ORIGINAL ARTICLE

Point-of-Use Membrane Filtration and Hyperchlorination to Prevent Patient Exposure to Rapidly Growing Mycobacteria in the Potable Water Supply of a Skilled Nursing Facility

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BACKGROUND. Healthcare-associated outbreaks and pseudo-outbreaks of rapidly growing mycobacteria (RGM) are frequently associated with contaminated tap water. A pseudo-outbreak of *Mycobacterium chelonae–M. abscessus* in patients undergoing bronchoscopy was identified by 2 acute care hospitals. RGM was identified in bronchoscopy specimens of 28 patients, 25 of whom resided in the same skilled nursing facility (SNF). An investigation ruled out bronchoscopy procedures, specimen collection, and scope reprocessing at the hospitals as sources of transmission.

OBJECTIVE. To identify the reservoir for RGM within the SNF and evaluate 2 water system treatments, hyperchlorination and point-of-use (POU) membrane filters, to reduce RGM.

DESIGN. A comparative in situ study of 2 water system treatments to prevent RGM transmission.

SETTING. An SNF specializing in care of patients requiring ventilator support.

METHODS. RGM and heterotrophic plate count (HPC) bacteria were examined in facility water before and after hyperchlorination and in a subsequent 24-week assessment of filtered water by colony enumeration on Middlebrook and R2A media.

RESULTS. *Mycobacterium chelonae* was consistently isolated from the SNF water supply. Hyperchlorination reduced RGM by 1.5 $\log_{10}$ initially, but the population returned to original levels within 90 days. Concentration of HPC bacteria also decreased temporarily. RGM were reduced below detection level in filtered water, a 3-$\log_{10}$ reduction. HPC bacteria were not recovered from newly installed filters, although low quantities were found in water from 2-week-old filters.

CONCLUSION. POU membrane filters may be a feasible prevention measure for healthcare facilities to limit exposure of sensitive individuals to RGM in potable water systems.

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Rapidly growing mycobacteria (RGM) have been associated with pulmonary colonization and pseudo-outbreaks from contaminated bronchoscopy equipment.[1-5] In July 2006, the Pennsylvania Department of Health was notified that 2 neighboring hospitals had seen an increase since January 2002 in the number of *Mycobacterium chelonae–M. abscessus*-positive cultures from bronchoscopies. The reference laboratory used by 1 hospital did not differentiate between *M. chelonae* and *M. abscessus*. An investigation was conducted to determine whether an outbreak was occurring and to determine the source to prevent colonization in patients at risk. The epidemiologic investigation identified 28 patients (21 from hospital A and 7 from hospital B) that accounted for 120 positive *M. chelonae–M. abscessus* bronchoscopy cultures during January 1, 2002–August 24, 2006. Of these patients, 15 (54%) were male, 23 (82%) were white, and median age at first positive *M. chelonae–M. abscessus* culture was 68 years (range, 21–85 years); 25 (89%) were chronically ventilator dependent at the time of first positive culture and resided in 1 skilled nursing facility (SNF A) specialized in respiratory care. The investigation did not identify a source of contamination of bronchoscopes at either hospital, including reprocessing procedures. Tap water was used for routine oral care for SNF A patients, and some patients were able to take ice chips and other liquids, including tap water, by mouth. During January 2002–June 2006, SNF A patients with a first positive acid-

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fast bacilli smear result were routinely admitted to local acute care hospitals under airborne isolation precautions to rule out *M. tuberculosis* infection; no tuberculosis cases were identified in this group. In July 2006, SNF A began screening new admissions for latent tuberculosis infection by using an interferon gamma release assay. Treating physicians at SNF A determined that most instances of positive *M. chelonae–M. abscessus* cultures likely represented colonization; however, 4 patients had findings consistent with American Thoracic Society criteria for lung disease caused by nontuberculous mycobacteria,[6] received antibiotic therapy, and recovered. Investigators concluded that the increase in positive cultures was due to antecedent colonization of SNF A patients who were frequently tested for mycobacteria during serial therapeutic bronchoscopies.[7]

Environmental mycobacteria, including *M. abscessus*, *M. chelonae*, *M. fortuitum*, *M. gordonae*, *M. paraffinicum*, *M. scrofulaceum*, *M. simiae*, *M. terrae*, and *M. xenopi*, have previously been associated with pseudo-outbreaks among hospitalized patients.[8-19] Frequently, systemwide contamination of hospital tap water with mycobacteria is the source for pseudo-outbreaks. The current investigation included an assessment of potential exposures for SNF A patients and an evaluation of interventions to reduce exposures to nontuberculous mycobacteria.

An environmental investigation was conducted at SNF A to describe the prevalence of RGM within the plumbing system, and a general assessment of water microbial quality was performed by measuring heterotrophic plate count (HPC) bacteria. Two water treatment interventions were also assessed in an effort to reduce potential exposures of ventilated patients to RGM. The 3 goals of this study were to identify the reservoir for RGM within the SNF, to assess the ability of hyperchlorination to reduce RGM and HPC bacteria in SNF A's plumbing system, and to assess the efficacy of point-of-use (POU) filters in reducing HPC bacteria and RGM in SNF A's tap water over a 24-week period.

METHODS

Facility Description

SNF A, located in Pennsylvania, specializes in subacute respiratory care of chronically ventilator-dependent patients. The potable water supplied to SNF A carried free chlorine (hypochlorite) residual.

Water Quality Testing

The free chlorine residual in water samples was measured on site by using the ferrous ammonium sulfate–N,N-diethyl-p-phenylenediamine[20] method (kit K-1515-C, Taylor Technologies). Temperature and pH also were measured.

Water Sampling and Processing

Sampling was performed before and after hyperchlorination and during a subsequent 24-week evaluation of POU membrane filters. Water samples were collected inside and outside of the facility in sterile 1-L Nalgene bottles containing sodium thiosulfate (0.01% final concentration; Fisher Scientific). Ice was obtained from the facility ice machine, placed in a 1-L Nalgene bottle, and allowed to melt during transport. Bottles were capped, sealed with parafilm, and shipped overnight to the Centers for Disease Control and Prevention (CDC) in a cooler containing cold packs. Samples were processed at CDC within 48 hours of collection.

Aliquots of water (300 mL) were decontaminated with 0.005% cetyl pyridinium chloride (final concentration) for 30 minutes at room temperature to reduce background bacteria. Decontaminated samples were filtered through mixed cellulose acetate membrane filters (diameter, 47 mm; pore size, 0.45 μm; Fisher Scientific). Filters were placed in 5 mL phosphate-buffered saline, pH 7.2, containing 0.1% polysorbate 80; bacteria were resuspended by 3 alternating cycles of sonication (42 kHz [$\pm$6%]) in a bath sonicator (Branson Ultrasonics) for 1 minute and vortexing at maximum setting for 30 seconds. Aliquots of 100 and 500 μL were spread-plated in triplicate on Middlebrook 7H11 + OADC selective agar containing per liter polymixin B, 200,000 units; carbenicillin, 50 mg; amphotericin B, 10 mg; and trimethoprim lactate, 20 mg (Becton, Dickinson [BD]); incubated at 30°C; and examined on days 3, 5, and 7 for the presence of RGM. HPC was also determined by plating additional water aliquots on R2A medium (BD) and incubating at room temperature for 7 days before colony enumeration.[20]

Facility Hyperchlorination Intervention

In January 2008, SNF A's potable water system was hyperchlorinated, increasing the hypochlorite concentration to a target of 25–35 mg/L for 2 hours and then reducing it to normal chlorine residual level within a 24-hour period. Chlorine was fed initially on the basis of estimated mass balance from average daily water consumption by adding sodium hypochlorite solution, 12.5%, to the incoming city water line. Once chlorine feed levels were developed in the building, chlorine feed was adjusted on the basis of actual free chlorine residual measured at the end of the distribution system. Chlorine residual was monitored at the farthest distal site initially every 15 minutes and then every 30 minutes until residuals were stable within developed parameters. After 2 hours, chlorine residual was returned to normal by flushing all water taps in the facility.

Water samples were collected immediately before and after hyperchlorination from 3 nurses' station sinks, a sink in a food preparation area, a patient unit bathroom sink, and an ice machine. The same sinks, as well as a fourth nurses' station sink, were sampled 90 days after hyperchlorination, in April

2008. Water samples were also collected from the main water supply in a shed near SNF A.

Facility Filter Installation and Sampling Schedule

POU membrane filters (0.2 μm; model AQ14F1S, Pall Medical), were installed on 3 of the same nurses' station sink faucets that were included in the hyperchlorination study and changed every 2 weeks. Water samples were collected every 4 weeks for 24 weeks, beginning in June 2008. At each sampling time point, water was collected before filtration, after filtration immediately after filter installation, and after filtration 2 weeks after filter installation. Water was also collected from the main supply in the facility basement and from a service station sink located 1 block away.

An inline filter was also placed on the supply line to the ice machine and changed every 2 weeks. The ice machine malfunctioned and was not sampled during the first 8 weeks of the filtration study. The new ice machine was in service for 2 weeks before an inline filter was installed. Ice was sampled during the last 4 samplings as described above.

Screening and Identifying RGM

Colonies on Middlebrook 7H11 selective agar that were transparent to opaque, were cream to buff colored, and exhibited smooth or rough morphology were enumerated as possible RGM. A subset of morphologically distinct colonies from each sample were subcultured onto Middlebrook 7H10 (BD) to screen for acid fastness by the Kinyoun method.[21] High-performance liquid chromatography (HPLC) analysis was used to identify representative acid-fast bacteria by a previously described method for extraction and separation of bromophenacyl-esterified mycolic acids.[22,23] When large numbers of isolates were obtained, they were screened by PCR restriction fragment length polymorphism analysis (RFLP, PRA) of a fragment of the 65-kD heat shock protein gene (hsp65).[24,25] Results of PRA screening were confirmed on a subset of isolates representing each RFLP type with sequence analysis of rpoB partial gene fragments.[26,27]

Statistical Analysis

Bacterial plate counts were $\log_{10}$ transformed and mean counts from different sampling points were compared by a 2-tailed t test. A P value less than .05 was considered statistically significant.

RESULTS

Hyperchlorination Intervention

Hyperchlorination of SNF A's potable water system reduced RGM and HPC bacteria by 1.5 and 2.0 logs, respectively (Figure 1). RGM were reduced from 3.39 to 1.91 $\log_{10}$ colony-forming units (CFU)/L ($P = .031$) and HPC bacteria decreased from 2.74 to 0.77 $\log_{10}$ CFU/mL ($P < .01$) 24 hours after the commencement of hyperchlorination. However, bacteria returned to pretreatment levels within 90 days of the intervention. RGM and HPC counts 90 days after intervention were significantly higher than 24-hour counts ($P < .01$) but not significantly different from pretreatment counts, demonstrating the temporary effect of hyperchlorination on the microbial population in the facility's water system.

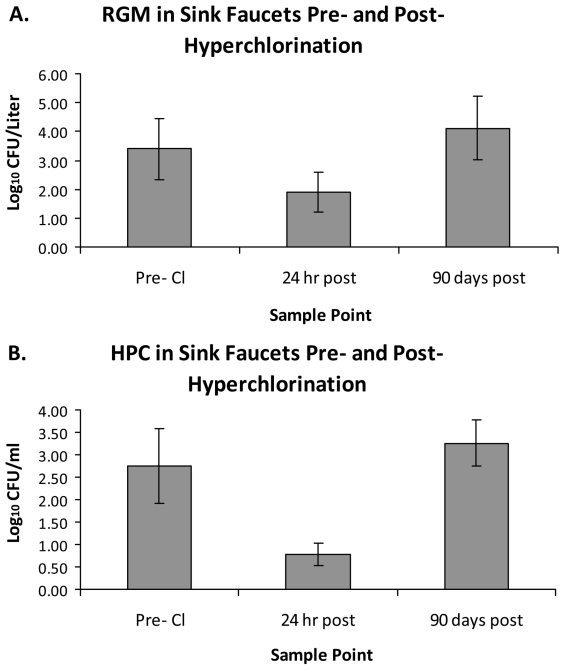

FIGURE 1. Effect of hyperchlorination on rapidly growing mycobacteria (RGM; *A*) and heterotrophic plate count (HPC) bacteria (*B*) in water collected from sink faucets in skilled nursing facility (SNF) A. Samples were collected before (Pre-Cl), 24 hours after, and 90 days after hyperchlorination. Error bars indicate standard deviation; $n = 5$, before chlorination and 24 hours after; $n = 6$, 90 days after. RGM concentration is expressed as $\log_{10}$ colony-forming units (CFU)/L; HPC bacteria concentration is expressed as $\log_{10}$ CFU/mL.

Water Quality during Hyperchlorination and Filtration Assessments

Potable water provided to the facility during both assessments contained residual free chlorine of 0.37 mg/L (range, 0.17–0.47 mg/L). Water within the SNF, measured at nurses' station sink taps, contained 0.4 mg/L free chlorine immediately before and 24 hours after the hyperchlorination. Residual free chlorine measured in water from the same nurses' station sinks during the 24-week filtration assessment was sometimes near the limit of detection, averaging 0.2 mg/L over several months (range, 0.02–0.43 mg/L; SD, 0.11; $N = 18$). Similarly, water from the SNF basement main supply and a nearby service station shop sink contained 0.21 and 0.18 mg/L free chlorine, respectively (basement range,

0.16–0.33 mg/L; SD, 0.08; $N = 4$; shop sink range, 0.09–0.33 mg/L; SD, 0.09; $N = 5$). Water pH at the facility sink taps and basement main supply and in the nearby shop sink tap ranged from 7.5 to 8.2. The mean temperature was 20.6°C (SD, 5.72; $N = 18$).

POU Membrane Filter Efficacy

RGM levels in unfiltered water from sink faucets at the nurses' stations ranged from 3.16 to 3.88 $\log_{10}$ CFU/L (mean values; Table 1, RGM). RGM were not detected in water obtained through newly installed filters or filters in place for 2 weeks (detection limit was 3 CFU/L). RGM CFU enumeration was significantly higher in unfiltered water at the nurses' station sinks than in water from the basement of the facility or from the service station sink ($P < .001$).

HPC bacteria levels ranged from 2.69 to 3.33 $\log_{10}$ CFU/mL (Table 1, HPC) in unfiltered water during the filtration assessment. In contrast to RGM, HPC bacteria in the nurses' station sink water were not statistically different from the service station or SNF basement samples. Although RGM were not recovered from old or new filters, HPC bacteria were detected in water samples collected from the effluents of 2-week-old filters but in lower amounts than found in unfiltered water ($P < .001$). A single new filter on 1 nurses' station sink faucet contained 97 CFU/L bacteria.

RGM and HPC bacteria were recovered from ice obtained from the facility ice machine during prehyperchlorination sampling (Table 2). Although hyperchlorination reduced RGM and HPC bacteria by 2.9 and 1.4 $\log_{10}$, respectively, bacteria were not eliminated from ice. RGM and HPC bacteria increased within 90 days, though not back to baseline. Even the addition of inline filters, changed every 2 weeks, did not eliminate RGM and HPC bacteria in the downstream ice. During the study, the ice machine malfunctioned and was replaced. The first ice sample from the new machine, obtained at week 12 of the filtration assessment, did not contain detectable RGM. However, RGM were recovered from subsequent samples, in increasing amounts, during weeks 16, 20, and 24, to 2.54 $\log_{10}$ CFU/L at 24 weeks.

RGM Isolate Identification

From SNF A water from sinks, the ice machine, the main water supply, and a nearby service station, 112 RGM isolates were recovered (Table 3). *Mycobacterium chelonae* was common to all sampling locations throughout the hyperchlorination and filtration studies (50/112 isolates) and was the only RGM species isolated from SNF A's water system immediately after hyperchlorination. Other RGM isolates analyzed by HPLC or PRA demonstrated group-level similarity to *M. mucogenicum*, *M. phocaicum*, *M. fortuitum*, and *M. peregrinum*. Species identification was confirmed by *rpoB* gene sequence analysis. The most frequently recovered isolate related to the *M. mucogenicum/phocaicum* complex was *M. llatzerense*. An unidentified species shared 94% sequence similarity to *M. fortuitum*, *M. peregrinum*, and related species. While the aforementioned isolates were recovered from drinking water inside and outside the facility, *M. immunogenum* was found only in ice from the new ice machine.

DISCUSSION

POU membrane filters provided a successful barrier against patient exposure to RGM residing in SNF A's plumbing. The facility contained significantly higher concentrations of RGM in its premise plumbing than found in the main water supply entering the facility or in a service station shop located 1

TABLE 1. Effect of Point-of-Use Filter on Rapidly Growing Mycobacteria (RGM) and Heterotrophic Plate Count (HPC) Bacteria during a 24-week Assessment of Sink Water in a Long-Term Care Facility

	Week					
	4	8	12	16	20	24
RGM counts, $\log_{10}$ CFU/L (SD)						
SS	2.60	2.64	ND	2.30	2.85	ND
Basement	2.60	2.43	2.60	2.24	2.75	2.11
NS sinks, unfiltered (SD)	3.28 (0.94)	3.78 (0.85)	3.77 (0.64)	3.16 (1.39)	3.88 (0.17)	3.45 (0.39)
NS sinks, 2-week-old filter	ND	ND	ND	ND	ND	ND
NS sinks, new filter	ND	ND	ND	ND	ND	ND
HPC counts, $\log_{10}$ CFU/mL (SD)						
SS	4.17	2.59	...	3.18	2.20	2.18
Basement	2.69	3.22	...	3.07	1.54	2.31
NS sinks, unfiltered (SD)	3.19 (0.20)	3.33 (0.71)	...	2.97 (1.62)	3.06 (0.83)	2.69 (0.35)
NS sinks, 2-week-old filter	NC	0.91 (1.00)	...	1.81 (1.16)	1.95 (0.41)	0.97 (0.68)
NS sinks, new filter	ND	ND	...	ND	ND	ND

NOTE. For facility nurses' station (NS) sinks, $n = 3$, except for the 2-week-old filters during week 8, from which water was collected from 1 filter only. HPC bacteria were not measured in the week 12 samples. NC, not countable; ND, not detected (detection limit <7 colony-forming units [CFU]/L for service station [SS] sink water, <3 CFU/L for facility water); SD, standard deviation.

TABLE 2. Rapidly Growing Mycobacteria (RGM) and Heterotrophic Plate Count (HPC) Bacteria in Ice from Old and New Ice Machines

Sample	RGM, $\log_{10}$ CFU/L	HPC bacteria, $\log_{10}$ CFU/mL
Old ice machine hyperchlorination study		
Before hyperchlorination	4.45	3.85
After hyperchlorination, 24 hours	1.52	2.47
After hyperchlorination, 90 days	2.67	>3.48
New ice machine POU filtration study, weeks		
12	Below detection (<7/L)	Not tested
16	1.52	>2.48
20	1.84	3.82
24	2.54	3.57

NOTE. An inline filter was installed in the ice machine water supply line and changed every 2 weeks. The new ice machine was in service for 2 weeks before an inline filter was installed. POU, point-of-use.

block from the facility. Conditions that may have enhanced growth of RGM in SNF A's plumbing include low residual free chlorine and low water usage. US Environmental Protection Agency regulation requires residual disinfectant to be equal to or greater than 0.2 mg/L at the entrance to the water distribution system, and it must be detectable throughout the system.[28] Although SNF A water met this requirement during both assessments, residual disinfectant level was lower inside the facility than in the water distribution system. Additionally, low flow or stagnation in potable water lines can lead to increased bacterial growth.[29] Possibly, a flushing regime could improve microbial water quality in facilities with low water usage.[30]

Temporary efficacy of hyperchlorination was not surprising after chlorine returned to normal residual level. As a frequently repeated treatment, hyperchlorination presents challenges because the facility may not use its drinking-water system during the 24-hour treatment. Other alternative treatments include supplemental chlorination of the facility water system, as is performed in some hospitals.[31] Previous research demonstrated that most RGM are highly tolerant to chlorine levels normally found in potable water,[32,33] suggesting that low-level, long-term supplemental chlorination may not be an effective solution for SNF A. Also, in a small facility, providing salary for a licensed engineer to treat the water system would be greater than monthly replacement of 4 POU filters in nurses' station sinks, with an estimated cost of $6,500/year (manufacturer list price, March 2011).

Positive *M. chelonae–M. abscessus* cultures obtained in patients at this facility resulted in acute care hospitalizations to rule out *M. tuberculosis*, and several patients received antibiotic therapy for RGM infection. During the initial investigation, public health authorities advised that SNF A use sterile water instead of tap water for oral care and other purposes where respiratory exposure might occur. This investigation was limited to a single water system within an SNF that serves a unique population of ventilator-dependent residents. Other SNFs may serve residents with different levels of susceptibility to colonization or infection. Occurrence of particular RGM species may vary between geographic regions and source waters, suggesting that choice of supplemental water treatment should be tailored to each facility.

In contrast to RGM, the quantity of HPC bacteria was similar inside and outside the facility. Although not currently regulated in American drinking water, HPC bacteria are used as a general indication of microbial water quality.[28] Others have observed more HPC bacteria in drinking water within residential premise plumbing than found in the main water distribution system.[34] It was not clear in this study what conditions led to increased RGM but not HPC bacteria within premise plumbing relative to the main water supply. Possibly flow and nutrient conditions favored RGM biofilm formation in facility plumbing.

HPC bacteria were recovered from a single newly installed POU filter. Since no other new filter water sample during any sampling point contained HPC bacteria, it was thought to be carryover contamination during filter change, water sampling, or laboratory processing. Less than 2.70 $\log_{10}$ CFU/mL HPC bacteria were found in samples obtained through 2-week-old filters. Presence of HPC bacteria in older filters may have been due to backsplash from the sink into the outlet of the filters, sample contamination during sampling or processing, or breakthrough contamination. The cause of HPC bacterial contamination of POU filters was not determined in this study.

RGM were enumerated by a semiselective protocol. Although some environmental mycobacteria studies involve incubating plate medium for 8 weeks to encourage growth of the most slowly growing isolates,[35] 7 days of incubation at 30°C was chosen to maximize recovery of RGM only. Limitations of the current isolation protocol include possible reduction of RGM along with HPC bacteria during the decontamination step and the difficulty in screening a representative group of environmental isolates to find the species of interest. Some mycobacteria can have variable colonial morphologies within a single strain.[36]

As this was a retrospective study, clinical *M. chelonae* isolates were not available for comparison with environmental

TABLE 3. Species Identification of Rapidly Growing Mycobacteria (RGM) Isolates Obtained from Water Samples during the Hyperchlorination and Filtration Interventions

	Species, no. of isolates			
Sample location	Before hyperchlorination	After hyperchlorination (24 hours)	After hyperchlorination (90 days)	Filtration study (compilation of 6 sampling times during 24-week study)
Nurses' station sinks	*M. chelonae*, 1; *M. mucogenicum/phocaicum* complex, 1	*M. chelonae*, 1	*M. chelonae*, 3; *M. fortuitum/peregrinum* complex, 3; *M. mucogenicum/phocaicum* complex, 2	*M. chelonae*, 22; *M. fortuitum/peregrinum* complex, 6; *M. llatzerense*, 9; *M. mucogenicum*, 1; unknown species, 18
Food prep and bathroom sinks (hyperchlorination study only)	*M. chelonae*, 1	*M. chelonae*, 1	*M. chelonae*, 4	Not sampled
Main shed (initial hyperchlorination), basement water supply (filtration study)	*M. chelonae*, 2		Not sampled	*M. chelonae*, 9; *M. fortuitum/peregrinum* complex, 2; unknown species, 5
Service station, 1 block away	Not sampled during hyperchlorination study	Not sampled during hyperchlorination study	Not sampled during hyperchlorination study	*M. chelonae*, 2; *M. mucogenicum/phocaicum* complex, 1; unknown species, 3
Old ice machine, ice	*M. chelonae*, 1; *M. llatzerense*, 2	No isolate was examined	*M. chelonae*, 1; *M. fortuitum/peregrinum* complex, 1	Not examined, no longer functioning
New ice machine, ice	Not yet installed	Not yet installed	Not yet installed	*M. chelonae*, 1; *M. immunogenum*, 3
New ice machine, unfiltered supply water	Not examined	Not examined	Not examined	*M. chelonae*, 1; unknown species, 2
New ice machine, filtered supply water	Not examined	Not examined	Not examined	*M. chelonae*, 1; unknown species, 2

isolates. However, *M. chelonae* was recovered throughout the facility during the entire course of the study, suggesting facility tap water as possible source of the pseudo-outbreak.

Another RGM isolated from facility premise plumbing, *M. llatzerense*, is a newly recognized rapidly growing *Mycobacterium* related to *M. mucogenicum* and *M. phocaicum*, first isolated in the water supply of a hemodialysis unit in a Spanish hospital.[37,38] This is the first report of isolating *M. llatzerense* from a North American potable water system. It was isolated from 4 of the 6 sampling time points during the filtration study (data for time points not shown). Finding the same species of RGM consistently throughout the study suggests that these organisms are long-term inhabitants of biofilm within the main water distribution system and SNF premise plumbing.

Ice machines in healthcare facilities can be important sources of infection or pseudo-infection.[13,39] Without proper maintenance, filtering the supply water will not eradicate the microbial biofilms already present in the ice machine plumbing, as observed during this study. Hyperchlorination led to similar temporary effects on ice microbial quality as it did on the rest of the premise plumbing. RGM in the new ice machine increased from nondetectable to 2.54 $\log_{10}$ CFU/L within 3 months, reinforcing the importance of following cleaning or disinfection schedules as part of routine maintenance.[40]

In conclusion, POU membrane filtration may lower the exposure risk of sensitive patients to RGM in healthcare facilities, providing 1 infection prevention strategy. This option proved effective in a small facility treating patients limited in water usage or exposure. Hyperchlorination is not an effective long-term control measure for mycobacteria residing in healthcare premise plumbing.

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