Mycobacterium bolletii/Mycobacterium massiliense Furunculosis Associated With Pedicure Footbaths
A Report of 3 Cases

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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 suppurative 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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Ontuberculous mycobacteria (NTM) are significant human pathogens causing systemic and cutaneous disease. Recently, the incidence of cutaneous infections associated with NTM has risen because of increased incidence of immunosuppression, increased number of surgical procedures, and improved detection methods.1,2 The most common NTM associated with cutaneous infections are Mycobacterium marinum and the rapidly growing mycobacteria Microbacterium fortuitum, Mycobacterium chelonae, and Mycobacterium abscessus.3-5 Rapidly growing mycobacteria are ubiquitous in soil and water6,7 and have been detected in chlorinated municipal water systems in the United States.8 They can be difficult to eradicate because they are resistant to many disinfectants.2 Infections have been most commonly reported following invasive cosmetic procedures such as liposuction and mammoplasty,3 intramuscular injections such as mesotherapy,9,10 and penetrating trauma predisposing to environmental contamination10,11; immunocompetent patients are increasingly affected.7 The diagnosis is often overlooked,2 and prolonged treatment can be necessary.4,12

We report 3 cases of lower extremity furunculosis caused by Mycobacterium bolletii/M massiliense, both recently described species.13 The patients were patrons of the same nail salon in North Carolina where they received footbaths and pedicures prior to the development of skin lesions. Mycobacterium bolletii/M massiliense was isolated from the lesions of 2 of the patients and identified by sequencing the 16S ribosomal RNA (rRNA) and hsp65 (65-kDa heat-shock protein) genes. Mycobacterial cultures from the third patient showed no growth; however, similar lesions and patronage of the same nail salon as the first 2 patients suggested M bolletii/M massiliense as the likely infectious agent.

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, doxycycline 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 therapy was continued for 6 months, during which time the patient’s lesions completely resolved.

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 rRNA 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
Mycobacterium bolletii is a recently described\textsuperscript{13} species of NTM classified as a member of the \textit{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,\textsuperscript{1,14} as well as an opportunistic pathogen in patients with underlying pulmonary disorders.\textsuperscript{2} \textit{Mycobacterium bolletii} was first identified in 2006 and reported in 2009,\textsuperscript{15} isolated from sputum from a patient with chronic pneumonia and later from a group of patients with cystic fibrosis. The species was initially described\textsuperscript{13} as resistant to clarithromycin. \textit{Mycobacterium bolletii} shares 100\% 16S rRNA and 95.6\% \textit{rpoB} gene sequence similarity with \textit{M abscessus}.\textsuperscript{13} It is also closely related to \textit{M massiliense}, which was identified and classified as a member of the \textit{M chelonae–M abscessus} complex in 2004.\textsuperscript{16} Accurate identification and discrimination between \textit{M abscessus}, \textit{M massiliense}, and \textit{M bolletii} is difficult and requires sequencing of multiple gene targets beyond their identical 16S rRNA sequences.\textsuperscript{17} Zelazny et al\textsuperscript{17} demonstrated a multilocus sequence analysis technique that included sequencing of \textit{rpoB}, \textit{hsp65}, and \textit{secA} for the molecular identification of 42 clinical isolates that had previously been classified as \textit{M abscessus}. The authors found that 7 isolates were \textit{M massiliense} and 2 were \textit{M bolletii}. Clinical manifestations were similar among the patients. The authors recommended this approach for identification of \textit{M abscessus} and closely related species, and it has recently been used\textsuperscript{10,18} in the classification of nosocomial outbreaks of \textit{M bolletii} and \textit{M massiliense}.

Few reported cases of \textit{M bolletii} and \textit{M massiliense} infection exist because of their recent identification. In 2008,\textsuperscript{14} both organisms were identified among South Korean isolates that were previously misclassified as \textit{M chelonae} or \textit{M abscessus}; of 144 isolates studied, 2 were reidentified as \textit{M bolletii} and 59 were reidentified as \textit{M massiliense}. Identification required sequencing of \textit{rpoB} and \textit{hsp65} regions.\textsuperscript{14} In Brazil, 311 immunocompetent individuals developed cutaneous infections attributed to \textit{M abscessus} after undergoing invasive cosmetic procedures.\textsuperscript{10} Reidentification of the isolates using \textit{hsp65} and \textit{rpoB} sequencing classified 59 of 67 isolates, all from surgical and postsurgical abscesses, as \textit{M massiliense} and the remaining 8, all from patients who had undergone mesotherapy, as \textit{M bolletii}. To our knowledge, our case series represents the first documented outbreak of \textit{M bolletii}/\textit{M massiliense} furunculosis, identified by \textit{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 \textit{M bolletii} and \textit{M massiliense}.

Mycobacterial furunculosis is characterized clinically by an initially benign nodular appearance, with a
protracted course that can result in scarring. 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. Lesions typically develop 1 to 2 months after introduction of the organism. Rapidly growing mycobacteria are ubiquitous in municipal water supplies, and recently, footbaths preceding pedicures at nail salons have been implicated as reservoirs of infection, where the organisms thrive in the nutrient-rich water. Case reports in 2005 and 2006 determined, by pulsed-field gel electrophoresis, to match the patients’ isolates. Case reports in 2005 and 2006 determined, by pulsed-field gel electrophoresis, to match the patients’ isolates.

### 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>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>M boltetii/M massilisense</td>
<td>AFB cultures</td>
<td>S, Clarithromycin, azithromycin</td>
</tr>
<tr>
<td>Christie,20 2006</td>
<td>1</td>
<td>1 (100)</td>
<td>M fortuitum</td>
<td>AFB culture</td>
<td>R, TMP-SMX, ciprofloxacin</td>
</tr>
<tr>
<td>Redbord et al,20 2006</td>
<td>4</td>
<td>4 (100)</td>
<td>M fortuitum/M peregrinum</td>
<td>AFB culture</td>
<td>Unknown</td>
</tr>
<tr>
<td>Gira et al,11 2004</td>
<td>2</td>
<td>2 (100)</td>
<td>M chelonae</td>
<td>Bacterial culture (non-AFB)</td>
<td></td>
</tr>
<tr>
<td>Grubb,21 2005</td>
<td>1</td>
<td>1 (100)</td>
<td>M mageritense</td>
<td>AFB culture</td>
<td>S, Clarithromycin, amikacin</td>
</tr>
<tr>
<td>Sniezek et al,2 2003</td>
<td>3</td>
<td>2 (67)</td>
<td>M chelonae/M fortuitum</td>
<td>Bacterial and AFB cultures</td>
<td></td>
</tr>
<tr>
<td>Winthrop et al,8 2002</td>
<td>34</td>
<td>24 (71)</td>
<td>M fortuitum (32) Unidentified (2)</td>
<td>Bacterial and AFB cultures followed by HPLC, PFGE, and MLEE</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** AFB, acid-fast bacilli; HPLC, high-performance liquid chromatography; hsp65, 65-kDa heat-shock protein gene; I, intermediate; MLEE, multilocus enzyme electrophoresis; PFGE, pulsed-field gel electrophoresis; R, resistant; S, sensitive; TMP-SMX, trimethoprim-sulfamethoxazole.

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 *M boltetii/M massilisense* from contaminated whirlpool footbaths. Our report further documents the pathogenicity of these newly identified species of rapidly growing mycobacteria. Accurate identification of *M boltetii* and *M massilisense* and differentiation from the other species of the *M chelonae-M abscessus* complex often depends on multilocus gene sequencing and is important given the differences in antibiotic resistance patterns. *Mycobacterium boltetii* 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. 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 *M boltetii* is thought to be resistant. However, in vitro susceptibilities 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.

However, Winthrop et al 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. All patients in our series responded to a combi-
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nalization of clarithromycin, doxycycline, azithromycin, and moxifloxacin.

It is likely that mycobacterial infections from whirlpool footbaths are underrecognized and underreported. Vugia et al. 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. 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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Author Contributions: Ms Wertman and Drs Morrell and Culton had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Morrell and Culton. Acquisition of data: Wertman, Miller, Morrell, Groben, and Culton. Analysis and interpretation of data: Wertman, Miller, Groben, and Culton. Drafting of the manuscript: Wertman, Morrell, and Culton. Critical revision of the manuscript for important intellectual content: Miller, Morrell, Groben, and Culton. Administrative, technical, or material support: Miller, Morrell, Groben, and Culton. Study supervision: Morrell, Groben, and Culton.

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REFERENCES