TITLE: Use of Copper-Silver Ionization for the Control of Legionellae in Alkaline Environments at Health Care Facilities

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PEER REVIEW SNAPSHOT

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INTRODUCTION

This study, published in The American Journal of Infection Control in 2015, demonstrated the efficacy of copper-silver ionization under alkaline water conditions in two health care facilities. The facilities kept the water supply at a high pH in order to control biofilm production and corrosion.

PURPOSE

The purpose of this study was to evaluate the success of copper-silver ionization (CSI) in maintaining a *Legionella*-free environment when installed on the hot water system of a facility in alkaline conditions (high pH).

METHOD

- The study was conducted on two separate building, an acute care facility (ACF), and a long-term care facility (LTCF). Prior to the start of the study, both were populated with *Legionella*.
- CSI systems were installed on the hot water recirculation lines of both buildings.
- Both buildings were tested regularly for *Legionella,* copper, silver, conductivity, and chlorine residual from previous unsuccessful treatment.
- Copper and silver levels in the water were monitored and kept well under EPA limits.

RESULTS

- The research found that the copper-silver ionization system brought the *Legionella* levels to non-detect.
- In both buildings, the copper and silver ion levels remained stable despite the high pH (average of 9 between the two buildings).
- Two years after the initiation of the CSI system, no further cases of Legionnaires' disease were reported at the studied facilities.

NOTEWORTHY

- Prior to the installation of a copper-silver ionization system, the hospital had previously attempted to use superheat and flush, chlorine dioxide treatment, as well as shock hyperchlorination, and all methods failed to bring *Legionella* levels to non-detect.
- The LiquiTech copper-silver ionization system proved to be successful in eliminating *Legionella* bacteria.
- The researchers noted that the advantages of the copper-silver ionization system included its easy installation and maintenance, nontoxic byproducts, its long residual protection time, and its success regardless of temperature, unlike chlorine and ozone, which degrade at high temperatures.

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Major article

Use of copper-silver ionization for the control of legionellae in alkaline environments at health care facilities

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Key Words:
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Percentage positivity

Background: There are multiple treatment options for the control of legionellae in premise hot water systems. Water chemistry plays a role in the efficacy of these treatments and should be considered when selecting a treatment. This study demonstrated the efficacy of copper-silver ionization (CSI) under alkaline water conditions in 2 health care facilities.

Methods: Monitoring for copper (Cu) and silver (Ag) ions was performed, and the corresponding percentage of positive *Legionella* cultures was monitored. Low *Legionella* colony forming units (CFU), with a mean <10 CFU/100 mL, and ≤30% positive culture for each sampling period, along with no recurrent disease, were considered indicative of control.

Results: CSI treatment was shown to reduce both the number of CFU found and the percentage of samples found to be culture positive. After treatment was established, culture positivity was, for example, reduced from 70% (>10^3 CFU/100 mL) to consistently <30% (38 CFU/100 mL).

Conclusion: Control of legionellae in premise water systems may be a complex process requiring long-term assessments for adequate control. This work found that CSI could be successful in controlling *Legionella* under alkaline water conditions, and the evidence suggests that Ag ions are responsible for the control of *Legionella pneumophila* 1, *L pneumophila* 6, and *L anisa*.

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Legionella spp cause disease in both acute and long-term health care settings under a variety of conditions.[1,2] Recognizing premise water systems as a source of *Legionella pneumophila* has led to extensive work in the control of colonization using physical and chemical agents. Acute, short-term treatments often rely on hyperchlorination,[3] heat,[3,4] or point-of-use filtration[5] for immediate control, whereas long-term control relies on more extensive in-house equipment and treatment. Included in the suite of choices are continuous chlorination,[6] chlorine dioxide,[7] chloramination,[8] and copper-silver ionization (CSI).

The long-term use of CSI was shown to be effective in 16 hospitals over a 5-year period,[9] whereas other workers have found that there was limited *Legionella* control for a period of <2 years.[10] In the latter work it was suggested that the legionellae developed a tolerance to silver (Ag) ions, and higher concentrations of Ag ions were needed for effective control. Lin et al[11] reported that copper (Cu) ions precipitated at alkaline pH (8.5-9.0), and there was a concomitant decline in the control of *Legionella*. In addition, increased levels of chloride could reduce the availability of Ag ions and potentially reduce their efficacy as a biocide. More recent work also found CSI was effective for the control of both planktonic and biofilm-associated legionellae.[12]

The work reported here originally involved 1 acute care facility that had 6 cases of legionellosis. Ultimately, a technical intervention was developed by the New York State Department of Health (NYSDOH) to understand the efficacy of CSI for controlling various legionellae in the premise water systems with alkaline water.

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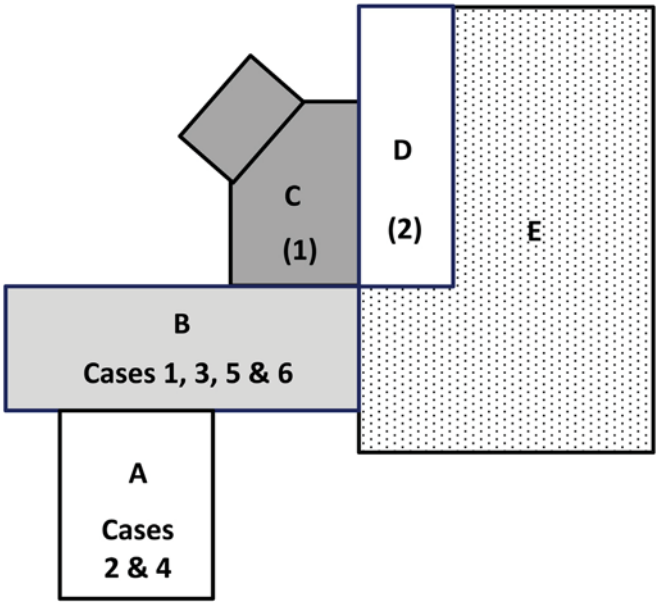

Fig 1. Simplified schematic of the complex structure of the acute care facility showing disease occurrence. Numbers in parentheses indicate additional locations of case patient exposures. Location B is discussed in the narrative as an example of treatment efficacy.

The high pH was used by the water purveyor to control corrosion and limit biofilm. The hospital was a complex assemblage of 7 in-house premise hot water systems (Fig 1). To gain control of colonization, several buildings of the hospital were treated with chlorine dioxide (ClO_2). The selection of ClO_2 was because of its demonstrated effectiveness over a broad pH range (6.0-8.5).[7] Work was then extended to an associated long-term care facility (LTCF) managed by the same group, in the same neighborhood, that also had alkaline water in its distribution system. Unlike the ACF, the LTCF had used no prior treatment other than an emergency hyperchlorination as an acute response to 2 cases of legionellosis. Ultimately, CSI was used for long-term control in both locations. The efficacy of the treatment methods and their impact on recurrent illness are reported.

MATERIALS AND METHODS

Approach

The ACF had 10 sample locations, and the LTCF had 11. The sample locations included case rooms, existing sample locations that had a history of positive culture data, outlying areas that represented the hot water distribution as a whole (low, average, and maximum water age), and the hot water return. The cold water inlet was also sampled as a control data point. Analyses included *Legionella* culture, Cu, Ag, pH, conductivity, temperature, and chlorine residual. Culture monitoring, to determine the effectiveness of ionization, relied on determining the number of *Legionella*-positive sites (percentage positivity) to follow the persistence, or control, of the *Legionella* populations. The convention of determining the percentage of positive sites as a reliable measure of the extent of colonization was used[1,2,9,13] along with colony forming units (CFU) enumeration for verification of control.

Sample collection

All hot water samples were first draw samples. Depending on the destination laboratory, 100 mL (NYSDOH, Wadsworth Laboratory), 110 mL, or 1 L (contract laboratories) of sample was aseptically collected after faucet aerators were removed. Sample bottles contained thiosulfate to inactivate free chlorine and other oxidants. Samples were capped and stored on synthetic ice bricks (0°C-4°C) in coolers and then transported to the appropriate laboratory. An additional 100 mL was subsequently collected for heterotrophic plate counts, and a separate sample was collected for total organic carbon analysis. The next 200 mL were for additional on-site wet chemical analysis, including pH, conductivity, temperature, turbidity, and Cu.

Methods: contract laboratories

Legionella sp culture was performed using direct and concentrated culture methodology. Direct cultures were performed by plating 100 μL of water directly onto buffered charcoal yeast extract (BCYE) agar and BCYE selective media with dye, glycine, vancomycin, and polymyxin B (DGVP). For concentrated cultures, 100 mL of the original water sample were filtered through a 0.2-μm polycarbonate filter (Whatman; VWR Scientific, Chester, PA), resuspended in 10 mL of the original unfiltered water sample, and vortexed; 100-μL aliquots were subsequently plated onto BCYE agar and BCYE with DGVP agar. All plates were incubated at 37°C for 7 days. Colonies suspected of being *Legionella* spp were tested using latex agglutination followed by direct fluorescent antibody staining (m-TECH, Alpharetta, GA) to confirm the presence of *Legionella* spp. All cultures handled in this fashion were performed at the Special Pathogens Laboratory (Pittsburgh, PA).

A second vendor laboratory (EM P&K Laboratory, Marlton, NJ) also used direct plating or filtration for potable water samples (1,000 mL). Samples were concentrated by using 0.2-μm polycarbonate filters and then resuspended in 5.0 mL of sterile water. Samples were plated on both PCV and GPVC. The change does not make that clear. Suggest: Samples of water or resuspended pellet (100 μL) were plated on BCYE supplemented with polmyxin B, cycloheximide and vancomycin (PCV) and GPVC (PCV with glycine).

Heterotrophic plate counts were performed according to method 9215.[14]

Methods: NYSDOH Laboratory

Samples processed by the NYSDOH were plated directly or after concentration. Potable environmental samples (15 mL) were concentrated 1:30 by centrifugation (4,000 rpm Beckman CS-6 with GH3.8 horizontal rotor for 20 minutes; Beckman Coulter, Inc, Brea, CA). All but 0.5 mL were carefully removed, and 0.05-mL aliquots of samples were plated on blood agar; BCYE with 0.1% alpha-ketoglutarate (BCYEα); and BCYE with cefamandole, polymyxin B, and anisomycin plates and streaked for isolation. In some instances BCYE agar with DGVP was used.

The concentrated pellet was tested by polymerase chain reaction for the presence of the 23S rRNA genus-wide gene and the *L pneumophila*-specific *MIP* gene. Results of the polymerase chain reaction analysis were used to guide culture steps. Plates were placed in plastic bags to maintain moisture, incubated in ambient air at 35°C-37°C, and examined every other day for 10 days. Typical colonies that grew on BCYEα slants with L-cysteine and failed to grow (or grew poorly) on BCYEα without L-cysteine were considered presumptive *Legionella* positive and were confirmed using the following tests: direct fluorescent antibody (m-Tech, Alpharetta, GA), auto-fluorescence, catalase, oxidase, urease, gelatinase, and browning on Feeley Gorman Agar (Sigma-Aldrich Corp., St. Louis, MO).[15]

Table 1
Water characteristics

Parameter	Distribution system water range*	Acute care facility†				Long-term care facility			
		No. of samples (n)	Range	Median	Mean ± SD	No. of samples (n)	Range	Median	Mean ± SD
pH	8.6-9.4	58	8.7-9.9	9.4	9.4 ± 0.3	66	8.9-9.7	9.3	9.4 ± 0.2
Hot water temperature (°C)‡	NA	53	23.0-42.0	34.0	33.2 ± 5.0	69	21.0-50.0	30.0	29.9 ± 4.2
Free chlorine (ppm)	1.4-1.7	57	0.0-1.19	0.12	0.24 ± 0.28	77	0.0-1.31	0.17	0.27 ± 0.31
Conductivity (μmhos/cm)	—	58	99.6-211.4	146.6	142.3 ± 22.9	66	119.6-217.3	145.4	146.1 ± 16.3
Turbidity (NTU)	0.11-023	58	0.2-7.1	0.86	1.4 ± 1.6	33	0.27-34.2	0.60	1.9 ± 5.9
Heterotrophic plate count (CFU/mL)	—	51	1.0-7.2 × 10^4	14.0	1.4 × 10^3 ± 1.0 × 10^4	48	1.0-8.4 × 10^2	7.5	61.7 ± 150.6

Note: dash indicates data not provided in distribution monitoring report.
CFU, colony forming units; *NA*, not applicable; *NTU*, nephelometric turbidity units.
*Data are from a typical July distribution system monitoring report.
†Example data are from location E in Figure 1.
‡Temperature data are first draw and final flush samples.

CSI systems

CSI units manufactured by Enrich Products (Model CU-EPI-3; Pittsburgh, PA) were used in all hot water locations. After commissioning, the target Ag concentrations were generally in the range of 0.01-0.08 mg/L. Frequent examination of the electrodes for scaling was needed for proper maintenance and continued disinfection. Electrodes were used for up to 2 years and replaced when they were 25%-50% of their original size and the desired amperage could no longer be attained. Cu and Ag analyses were performed weekly. All data presented here are for values determined from the hot water recirculation return line.

Cu and Ag analyses

Water samples were immediately preserved with nitric acid on receipt at the laboratory. After preservation and nitric acid digestion, the samples were analyzed using inductively coupled plasma-mass spectrometry using USEPA method 200.8, Determination of Trace Elements in Water and Wastes by Inductively Coupled Plasma-Mass Spectrometry.

On-site analyses

Temperature, pH, and conductivity were measured using a HACH HQ40d combination meter (58,258-00; HACH, Loveland, CO). Water temperature was confirmed using a HACH Pocket Pal Temperature Tester (44,450-01; HACH, Loveland, CO). Cu was measured on-site with a portable colorimeter (Pocket Colorimeter II, 58,700-19; HACH, Loveland, CO) using the manufacturer's premeasured reagents (CuVer 1, 21,058-69; HACH, Loveland, CO). Free chlorine was determined using a portable colorimeter and the N,N-diethyl-*p*-phenylenediamine (DPD) method (Pocket Colorimeter II, PCII-CHLOR; HACH, Loveland, CO). Turbidity readings were done using a HACH 2100Q Portable Turbidimeter (2100Q01; HACH, Loveland, CO).

Data analysis

Data were accumulated in, and rudimentary analyses were performed using, Microsoft Excel 14.0 (Microsoft, Redmond, WA). Descriptive statistics were performed using SYSTAT 13.1 (Systat Software, Chicago, IL), whereas additional statistical analyses were done using SAS 9.4.0 (SAS Institute, Cary, NC).

RESULTS

Acute care hospital

A total of 6 cases were reported between the period of April 2009 and October 2010. The initial case of legionellosis was reported in a patient with exposures to several locations (Fig 1). Once the first case had occurred, both retrospective and prospective patient surveillance were initiated. Initial culture results indicated the presence of *L pneumophila* 1. However, *L pneumophila* 6 or *L anisa* were codominant organisms in other hot water systems in the complex.

The facility hired consultants to evaluate treatment options that included periodic heat and flush or hyperchlorination and continuous treatment with ClO_2, low-level continuous chlorination, and CSI. Characterization of the hot water system (Table 1) showed that the pH range (8.7-9.9) of the acute care hospital discouraged the use of chlorine and suggested the use of ClO_2. The latter would be more effective at these alkaline pH values. A heat and flush routine[4] was performed as an acute response in 2 units in August 2009, but regrowth of legionellae occurred fairly rapidly in location B of the ACF, therefore confirming the need for long-term treatment. Based on culture results for legionellae, ClO_2 systems were installed throughout the hospital in October 2009 (locations B-D; Fig 1).

The metric for culture results was the number of positive sample sites as a percentage of the total number of sites sampled; also known as percentage positivity.[1,2,13,16] Although this current study was ongoing, the 30% threshold was questioned by other workers[17]; CFU were also used to determine efficacy, and prospective surveillance of the patient-resident populations continued in parallel.[18] These interventions were started because of the high level of positive samples experienced in several locations of the ACF during ongoing ClO_2 treatment from October 2009-January 2011 (eg, <1-3.6 × 10^4 CFU/100 mL, with a median of 1,200 CFU/100 mL, n = 66). In addition, unit maintenance was performed on the ClO_2 units in January 2011, but regrowth occurred in June and continued to be problematic through August 2011. Because of the issues of regrowth, the initial intervention visit was made in August.

The first indication that CSI might be an effective treatment occurred in January 2011. A CSI unit was installed as a short-term treatment[19] in location A of the ACF (Fig 1). It effectively reduced the *Legionella* populations to undetectable levels and, as planned, the unit was removed after 3 months. Three months after removal of the unit, culture results increased and did not decline again until the ionization unit was permanently reinstalled. Consequently, CSI was installed in multiple areas of the ACF, but location B (Fig 1) will be used as an example of treatment efficacy.

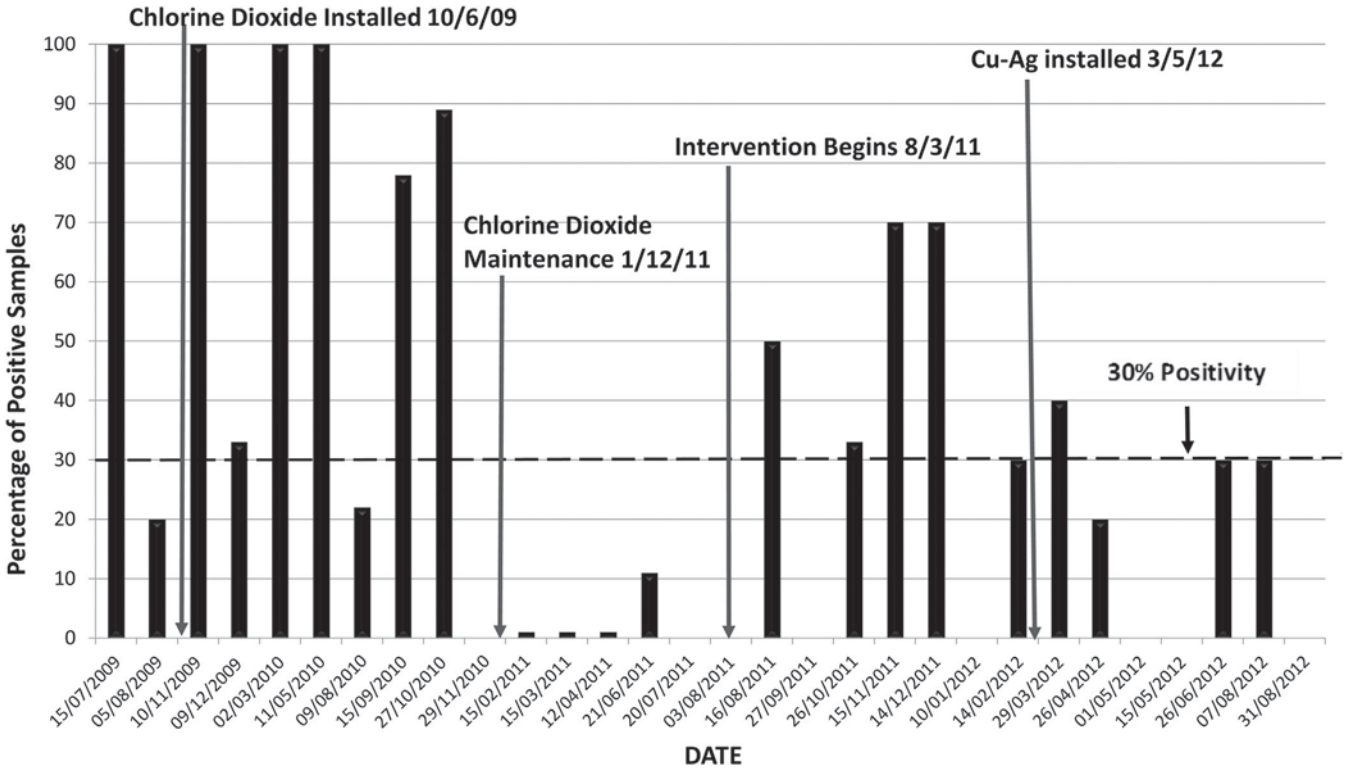

Fig 2. Percentage of culture positive sites in location B (Fig 1) prior to and during an intervention at the acute care facility. Arrows indicate key events during the investigation and treatment. Initial treatment at this location was chlorine dioxide. *Ag*, silver; *Cu*, copper.

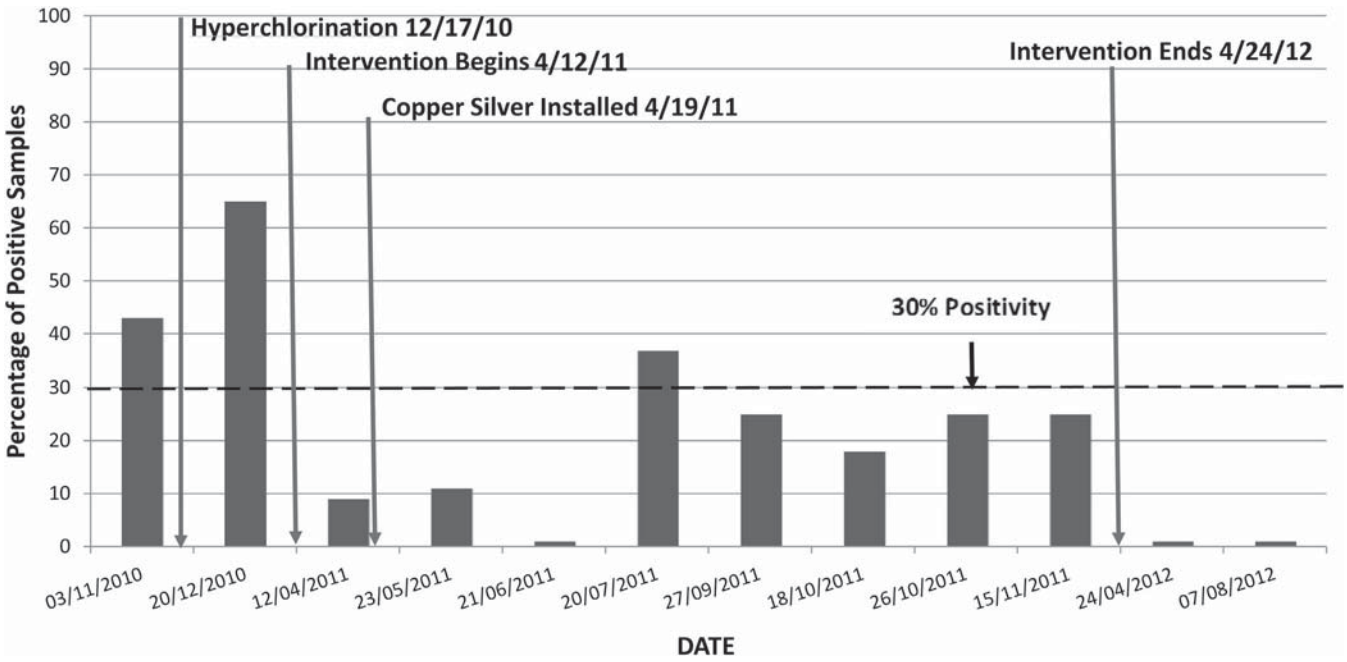

Fig 3. Percentage of culture positive sites prior to and during the intervention at the long-term care facility. Arrows indicate key events during the investigation and treatment. The only treatment that predated copper-silver ionization was the hyperchlorination event that occurred in December 2010.

After ClO_2 treatment was installed, the positivity levels varied from 22%-100% (<1.0- >10^5 CFU/100 mL, n = 79). Maintenance of the treatment units in January 2011 resulted in low levels of legionellae being detected from February 2011 to early August 2011 (<10% positive represented by a single recovery of 120 CFU/100 mL) (Fig 2). Starting in mid-August, on-going monitoring showed positivity levels from just above 30%-70% (mean of 282.5 CFU/100 mL, with a range of <1.0-7,900 CFU/100 mL, n = 49). CSI was initiated in March 2012 and from April 2012 until the end of the intervention in August 2012, the percentage of positive sites remained ≤30% (mean of 2.6 CFU/100 mL, range of <1.0-38 CFU/100 mL, n = 40).

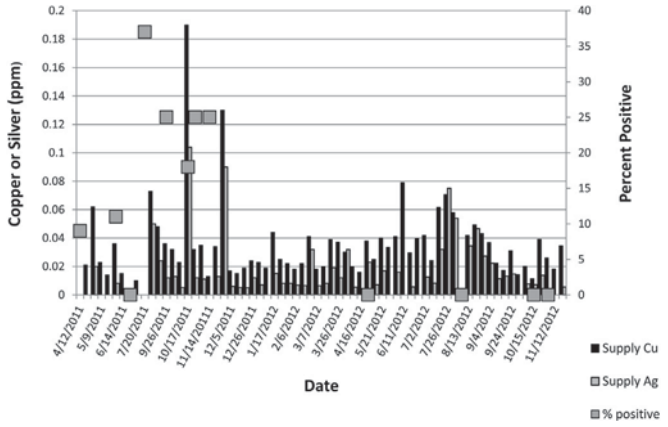

Fig 4. Copper and silver ion concentrations during start-up and stabilization of copper-silver ionization. Superimposed on the bars are the corresponding percentages of positive cultures (light gray squares). Early low transients (July, October, and November 2011) resulted in rapid increases in positive cultures, whereas later transients did not (April, July, and November 2012).

Locations that switched from ClO_2 to CSI responded in a fashion very similar to, or better than, location B. The ClO_2 systems installed in locations C and D were functioning normally and effectively; regular sampling that paralleled the monitoring of the CSI installation resulted in no recovery of any legionellae at these locations. During the period of this intervention and follow-up through March 2015, prospective surveillance showed that there was no recurrence of legionellosis in any of the buildings associated with the hospital.

LTCF

This intervention compared the relatively untreated LTCF with sections of the ACF that had been subjected to ClO_2 and, eventually, CSI. This facility was never treated with ClO_2 but was treated using a short-term emergency hyperchlorination. There was disagreement among the concerned parties about the occurrence of *L anisa* and the potential for disease. The literature cites that these organisms rarely cause disease.[20] These blue-white fluorescent *Legionella* spp are often regarded as a minor threat, but they may be an indicator of *L pneumophila* presence.[21] The history of *L anisa* in this location and the occurrence of *L anisa* pneumonia in the affiliated LTCF made its control a prudent step. Various species of *Legionella*, including *L anisa*, elicit a heightened concern for the NYSDOH because they either have previously caused disease in New York State or they dominate the *Legionella* populations in a particular facility. Occurrence (ie, positivity) was approximately 40%-80%, and culture data indicated colony counts in the range of <1.0-350 CFU/100 mL (mean = 84.5 CFU/100 mL, n = 14) for *L anisa*, which exceeded the level of concern (ie, any double-digit CFU/100 mL; NYSDOH, Environmental Health Information Related to Legionellosis in Healthcare Facilities; 2012, unpublished data). In September 2010 there was 1 case of legionellosis, and the sputum sample was positive for *L anisa*. A second case occurred in October 2010; the agent involved was *L pneumophila* 1, and the patient died. This is the point at which a short-term hyperchlorination was implemented (Fig 3; December 2010). This treatment was followed in January 2011 by water restrictions for residents that included point-of-use filters for showers (Pall-Aquasafe; Pall Corp, Port Washington, NY). The LTCF staff attended to the cleaning and disinfection of the ice machines and their water dispensers, which had shown very high concentrations of *L anisa* (320 CFU/100 mL). During this time decisions were made for long-term treatment; CSI was installed in April 2011 (Fig 3).

Results indicated that for the first full year of CSI treatment the positivity was <30% (range, <1.0-60 CFU/100 mL, with mean CFU of 3.8/100 mL, n = 56), and by the end of the formal intervention in April 2012 legionellae were not routinely detected (Figs 3 and 4). To define treatment conditions, Cu and Ag concentrations were monitored weekly. Figure 4 shows the Cu and Ag levels detected on the supply side of the LTCF hot water system for the period between April 2011 and November 2012. Typically, Cu and Ag ions are applied at the 0.2 and 0.02 mg/L (or ppm) levels, respectively, but lower levels are often attempted to limit electrode consumption. During this work, the acceptable range of Ag concentrations established by the vendor was from 0.01-0.08 ppm for both facilities. The range of Ag ion concentrations detected was <0.01-0.104 ppm, with a mean of 0.015 ± 0.021 ppm and a median of 0.03 ppm; based on 0.02 ppm, the mean is approximately 75% of the suggested industry target. Cu was recovered in the range of <0.01-0.19 ppm, with a mean of 0.035 ± 0.027 ppm. This represents >80% loss of Cu based on a 0.2 ppm target concentration. In addition, on-site wet chemical analyses for Cu during the same time period averaged 0.07 ± 0.2 ppm. During the period of the intervention the output current of the ionization unit averaged 1.65 ± 0.15 A. This indicates that conditions were relatively stable, and the amperage did not have to be increased to maintain ion levels. In Figure 4, the data for the percentage of positive *Legionella* cultures are superimposed on the plot (black squares) of Cu and Ag values. During the early stages of treatment, instabilities in both Cu and Ag concentrations occurred and were attributed to electrode scaling. This was followed by an upshift in the level of legionellae in both June and October 2011. As treatment progressed, ion concentrations stabilized and there was no recurrence of legionellosis in the resident population.

DISCUSSION

Through the summer of 2013, legionellae had not been detected in either the LTCF or the ACF. Patient surveillance has shown that since the intervention and stabilization of the ionization units, *Legionella* control remained good and there has been no recurrence of disease through August 2014. Early in the treatment cycle there were sporadic incidences where the numbers of positive culture samples were >30%. However, colony counts of positive sites were low (4.0-38 CFU/100 mL) when compared with the pretreatment conditions. Sporadic occurrence of ions was resolved by localized flushing to target individual fixtures or limited areas of concern.

During the intervention the average output current for the ionization units was stable. The very high value of 2.25 A was associated with unplanned high ion transients and the stabilization of the CSI unit that is clearly seen in Figure 4 (October 2011). Similar stable, long-term results were found in the ACF. There has been no need to progressively increase the output current over time to meet the targeted Ag concentration. Other workers have indicated that over time higher concentrations of Ag were required for reasonable control and that *Legionella* tolerance to Ag was a possibility.[10]

Weekly monitoring allowed detection of low levels of Ag, and fine adjustments could be made to the CSI unit, therefore preventing long-lasting low transients in ion concentrations, which could lead to regrowth of legionellae. For examples of low transient conditions, refer to July 2011 and August 2012 in Figure 4.

Table 1 shows some water quality parameters, including those that could negatively impact both Cu and Ag. The pH of this premise water system generally is >9.0, with median values for the ACF and LTCF at pH values of 9.4 and 9.3, respectively. As expected, Cu concentrations were lower than targeted as a result of precipitation.[11]

From the data presented, it appears that Cu was not the major ion that affected control of legionellae in these facilities. Most Cu

was precipitated, and the ability to control legionellae, including *L pneumophila* 1, *L pneumophila* 6, and *L anisa*, should be attributed to Ag.[22,23] Chloride has been implicated in complexation with, and removal of, Ag ions. To estimate the extent of this issue an analysis of conductivity was done. The median value of conductivity for the potable water in the LTCF and ACF was approximately 145 μmhos/cm, respectively. Converting conductivity to total dissolved solids,[24,25] it was determined that the maximum amount of chloride available for complexing Ag ions would be approximately 53 mg/L. This is a conservatively high value because it does not consider the other ions that contribute to conductivity. Referring to Figure 1C in the Lin et al article,[11] an estimate of the minimum percentage of Ag as Ag^+ under the conditions studied would be approximately 25% of the total Ag available, which was sufficient for control. Therefore, water quality conditions did not appear to negatively impact the biocidal capability of Ag.

This work was designed as an intervention to determine, by surveillance of chemical and biologic parameters, whether different treatments succeeded. However, because many water measurements were conducted on samples collected from the same locations (ie, sampling points), such observations were not considered independent. Because the assumption of independence between the measurements did not hold, simple bivariate analysis, such as χ^2 tests, are not communicated here.

Remediating the occurrence of *Legionella* sp at a health care facility can be a complex process that may require long-term assessments to result in adequate control. ClO_2 was successful in certain areas of a complex hospital environment, whereas successful control of *L pneumophila* 1, *L pneumophila* 6, and *L anisa* was demonstrated with CSI.[26] During this intervention, both treatments established excellent control in their respective application locations.

Some future considerations for the application of CSI should include intermittent chlorination to control regrowth events or bacterial resistance,[27] determining the role of Cu deposition in any potential corrosion events,[28] and possibly reconfiguring electrode needs for high pH environments.

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