American Journal of Infection Control 43 (2015) 971-6

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major article

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

David M. Dziewulski PhD [a,b,*], Erin Ingles PE [c], Neculai Codru MPH [a], John Strepelis PE [c], Dianna Schoonmaker-Bopp MS [d]

[a] Bureau of Water Supply Protection, New York State Department of Health, Albany, NY
[b] School of Public Health, Department of Environmental Health Sciences, State University at Albany, Rensselaer, NY
[c] Central Regional Office, New York State Department of Health, Syracuse, NY
[d] Wadsworth Center, New York State Department of Health, Albany, NY

Key Words:
Copper-silver ionization (CSI)
Legionellosis
Legionella
Hospital
Nursing home
Alkaline environment
Chlorine dioxide
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 $\leq$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*.

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.

* Address correspondence to David M. Dziewulski, PhD, New York State Department of Health, Empire State Plaza – Corning Tower, Bureau of Water Supply – Rm 1110, Albany, NY 12237.
E-mail address: dmd14@nyhealth.gov (D.M. Dziewulski).

Funding/Support: Supported by the Environmental Health Specialist Network cooperative agreement (no. 5UE2EH000725) from the Centers for Disease Control and Prevention.

Disclaimer: These contents are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention.

Conflicts of interest: None to report.

http://dx.doi.org/10.1016/j.ajic.2015.05.018

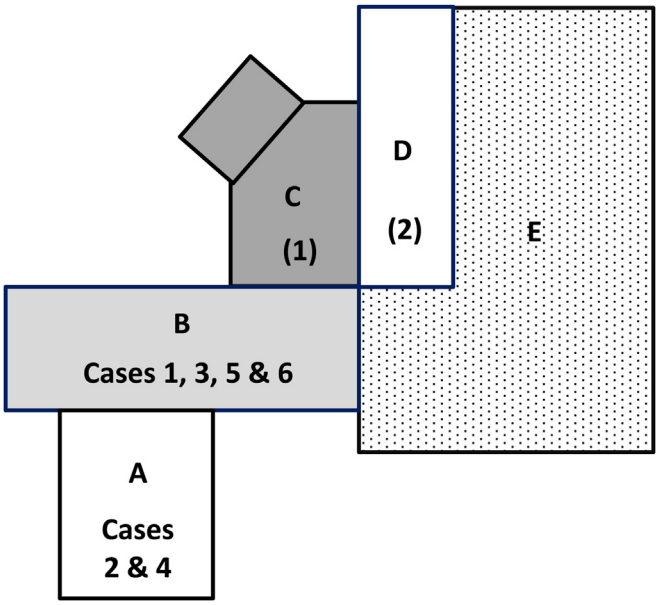

Fig 1. Simplified schematic of the complex structure of the acute care facility showing disease occurrence. Numbers in parentheses indicate additional locations of case patient exposures. Location B is discussed in the narrative as an example of treatment efficacy.

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]