

Disease Attributed to *Mycobacterium chelonae* in South African Clawed Frogs (*Xenopus laevis*)

Sherril L. Green,^{1*} Barry D. Lifland,¹ Donna M. Bouley,¹ Barbara A. Brown,² Robert J. Wallace, Jr.,² and James E. Ferrell, Jr.³

Abstract | The fast-growing nontuberculous mycobacterial species *Mycobacterium chelonae* was isolated from six captive South African clawed frogs (*Xenopus laevis*) with chronic weight loss and nonhealing ulcerative skin lesions. Three of the *M. chelonae* isolates were evaluated to confirm the species identification using polymerase chain reaction restriction analysis. Disease associated with *M. chelonae* is reported mainly in people and in fish. To our knowledge, this is the first report of disease associated with *M. chelonae* in a colony of captive *Xenopus* sp.

Mycobacterium chelonae is a nontuberculous rapidly growing mycobacterium, and is one of the most common causes of nontuberculous cutaneous disease in humans (1–3). The organism is an acid-fast, free-living, ubiquitous species that is present in soil and water (4). It is one of three common species included in the *M. fortuitum* complex, with the other two being *M. fortuitum* and *M. abscessus*. *Mycobacterium chelonae* is an opportunistic pathogen, and is an increasingly recognized cause of disease in immunocompromised patients, particularly patients on long-term corticosteroid therapy (3, 5–7). Community-acquired and nosocomial infections are suspected to be acquired directly from the environment, via inhalation through the respiratory tract (8–10) or through contamination of broken epithelial surfaces of the skin (1, 6, 7, 11–13). After cutaneous inoculation, clinical disease is generally evident within four to six weeks. Localized cutaneous infections after surgery, other medical procedures, including medical injections and catheter placement, and minor trauma have been described (1, 7, 13, 14). Skin infections often appear as chronic draining abscesses or nonhealing erosive ulcers (12). In immunocompromised patients, *M. chelonae* may induce disseminated disease (6, 7). *Mycobacterium chelonae* is highly resistant to most antimicrobials other than clarithromycin and tobramycin (5, 7, 14–16). Infections attributable to *M. chelonae* can, therefore, be difficult to treat, and have been associated with development of drug resistance and treatment failures (17).

Infections attributed to *M. marinum* have been reported to induce infectious granulomas in *Xenopus laevis* (18), but to the authors' knowledge, disease in this species due to *M. chelonae* has not been described. Although mycobacteriosis resulting from *M. chelonae* infection has been reported principally in humans and diseased turtles (19) and is an important problem in the fish industry (20–23), it is rarely recognized as a pathogen in other animal species (21, 22). Here we describe the clinical findings, pathologic changes, and molecular characterization of *M. chelonae* associated with disease in a colony of captive South African clawed frogs (*Xenopus laevis*).

Materials and Methods

Case histories: Between January 1997 and March 1999, *M.*

chelonae was isolated from six sexually mature female frogs from the *Xenopus laevis* colonies at Stanford University's School of Medicine research animal facility. The affected frogs were purchased from a single commercial supply house (Nasco, Madison, WI), and had been present in the facility for three weeks to eight months. The affected frogs were culled from an approximately 1,100-member colony maintained at Stanford University for cell cycle research, using *Xenopus laevis* oocytes and eggs under an animal use protocol approved by the Stanford Animal Use and Care Committee. Animal care and housing were conducted according to recommendations in the NIH Guide for general animal housing and the local, state, and federal fish and game regulations. Frogs were housed in self-flushing dark green opaque bathtub-style 200-L tanks, 100 frogs/tank, and were fed trout chow 3 times/wk, 3 hours prior to a filtered water flush, replacing a third of the total water volume. Tank water temperature was maintained at approximately 21°C.

The treated, chlorinated, potable water from the municipal water supply is filtered by use of an in-pipeline charcoal filter system at the Stanford frog-housing rooms. Annual reports on the municipal water quality (on the basis of weekly and bimonthly tests on water samples collected from campus water supplies) are provided to the animal facility by the Stanford University Water Utilities Division and the city of Stanford's Public Utilities Commission, which is in compliance with the US Environmental Protection Agency and the California Department of Health Services. These agencies test for inorganic, organic, and radioactive water contaminants, water turbidity, and microbial contaminants (coliforms, and *Cryptosporidium* and *Giardia* spp.), pH, and water hardness, pesticide, herbicide, and heavy metal contents. All results met State and Federal drinking water regulations. The average values for water quality parameters in samples collected from sources supplying the animal facility during the study period were: pH 8.7; hardness (as CaCO₃) 60 ppm; total coliform bacteria 0 (% positive samples); fluoride 0.89 ppm; magnesium 6 ppm; potassium 0.8 ppm; copper 70 ppb; lead 2.8 ppb; aluminum < 50 ppb, chlorine 0.8 ppm. In addition, the Stanford Veterinary Service Center Diagnostic Laboratory monitors frog tank water quality (after the water has been filtered through the pipeline charcoal filter system in the frog-housing rooms) using a commercially available water analysis kit (Voluette™ Analytical Standards, Hatch Company, Loveland,

From the Departments of ¹Comparative Medicine, and ³Molecular Pharmacology, Stanford University, Stanford, CA 94305, and the ²Department of Microbiology, University of Texas Health Center, Tyler, TX 75708.

* Corresponding author

CO). During the study period (water samples collected biweekly during the months of the frog deaths), the average values for the following parameters, using this test kit were: pH 8.2; chlorine 0.10 ppm; ammonia 0.25 mg/L; nitrite 0.25 mg/L; nitrate 0.0 mg/L, copper 0.25 g/L, water fecal coliform count < 2,000/100 ml. All values were within ranges considered safe for aquatic amphibians.

The facility has maintained an average daily census of 1,100 *Xenopus laevis* under these conditions for the past 4 years. Between 1997 and 2000, morbidity and mortality in the colony has been attributed principally to sporadic infection with *Aeromonas hydrophila* ("red leg" syndrome), fungal infections (*Saprolegnia* sp.) or infection with *Flavobacterium meningosepticum* (24).

In accordance with the investigator's institutionally approved animal use protocol, egg laying had been induced in all 6 of the frogs by administration of 30 IU of pregnant mare serum gonadotropin given subcutaneously in the dorsal lymph sac, followed by induction of ovulation four days later by injection of 800 IU of human chorionic gonadotropin (into the dorsal lymph sac). The frogs were subsequently housed for the short term (24 to 72 hours) individually in approximately 2 to 3 L of de-chlorinated water in 5-gallon buckets so that the freshly laid eggs could be easily collected. On return to regular housing, four of the six frogs had focal ulcerations/abrasions at the points of the hocks and elbows, presumably related to trauma when attempting to swim or jump in the 5-gallon buckets. All frogs were noticeably lethargic and were in poor body condition. Affected frogs were isolated and maintained in 50 × 40 × 20-cm polycarbonate cages, 1 to 2 frogs/cage, containing 2 L of isotonic saline. After 10 to 14 days, there was no evidence that the skin wounds were healing and all 6 frogs were euthanized by administration of 3 ml of a 10% solution of tricaine, given intraperitoneally, and necropsy was performed.

Three years prior to this report, *M. chelonae* had been isolated from a diseased goldfish (*Carassius auratus*) in the animal facility, but the organism had not been isolated from any of the other aquatic species that share the same water supply, including leopard frogs (*Rana pipiens*), giant toads (*Bufo marinus*), red-bellied newts (*Cynops pyrrhogastor*), and spotted salamanders (*Ambystoma maculatum*).

Necropsy findings: Four of six frogs had focal ulcerative wounds ranging from 0.1 to 0.5 cm on the points of the hocks and/or elbows, and in one case, the focal ulcerative lesions extended up the dorsum of the hind limbs and forelimbs (Figure 1, A and B). All affected frogs were in poor body condition and appeared thin. There were no grossly visible lesions in any of the internal viscera, with the exception of one frog, in which there was a large mildly cystic mass associated with the spleen. All frogs had histopathologic evidence of multifocal, mild to moderate granulomatous hepatitis, nephritis, splenitis, and myositis (Figure 2, A and B). A uniform population of large mononuclear cells with abundant, mildly vacuolated eosinophilic cytoplasm and large, oval to round, centrally located nuclei (epithelioid macrophages) and randomly infiltrated renal, splenic, and hepatic parenchyma. Marked infiltration with similar cells in the endomysium markedly displaced myofibers and disrupted the normal histoarchitecture (Figure 2). A few muscle fibers, surrounded by inflammatory cells, had typical hyaline degeneration (Zenker's necrosis). The splenic mass seen grossly in one frog was determined to be consistent with the lesion described as "infectious granuloma" seen in *Xenopus laevis* infected with

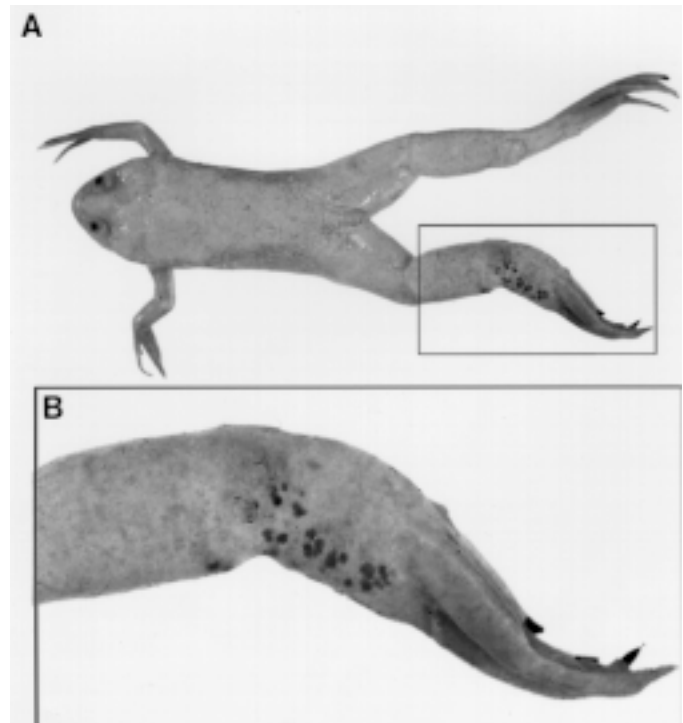


Figure 1. (A) Albino South African clawed frog with weight loss (notice the concave, collapsed appearance of the abdomen) and cutaneous lesions attributed to *Mycobacterium chelonae*. (B) Multifocal ulcerated lesions spread in a sporotrichoid-like manner on the dorsum of the swollen distal portion of the extremity.

M. marinum (18). Lesions were not apparent in the heart, lungs, brain, stomach, intestines, or pancreas.

Microbiological examination: Tissue specimens (skin, liver, spleen, and/or heart blood) were incubated at 29 to 30°C for 18 to 48 hours on agar plates, including: 5% sheep blood agar, chocolate agar, MacConkey agar, phenylethyl alcohol (PEA) with 5% sheep blood and Rimler-Shotts agar. A Middlebrook 7H11/Mitchison 7H11 selective biplate (Remel, Lenexa, KS) was added to the routine panel when acid-fast organisms were suspected.

Results

Mycobacterium chelonae was isolated from the skin, liver, spleen and/or heart blood of all six frogs, and the organism was identified on the basis of biochemical characteristics, results of high-performance liquid chromatography (HPLC), and molecular characterization. In three of the six frogs that were culture positive for *M. chelonae*, rare acid-fast bacilli were observed in liver and spleen, and numerous organisms were seen in the skeletal muscle lesions subjacent to the chronic skin ulcerations. Other organisms were not isolated from the tissues of the affected frogs.

A light growth of acid-fast bacilli was first noted after the fifth to seventh day of incubation. Preliminary classification of the mycobacteria species was based on growth characteristics including: colony morphology (rough), primary growth at 30°C in 5 to 7 days, no pigmentation or photoreactivity on LJ egg-based medium, and positive growth on MacConkey agar without crystal violet (25).

Isolates were sent to the National Jewish Medical Research Center (Denver, CO) for confirmatory biochemical testing. One of the isolates was also sent to the San Mateo County Public

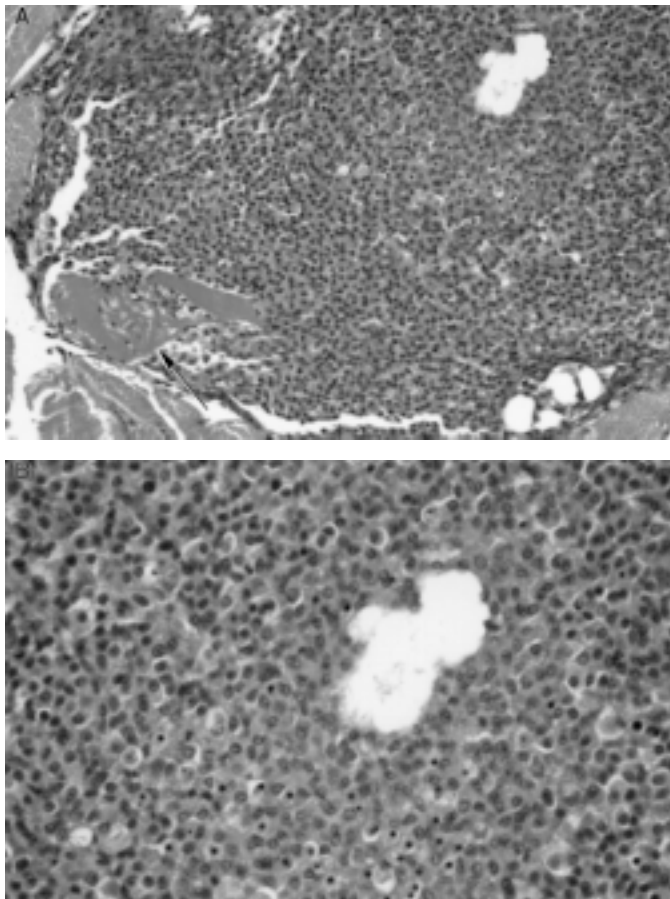


Figure 2. (A) Photomicrograph of a section of skeletal muscle from the distal portion of the extremity of the frog in Figure 1. Dense granulomatous inflammation markedly displaces myofibers and surrounds an isolated necrotic myofiber (arrow). H&E stain; original magnification, $\times 11.5$. (B) Higher magnification of the monomorphic population of macrophages that comprised the inflammatory infiltrate. H & E stain; original magnification, $\times 46$.

Health Laboratory (San Mateo, CA) for chromatographic analysis of the cell wall lipids by use of HPLC for species confirmation of mycobacteria.

Species identification by use of polymerase chain reaction (PCR) restriction analysis and DNA fingerprinting:

Four of the aforementioned *M. chelonae* isolates were referred to the Mycobacteria/Nocardia Laboratory at the University of Texas Health Center (Tyler, TX) to confirm species identification by use of PCR restriction analysis. A 439-bp segment of the 65-kDa heat shock protein gene was amplified from cell supernatants of the four isolates of *M. chelonae*, together with appropriate positive and negative controls, as recommended by Telenti et al. (26) and Steingrube et al. (27) (Figure 3, A and B). Restriction fragment analysis was performed, using commercially available endonucleases and agarose gels stained with ethidium bromide. Band sizes in base pairs were estimated by use of a computerized Bio Image system (27). Restriction fragment length polymorphisms (RFLP) pattern analysis of genomic DNA, using pulsed field gel electrophoresis (PFGE), was performed as described (28) on isolates from two of the affected frogs and the diseased goldfish (Figure 4) (29, 30). One of the three frog isolates was nonviable on subculture.

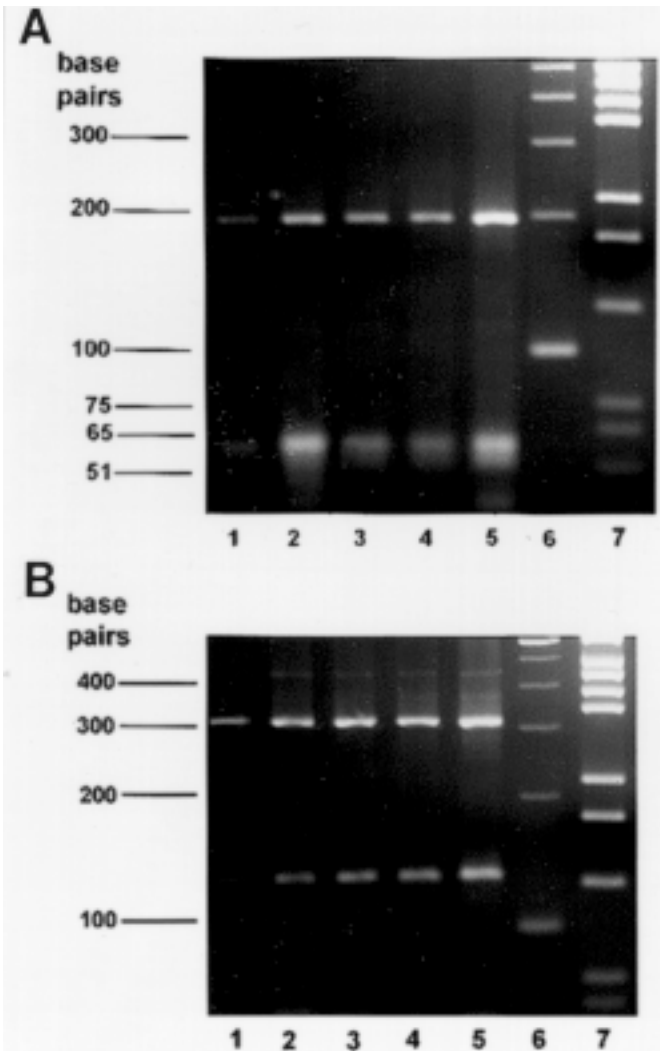


Figure 3. Comparison of *Hae*III and *Bst*EII restriction fragment length polymorphism (RFLP) patterns of *M. chelonae* isolates associated with clinical disease in *Xenopus laevis* and a goldfish housed in the animal research facility. (A) The *Hae*III digests. Lanes: 1 to 3, *M. chelonae* isolates from diseased *Xenopus laevis*; 4, *M. chelonae* isolate from a diseased goldfish; 5, *M. chelonae* ATCC 35752 isolate; 6, 100-bp ladder; 7, pGEM ladder. (B) The *Bst*EII digests. Lane designations are the same as those for panel A.

Discussion

The historical, clinical, and histopathologic features of disease attributed to *M. chelonae* in captive *Xenopus laevis* are remarkably similar to those described in human infections. In particular, focal nonhealing ulcerated skin wounds in the frogs were striking, and in one frog, spread in the sporotrichoid-like manner that has been described in human dermatologic infection with *M. chelonae* (6, 11). As is typical of nontuberculous mycobacterial infection, the histologic lesions in the frogs were characterized by granulomatous inflammation, but caseation was not observed (1). Special stains detecting acid-fast bacilli can be insensitive detectors of the organisms. However, we observed acid-fast bacilli in tissue sections from three of six frogs. The acid-fast stains yielded our provisional diagnosis of infection with a mycobacterial species.

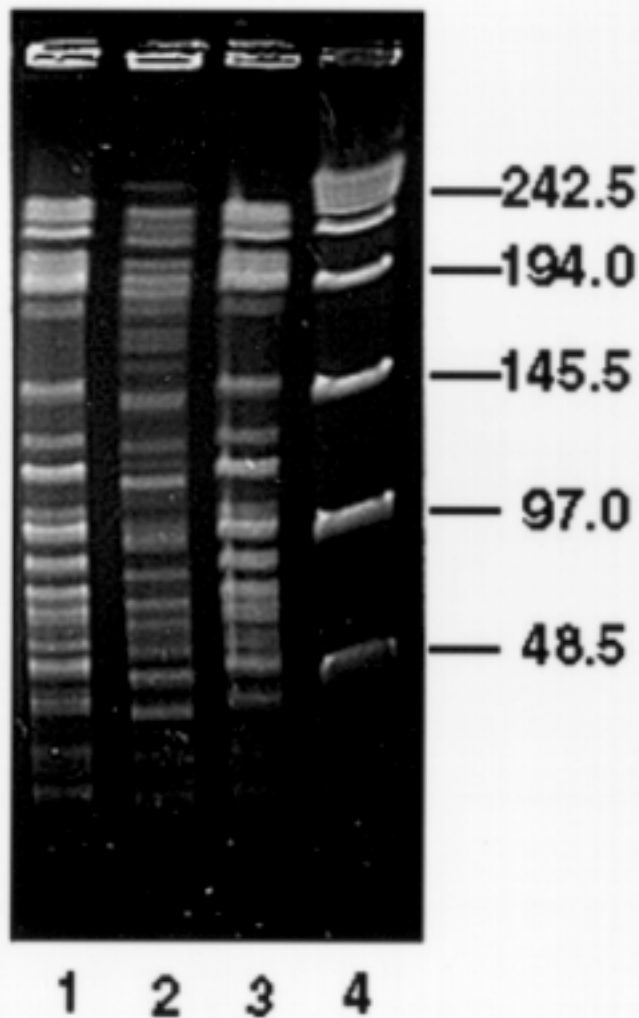


Figure 4. Pulsed field gel electrophoresis (PFGE) patterns of *M. chelonae* isolates associated with clinical disease in a goldfish and two *Xenopus laevis*. Isolates were digested by use of *Xba*I and *Dra*I. Lanes: 1, *M. chelonae* isolate from a diseased *Xenopus laevis*, 1998; 2, *M. chelonae* isolate from a diseased goldfish, 1997; 3, *M. chelonae* isolate from a diseased *Xenopus laevis*, 1999; 4, lambda DNA standard.

On microbial culture we were able to distinguish the organisms as members of the Runyon classification group IV, a subtype of nontuberculous mycobacteria termed rapidly growing because they typically mature easily and produce visible colonies within three to five days in temperatures ranging from 25 to 45°C (4, 31). Other groups of mycobacteria require at least two weeks to produce mature growth. In addition, we were able to grow sufficient amounts of one of the frog isolates for analysis by use of HPLC.

Although the biochemical test and HPLC results supported our suspicion that *M. chelonae* was the disease-causing organism in the frogs, these techniques are limited by the time it takes to complete them and the amount of material (for HPLC) required. Molecular characterization by use of PCR restriction analysis and PFGE is a more definitive method to consistently speciate mycobacteria and determine their relatedness, if any (27, 29).

Three of the six original *M. chelonae* isolates from the frogs were available and viable for speciation by use of PCR restric-

tion analysis. Two of the three were evaluable by use of RFLP analysis of genomic DNA, using PFGE. The PCR RFLP pattern was identical to that reported for *M. chelonae* (7, 16, 22, 26, 27). The RFLP/ PFGE banding pattern of *M. chelonae* from one of the diseased frogs (from 1998) and from the diseased goldfish (from 1997) were similar. The isolate from the other frog (1999) clearly differed from the other two. This suggests that the three isolates were not related epidemiologically.

We have not isolated this organism from any other healthy or diseased animal species in our facility. The source of the infection in this report was not identified, but *M. chelonae* is likely present in the animal facility environment. *Mycobacterium chelonae* infection in the *Xenopus laevis* may have been acquired via penetration of environmental organisms at the injection sites, or through the skin wounds on the distal portion of the extremities. It is also possible that the frogs of this report were harboring an infection that was acquired in the wild, because several were wild caught by the supplier. *Mycobacterium chelonae* has been isolated from apparently healthy wild-caught Amazonian toads (*Bufo marinus* and *B. granulosus*), amphibian species that share a similar habitat (32). The stress of the experimental manipulations and physical handling (e.g., disruption of the protective amphibian "slime coating" of the skin), were possible factors contributing to infection and disease in the *Xenopus laevis* described here (14, 16, 33).

Several recent reports have documented the efficacy of the newer macrolides, clarithromycin and azithromycin (7, 14, 15, 28), for treatment of *M. chelonae* infection. However, we did not determine the antimicrobial susceptibility patterns for the *M. chelonae* isolates or attempt to treat the affected frogs with anti-mycobacterial drugs. Minimal inhibitory concentrations have not been established in *Xenopus laevis* for most drugs, including clarithromycin or azithromycin.

Although the disease attributable to *M. chelonae* is sporadic and infrequent in the *Xenopus* colony (the six cases reported here were collected over a three-year period), frogs with signs of infection are now immediately culled. The infection is not generally transmitted from human to human or from frog to frog, and there has been no transmission of *M. chelonae* from infected frogs to any human handlers in our facility.

However, the organism is considered a potential zoonotic pathogen. Other mycobacterial species have been recovered from swimming pools and fish tank water that were apparent sources of human infection (8). We have grown several mycobacterial species, including *M. chelonae*, from frog water tank samples, but have not yet confirmed the species identification of the water mycobacteria by use of PCR restriction analysis. Nevertheless, laboratory research personnel and husbandry staff are advised to wear gloves when handling the frogs, frog water, and related equipment. There is some evidence that *M. chelonae* is resistant to 2% alkaline activated glutaraldehyde and that other disinfectants, such as peracetic acid, chlorine dioxide, and alcohol, are more effective mycobactericidals (34, 35). Use of these disinfectants is advised in animal facilities housing *Xenopus laevis*, particularly where disease attributable to *M. chelonae* has been documented.

Acknowledgments

We thank Yansheng Zhang, Linda Mann, and Desiree Monteiro for their contributions.

References

1. **Wallace, R. J., Jr., J. M. Swenson, V. A. Silcox, et al.** 1983. Spectrum of disease due to rapidly growing mycobacteria. *Rev. Infect. Dis.* **5**:657–679.
2. **Horsburgh, C. R., Jr., D. L. Hanson, J. L. Jones, et al.** 1996. Protection from *Mycobacterium avium* complex disease in human immunodeficiency virus-infected persons with a history of tuberculosis. *J. Infect. Dis.* **174**:1212–1217.
3. **O'Brien, R. J., L. J. Geiter, and D. E. J. Snider.** 1987. The epidemiology of nontuberculous mycobacterial diseases in the United States. Results from a national survey. *Am. Rev. Respir. Dis.* **135**:1007–1014.
4. **Sanders, Jr., W. E., and Horowitz, E. A.** 1990. Other *Mycobacterium* species, p. 1914–1926. *In* G. L. Mandel, R. G. Douglas, Jr., and J. E. Bennett (ed.), Principles and practice of infectious disease. Churchill, Livingston, New York.
5. **Schutt-Gerowitz, H.** 1995. On the development of mycobacterial infections. I. A review concerning the common situation. *Zentralbl. Bakteriologie*. **283**:5–13.
6. **Jopp-McKay, A. G., and P. Randell.** 1990. Sporotrichoid cutaneous infection due to *Mycobacterium chelonae* in a renal transplant patient. *Australas. J. Dermatol.* **31**:105–109.
7. **Wallace, R. J., Jr., B. A. Brown, and G. O. Onyi.** 1992. Skin, soft tissue, and bone infections due to *Mycobacterium chelonae chelonae*: Importance of prior corticosteroid therapy, frequency of disseminated infections, and resistance to oral antimicrobials other than clarithromycin. *J. Infect. Dis.* **166**:405–412.
8. **Wolinsky, E.** 1979. Nontuberculous mycobacteria and associated diseases. *Am. Rev. Respir. Dis.* **119**:107–159.
9. **Griffith, D. E., W. M. Girard, and R. J. Wallace, Jr.** 1993. Clinical features of pulmonary disease caused by rapidly growing mycobacteria. An analysis of 154 patients. *Am. Rev. Respir. Dis.* **147**:1271–1278.
10. **Wenger, J. D., J. S. Spika, R. W. Smithwick, et al.** 1990. Outbreak of *Mycobacterium chelonae* infection associated with use of jet injectors. *JAMA* **264**:373–376.
11. **Kelley, L. C., K. C. Deering, and E. T. Kaye.** 1995. Cutaneous *Mycobacterium chelonae* presenting in an immunocompetent host: Case report and review of the literature. *Cutis* **56**:293–295.
12. **Hautmann, G., and T. Lotti.** 1994. Atypical mycobacterial infections of the skin. *Dermatol. Clin.* **12**:657–668.
13. **Safranek, T. J., W. R. Jarvis, L. A. Carson, et al.** 1987. *Mycobacterium chelonae* wound infections after plastic surgery employing contaminated gentian violet skin-marking solution. *N. Engl. J. Med.* **317**:197–201.
14. **Saluja, A., N. T. Peters, L. Lowe, et al.** 1997. A surgical wound infection due to *Mycobacterium chelonae* successfully treated with clarithromycin. *Dermatol. Surg.* **23**:539–543.
15. **Tartaglione, T.** 1997. Treatment of nontuberculous mycobacterial infections: role of clarithromycin and azithromycin. *Clin. Ther.* **19**:626–638.
16. **Telenti, A., P. Imboden, F. Marchesi, et al.** 1993. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. *Lancet* **341**:647–650.
17. **Griffith, D. E., and R. J. Wallace, Jr.** 1996. New developments in the treatment of nontuberculous mycobacterial (NTM) disease. *Semin. Respir. Infect.* **11**:301–310.
18. **Asfari, M.** 1988. Mycobacterium-induced infectious granuloma in *Xenopus*: histopathology and transmissibility. *Cancer Res.* **48**:958–963.
19. **Friedmann, F. F.** 1930. Spontane Lungentuberkulose bei Schildkröten und die Stellung des Tuberkelbazillus im System. *Z. Tuberk.* **4**:439–451.
20. **Vogel, H.** 1958. Mycobacteria from cold-blooded animals. *Am. Rev. Tuberc.* **77**:823–838.
21. **Bruno, D. W., J. Griffiths, C. G. Mitchell, et al.** 1998. Pathology attributed to *Mycobacterium chelonae* infection among farmed and laboratory-infected Atlantic salmon *Salmo salar*. *Dis. Aquat. Organ.* **33**:101–109.
22. **Talaat, A. M., R. Reimschuessel, and M. Trucksis.** 1997. Identification of mycobacteria infecting fish to the species level using polymerase chain reaction and restriction enzyme analysis. *Vet. Microbiol.* **58**:229–237.
23. **Adams, A., K. D. Thompson, H. McEwan, et al.** 1996. Development of monoclonal antibodies to *Mycobacterium* spp. isolated from chevron snakeheads and siamese fighting fish. *J. Aqua. Anim. Health* **8**:208–215.
24. **Green, S. L., D. M. Bouley, R. J. Tolwani, et al.** 1999. Identification and management of an outbreak of *Flavobacterium meningosepticum* infection in a colony of South African clawed frogs (*Xenopus laevis*). *J. Am. Vet. Med. Assoc.* **214**:1833–1838.
25. **Metchock, B. G., F. S. Nolte, and R. J. Wallace, Jr.** 1999. *Mycobacterium*, p. 399–437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, et al (ed.), Manual of clinical microbiology. American Society for Microbiology Press, Washington.
26. **Telenti, A., F. Marchesi, M. Balz, et al.** 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. *J. Clin. Microbiol.* **31**:175–178.
27. **Steingrube, V. A., J. L. Gibson, B. A. Brown, et al.** 1995. PCR amplification and restriction endonuclease analysis of a 65-kilodalton heat shock protein gene sequence for taxonomic separation of rapidly growing mycobacteria [published erratum appears in *J. Clin. Microbiol.* 1995 Jun;33(6):1686]. *J. Clin. Microbiol.* **33**:149–153.
28. **Wallace, R. J., Jr., V. A. Silcox, M. Tsukamura, et al.** 1993. Clinical significance, biochemical features, and susceptibility patterns of sporadic isolates of the *Mycobacterium chelonae*-like organism. *J. Clin. Microbiol.* **31**:3231–3239.
29. **Wallace, R. J., Jr., Y. Zhang, B. A. Brown, et al.** 1993. DNA large restriction fragment patterns of sporadic and epidemic nosocomial strains of *Mycobacterium chelonae* and *Mycobacterium abscessus*. *J. Clin. Microbiol.* **31**:2697–2701.
30. **Hector, J. S., Y. Pang, G. H. Mazurek, et al.** 1992. Large restriction fragment patterns of genomic *Mycobacterium fortuitum* DNA as strain-specific markers and their use in epidemiologic investigation of four nosocomial outbreaks. *J. Clin. Microbiol.* **30**:1250–1255.
31. **Grange, J. M.** 1981. *Mycobacterium chelonae*. *Tubercle.* **62**:273–276.
32. **Mok, W. Y., and C. M. Carvalho.** 1984. Occurrence and experimental infection of toads (*Bufo marinus* and *B. granulatus*) with *Mycobacterium chelonae* subsp. *abscessus*. *J. Med. Microbiol.* **18**:327–333.
33. **Telenti, A., P. Imboden, F. Marchesi, et al.** 1993. Direct, automated detection of rifampin-resistant *Mycobacterium tuberculosis* by polymerase chain reaction and single-strand conformation polymorphism analysis. *Antimicrob. Agents Chemother.* **37**:2054–2058.
34. **Griffiths, P. A., J. R. Babb, and A. P. Fraise.** 1999. Mycobactericidal activity of selected disinfectants using a quantitative suspension test. *J. Hosp. Infect.* **41**:111–121.
35. **Manzoor, S. E., P. A. Lambert, P. A. Griffiths, et al.** 1999. Reduced glutaraldehyde susceptibility in *Mycobacterium chelonae* associated with altered cell wall polysaccharides. *J. Antimicrob. Chemother.* **43**:759–765.