Medical Sciences, Isfahan, Iran (H. Shojaei, A. Hashemi, A.D. Naser); and Tehran University of Medical Sciences, Tehran, Iran (P. Heidarieh)

DOI: 10.3201/eid1703.101400

References

1. Falkinham JO III. Epidemiology of infection by nontuberculous mycobacteria. Clin Microbiol Rev. 1996;9:177–215.
2. Jarzembowski JA, Young MB. Nontuberculous mycobacterial infections. Arch Pathol Lab Med. 2008;132:1333–41.
3. Shojaei H, Magee JG, Freeman R, Yates M, Horadagoda NU, Goodfellow M. *Mycobacterium novocastrense* sp. nov., a rapidly growing photochromogenic *Mycobacterium*. Int J Syst Bacteriol. 1997; 47:1205–7. DOI: 10.1099/00207713-47-4-1205
4. N'Guessan K, Vincent V, Nahoua G, Kamate M, Dosso M. *Mycobacterium novocastrense* isolated from sputum: contamination or pulmonary infection? [in French]. Med Mal Infect. 2006;36:180. DOI: 10.1016/j.medmal.2005.11.011
5. Kent PT, Kubica GP. Public health mycobacteriology: a guide for the level III laboratory. Atlanta: Centers for Disease Control, US Department of Health and Human Services; 1985.
6. National Committee for Clinical Laboratory Standards. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes: approved standard. Wayne (PA): The Committee; 2003.
7. Khan IU, Yadav JS. Development of a single-tube, cell lysis-based, genus-specific PCR method for rapid identification of mycobacteria: optimization of cell lysis, PCR primers and conditions, and restriction pattern analysis. J Clin Microbiol. 2004;42:453–7. DOI: 10.1128/JCM.42.1.453-457.2004
8. Kim H, Kim SH, Shim TS, Kim MN, Bai GH, Park YG, et al. Differentiation of *Mycobacterium* species by analysis of the heat-shock protein 65 gene (*hsp*65). Int J Syst Evol Microbiol. 2005;55:1649–56. DOI: 10.1099/ijs.0.63553-0
9. Adékambi T, Colson P, Drancourt M. *rpo*B-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. J Clin Microbiol. 2003;41:5699–708. DOI: 10.1128/JCM.41.12.5699-5708.2003

Address for correspondence: Hasan Shojaei, Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran; email: hasanshojaei@msn.com

Geographic Expansion of Buruli Ulcer Disease, Cameroon

To the Editor: Buruli ulcer disease (BU) is a necrotizing skin disease caused by *Mycobacterium ulcerans* that affects mostly children in humid, tropical areas (*1*). The exact mode of *M. ulcerans* transmission remains unclear, although the role of water bugs has been supported by various observations and experimental studies (*2*,*3*). We report the identification of a new BU-endemic area in Cameroon, the Bankim district, and specify ecologic and clinical characteristics of *M. ulcerans* infection in this area. These characteristics hint at the possible role of environmental changes (building of a dam several years ago) in the expansion of BU in this area.

Since 1969, only 1 BU-endemic area in Cameroon has been described: the Nyong River basin, where equatorial forest predominates (*4*). In 2004, clinically suspected cases of BU in the district of Bankim have been reported (*5*). This region differs from the first BU-endemic area by geography and climate. Representing a transition between forested south and savanna north, this area has benefited from the building of a dam on the Mape River in 1989, which created an artificial lake of 3.2 billion m^3 capacity.

From January 2007 through June 2009, all cases of skin lesions evocative of active BU were recorded as BU probable cases according to World Health Organization guidelines (*6*). During this period, 195 clinically suspected cases were reported from the Bankim health district (Figure). The overall median age for these 195 patients was 19.5 years (interquartile range 10–37 years). No significant difference in age was found according to gender, but a significant trend of decreasing overall median age was found (20 years in 2007 to 12 years in 2009. The most frequent type of lesion was ulcer. Since March 2009, the Centre Pasteur of Cameroon has performed laboratory confirmation for suspected BU cases: microscopic examination for acid-fast bacilli, culture, and *M. ulcerans* DNA detection by PCR (*6*). From April through June 2009, of 34 consecutive samples tested in the reference laboratory, 10 were positive for *M. ulcerans* by at least microscopy and PCR.

Whether BU is emerging in Bankim or is just a newly recognized preexisting disease is difficult to establish. However, that the incidence of BU in the region is increasing is unquestionable. The decreasing median age of patients since 2007 might be consistent with emergence of BU as a new disease in Bankim. This observation could suggest either an increasing level of acquired immunity in the population, leading to protection correlated with age, or the expansion of risky sites for human infection with *M. ulcerans*.

During 1 week in January 2008, water bugs were collected from the artificial lake and water bodies located within or close to each community. A previously described sampling method was used (*2*). To detect *M.*