Case of *Mycobacterium marinum* Infection with Unusual Patterns of Susceptibility to Commonly Used Antibiotics

Nicole Parrish, Ronald Luethke, Kim Dionne, Karen Carroll, and Stefan Riedel*

Johns Hopkins Medical Institutions, Baltimore, Maryland

Received 6 October 2010/Returned for modification 28 October 2010/Accepted 15 March 2011

*Mycobacterium marinum*, found commonly in salt water and freshwater, is the causative agent of disease in many species of fish and occasionally in humans. MICs to most antimicrobial agents are relatively low. Susceptibility testing is not routinely performed, and single-drug therapy is used for the treatment of most infections. Here, we report an infection caused by a drug-resistant *M. marinum* strain in an otherwise healthy patient.

**CASE REPORT**

A 45-year-old healthy man sought medical care following a fish hook injury to the right third metacarpal area. Over the course of 10 to 14 days, he developed a tender lump over the right third metacarpal joint, which became increasingly tender to the touch. Otherwise, the patient was in good health, and his past medical history was unremarkable. On physical examination, he appeared well and was afebrile and in no acute distress. The examination of the hand was pertinent for no gross peritendinous fluid collection, tenosynovial thickening, or evidence of cellulitis. A magnetic resonance image (MRI) with and without contrast was obtained, indicating soft tissue swelling over the distal third metacarpal and proximal phalanx, with no evidence of osteomyelitis or abscess. Given the patient’s history and recent fish hook injury, the possibility of an atypical mycobacterial infection was raised. An incisional biopsy was performed, and a small full-thickness ellipse of the inflamed skin was removed and sent to the laboratory for microscopy and culture. Additional skin samples were sent to pathology for histology and special stains. The patient was treated with doxycycline (100 mg orally [p.o.], twice daily) and topical mupirocin.

Two weeks later, the patient returned for a follow-up visit. Physical examination revealed a residual, patchy, erythematous rash over the right third metacarpal, a small circular patchy rash over the right fourth metacarpal, and a similar rash over the dorsum of the right hand. These findings were clinically consistent with and interpreted as psoriasis. Examination of his trunk and extremities showed no other patches or lesions similar to those present on the right hand. He was advised to continue the doxycycline and follow up in 2 to 3 weeks. The patient was also given a prescription for 15 g of 0.05% fluocinonide (Lidex) ointment and instructed to use it sparingly on the affected area.

Initial cultures of the wound material grew an acid-fast bacillus after 18 days of incubation at 37°C using Lowenstein-Jensen medium. Probes for *M. tuberculosis*, *M. kansasii*, *M. avium*, and *M. gordonae* were negative (AccuProbe; Genprobe). Subsequently, growth was obtained from Middlebrook 7H11 selective plates at both 30°C and 37°C and used for identification by high-performance liquid chromatography (HPLC) (Sherlock Mycobacteria Identification System; MIDI, Newark, DE). By this method, the isolate was identified as *M. marinum*, with a similarity index of 0.65 and no second match. DNA sequencing of a portion of the hypervariable region of the 16S rRNA gene confirmed the identification of *M. marinum/M. ulcerans*.

The patient returned 2 weeks after the prior visit and presented with a persistent, nonhealing, irregular erythematous nodule with a diminished plaque and an individual punctate elevation of the skin. He was advised to continue the doxycycline twice daily, and the fluocinonide was discontinued. Susceptibility testing on this isolate was requested and performed by a large reference laboratory according to established protocols using broth microdilution and a quality control strain of *M. avium* (ATCC 700898) susceptible to both doxycycline (MIC, 2 μg/ml) and rifampin (MIC, 1 μg/ml). Assays were conducted as recommended by the Clinical and Laboratory Standards Institute (CLSI) (5). Based on current CLSI resistance breakpoints established for broth microdilution and a quality control strain of *M. avium* (ATCC 700898) susceptible to both doxycycline (MIC, 2 μg/ml) and rifampin (MIC, 1 μg/ml), susceptibility testing results were comparable between the two methods.

The prototype multidrug-resistant *M. tuberculosis* (MDR-TB) PCR TruArray kit (Akonni Biosoftware, Frederick, MD) was used to determine if the most common *rpoB* mutations associated with rifampin resistance were present in this isolate. Represented in this assay are predominant mutations found at codons 516, 526, 531, and 533 in the rifampin resistance-determining region (RRDR). Published sequences for *rpoB* (H37Rv, ATCC 27294) and *M. marinum* (ATCC BAA-535) indicated a >80% identity within the RRDR between the two species. PCR amplification of this region was obtained with the ATCC strains of *M. tuberculosis* and *M.
* Mycobacterium marinum* is a nontuberculous photochromogenic *Mycobacterium* (NTM) species belonging to group I of Runyon’s classification (12). The natural habitats for this organism are freshwater and salt water (17, 18). The organism is a photochromogenic *Mycobacterium* species with optimal growth at 30°C. The organism is known to cause typical disease in humans after traumatic inoculation and waterborne exposures, often referred to as “fish-tank granuloma” (2). Infections are generally limited to disease in skin and soft tissue, resembling sporotrichosis; however, cases of tenosynovitis, bursitis, arthritis, osteomyelitis, and disseminated infection in immunocompromised patients have been documented (3, 6, 8, 10, 20, 21). In undiagnosed or underrecognized cases of infection due to *M. marinum*, the disease process can slowly progress and involve contiguous sites of soft tissue, tendons, joints, and bones. In rare cases, disseminated disease has been reported (14).

To our knowledge, no controlled studies have been conducted to evaluate optimal treatment regimens for *M. marinum* infection, because studies for susceptibility testing involving large numbers of strains are rare. Localized cutaneous infections may resolve spontaneously over a prolonged period of time, extending months to years (7). Furthermore, the MIC to most drugs is relatively low. However, such findings have been hampered by the limited numbers of strains and antibiotics tested and the lack of a standardized susceptibility test method specific for *M. marinum* (13, 15, 16).

Antibiotic monotherapy is usually reserved for patients with uncomplicated, localized cutaneous infections. Combinations of two or more antibiotics are used for more serious infections and typically contain combinations of clarithromycin and minocycline-doxycycline or rifampin-ethambutol and trimethoprim-sulfamethoxazole (1). Regardless of the drug(s) utilized, clinical response is generally slow, requiring 3 to 6 months of therapy for localized disease and >12 months for disseminated disease (4, 7, 9).

To date, few studies have been conducted examining the antibiotic susceptibility of *M. marinum* strains isolated from clinical specimens or natural sources. What has been shown is that antibiotic resistance in *M. marinum* is relatively rare in strains isolated from nature (19). In addition, most isolates are intrinsically resistant to isoniazid and pyrazinamide and produce β-lactamase (19). In the most comprehensive studies conducted to date, higher MICs to doxycycline have been reported. In these studies multiple clinical isolates (*n* = 98) of *M. marinum* were tested to determine the MIC50 and MIC90 values to doxycycline and other antibiotics, including rifampin. The MIC50 values for doxycycline ranged from 2 μg/ml to 8 μg/ml, and the MIC90 values ranged from 6 μg/ml to 16 μg/ml. In comparison, MICs to rifampin were consistently low, ranging from 0.25 μg/ml to 0.50 μg/ml (MIC50) and 0.5 μg/ml to 3 μg/ml (MIC90). Resistance to rifampin was not observed in either study (1, 2).

To our knowledge, intrinsic resistance to rifampin has not been definitively described in *M. marinum*. Most antibiotic resistance in the genus *Mycobacterium* is mediated by a single chromosomal mutation or accumulation of mutations. These mutations may be spontaneous or occur due to direct exposure of the organism to a particular drug. In this case, the MIC to rifampin (≥16 μg/ml) is unusual in that the patient had no prior exposure to rifampin or any other rifamycins. This suggests that selective pressure in the conventional sense may not have played a role in the development of resistance. However, unexplained rifampin resistance is not without precedent in various bacteria and possibly *M. marinum*. Recent environmental surveys of both freshwater- and salt water-borne bacteria have documented unexplained antibiotic resistance to various antimicrobial drugs, including rifampin. Although it is not proven, Manjusha and coworkers speculated that exposure to agricultural runoff or human waste containing antibiotics may play a role in the recent increase in drug resistance in bacteria found in natural environments (11). In the present study, the RRDR of rpoB failed to amplify in the clinical strain, suggesting that significant differences exist in this region versus the type strain of *M. marinum*, for which the sequence is known. Although the nature of these differences and the exact mechanism of resistance remain unknown, questions remain with respect to antibiotics used for treatment of human and animal infections and those associated with current aquaculture practices.

Here, we describe an isolate of *M. marinum* as a cause of a prolonged skin and soft tissue infection after peripheral traumatic injury by a fish hook. The infection did not resolve upon treatment with doxycycline. In addition, the *in vitro* susceptibility studies demonstrated resistance to doxycycline and rifampin. Our patient had no prior rifampin exposure. However, such exposure might be considered less relevant, as *M. marinum* is not part of normal or resident flora in humans and therefore not subject to prior or ongoing antimicrobial treatment. This case raises potential questions as to the impact of antibiotic use in aquaculture and exposure of open water bodies to agricultural runoff and human waste containing unmetabolized antibiotics or metabolic by-products. Further studies are necessary to evaluate the presence of antimicrobial resistance in such organisms, as continuation of current practices may have serious public health implications.

We thank Yvonne Linger and Charles Daitch at Akonni Biosystems, Frederick, MD, for their valuable assistance with the rpoB genetic analysis used in this study.
REFERENCES


