**Background:** Mycobacterium bolletii and Mycobacterium massiliense are recently described species of nontuberculous mycobacteria. Footbaths preceding pedicures at nail salons have been implicated as reservoirs of infection with nontuberculous mycobacteria. To our knowledge, this case series represents the first documented outbreak of *M. bolletii/M. massiliense* furunculosis, identified by heat-shock protein 65 gene, *hsp65*, sequencing, occurring in immunocompetent patrons of a North Carolina nail salon.

**Observations:** We describe 3 cases of lower extremity furunculosis caused by *M. bolletii/M. massiliense* associated with pedicure footbaths from the same North Carolina nail salon. Lesions developed within 1 month of the salon visit and were characterized by erythematous, indurated papules and plaques. Histologic examination revealed supplicative granulomatous dermatitis. *Mycobacterium bolletii/M. massiliense* was identified by sequencing the 16S ribosomal RNA (rRNA) and *hsp65* genes. All 3 patients responded to different combinations of clarithromycin, doxycycline hydrochloride, azithromycin, and moxifloxacin hydrochloride for complete lesion resolution.

**Conclusions:** Clinicians should elicit a history of pedicure footbaths and maintain a high level of suspicion when faced with skin lesions of the lower extremities that are culture negative or are refractory to conventional antibiotic therapy. Accurate identification and discrimination of *M. massiliense* and *M. bolletii* is difficult and requires sequencing of multiple gene targets beyond their identical 16S rRNA sequences.

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**REPORT OF CASES**

**CASE 1**

A 42-year-old white woman was referred to our dermatology clinic for a 2-month history of a plaque on her lower left leg. The lesion began as a small area that progressively enlarged. By the time of her visit to our clinic, she had developed a second...
Lesion on the leg. She denied any fever, drainage from the site, or other skin lesions; the remainder of the review of systems was negative and she was in good health. She reported having had 3 pedicures with footbaths on a monthly basis at the same nail salon before the lesions appeared, but could not recall the exact timing of the lesion’s appearance. She reported shaving her legs approximately once per week.

On physical examination, a 2-cm verrucous erythematous plaque on her left shin and a more distal 4-mm erythematous papule (Figure 1) were present. No other lesions were noted. An 8-mm punch biopsy revealed suppurative and granulomatous dermatitis with dilation and rupture of multiple follicles. Special stains did not show any fungal or acid-fast organisms. Mycobacterial culture was performed on the tissue, and *M. bolletii/M. massiliense* was isolated and identified by sequencing the 16S rRNA and hsp65 genes.

Clarithromycin treatment was initiated, but no improvement was apparent after 3 weeks. The woman was subsequently seen by an infectious disease physician and was instructed to stop taking clarithromycin, as *M. bolletii* is frequently resistant to that drug; moxifloxacin hydrochloride, and azithromycin were prescribed at that time. She stopped shaving her legs and refrained from pedicures. Results of antimicrobial susceptibility tests showed the organism to be resistant to doxycycline; it was therefore discontinued. Moxifloxacin and azithromycin were prescribed. She subsequently visited an infectious disease physician, who added moxifloxacin to her medication regimen to be continued for 6 months. In the absence of improvement, the patient returned to the clinic approximately 1 month later reporting no clinical improvement. At that time multiple indurated, erythematous to violaceous nodules and plaques were noted, with some central ulceration on the bilateral distal portions of her legs. An 8-mm punch biopsy was performed, revealing an abscess in the deep dermis with surrounding granulomatous inflammation shown in the hematoxylin-eosin–stained sections (Figure 2). Several acid-fast rods were found in the microabscess (Figure 2, inset). Mycobacterial cultures were performed on the tissues, and azithromycin was prescribed.

The patient returned to our clinic 2 weeks later with fluctuant nodules, some of which had spontaneously drained. Three of the nodules were incised and drained, expressing thick, purulent material. *Mycobacterium bolletii/M massiliense* was isolated from the original tissue culture and identified by sequencing the 16S RNA and hsp65 genes. Doxycycline was added to the azithromycin treatment course. She subsequently visited an infectious disease physician, who added moxifloxacin to her medication regimen to be continued for 6 months. At the time of her last visit at completion of therapy, the patient reported that the lesions were healing well.

### CASE 2

A 23-year-old white woman was referred to our dermatology clinic for lesions that had been present on the bilateral lower legs for 4 months. The lesions began with 1 small pustule, which gradually expanded into multiple pustules. These pustules did not drain and resolved spontaneously within a few weeks, with residual erythematous macules. The woman applied a corticosteroid cream without improvement. She reported no pruritus or drainage but stated that the lesions were tender. She was otherwise in good health. The patient reported regularly patronizing a nail salon for pedicures with footbaths and recalled that her most recent pedicure had been 2 weeks before the appearance of the pustules. She reported shaving her legs regularly and had continued to shave despite the pustules.

Approximately 8 papules in various stages of development were present on both legs from her knees to her ankles. The papules were firm, nonfluctuant, and without drainage, with an osteumlike opening and surrounding erythema. The remainder of the physical examination was unremarkable. Treatment with clobetasol ointment, 0.05%, and clindamycin lotion, 1%, was initiated; however, the patient returned to the clinic approximately 1 month later reporting no clinical improvement. At that time multiple indurated, erythematous to violaceous nodules and plaques were noted, with some central ulceration on the bilateral distal portions of her legs. An 8-mm punch biopsy was performed, revealing an abscess in the deep dermis with surrounding granulomatous inflammation shown in the hematoxylin-eosin–stained sections (Figure 2). Several acid-fast rods were found in the microabscess (Figure 2, inset). Mycobacterial cultures were performed on the tissues, and azithromycin was prescribed.

The patient returned to our clinic 2 weeks later with fluctuant nodules, some of which had spontaneously drained. Three of the nodules were incised and drained, expressing thick, purulent material. *Mycobacterium bolletii/M massiliense* was isolated from the original tissue culture and identified by sequencing the 16S RNA and hsp65 genes. Doxycycline was added to the azithromycin treatment course. She subsequently visited an infectious disease physician, who added moxifloxacin to her medication regimen to be continued for 6 months. At the time of her last visit at completion of therapy, the patient reported that the lesions were healing well.

### CASE 3

A 14-year-old African American girl was referred to our clinic for eruptions on her bilateral lower extremities. The patient was initially seen by an outside dermatologist with
a 1-month history of tender, pruritic red papules. She stated that she was continuously developing new lesions that spontaneously resolved; at one time she had approximately 20 on each leg. She was treated with minocycline for presumed furunculosis but had no clinical improvement after 2 weeks. A biopsy was performed, and histologic examination revealed a necrotizing suppurative granulomatous dermatitis; acid-fast and fungal stains, as well as all cultures (bacterial, fungal, and mycobacterial), were negative. The patient next tried clobetasol ointment, 0.05%, without improvement and then topical corticosteroid tape, which flattened the existing lesions but did not prevent new lesions.

At the patient’s initial visit to our clinic, the lesions had been present for approximately 4 months. She was otherwise healthy. She reported patronizing a nail salon for pedicures with footbaths about 1 month before the appearance of the lesions.

Multiple papules on both legs with surrounding erythema and crusting were noted, as well as several hyperpigmented macules in locations where lesions had been present (Figure 3A). Empirical treatment with clarithromycin and doxycycline was begun for a suspected mycobacterial infection until final results from the cultures performed by an outside laboratory were available.

The patient returned to our clinic 1 month later and reported overall improvement, including flattening of the lesions and no further lesion development. She was given hydroquinone, 4% cream, to decrease pigmentation of lesions and no further lesion development. She was given betasol ointment, 0.05%, without improvement and then postinflammatory hyperpigmentation at sites of previous involvement following multidrug treatment.

**Figure 3.** Lower leg of 14-year-old African American girl (case 3). A, The patient presented with multiple papules on bilateral lower extremities with surrounding erythema and crusting. B, Postinflammatory hyperpigmentation at sites of previous involvement following multidrug treatment.

Mycobacterium bolletii is a recently described species of NTM classified as a member of the *M chelonae–M abscessus* complex of rapidly growing mycobacteria. This complex is frequently a cause of cutaneous infections, notably after injections or minor surgical procedures, as well as an opportunistic pathogen in patients with underlying pulmonary disorders. Mycobacterium bolletii was first identified in 2006 and reported in 2009, isolated from sputum of a patient with chronic pneumonia and later from a group of patients with cystic fibrosis. The species was initially described as resistant to clarithromycin. Mycobacterium bolletii shares 100% 16S rRNA and 95.6% rpoB gene sequence similarity with *M abscessus*. It is also closely related to *M massiliense*, which was identified and classified as a member of the *M chelonae–M abscessus* complex in 2004. Accurate identification and discrimination between *M abscessus*, *M massiliense*, and *M bolletii* is difficult and requires sequencing of multiple gene targets beyond their identical 16S rRNA sequences. Zelazny et al demonstrated a multilocus sequence analysis technique that included sequencing of rpoB, hsp65, and secA for the molecular identification of 42 clinical isolates that had previously been classified as *M abscessus*. The authors found that 7 isolates were *M massiliense* and 2 were *M bolletii*. Clinical manifestations were similar among the patients. The authors recommended this approach for identification of *M abscessus* and closely related species, and it has recently been used in the classification of nosocomial outbreaks of *M bolletii* and *M massiliense*.

Few reported cases of *M bolletii* and *M massiliense* infection exist because of their recent identification. In 2008, both organisms were identified among South Korean isolates that were previously misclassified as *M chelonae* or *M abscessus*. Of 144 isolates studied, 2 were reidentified as *M bolletii* and 59 were reidentified as *M massiliense*. Identification required sequencing of rpoB and hsp65 regions. In Brazil, 311 immunocompetent individuals developed cutaneous infections attributed to *M abscessus* after undergoing invasive cosmetic procedures. Reidentification of the isolates using hsp65 and rpoB sequencing classified 59 of 67 isolates, all from surgical and postsurgical abscesses, as *M massiliense* and the remaining 8, all from patients who had undergone mesotherapy, as *M bolletii*. To our knowledge, our case series represents the first documented outbreak of *M bolletii/M massiliense* furunculosis, identified by hsp65 sequencing, occurring in immunocompetent patrons of a nail salon in North Carolina. Additional sequencing studies would have to be performed to further identify these isolates and differentiate between *M bolletii* and *M massiliense*.

Mycobacterial furunculosis is characterized clinically by an initially benign nodular appearance, with a
protracted course that can result in scarring.\textsuperscript{19} Diagnosis is often delayed by patients not seeking medical treatment soon after appearance of the lesions and by a lack of suspicion by the physician.\textsuperscript{19} Lesions typically develop 1 to 2 months after introduction of the organism.\textsuperscript{19} Rapidly growing mycobacteria are ubiquitous in municipal water supplies,\textsuperscript{6,8} and recently, footbaths preceding pedicures at nail salons have been implicated as reservoirs of infection, where the organisms thrive in the nutrient-rich water.\textsuperscript{2,8,11,19-21} In 2002, Winthrop et al\textsuperscript{8} documented a community outbreak of lower extremity furunculosis in 110 patrons of a nail salon in California. The outbreak was linked to whirlpool footbaths contaminated with \textit{M fortuitum} used before pedicures. Sniezek et al\textsuperscript{2} later reported 2 cases of \textit{M chelonae} and 1 case of \textit{M abscessus} infection of the lower extremities following whirlpool footbaths and pedicures at 2 nail salons in southern California. Gira et al\textsuperscript{11} reported 2 cases of \textit{Mycobacterium mageritense} furunculosis in women receiving footbaths and pedicures at a salon in Atlanta. Isolates recovered from the footbath drains at the salon were determined, by pulsed-field gel electrophoresis, to match the patients’ isolates. Case reports in 2005\textsuperscript{21} and 2006\textsuperscript{20} documented lower extremity furunculosis resulting from footbaths and pedicures attributed to \textit{M chelonae} and \textit{M fortuitum}, respectively. Redbord et al\textsuperscript{19} reported 4 cases of \textit{M fortuitum} furunculosis after footbaths and pedicures from 3 different salons around Cincinnati, Ohio.

Leg shaving is known to be a risk factor for acquiring infection from footbaths. Winthrop et al\textsuperscript{8} found that leg shaving is significantly associated with infection (odds ratio, 4.8; 95% confidence interval, 2.1-11.1). Sniezek et al\textsuperscript{2} reported that 2 of 3 patients had shaved their legs prior to the pedicure and Gira et al\textsuperscript{11} and Redbord et al\textsuperscript{19} reported that 100% of patients had shaved prior to the pedicures.

Skin microtrauma from shaving facilitates entry of the organisms from the contaminated footbaths. All 3 of our patients reported leg shaving prior to their pedicures.

We report 3 cases of furunculosis caused by \textit{M bolletii}/\textit{M massiliense} from contaminated whirlpool footbaths. Our report further documents the pathogenicity of these newly identified species of rapidly growing mycobacteria. Accurate identification of \textit{M bolletii} and \textit{M massiliense} and differentiation from the other species of the \textit{M chelonae–M abscessus} complex often depends on multilocus gene sequencing and is important given the differences in antibiotic resistance patterns. \textit{Mycobacterium bolletii} is known to be multidrug resistant; thus, cultures and sensitivities should be performed. Punch biopsy samples are thought to be more sensitive in capturing organisms for culture and are preferred over wound swab samples.\textsuperscript{22} Notably, cultures in 2 of our 3 patients identified the organisms, but culture in the third patient did not; antimicrobial susceptibility tests showed the organisms in the first 2 patients to be susceptible to clarithromycin, although \textit{M bolletii} is thought to be resistant. However, in vitro sensitivities may not accurately reflect in vivo activity, as the lesions in one of our patients did not clinically improve during clarithromycin treatment. Current treatment guidelines recommend debridement of abscesses and 2-agent treatment with clarithromycin in combination with ciprofloxacin, doxycycline, cefoxitin, or amikacin if the organisms are shown to be susceptible to those drugs.\textsuperscript{1,2} However, Winthrop et al\textsuperscript{22} demonstrated that infections can be treated effectively with oral antibiotic therapy tailored to the organism and surgical resection of the area can be avoided. Clinicians should prescribe 2-agent antimicrobial coverage to avoid acquired drug resistance.\textsuperscript{22} All patients in our series responded to a combi-

### Table. Reported Cases of Mycobacterial Furunculosis Occurring After Pedicures

<table>
<thead>
<tr>
<th>Source</th>
<th>Patients, No.</th>
<th>Shaved Legs, No. (%)</th>
<th>\textit{Mycobacterium} species (No. of Patients)</th>
<th>Diagnostic Method</th>
<th>Sensitivity Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>3</td>
<td>3 (100)</td>
<td>\textit{M bolletii}/\textit{M massiliense}</td>
<td>AFB cultures followed by sequencing of \textit{hsp65}</td>
<td>S: Clarithromycin, azithromycin</td>
</tr>
<tr>
<td>Christie,\textsuperscript{20} 2006</td>
<td>1</td>
<td>1 (100)</td>
<td>\textit{M fortuitum}</td>
<td>AFB culture</td>
<td>Unknown</td>
</tr>
<tr>
<td>Redbord et al,\textsuperscript{21} 2006</td>
<td>4</td>
<td>4 (100)</td>
<td>\textit{M fortuitum}/\textit{M peregrinum}</td>
<td>AFB culture</td>
<td>\textit{S}: Amikacin, imipenem, doxycycline, ciprofloxacin, gatifloxacin, moxifloxacin, \textit{R}: Tobramycin, azithromycin</td>
</tr>
<tr>
<td>Grubb,\textsuperscript{21} 2005</td>
<td>1</td>
<td>1 (100)</td>
<td>\textit{M chelonae}</td>
<td>AFB culture</td>
<td>Unknown</td>
</tr>
<tr>
<td>Gira et al,\textsuperscript{11} 2004</td>
<td>2</td>
<td>2 (100)</td>
<td>\textit{M mageritense}</td>
<td>Bacterial culture (non-AFB)</td>
<td>S: Clarithromycin, amikacin</td>
</tr>
<tr>
<td>Sniezek et al,\textsuperscript{2} 2003</td>
<td>3</td>
<td>2 (67)</td>
<td>\textit{M chelonae} &amp; \textit{M fortuitum}</td>
<td>Bacterial and AFB cultures</td>
<td>S: Clarithromycin, ciprofloxacin, R: Tobramycin, azithromycin</td>
</tr>
<tr>
<td>Winthrop et al,\textsuperscript{8} 2002</td>
<td>34</td>
<td>24 (71)</td>
<td>\textit{M fortuitum} (32)</td>
<td>Bacterial and AFB cultures followed by HPLC, PFGE, and MLEE</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Abbreviations: AFB, acid-fast bacilli; HPLC, high-performance liquid chromatography; \textit{hsp65}, 65-kDa heat-shock protein gene; I, intermediate; MLEE, multilocus enzyme electrophoresis; PFGE, pulsed-field gel electrophoresis; R, resistant; S, sensitive; \textit{TMP-SMX}, trimethoprim-sulfamethoxazole.
nation of clarithromycin, doxycycline, azithromycin, and moxifloxacin.

It is likely that mycobacterial infections from whirlpool footbaths are underrecognized and underreported. Vugia et al\textsuperscript{23} conducted a survey of nail salons in the 5 largest counties across California and found that 97% of footbaths (29 of 30) were contaminated with NTM and 50% carried more than 1 species. Mycobacterium fortuitum was the most prevalent. The study resulted in the adoption of regulations by the California Board of Barbering and Cosmetology for disinfection of the footbaths.\textsuperscript{23} In light of the prevalence of NTM organisms in whirlpool footbaths, clinicians must have a high level of suspicion when patients develop skin lesions of the lower extremities that show no organisms on culture or are refractory to conventional antibiotic therapy and must correctly identify any NTM species cultured from lesions. Public health measures, including educating the public about the risks of infection after pedicure and increased regulation of the nail salon industry, can have a major role in the prevention of mycobacterial outbreaks.

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