

in Nicaragua in 2006 and 2008. The bootstrap support for this grouping was 100%. Phylogenetic analyses with neighbor-joining, maximum-parsimony, and Bayesian methods gave trees with similar topologies, including clear separation of most recent Central American isolates into 1 clade, as well as grouping of the Key West sequence with the same 2 isolates from Nicaragua (data not shown). No protein coding changes between these strains were identified, which suggests purifying selection for an optimum phenotype. There were 8 synonymous differences over the 1,708-nt amplified region between the Key West and Nicaragua 2008 sequences. Previous molecular clock determinations for DENV-1 provided a range of  $2.5\text{--}7.0 \times 10^{-4}$  substitutions per nucleotide per year (8). This calculation produced an estimate of a 6.7–18.7-year divergence time between the Key West virus and the most closely related Nicaragua strain. When during this time the ancestor of Key West DENV was introduced to Florida is unknown.

Analysis of the entire Key West DENV-1 genome may help pinpoint the origin and address the possibility of selective pressure on other genes or recombination events (9). Given the recent reports of DENV in residents of other Florida counties who had no travel histories (2), monitoring of Key West and other nearby urban areas for evidence of local DENV transmission should continue.

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#### References

- Centers for Disease Control and Prevention. Locally acquired dengue—Key West, Florida, 2009–2010. *MMWR Morb Mortal Wkly Rep.* 2010;59:577–81.
- Anil L, Stanek D, Blackmore C, Stark L, Mock V. Florida arbovirus surveillance week 52: December 26–January 1, 2011 [cited 2011 Mar 18]. [http://www.doh.state.fl.us/Environment/medicine/arboviral/pdfs/2010/2010Week52ArbovirusReport\\_1\\_1\\_2011.pdf](http://www.doh.state.fl.us/Environment/medicine/arboviral/pdfs/2010/2010Week52ArbovirusReport_1_1_2011.pdf)
- Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase–polymerase chain reaction. *J Clin Microbiol.* 1992;30:545–51.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 1997;24:4876–82. doi:10.1093/nar/25.24.4876
- Resch W, Zaslavsky L, Kiryutin B, Rozanov M, Bao Y, Tatusova TA. Virus variation resources at the National Center for Biotechnology Information: dengue virus. *BMC Microbiol.* 2009;9:65. doi:10.1186/1471-2180-9-65
- Gouy M, Guindon S, Gascuel O. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol.* 2010;27:221–4. doi:10.1093/molbev/msp259
- Zhang C, Mammen MP Jr, Chinnawirotpisan P, Klungthong C, Rodpradit P, Monkongdee P, et al. Clade replacements in dengue virus serotypes 1 and 3 are associated with changing serotype prevalence. *J Virol.* 2005;79:15123–30. doi:10.1128/JVI.79.24.15123-15130.2005
- Twiddy SS, Holmes EC, Rambaut A. Inferring the rate and time-scale of dengue virus evolution. *Mol Biol Evol.* 2003;20:122–9. doi:10.1093/molbev/msg010
- Carvalho SES, Martin DP, Oliveira LM, Ribeiro BM, Nagata T. Comparative analysis of American dengue virus type 1 full-genome sequences. *Virus Genes.* 2010;40:60–6. doi:10.1007/s11262-009-0428-0

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## *Mycobacterium doricum* Osteomyelitis and Soft Tissue Infection

**To the Editor:** Infections with nontuberculous mycobacteria (NTM) are being increasingly identified. Several factors may contribute to this finding, including increased awareness of these organisms as pathogens, improved ability of laboratories to isolate and identify these organisms, and increasing prevalence (1). We describe a case of osteomyelitis and soft tissue infection with *Mycobacterium doricum* after trauma in a previously healthy adult.

A 21-year-old man sustained an open right femur fracture with gross contamination of the wound with dirt and gravel. The wound was irrigated and debrided, the fracture was fixed by intramedullary nailing, and the wound was closed.

Sixteen weeks later, pain, swelling, and erythema developed in the right thigh of the patient.

Radiographs showed incomplete union of the fracture and possible loosening of the nailing hardware. Repeat irrigation and debridement of the right thigh was performed. Thirty milliliters of purulent material was drained. Bone was not visible, and the hardware was not removed or replaced. Gram staining of the purulent material showed 1 polymorphonuclear cell per oil-immersion microscopic field and no bacteria. Routine bacterial cultures were negative. Results of acid-fast staining were also negative. Three operative specimens were tested for acid-fast organisms. The patient was discharged and prescribed a 6-week course of vancomycin, 750 mg intravenously every 8 hours, and ciprofloxacin, 500 mg orally 2×/d.

Four weeks later, all 3 operative cultures grew an acid-fast bacillus, which was identified by DNA sequencing and high-performance liquid chromatography as *M. doricum* (Centers for Disease Control and Prevention, Atlanta, GA, USA). While in vitro susceptibility testing results were pending (2), recurrent swelling, erythema, and warmth developed in the patient at the previous injury site. A computed tomography scan showed multiple small abscesses adjacent to the nonunited fracture, an irregular periosteal reaction around the fracture site, and lucency surrounding the medullary rod, suggestive of osteomyelitis (online Appendix Figure, [wwwnc.cdc.gov/EID/article/17/11/11-0460-FA1.htm](http://wwwnc.cdc.gov/EID/article/17/11/11-0460-FA1.htm)).

The patient was treated with irrigation and debridement of the abscesses and exchange of hardware. Two specimens were tested for acid-fast culture, 1 of which grew *M. doricum* after 3 weeks of incubation. The patient was discharged and empirically treated with amikacin, 1,250 mg/d intravenously for 3 weeks, and levofloxacin, 750 mg/d orally for 3 months, as therapy for infection with *M. doricum*, pending susceptibility testing results.

In vitro susceptibility results showed susceptibility to all drugs tested: MIC <1 µg/mL for amikacin, 0.25 µg/mL for ciprofloxacin, 4 µg/mL for clarithromycin, 2 µg/mL for ethambutol, <0.12 µg/mL for rifampin, <0.25 µg/mL for rifabutin, <0.5 µg/mL for streptomycin, and <0.12/2.4 µg/mL for trimethoprim/sulfamethoxazole (Associated Regional and University Pathologists, Salt Lake City, UT, USA). The antimicrobial drug regimen was changed to trimethoprim/sulfamethoxazole (800 mg of trimethoprim and 400 mg of sulfamethoxazole) orally 2×/d, and doxycycline, 100 mg orally 2×/d. After 10 months of therapy, the patient stopped taking these antimicrobial drugs. Six weeks after discontinuation of therapy, he had no signs or symptoms of recurrent infection.

*M. doricum* was first identified in a cerebrospinal fluid sample from a 50-year-old man with AIDS (CD4+ lymphocyte count 28 cells/mm<sup>3</sup>). *Cryptococcus neoformans* was also isolated from the same specimen. This patient was treated for *C. neoformans* infection with amphotericin B and 5-fluorocytosine but died 6 weeks later, before isolation of *M. doricum*. The isolate was susceptible to all antimicrobial drugs tested in vitro, including amikacin, azithromycin, clarithromycin, ciprofloxacin, clofazimine, ethambutol, isoniazid, ofloxacin, rifabutin, rifampin, sparfloxacin, and streptomycin (3).

The American Thoracic Society and Infectious Diseases Society of America have published guidelines on the diagnosis, treatment, and prevention of NTM diseases (1). However, there are no recommendations for treatment of *M. doricum* infection. At least 2 antimicrobial drugs are recommended for treating infections with NTM, and surgery is indicated for extensive disease, abscess formation, or contraindications/intolerance to medical therapy. When possible, it is recommended that foreign bodies

be removed. For skin and soft tissue infections, ≥4 months of therapy is recommended, with extension to 6–12 months for cases of bone involvement (1). In this instance, trimethoprim/sulfamethoxazole and doxycycline were chosen because of availability of oral formulations, in vitro susceptibility testing results, and affordability given the patient's lack of medical insurance.

Disease in the patient was eradicated by surgical debridement and prolonged antimicrobial drug therapy. We speculate that the organism was introduced from the soil at the time of the open fracture. Given increased awareness of NTM as pathogens and improvement in the ability of laboratories to isolate and identify these organisms from clinical specimens, this *Mycobacterium* species might be increasingly identified as a cause of disease. Additional reports of treating disease caused by *M. doricum* will be valuable so that in vitro susceptibility testing can be correlated with clinical responses, eventually enabling development of guidelines for therapy.

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#### References

1. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med*. 2007;175:367–416. doi:10.1164/rccm.200604-571ST
2. National Committee for Clinical Laboratory Standards. Susceptibility testing of mycobacteria, *Nocardia*, and other aerobic actinomycetes. Approved Standard. Document no. M24-A. Wayne (PA): The Committee; 2003.
3. Tortoli E, Piersimoni C, Kroppenstedt RM, Montoya-Burgos JI, Reischl U, Giacometti A, et al. *Mycobacterium doricum* sp. nov. *Int J Syst Evol Microbiol*. 2001;51:2007–12. doi:10.1099/00207713-51-6-2007

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## Disseminated *Mycobacterium* *abscessus* Infection and Showerheads, Taiwan

**To the Editor:** Diseases caused by nontuberculous mycobacteria (NTM) in patients with Sjögren syndrome have rarely been reported (1,2). In addition, showerheads in residential bathrooms as a source of *Mycobacterium abscessus*-induced disseminated disease have never been reported (3–5).

A 65-year-old woman with Sjögren syndrome sought treatment at National University Taiwan hospital with fever (38.6°C) and a 3-month history of lymphadenopathy over the left neck, left submandibular, and bilateral inguinal areas. Active Sjögren syndrome with lymphadenitis was considered because of progressive hypergammaglobulinemia (IgG 3,030 mg/dL, reference range 700–1,600 mg/dL) and high titers of anti-Sjögren syndrome (SS) A (561 AU/mL) and anti-SSB antibodies (220 AU/mL; positive >120 AU/mL). A chest radiograph obtained 1 month before admission showed no active lung lesions; however, cultures of 3 samples of sputum all yielded *M. abscessus* bacteria (isolate A). Pathologic examination of excised lymph nodes of the bilateral inguinal area showed reactive lymphoid proliferation and granulomatous inflammation with

multinucleate giant cell formation, suggestive of mycobacterial disease; however, there was no evidence of caseating necrosis or acid-fast bacilli. ELISA results were negative for antibodies to HIV-1, HIV-2, HTLV-1, and HTLV-2.

Parenteral antimicrobial drugs (imipenem, 500 mg every 8 h) and amikacin (250 mg 2×/d) along with oral clarithromycin (500 mg 2×/d) were administered. Fever subsided 3 days after lymph node excision with concomitant administration of antimycobacterial agents. The patient was treated successfully with intravenous antimicrobial drugs for a total of 14 days, followed by oral clarithromycin (500 mg 2×/d) and doxycycline (100 mg 2×/d) therapy for 4 months. Follow-up blood cultures 10 weeks after initiation of antimycobacterial agents were negative for the organism. *M. abscessus* bacteria grew on cultures of the excised lymph nodes (isolate B) and 2 sets of blood cultures (isolate C).

A total of 6 swab specimens taken from the interior surface of the showerheads from the 6 bathrooms

of the patient's 2 houses (3 in each house), 1 in Taichung (central Taiwan) and the other in Taipei (northern Taiwan), and 6 shower water samples of the 6 bathrooms were submitted for mycobacterial cultures. Four of the 6 swab samples (isolates D–G), 2 (isolates D and E) from Taichung and 2 (isolates F and G) from Taipei, grew *M. abscessus* bacteria. Cultures of shower water from the 6 bathrooms were all negative for the organism. These isolates were identified as *M. abscessus* by conventional biochemical methods and confirmed by 16S rRNA gene sequencing analysis and a PCR–restriction fragment length polymorphism–based method targeting a 439-bp fragment of the 65-kDa HSP gene as previously described (6,7). Random amplified polymorphic DNA patterns of these isolates (isolates A–G) as determined by means of arbitrarily primed PCR using 3 different random primers were identical (i.e., they shared every band) (Figure). Three unrelated isolates of *M. abscessus* recovered from cutaneous lesions of 3 patients who were treated at the same hospital in

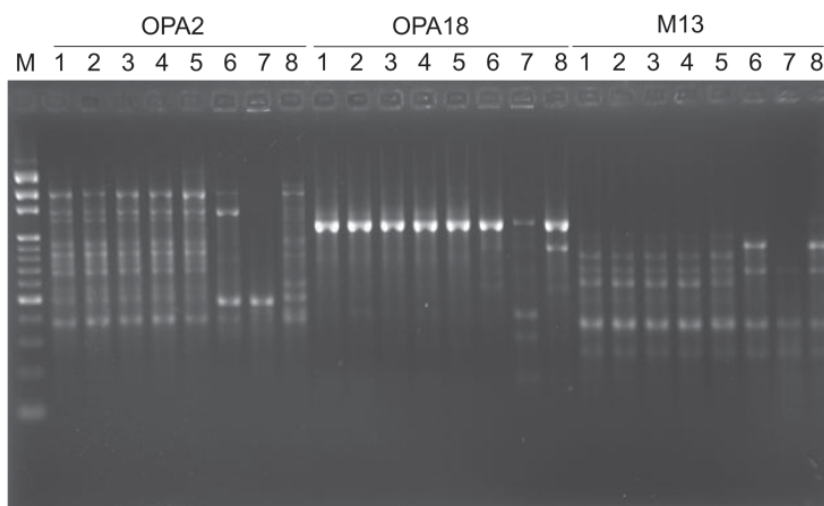


Figure. Random amplified polymorphic DNA patterns of 8 isolates of *Mycobacterium abscessus* generated by arbitrarily primed PCR with the primers OPA2, OPA18, and M13 (Operon Technologies, Inc., Alameda, CA, USA). Lanes: M, molecular size marker (1-kb ladder; Gibco BRL, Gaithersburg, MD, USA); 1, isolate A; 2, isolate B; 3, isolate C; 4, isolate D; 5, isolate F; 6–8, three unrelated isolates of *M. abscessus* recovered from cutaneous lesions of 3 patients who were treated at National Taiwan University hospital in 2010 (see text for designation of isolates).