

Isolation of Nontuberculous Mycobacteria (NTM) from Household Water and Shower Aerosols in Patients with Pulmonary Disease Caused by NTM

Rachel Thomson,^a Carla Tolson,^b Robyn Carter,^b Chris Coulter,^b Flavia Huygens,^c Megan Hargreaves^d

Gallipoli Medical Research Centre, Greenslopes Private Hospital, Brisbane, Queensland, Australia^a; Queensland Mycobacterium Reference Laboratory, Pathology Queensland, RBWH Campus, Herston, Queensland, Australia^b; Queensland University of Technology, Institute of Health and Biomedical Innovation, Kelvin Grove Campus, Brisbane, Queensland, Australia^c; Queensland University of Technology, Faculty of Science and Technology, Brisbane, Queensland, Australia^d

It has been postulated that susceptible individuals may acquire infection with nontuberculous mycobacteria (NTM) from water and aerosol exposure. This study examined household water and shower aerosols of patients with NTM pulmonary disease. The mycobacteria isolated from clinical samples from 20 patients included *M. avium* (5 patients), *M. intracellulare* (12 patients), *M. abscessus* (7 patients), *M. gordonae* (1 patient), *M. lentiflavum* (1 patient), *M. fortuitum* (1 patient), *M. peregrinum* (1 patient), *M. chelonae* (1 patient), *M. triplex* (1 patient), and *M. kansasii* (1 patient). One-liter water samples and swabs were collected from all taps, and swimming pools or rainwater tanks. Shower aerosols were sampled using Andersen six-stage cascade impactors. For a subgroup of patients, real-time PCR was performed and high-resolution melt profiles were compared to those of ATCC control strains. Pathogenic mycobacteria were isolated from 19 homes. Species identified in the home matched that found in the patient in seven (35%) cases: *M. abscessus* (3 cases), *M. avium* (1 case), *M. gordonae* (1 case), *M. lentiflavum* (1 case), and *M. kansasii* (1 case). In an additional patient with *M. abscessus* infection, this species was isolated from potable water supplying her home. NTM grown from aerosols included *M. abscessus* (3 homes), *M. gordonae* (2 homes), *M. kansasii* (1 home), *M. fortuitum* complex (4 homes), *M. mucogenicum* (1 home), and *M. wolinskyi* (1 home). NTM causing human disease can be isolated from household water and aerosols. The evidence appears strongest for *M. avium*, *M. kansasii*, *M. lentiflavum*, and *M. abscessus*. Despite a predominance of disease due to *M. intracellulare*, we found no evidence for acquisition of infection from household water for this species.

Nontuberculous mycobacteria (NTM) are ubiquitous organisms responsible for progressive pulmonary disease. Water is a documented source of NTM infection in humans, although it is not the only source. The incidence of pulmonary disease due to environmental mycobacteria is increasing in many parts of the world, including Australia (1). Reasons include increased awareness of mycobacteria as pulmonary pathogens, improvements in methods of detection and culture, and an aging population (as this is often a disease of the elderly). An increase in exposure is also possible as people are exposed to mycobacteria in water through drinking, swimming, and bathing. Aerosols generated during these activities can be inhaled, and water can be aspirated when swallowed (2). Evidence linking human infection due to exposure to *Mycobacterium intracellulare* and household dust (3), soil and potting mix (4), and peat moss (5) also exists.

There is some evidence that susceptible individuals may acquire infection from potable water in their own homes. To date this evidence has been largely reported for *Mycobacterium avium* (6–8). We recently documented NTM in the Brisbane, Australia, potable water distribution system (9) and have shown that strains of *M. lentiflavum* (10) and *M. abscessus* (11) are highly related or identical to those found in patients with disease. Despite frequent identification of *Mycobacterium kansasii* from water samples, pathogenic strains were found infrequently (unpublished data). *M. intracellulare* and *M. avium* were found infrequently in potable water. It has been suggested that the low yield of *M. intracellulare* from potable water samples is the result of insufficient sample volume, difficulties with culture techniques, and the fact that perhaps this species resides in the biofilm lining pipes and is released

only intermittently into water (12). Previous investigators have documented the presence of mycobacteria in soil and house dusts in Queensland (QLD) and have shown that ~50% of environmental strains were also found in human specimens, such as sputum (3, 13, 14).

The aim of this study was to compare NTM found in household water, biofilm, and aerosols generated by showering with the species or strains causing infection in patients with known nontuberculous mycobacterial pulmonary disease in Brisbane, Australia.

MATERIALS AND METHODS

Patients were recruited by the principal investigator (R.T.) through the Respiratory Outpatient Department at The Prince Charles Hospital, through clinics at the Queensland Tuberculosis Control Centre, and at Greenslopes Private Hospital. Prevalent patients who had been resident in the same dwelling for greater than 5 years prior to the diagnosis of NTM disease were eligible for home sampling. Following signed informed consent, the PI and assistant visited the homes to collect 1-liter samples of water into sterile containers directly from kitchen, bathroom, and shower

Received 5 April 2013 Returned for modification 28 May 2013

Accepted 9 July 2013

Published ahead of print 10 July 2013

Address correspondence to Rachel Thomson, R.Thomson@uq.edu.au.

Supplemental material for this article may be found at <http://dx.doi.org/10.1128/JCM.00899-13>.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JCM.00899-13

taps, rainwater tanks, and swimming pools (if applicable). Swabs were taken from inside all taps and showerheads.

Aerosols from showers were sampled using Andersen six-stage cascade impactors for viable air sampling (Grasby-Andersen, Smyrna, GA), which provide validated particle size distribution data (15) and which have been used previously to collect mycobacterial aerosols (16–18). The impactors are calibrated at a flow rate of 28.3 liters/min, and samples were collected onto petri dishes containing Mycobacteria 7H11 agar, supplemented with OADC (oleic acid-albumin-dextrose-catalase) enrichment (100 ml/liter), malachite green (25 mg/liter), and cycloheximide (500 mg/liter) (17). The impactor was placed as close as practicable to the shower recess, and the shower hot tap was run. Windows were left as the patient would normally have them during showering, and exhaust fans were left off. The collection time was 20 min. After collection, mycobacterial plates were sealed with parafilm, packed in a plastic bag, and incubated at 30°C for 3 to 6 months. Plates were read weekly, and purity plate subcultures were performed using 7H11 agar.

Water samples were processed as previously described (9, 19). After analysis of water sampling revealed an increased yield for rapidly growing mycobacteria using liquid medium (MGIT tubes) (9), these were included for the last 6 houses that were sampled. Swabs were placed in 2 ml sterile distilled water (SDW) at time of collection, sealed, and transported to the lab at room temperature. Each swab container was vortexed for 2 min and inoculated as per the water samples. Following initial inoculation (M7H11 plates and MGIT tubes), the samples were decontaminated with 0.005% cetylpyridium chloride (CPC) for 30 min and further inoculated for culture.

In a subgroup of seven patients, once samples were processed for culture, the remaining samples for each house were pooled according to source (e.g., hot water, cold water, or swabs) and frozen for later PCR and high-resolution melt curve (HRM) analysis.

HRM methodology. Frozen samples were thawed in a water bath at 65°C. One hundred milliliters of each sample was filtered through a 0.45- μ m-pore-size (47-mm-diameter) membrane (Advantec, Tokyo, Japan). Each filter was aseptically transferred into a separate sterile 50-ml tube containing 10 ml sterile distilled water (SDW). The tubes were vortexed for 8 to 10 min at 2,200 rpm, membranes were aseptically removed, and the tubes were centrifuged at 4,750 rpm for 30 min at 4°C in a swinging-bucket rotor.

The supernatant was discarded, and DNA was extracted using the Power Water DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA), as follows. The pellet was transferred to the supplied Power Bead tube and processed in a Mini-BeadBeater (BioSpec, Bartlesville, OK) for 5 min with 60 μ l kit-supplied C1 lysis buffer. The kit's "Experienced user protocol" was followed from the second step onwards, with the exception of a primary elution performed with 50 μ l DNase- and RNase-free deionized water and a secondary elution performed with 100 μ l kit-supplied C6 elution buffer. The DNA extracts were stored at –80°C until use. All samples and NTM ATCC controls (*M. peregrinum* ATCC 700686, *M. gordonae* ATCC 14470, *M. terrae* ATCC 15755, *M. scrofulaceum* ATCC 19981, *M. chelonae* NCTC 946, *M. fortuitum* NCTC 10394, *M. abscessus* ATCC 19977, *M. avium* 8867/GI/97, *M. intracellulare* ATCC 13950, *M. smegmatis* ATCC 19420, *M. kansasii* TMC 1201, and *M. malmoense* ATCC 29571) were quantified using Nano Drop 1000 (Thermo Scientific, Australia). The real-time PCRs were prepared to a volume of 20 μ l; their constituents are shown in Table S1 in the supplemental material. Amplification was performed on the Rotor-gene Q real-time PCR instrument (Qiagen Inc., Germantown, MD). Real-time PCR followed by HRM was performed using the following conditions: step 1, hold at 95°C for 10 min; step 2, cycling at 95°C for 10 s, 52°C for 15 s, and 72°C for 30 s and repeated 40 times; step 3, hold at 72°C for 5 min; and step 4, HRM, melt from 72 to 95°C rising by 0.05°C at each step. In all amplification reactions, negative samples (whose constituents are listed in Table S2 in the supplemental material) were used to avoid any possible contamination. Each assay in-

cluded 12 reference strains as positive controls to validate the characterization of the tested samples. All reactions were performed in duplicate.

Human samples were digested and decontaminated using 4% NaOH, neutralized with phosphoric acid, and centrifuged at 3,000 \times g to concentrate the acid-fast bacilli (AFB). Smears were prepared from the sediment and stained by the Ziehl-Neelsen (ZN) method. One Lowenstein-Jensen slope (\pm pyruvate) and a 7-ml MGIT tube were inoculated and incubated at 35°C until growth was detected. ZN staining of colonies confirmed AFB. Multiplex PCR (7) was performed to discriminate between *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. abscessus*, and other *Mycobacterium* spp. Isolates identified as other *Mycobacterium* spp. were further determined to the species level using Hain Life Sciences GenoType Mycobacterium AS (additional species) kit (2004 to 2007 only) and/or 16S rRNA sequencing in conjunction with phenotypic characteristics.

The clonality of clinical and water isolates was determined using a method based on repetitive element palindromic PCR (Rep-PCR) (Diversilab system; bioMérieux, Melbourne, Australia). DNA was extracted from clinical and water isolates using the Ultraclean microbial DNA isolation kit (Mo Bio Laboratories). The PCR mixture was prepared using AmpliTaq polymerase and PCR buffer (Applied Biosystems, Hammon, NJ) and Mycobacterium Diversilab primer mix according to the manufacturer's instructions. Separation and detection of Rep-PCR products were performed by microfluidic chips of the Diversilab System. Fingerprints were analyzed with Diversilab software v.3.4.38 using the Pearson correlation coefficient and unweighted-pair group method with arithmetic means to compare isolates and determine clonal relationships. A similarity index of \geq 97% was used to define isolates that were indistinguishable, based on the manufacturer's recommendations. Isolates that shared 95% similarity were considered similar, and those with <90% similarity were considered "different/unrelated."

The protocol was approved by the Queensland University of Technology Research Ethics Unit (approval no. 0900000085) and the Human Research Ethics Committee of the Prince Charles Hospital (EC 2617).

RESULTS

The species of mycobacteria isolated from clinical samples from 20 patients included *M. avium* (5 patients), *M. intracellulare* (12 patients), *M. abscessus* (7 patients), *M. gordonae* (1 patient), *M. lentiflavum* (1 patient), *M. fortuitum* (1 patient), *M. peregrinum* (1 patient), *M. chelonae* (1 patient), *M. triplex* (1 patient), and *M. kansasii* (1 patient). Patient details are summarized in Table 1.

Twenty patients' homes were sampled, and mycobacteria were cultured from all but one home. (Full details are provided in Table S1 in the supplemental material.) Species identified in the home matched that found in the patient in seven (35%) cases: *M. abscessus* (3 cases), *M. avium* (1 case), *M. gordonae* (1 case), *M. lentiflavum* (1 case), and *M. kansasii* (1 case). In an additional patient with *M. abscessus* infection, this species was isolated from potable water in the reservoir zone supplying the home.

In those instances where the environmental isolate was cultured (as opposed to direct detection using PCR), the strain type matched that of the patient's infecting strain in the patient with *M. kansasii* infection (Fig. 1A). The clinical isolate of *M. lentiflavum* was closely related to the *M. lentiflavum* isolate found in the patient's house and the local water supply. (Fig. 1B) The *M. gordonae* isolate from patient's sputum was unrelated to the isolate found in the home water. Despite repeated attempts, the *M. avium* isolate from the shower swab (patient P6) was unable to be regrown for comparison with the patient's clinical isolate. In one patient with infection due to *M. abscessus* subsp. *bolleti*, a different strain type of *M. abscessus* was grown from his shower water (Fig. 1C).

Results from Brisbane-wide sampling of water sites (distribution, reservoir, and trunk main samples) revealed a wide variety of

TABLE 1 Clinical details of patients whose homes were sampled^a

Patient ID (gender; age [yr]) ^b	Other patient/sampling environment characteristic(s)	Radiology	Microbiology and symptoms	Clinical notes
P1 (F; 72)	Gardener with ++ pots	BE	S++ bronchial wash in 2007, <i>M. abscessus</i> ; cough, sputum, hemoptysis	Treated for 6 wk, clinically stable but remained culture positive, died from cancer of the pancreas in 2011
P2 (F; 69)	Avid gardener	BE	Multiple S+ samples from 2004, progressive radiology, wt loss, hemoptysis; <i>M. intracellulare</i> in Sept and Dec 2004 (multiple), April 2006, June 2007, and June 2008	Treated for 4 yr (ethambutol, rifampin, clarithromycin, clofazimine, and amikacin), converted sputum in July 2009; culture negative from 2010 to June 2011
		BE, small cavities	New infiltrate, increased cough and hemoptysis; 2 positive sputa in June 2011 (1/3); in Sept 2011 (1/3), <i>M. kansasii</i>	Treated for 12 mo with ethambutol, rifampin, and clarithromycin, converted sputum, in remission in 2013
P3 (M; 72)		BE, nodules, large cavities	Multiple S+++ <i>M. intracellulare</i> , sputa from April 2006, significant wt loss, cough	Partial clinical response to treatment, but remains S++ C+, end-stage disease
P4 (F; 74)	>400 orchids, exposure to ++ peat moss	Nodules, BE, small cavity	S++ <i>M. intracellulare</i> bronchial wash in Feb 2009; <i>Pseudomonas aeruginosa</i> , <i>Aspergillus fumigatus</i> , <i>Trichosporin</i> sp.; cough, sputum, hemoptysis	Treated with ethambutol, rifampin, and clarithromycin, converted sputum in Aug 2009; ongoing hemoptysis; ceased treatment at end of 2011
			Relapse/reinfection in 2013, new infiltrate; 1/3 sputa S- C+/-; bronchial wash S- C+ in Jan 2013, <i>M. intracellulare</i>	Commenced retreatment in Feb 2013
P5 (F; 51)	Rainwater tanks	BE	Multiple sputa positive in 2007 for <i>M. avium</i> ; previous <i>S. aureus</i> , new radiology, symptoms	Treated for 18 mo, converted sputum, still in remission
P6 (F; 72)	Large fernery, rainwater tanks	BE	Cough, sputum, respiratory failure, cachexia, progressive radiology; <i>M. avium</i> in 2007; <i>M. intracellulare</i> in 2006 and 2008	Died from disease despite treatment in 2011
P7 (F; 85)	Rainwater tanks	Nodules, BE	Cough, sputum, wt loss, sweats; bronchial wash in April 2008, <i>Mycobacterium</i> species; OLB in Nov 2008, <i>M. lentiflavum</i>	Treated for 2 yr with ethambutol, rifampin, and clarithromycin, remains in remission; <i>P. aeruginosa</i>
P8 (F; 63)	Rainwater tanks	BE	Recurrent LRTIs, persistent cough; bronchial wash in Nov 2007; in July 2008 and May 2010, <i>M. intracellulare</i>	Treated for 2 yr with ethambutol, rifampin, and clarithromycin, in remission
P9 (F; 63)	Gardener	BE, nodules	Hemoptysis; bronchial wash in Jan 2006, in Aug 2007, <i>M. intracellulare</i>	Treated for 18 mo, RML lobectomy in 2011; relapse/reinfection in 2013
P10 (M; 81)		BE, nodules	Cough, sputum, SOB; bronchial wash in Oct 2007, <i>M. intracellulare</i> ; sputum, <i>M. abscessus</i> , in Jan 2008, Apr 2008, and Sept 2008 and in 2012	Treated for 6 wk with i.v. amikacin, cefoxitin, and clarithromycin in 2010 and for 2 wk in 2012
P11 (F; 75)	Didn't use shower, gardener, very dusty house	BE	Recurrent infections in RML, <i>S. aureus</i> ; <i>M. intracellulare</i> isolated most frequently; <i>M. intracellulare</i> in March 2006; slow grower in April 2008; <i>M. abscessus</i> in June 2008; <i>M. intracellulare</i> in July 2008; <i>M. peregrinum</i> in July 2008; <i>M. gordonae</i> in Aug 2008; <i>M. intracellulare</i> in Sept to Dec 2008 (multiple); <i>M. peregrinum</i> in Dec 2008; <i>M. intracellulare</i> in Feb 2009 (multiple); <i>M. peregrinum</i> in March 2009; <i>M. fortuitum</i> in March 2009; <i>M. fortuitum</i> in May 2009; <i>M. intracellulare</i> in Oct 2009; <i>M. fortuitum</i> complex in Dec 2009; <i>M. intracellulare</i> in Jan 2010	Treated for 24 mo with ethambutol, rifampin, clarithromycin, and ciprofloxacin, showed some symptomatic improvement, but didn't convert sputum; currently stable off treatment with chronic cough, sputum, and lethargy but reluctant for retreatment
P12 (M; 69)	Cactus house (>300 plants)	BE, nodules, cavities	<i>M. intracellulare</i> in 2003–2004; <i>M. triplex</i> in 2006; <i>M. avium</i> and <i>M. intracellulare</i> in 2007; <i>M. intracellulare</i> in 2008; <i>Nocardia farcinica</i> in 2009; <i>M. intracellulare</i> in 2009	Treated over 3–4 yr, drug intolerances, peripheral neuropathy; eventually converted sputum; remains in remission
P13 (F; 60)		BE, nodules	<i>M. intracellulare</i> repeatedly from 2000–2007; <i>M. avium</i> after 15-mo remission in March 2005; RGM single isolates while on treatment for SGM; <i>M. chelonae</i> in May 2005; <i>M. abscessus</i> in Jan 2007	RML lobectomy following 4 yr of treatment, drug side effects, and short relapse time; now deceased secondary to NTM disease
P14 (M; 85)		Cavities	<i>M. intracellulare</i> in Dec 2006, March 2007, and April 2007	Treated for 18 mo with ethambutol, rifampin, and clarithromycin, converted sputum
P15 (M; 25)		BE, nodules, bronchiolitis	Post-pertussis bronchiolitis with secondary infection, <i>M. abscessus</i> subsp. <i>bolletii</i> in July–Sept 2011	Treated for 6 mo with amikacin, cefoxitin, clarithromycin, and clofazimine
P16 (F; 60)		RML BE	<i>M. avium</i> bronchial wash in 2007	Treated for 6 mo, intolerant of medication; stable and culture negative off treatment
P17 (F; 77)		BE	<i>M. intracellulare</i> in 2000; <i>M. abscessus</i> (multiple), including Sept 2008, Oct 2009, and Feb 2011	<i>M. intracellulare</i> treated in 2000; in 2007, multiple courses of treatment for <i>M. abscessus</i> with partial response, limited by drug toxicity; respiratory failure secondary to disease, death

(Continued on following page)

TABLE 1 (Continued)

Patient ID (gender; age [yr]) ^b	Other patient/sampling environment characteristic(s)	Radiology	Microbiology and symptoms	Clinical notes
P18 (F; 53)		Large cavities, nodules, BE	<i>M. abscessus</i> sputum in Oct 2010, bronchial wash in Dec 2010 (with <i>S. aureus</i>) and Dec 2012 and multiple in between	Heavy smoker of cigarettes, marijuana, large cavities; treated with i.v. therapy, response when abstinent from cigarettes; died from cancer of the lung and progressive cavitory disease
P19 (F; 80)		BE	<i>M. abscessus</i> in March and April 2011	Treated for 2 wk with amikacin and ceftioxin and 6 wk with clarithromycin and clofazimine; in remission
P20 (F; 65)		Nodular BE	<i>M. intracellulare</i> in 2007, bronchial wash, granulomata on TBB	Progressive radiology and symptoms; treated with ethambutol, rifabutin, and clarithromycin in 2010; rash and hepatitis; treated with azithromycin, ethambutol, clofazimine, and amikacin, in remission

^a Abbreviations: F, female; M, male; BE, bronchiectasis; S, smear; C, culture; LRTIs, lower respiratory tract infections; SOB, shortness of breath; RGM, rapidly growing mycobacteria; SGM, slowly growing mycobacteria; OLB, open lung biopsy; TBB, transbronchial lung biopsy. For smear and culture results, + and – indicate positive and negative to the general degree shown (where + is positive and +++ is highly positive).

^b Age calculated from date of first isolate.

NTM (18). Some of these NTM (especially *M. kansasii*, *M. gordonae*, *M. fortuitum*, and *M. mucogenicum*), which were cultured from 16 homes, matched the species isolated from sampling sites near the patients' homes. In two instances (patients P10 and P19), *M. abscessus* was cultured from water close to the homes of these patients with *M. abscessus* disease and the strains were closely related to those found in their clinical samples (Fig. 1D). Similarly in the patient with *M. lentiflavum* infection, the municipal water isolates were similar to the home and clinical isolates (Fig. 1B). Of four other patients for which the infecting NTM species was isolated from their home samples (patients P1, P2, P9, and P11), the species was also isolated from local water sampling sites. (A full table that includes isolates cultured from reservoir zones supplying each patient's house and the distance from the house to the sampling point is given in Table S2 in the supplemental material.)

Aerosols were collected in 18 homes. The culture plates were overgrown in 6 patients, negative at 6 months in 3, but grew NTM in the remaining 9 (50%). The species identified included *M. abscessus* (3 homes, including one patient with disease due to *M. abscessus*), *M. gordonae* (2 patients), *M. kansasii* (1 patient), *M. fortuitum* complex (4 patients), *M. mucogenicum* (1 patient), and *M. wolinskyi* (1 patient).

DISCUSSION

In this study, we have demonstrated that NTM present in potable water distribution systems can also be found in the houses of patients with NTM disease and can be grown from aerosols generated by showering. Matching human and environmental strains were found for *M. abscessus*, *M. lentiflavum*, and *M. kansasii*. Other investigators have explored the possibility that patients with NTM disease (predominantly *M. avium* and *M. intracellulare*) may have acquired their infection from water sources in the home. A Japanese group (8) looked at the isolation of NTM from the homes of 49 patients with *M. avium* complex (MAC) pulmonary disease and 43 healthy volunteers without MAC disease. From each home, three 200-ml water samples (kitchen tap, showerhead, and used bathtub water) were collected. In addition, scale on the surface of showerheads, slime from 3 drains, and dust from air conditioners was sampled. Eleven samples contained MAC: 10

were from 9/49 patients' residences, and 1 was from the 43 volunteers' residences. Eight isolates were *M. avium*, and 3 were *M. intracellulare*—a similar ratio to the species causing pulmonary disease in the Japanese population. The isolates from the patients' bathrooms were compared with those from their sputum samples, and 2 had identical pulsed-field gel electrophoresis (PFGE) patterns and serotypes. For 4 patients the isolates were the same species but had different restriction fragment length polymorphism (RFLP) or PFGE profiles. Interestingly only bathroom samples were positive: MAC was not isolated from kitchen tap water, yet the same system was supplying water to the whole house. The authors postulate that favorable temperature and humidity allow MAC to preferentially colonize the bathroom. However, the frequency of use of kitchen taps may allow more flushing out of mycobacteria from pipes, and the possibility that expectorating patients may have contaminated bathroom drains should not be discounted. In the single patient who had *M. intracellulare* infection, where the pathogen was grown only from a drain site, this may certainly have been the case.

Falkinham (7) reported the results of home water sampling from 31 patients (with 37 homes) in the United States and Canada with NTM disease. Patients included in the study had infection with *M. avium* (9 patients), *M. intracellulare* (6 patients), MAC (11 patients), *M. abscessus* (4 patients), and *M. xenopi* (1 patient). Forty-nine percent of households sampled grew NTM, including *M. avium* (10 homes), *M. intracellulare* (10 homes), *M. malmoense* (5 homes), *M. szulgai* (3 homes), *M. gordonae* (6 homes), *M. chelonae* (2 homes), *M. scrofulaceum* (1 home), *M. triviale* (1 home), and *M. terrae* (1 home). In 17/37 (46%) homes, a species was isolated that matched the patient's infecting species, and in seven of these the strains had matching Rep-PCR patterns. Details of these matching species are not reported in the paper—although two of the matching strains illustrated were *M. avium*. In contrast to our findings, there were a greater yield of *M. intracellulare* and an absence of *M. abscessus* in home samples.

Aerosol sampling for NTM has been performed previously to investigate hot tubs and indoor pools as a source of NTM (20). It has also been used to document NTM in air samples collected

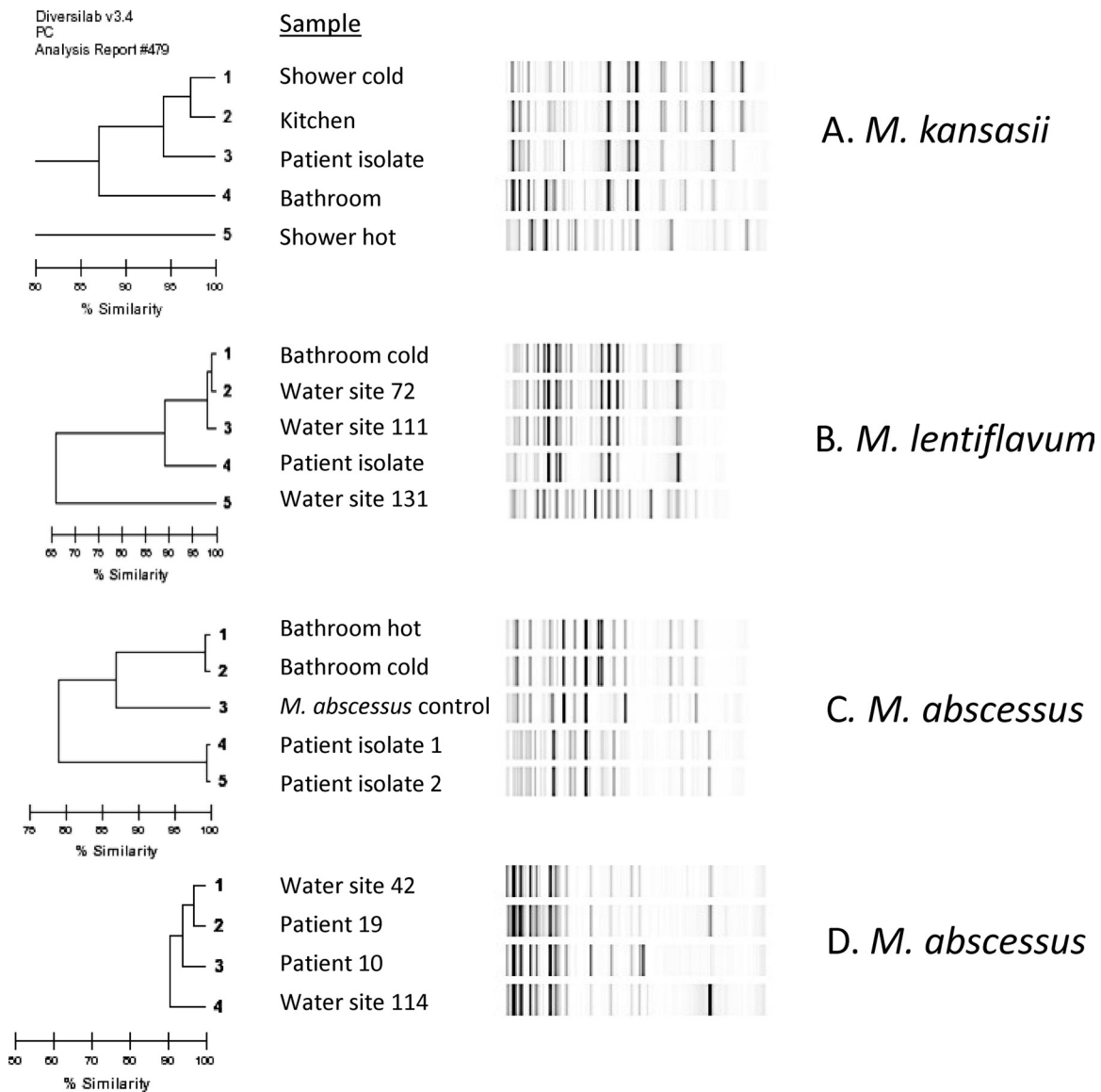


FIG 1 Rep-PCR dendrograms of (A) *M. kansasii* isolated from patient sputum (patient P2) and samples from the patient's home, (B) *M. lentiflavum* isolated from patient P7, her home water, and surrounding water distribution sites, (C) *M. abscessus* subsp. *bolletii* from one patient and *M. abscessus* subsp. *abscessus* grown from shower water, and (D) *M. abscessus* isolated from 2 patients and from the water supply close to their homes.

during the remediation of an old building (17) and from a peat moss processing plant (5). De Groote (4) analyzed aerosols generated by the pouring of commercial potting mix using the Andersen impactor. The isolates shown to be acid fast were presumptively called mycobacteria but not identified further.

Ours is the first study to examine the household shower aerosols as a potential source of NTM. We have demonstrated that a variety of species can be aerosolized and cultured from droplets of a respirable size. The most significant of these is *M. abscessus*, which was found in aerosols of patients with disease. We did not demonstrate the aerosolization of *M. intracellulare*, which was the main pathogen associated with disease in the patients studied.

The yield of *M. intracellulare* from both Brisbane water (9) and home samples was interesting. Falkinham (12) has suggested that in previous water studies the low yield was due to an insufficient

number of specimens or the lack of biofilm specimens. In our study, however, a large number of samples and relatively larger volume of water samples were collected, and biofilm samples were collected from every tap. There may be issues with detection, although the use of direct detection using HRM didn't increase the yield for *M. intracellulare* (yet did find other NTM). Faezel (21) reported extensive sampling of the biofilm of showerheads in the United States, and while *M. avium* was found, no *M. intracellulare* was reported. In both of the other home sampling papers, there was no evidence to support the proposal that patients with *M. intracellulare* disease had acquired infection from water sources.

These findings in combination with the earlier studies done by Dawson (3, 13, 14) on house dust and soil suggest that Queensland patients at least may be more likely to acquire *M. intracellulare* from soil or dust than from water.

More recently Fujita (22) conducted a large survey of soils from 100 patients with MAC infection. In those with high soil exposure (>2 h/week), 6/16 patients had matching soil and clinical isolates using variable-number tandem repeat (VNTR) profiles (5 *M. avium* and 1 *M. intracellulare*). This compared with none of the 19 patients with low or no soil exposure, again suggesting that residential soils may also be a source of exposure for patients with MAC disease.

A major limitation of home water and soil sampling studies relates to the long lag time between exposure and infection that can occur with NTM disease. Quite often a patient has had symptoms for many years prior to diagnosis, and hence, establishing the environment in which they were exposed is often very difficult. Patients are also potentially exposed to NTM outside their home. It is also worth considering the close relationship between soil and water in the environment, in activities such as gardening and in the situation of soil entering water distribution networks through cracks in pipes and rainwater tanks via household roofs.

In summary, we have shown that pathogenic NTM can be isolated from water, biofilm, and aerosols in the households of patients with NTM disease. The presence of matching clinical and patient isolates was demonstrated. From our work and other literature, the evidence supporting water as a source of infection would appear greatest for *M. avium*, *M. abscessus*, *M. lentiflavum*, and *M. kansasii*. However, there appears little evidence for *M. intracellulare*, and soil exposure may be more relevant.

REFERENCES

1. Thomson R. 2010. Changing epidemiology of pulmonary nontuberculous mycobacteria infections. *Emerg. Infect. Dis.* 16:1576–1582.
2. Thomson R, Armstrong J, Looke D. 2007. Gastroesophageal reflux disease, acid suppression, and *Mycobacterium avium* complex pulmonary disease. *Chest* 131:1166–1172.
3. Reznikov M, Leggo J, Dawson DJ. 1971. Investigation by seroagglutination of strains of the *Mycobacterium intracellulare*-*M. scrofulaceum* group from house dusts and sputum in Southeastern Queensland. *Am. Rev. Respir. Dis.* 104:951–953.
4. De Groot MA, Pace NR, Fulton K, Falkinham JO, III. 2006. Relationships between *Mycobacterium* isolates from patients with pulmonary mycobacterial infection and potted soils. *Appl. Environ. Microbiol.* 72:7602–7606.
5. Cayer M-P, Veillette M, Pageau P, Hamelin R, Bergeron M-J, Mériaux A, Cormier Y, Duchaine C. 2007. Identification of mycobacteria in peat moss processing plants: application of molecular biology approaches. *Can. J. Microbiol.* 53:92–99.
6. Embil J, Warren P, Yakrus M, Stark R, Corne S, Forrest D, Hershfield E. 1997. Pulmonary illness associated with exposure to *Mycobacterium avium* complex in hot tub water. Hypersensitivity pneumonitis or infection? *Chest* 111:813–816.
7. Falkinham JI. 2011. Nontuberculous mycobacteria from household plumbing of patients with nontuberculous mycobacteria disease. *Emerg. Infect. Dis.* 17:419–424.
8. Nishiuchi Y, Maekura R, Kitada S, Tamaru A, Taguri T, Kira Y, Hiraga T, Hirotsu A, Yoshimura K, Miki M, Ito M. 2007. The recovery of *Mycobacterium avium-intracellulare* complex (MAC) from the residential bathrooms of patients with pulmonary MAC. *Clin. Infect. Dis.* 45:347–351.
9. Thomson R, Carter R, Tolson C, Coulter C, Huygens F, Hargreaves M. 2013. Factors associated with isolation of nontuberculous mycobacteria (NTM) from a large municipal water system in Brisbane, Australia. *BMC Microbiol.* 13:89. doi:10.1186/1471-2180-13-89.
10. Marshall H, Carter R, Torbey M, Minion S, Tolson C, Sidjabat H, Huygens F, Hargreaves M, Thomson R. 2011. *Mycobacterium lentiflavum* in drinking water supplies, Australia. *Emerg. Infect. Dis.* 17:395–402.
11. Thomson R, Tolson C, Carter R, Huygens F, Hargreaves M. 2013. *Mycobacterium abscessus* isolated from potable water—a potential source of human infection. *BMC Infect. Dis.* 13:241. doi:10.1186/1471-2334-13-241.
12. Falkinham J, III, Norton C, Le Chavallier M. 2001. Factors influencing numbers of *Mycobacterium avium*, *Mycobacterium intracellulare*, and other mycobacteria in drinking water distribution systems. *Appl. Environ. Microbiol.* 67:1225–1231.
13. Reznikov M, Dawson D. 1971. Serological investigation of strains of *Mycobacterium intracellulare* (“Battey” bacillus) isolated from house-dusts. *Med. J. Aust.* 1:682–683.
14. Dawson D. 1971. Potential pathogens among strains of mycobacteria isolated from house-dusts. *Med. J. Aust.* 1:679–681.
15. Andersen A. 1958. New sampler for the collection, sizing, and enumeration of viable airborne particles. *J. Bacteriol.* 76:471–484.
16. Fennelly KP, Martyny JW, Fulton KE, Orme IM, Cave DM, Heifets LB. 2004. Cough-generated aerosols of *Mycobacterium tuberculosis*: a new method to study infectiousness. *Am. J. Respir. Crit. Care Med.* 169:604–609.
17. Rautiala S, Torvinen E, Torkko P, Suomalainen S, Nevalainen A, Kalliokoski P, Katila ML. 2004. Potentially pathogenic, slow-growing mycobacteria released into workplace air during the remediation of buildings. *J. Occup. Environ. Hyg.* 1:1–6.
18. Wendt SL, George K, Parker BC, Gruft H, Falkinham JO, III. 1980. Epidemiology of infection by nontuberculous mycobacteria. III. Isolation of potentially pathogenic mycobacteria from aerosols. *Am. Rev. Respir. Dis.* 122:259–263.
19. Thomson R, Carter R, Gilpin C, Coulter C, Hargreaves M. 2008. Comparison of methods for processing drinking water samples for the isolation of *Mycobacterium avium* and *Mycobacterium intracellulare*. *Appl. Environ. Microbiol.* 74:3094–3098.
20. Glazer CS, Martyny JW, Lee B, Sanchez TL, Sells TM, Newman LS, Murphy J, Heifets L, Rose C. 2007. Nontuberculous mycobacteria in aerosol droplets and bulk water samples from therapy pools and hot tubs. *J. Occup. Environ. Hyg.* 4:831–840.
21. Feazel L, Baumgartner L, Peterson K, Frank D, Harris J, Pace N. 2009. Opportunistic pathogens enriched in showerhead biofilms. *Proc. Natl. Acad. Sci. USA* 106:16393–16399.
22. Fujita K, Ito Y, Hirai T, Maekawa K, Imai S, Tatsumi S, Niimi A, Iinuma Y, Ichiyama S, Mishima M. 2013. Genetic relatedness of *Mycobacterium avium-intracellulare* complex isolates from patients with pulmonary MAC disease and their residential soils. *Clin. Microbiol. Infect.* 19:537–541.