

Mycobacterium simiae Pseudo-outbreak Resulting from a Contaminated Hospital Water Supply in Houston, Texas

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Various species of nontuberculous mycobacteria are known to cause nosocomial pseudo-outbreaks, but there have been no detailed reports of nosocomial *Mycobacterium simiae* pseudo-outbreaks. From April 1997 through February 2001, we recovered 65 *M. simiae* isolates from 62 patients at a community teaching hospital in Houston, Texas. The organism was grown in various water samples obtained in the hospital building and in professional building 1 but not in professional building 2, which has a separate water supply system. Thirty-one environmental and human outbreak-related *M. simiae* isolates had indistinguishable or closely related patterns on pulsed-field gel electrophoresis and were considered clonal. *M. simiae* can be a cause of nosocomial pseudo-outbreaks. The reservoir for this pseudo-outbreak was identified as a contaminated hospital water supply.

Nontuberculous mycobacteria (NTM) were described in clinical specimens as early as 1885. However, their importance as pathogens in humans has been overshadowed by *Mycobacterium tuberculosis*. With the improvements in techniques for the isolation and identification of mycobacteria and the control of tuberculosis, the importance of NTM, especially in immunocompromised hosts, has become recognized. One of the more important biological traits of NTM is their capacity to survive and grow in water, despite being

subjected to various forms of disinfection, including chlorine, glutaraldehyde, formaldehyde, and mercury [1–6]. In fact, municipal water supplies are known to be contaminated with NTM, with the biofilms that are formed in pipes identified as their major site of localization [7, 8]. As a result, NTM contaminate the water supplies of hospitals and cause nosocomial outbreaks of disease (especially wound infections after cardiac and plastic surgery), the development of postinjection abscesses, and pseudo-outbreaks mostly due to contaminated bronchoscopy and hemodialysis equipment [9–16]. Mycobacterial species that have been reported to cause such outbreaks include *Mycobacterium immunogenum*, *Mycobacterium abscessus*, *Mycobacterium fortuitum*, *Mycobacterium chelonae*, *Mycobacterium mucogenicum*, *Mycobacterium xenopi*, *Mycobacterium avium* complex, *Mycobacterium terrae* complex, and *Mycobacterium gordonae*. To our knowledge, no detailed descriptions of nosocomial *Mycobacterium simiae* outbreaks have appeared in the literature.

M. simiae isolates were first recovered in 1965 from

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Macacus rhesus monkeys imported from India [17]. *M. simiae* is a slowly growing mycobacterium that is weakly photochromogenic and is usually niacin positive and catalase positive. Given its isolation from sphagnum vegetation, its environmental niche is assumed to be water [18]. Wolinsky [19] mentioned that the organism had caused a pseudo-outbreak that resulted from contamination of a hospital's well water in Arizona, and the organism was reported to have been isolated from the water supply of a hospital in Gaza, although no description of the consequences of isolation of *M. simiae* was provided [20].

There are geographic variations in the frequency of isolation of *M. simiae*, with Cuba, the southwestern United States, Israel, and, recently, Guadeloupe reporting outbreaks and/or increased frequency of isolation of *M. simiae* [21–25]. Isolates recovered from humans are estimated to be clinically relevant in 9%–21% of specimens [21, 22, 26]. *M. simiae* can present as a pulmonary pathogen in patients with underlying pulmonary abnormalities and can cause disseminated disease in patients with AIDS [27, 28]. Treatment of *M. simiae* infection has not been standardized, but clarithromycin, quinolones, ethambutol, cycloserine, and ethionamide have been reported to have activity against the organism [20, 29].

Genotypic characterization of *M. simiae* isolates is needed to establish the epidemiologic relationships between environmental and clinical isolates. However, the molecular characterization of NTM, including *M. simiae*, remains an evolving field, and 2 genotyping methods are of interest. PFGE is considered the “gold standard” for comparison of NTM strains; however, it is a technically difficult and labor-intensive method, and some species show only minimal genetic differences between epidemiologically unrelated isolates. Amplification and sequencing of an *hsp65* gene fragment has proved useful in species identification of NTM in general and in strain identification of *Mycobacterium kansasii* and *M. avium*, but not with *M. simiae* strains reported to date [30, 31].

In March 1998, the City of Houston Mycobacteriology Laboratory confirmed that there was an increase in the number of *M. simiae* isolates recovered at a community teaching hospital. This finding prompted an epidemiologic and molecular investigation of the outbreak, which we describe in this report. We also compared the discriminatory ability of PFGE and *hsp65* in differentiating the outbreak-related *M. simiae* strain from various control strains.

METHODS

Hospital description. The index hospital is part of a private health care system that includes 5 hospitals in Houston, Texas. Mycobacterial cultures from the 5 hospitals are processed in a centralized laboratory. All of the *M. simiae* cultures originated

from 1 facility that consisted of a 600-bed community teaching hospital and 2 professional buildings (hereafter known as “PB1” and “PB2”). The hospital building and PB1 receive their water from a municipal water supply and a private well. Water is pumped into the energy dispatch building (EDB) and then is delivered via pipes to the hospital building and PB1. PB2 receives its water supply separately from the municipal water source.

Environmental sampling and microbiologic testing. Water samples obtained from the municipal water supply, the private well, and the EDB and from various locations in PB1, PB2, and the main building were cultured. Isolates were grown on Löwenstein-Jensen (LJ) medium and/or in Bactec 12B media (Becton Dickinson), and routine biochemical testing was performed. Grouping of the strains as *M. simiae* complex was further confirmed by high-performance liquid chromatography. *M. simiae* isolates that had been submitted from various geographic areas in the United States to the University of Texas Health Center at Tyler Mycobacteria/Nocardia Laboratory for identification or susceptibility testing were used as control strains in the molecular investigation of the outbreak.

Chart review. The medical records of patients with positive culture results were reviewed, and data on comorbidities, time from admission until culture was performed, reason for performing culture, and the presence of an alternative diagnosis were recorded.

Automated DNA sequencing of *hsp65*. Isolates were cultured on LJ slants for 2–4 weeks. Procedures were performed in a laboratory with level 3 biosafety. A sample of the culture was emulsified in an Eppendorf tube that contained 500 μ L of STET buffer (10 mM Tris-HCl, 1 mM EDTA, and 1% Triton X-100). The bacteria were harvested by centrifugation at 20,000 g for 10 min. The supernatant was discarded, and the cell pellet was resuspended in 100 μ L of 95% ethanol. After incubation overnight at 4°C, the suspension underwent centrifugation at 20,000 g for 10 min, and the supernatant was discarded. After the addition of 0.1 mL of STET buffer and 50 μ g of 0.1-mm zirconium oxide beads, the bacteria were lysed by agitation at 3920 g for 100 s with a mini-BeadBeater (Biospec Products). The supernatant that contained DNA was used for analyses. A 360-bp region of the *hsp65* gene was amplified with a DNA thermal cycler (Perkin-Elmer Cetus). The following nucleotides were used as primers: forward primer 5'-ACCAACGATGGTGTGTCCAT-3' and reverse primer 5'-CTTGTCGAACCGCATACCCT-3'. The 360-bp fragment was characterized using an automated DNA sequencer (ABI 377; Applied Biosystems).

PFGE. In brief, *M. simiae* organisms were incorporated into agarose plugs with a low melting point and then were lysed with lysozyme (1 mg/mL). DNA was digested with *DraI* and *XbaI* and was separated using the CHEF Mapper system (Bio-Rad Laboratories) at 14°C for 20 h at 6 V/cm. Pulse time was

ramped from 3 s to 12 s after *Xba*I digestion, from 5 s to 15 s for a 14-h period, and then from 60 s to 70 s for 6 h after *Dra*I digestion. Gels were photographed after they were stained with ethidium bromide [32, 33]. Strains were compared for relatedness by use of the method of Tenover et al. [34], with minor modification of the definitions based on in-house experience with this method. Basically, isolates were considered to be the same ("indistinguishable") if they showed no fragment differences with either restriction enzyme; closely related, if they differed by 2–3 bands (i.e., restriction fragments) with either or both enzymes; possibly related, if they differed by 4–6 bands but >50% of well-resolved bands were the same with either or both enzyme; or different or unrelated, if ≥ 7 bands were different and <50% of well-resolved bands were the same with both enzymes.

RESULTS

Microbiologic results. From April 1997 through February 2001, a total of 65 *M. simiae* isolates recovered from 62 patients were identified. The *M. simiae* isolates constituted 15.3% of all mycobacterial isolates recovered at the community teaching hospital, while, at the Houston Department of Health and Human Services Laboratory, *M. simiae* isolates constituted only 0.8% of all mycobacterial isolates recovered (95 of 620 isolates vs. 38 of 4610 isolates, respectively; $P = .001$). During the study period, 90% of all *M. simiae* isolates recovered in Houston came from the community teaching hospital. The colonies were smooth, dome shaped, and weakly photochromogenic. Biochemically, the isolates did not reduce nitrates, but they produced urease and catalase, had variable production of niacin, and had a negative Tween hydrolysis test result. High-performance liquid chromatography revealed a 3-peaked pattern, which is characteristic of *M. simiae* [35]. The isolates were resistant to isoniazid, streptomycin, ethambutol, and rifampin, but they were susceptible to amikacin, kanamycin, and ciprofloxacin.

Patient characteristics. The medical records of 58 patients were reviewed. The mean patient age was 58.9 years (median, 66 years; range, 11–94 years). Twenty-eight patients (48.3%) were male. Thirty-two patients (55.2%) were white, 12 (20.7%) were black, 8 (13.8%) were Hispanic, and 6 (10.3%) were Asian. Specimens for culture were obtained as part of a clinical workup performed for 33 patients with pneumonia, 4 with fever, 5 with pleural effusion, 5 with shortness of breath, 3 with diarrhea, and 1 each with hemoptysis, upper respiratory tract infection, and lung mass. Fifty-nine isolates (90.7%) were recovered from respiratory sources, 5 (7.7%) were recovered from feces, and 1 (1.5%) were recovered from urine. Cultures were performed a mean of 3.8 days after the patients were admitted to the hospital (median, 3 days; range, 1–24 days). All smear specimens were negative for acid-fast bacilli. The majority of patients

had ≥ 1 chronic medical condition diagnosed: 35 patients had cardiopulmonary disease, 8 had cancer, 6 had chronic renal failure, 4 had diabetes mellitus, 4 had AIDS, 4 had treated tuberculosis, 3 had systemic lupus erythematosus, 2 had inflammatory bowel disease, and 1 patient each had depression, paraplegia, or schizophrenia. None of the patients received specific antimicrobial treatment for *M. simiae* infection, and isolation of *M. simiae* was unrelated to the clinical presentation of the patients.

Environmental investigation. All isolates were recovered from patients in the main hospital building and PB1. Cultures of water samples obtained from the municipal water supply, the ground well, and the EDB did not yield *M. simiae*. Culture specimens obtained from the pipes that connected the EDB to the hospital building and PB1, as well as culture specimens obtained from heat exchangers, sinks, drinking fountains, and ice machines in the hospital building and PB1, were positive for *M. simiae*. Culture samples obtained from PB2 were negative for *M. simiae* (figure 1). The chlorine concentration in the water at the point of use was measured at all 3 locations and was found to be <1 ppm. In May 1999, the chlorination of the water in the EDB was increased to achieve a chlorine concentration of 1 ppm at various points of use in the hospital and PB1. This resulted in a transient decrease in the number of *M. simiae* isolates recovered (figure 2).

Molecular characterization. A total of 58 *M. simiae* isolates were characterized by sequencing of a 360-bp fragment of the *hsp65* gene: 44 isolates (37 isolates from 33 patients and 7 environmental isolates [5 from hospital water at various sites, 1 from a drinking fountain, and 1 from an ice machine]) were outbreak related, and 14 were control isolates from various areas of the United States. Genotyping of the *hsp65* frag-

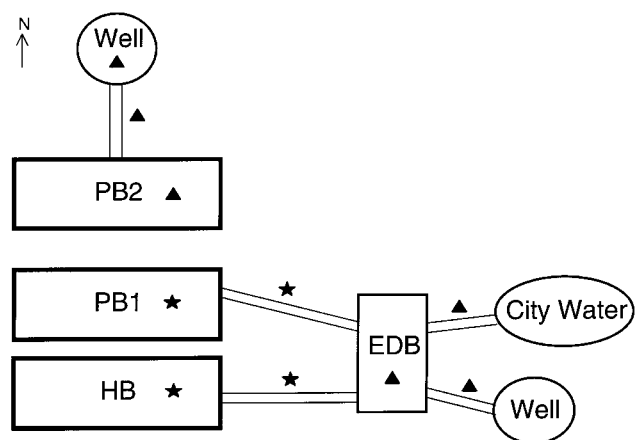


Figure 1. Schematic representation of the buildings, pipes, and locations of a community teaching hospital (Houston, TX) from which specimens obtained for culture had positive results. EDB, energy dispatch building; HB, hospital building; PB, professional building; ▲, cultures negative for *Mycobacterium simiae*; ★, cultures positive for *M. simiae*.

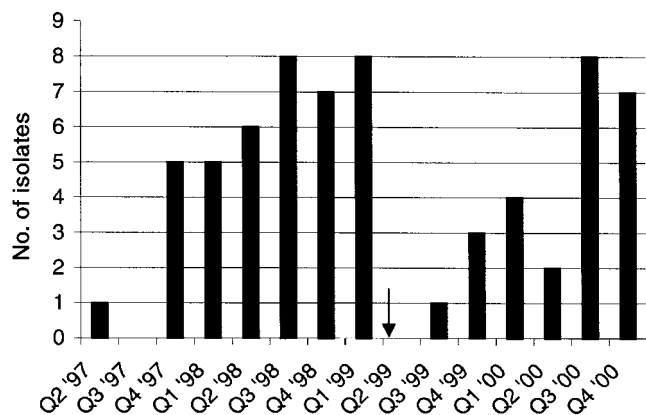


Figure 2. The number of *Mycobacterium simiae* isolates recovered over time at a community teaching hospital in Houston, Texas. The arrow denotes the time when chlorination was increased in the water system. Q, yearly quarter.

ment could not discern between outbreak-related and control isolates. All isolates were categorized as belonging to 1 of 5 groups that differed by only 1–2 nucleotides. In contrast, the PFGE genotyping method helped differentiate outbreak-related isolates from control isolates. Thirty-one outbreak-related isolates (24 from patients and 7 from the hospital environment) and 7 control isolates (including the ATCC [American Type Culture Collection] 27257 strain) were ge-

notyped using the PFGE method. With one exception, all outbreak-related isolates had either indistinguishable or closely related PFGE patterns. All control isolates had unique, unrelated PFGE patterns (figure 3).

DISCUSSION

We present the first study that, by use of molecular genotyping, traces the source of a nosocomial *M. simiae* pseudo-outbreak to a contaminated hospital water supply. The isolation of rare NTM from specimens obtained from humans has become increasingly recognized [36]. This is the result, in part, of an increase in the number of immunocompromised hosts and the improvement in mycobacterial isolation techniques. Previous studies have shown that genotyping of the *hsp65* gene is valuable in the differentiation of mycobacteria at the species and subspecies levels [37, 38]. The *hsp65* gene is present in all known mycobacteria, and enough allelic diversity is present within certain species, with the exception of the members of the *M. tuberculosis* complex, to allow for strain identification. To date, *hsp65* genotyping has been used to allow for the rapid identification of a large array of mycobacterial species on the basis of positive Bactec 12B specimens, to study the genetic diversity of *M. avium* complex and *M. kansasii* human isolates, and to identify a predominant *M. simiae* allelic variant in Guadeloupe [30, 31, 39]. However, in our study, *hsp65* genotyping failed to

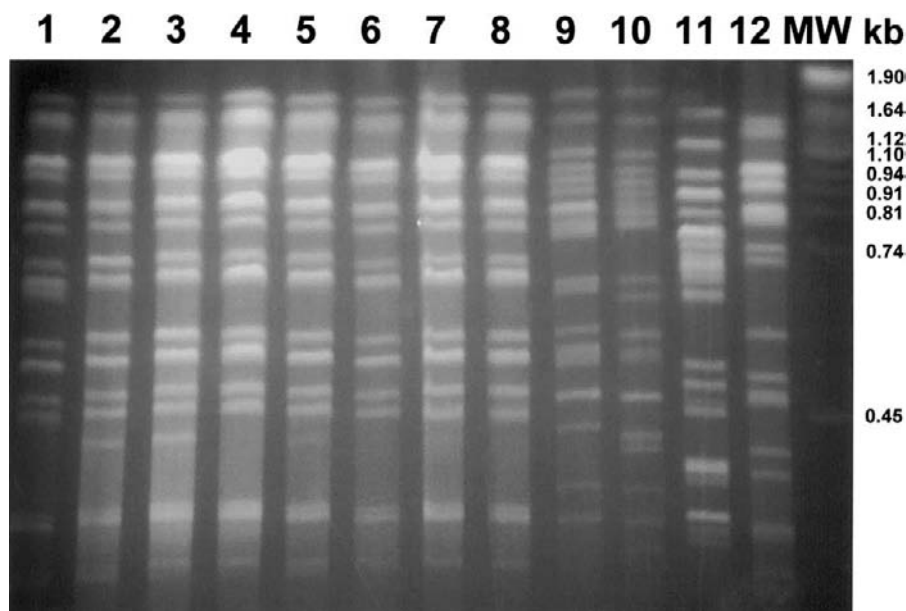


Figure 3. PFGE patterns of *Mycobacterium simiae* genomic DNA digested with *DraI*. Lanes 1–8, Houston epidemic isolates: lane 1, strain 18 (water); lane 2, strain 35 (drinking fountain); lane 3, strain 36 (ice machine); lane 4, strain 13 (sputum, recovered in 1998); lane 5, strain 34 (sputum; recovered in 1999); lane 6, strain 31 (sputum; recovered in 2000); lane 7, strain 6 (bronchial wash specimen); lane 8, strain 7 (gastric). Lanes 9–11, Random clinical isolates: lane 9, Mo 1265 (recovered in Madison, WI); lane 10, Mo 1046 (recovered in Bellevue, WA); lane 11, Mo 1355 (recovered in Tyler, TX). Lane 12, ATCC (American Type Culture Collection) strain 25275. Lane 13, Yeast DNA standards. MW, molecular weight standards; kb, kilobases.

differentiate the outbreak-related *M. simiae* strain from epidemiologically unrelated control strains because of the minimal intraspecies variability of the targeted *hsp65* fragment. In contrast, PFGE had a more sensitive discriminatory power and could differentiate between pseudo-outbreak-related strains and control *M. simiae* strains.

In the literature, there are no detailed reports of pseudo-outbreaks of *M. simiae* infection. *M. simiae* was the third most common mycobacterium identified in one laboratory in Arizona in 1994–1995 and at a university hospital in San Antonio, Texas, in 1983–1993 [21, 22]. It is unclear whether these situations represent a pseudo-outbreak situation, because no environmental source was identified or linked to the clinical isolates. In Israel, *M. simiae* accounted for 30% of all NTM isolated in 1975–1981, mostly in patients with prior or concomitant tuberculosis [27]. However, molecular characterization of these isolates was not available. In Guadeloupe, an increase in the frequency of recovery of *M. simiae* isolates from patients with AIDS was noted during 1992–1997 [24]. Molecular subtyping of these isolates with PFGE revealed that patients were infected with individual strains. In the present study, there was a significant increase in the reported frequency of isolation of *M. simiae* at one hospital, compared with that noted at other hospitals in Houston and compared with the incidence reported in previous years. Moreover, we provide evidence that these isolates were clonally related to each other and to a common water source in the hospital.

Like most NTM, aquatic environments constitute the natural habitat of *M. simiae*. The water in the pipes that connect the EDB to the hospital building and PB1 was identified as the source of the current outbreak. Biofilms that form in the plumbing systems constitute an environment that promotes the growth of mycobacteria and partially shields it from the effect of disinfectants [7, 40]. Controlling the pseudo-outbreak would require decreasing colonization of the water distribution system. However, different NTM have different susceptibilities to disinfectants. For example, *M. avium* is relatively more resistant to disinfection than are *M. terrae* complex, *M. tuberculosis*, *M. chelonae*, and *M. fortuitum* [41, 42]. The relative susceptibility of *M. simiae* to disinfectants has not been studied. Monochloramine penetrates biofilms better than chlorine, and it has been more effective in controlling infections due to *Naegleria fowleri* and *Legionella pneumophila* [43–45]. The effect of monochloramine, compared with that of chlorine, on controlling NTM colonization in water distribution systems remains unknown, and it is an important subject for investigation.

After conducting a national survey from 1981 through 1983, the Centers for Disease Control and Prevention (Atlanta) concluded that 21% of *M. simiae* isolates represented true disease [26]. In subsequent studies that used a more stringent definition of definitive disease, it was concluded that 8%–10% of cultures

that are positive for *M. simiae* denote the presence of disease [20, 21]. The patients usually have advanced AIDS or an underlying pulmonary abnormality, such as chronic obstructive pulmonary disease or bronchiectasis. In our study, *M. simiae* colonization occurred in the hospital, and isolation of the organism was unrelated to clinical presentation. The long-term consequences of this colonization have not yet been fully determined.

Although *M. simiae* is only weakly pathogenic, its frequency of isolation must be reduced in this large urban hospital. A trial of hyperchlorination of the water system for 3 months is underway, and if a sustained decrease in the frequency of isolation of *M. simiae* is not observed, a chlorine-releasing agent, which was reported by Griffiths et al. [42] to be highly effective in the eradication of mycobacteria in biofilms, will be used to control the pseudo-outbreak.

In brief, *M. simiae* was identified as a potential pathogen capable of producing health care-associated pseudo-outbreaks. The reservoir was identified in the hospital water-distribution system. Compared with *hsp65* genotyping, PFGE had a higher discriminatory power to differentiate *M. simiae* strains, and it remains the “gold standard” for investigating future outbreaks of *M. simiae*.

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