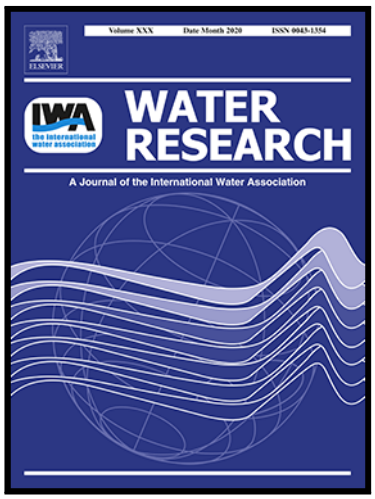


A Comprehensive Evaluation of Monochloramine Disinfection on Water Quality, Legionella and Other Important Microorganisms in a Hospital

Darren A. Lytle , Stacy Pfaller , Christy Muhlen , Ian Struewing , Simoni Triantafyllidou , Colin White , Sam Hayes , Dawn King , Jingrang Lu

PII: S0043-1354(20)31191-X
DOI: https://doi.org/10.1016/j.watres.2020.116656
Reference: WR 116656

To appear in: *Water Research*

Received date: 26 August 2020
Revised date: 15 November 2020
Accepted date: 16 November 2020

Please cite this article as: Darren A. Lytle , Stacy Pfaller , Christy Muhlen , Ian Struewing , Simoni Triantafyllidou , Colin White , Sam Hayes , Dawn King , Jingrang Lu , A Comprehensive Evaluation of Monochloramine Disinfection on Water Quality, Legionella and Other Important Microorganisms in a Hospital, *Water Research* (2020), doi: https://doi.org/10.1016/j.watres.2020.116656

Highlights

- Monochloramine was reliably added to a hospital's hot water supply.
- Adding monochloramine to a hospital's hot water significantly decreased *Legionella*.
- Monochloramine reduced HPCs, *Pseudomonas aeruginosa* and *Vermamoeba vermiformis*.
- No signs of nitrification were observed following monochloramine addition.
- Treatment did not have other negative unintended water quality consequences.

A Comprehensive Evaluation of Monochloramine Disinfection on Water Quality, *Legionella* and Other Important Microorganisms in a Hospital

By

Darren A. Lytle[1], Stacy Pfaller[1], Christy Muhlen[1], Ian Struewing [2], Simoni Triantafyllidou[1], Colin White[3], Sam Hayes[1], Dawn King[1], Jingrang Lu [2]

[1] U.S. Environmental Protection Agency, ORD, Center for Environmental Solutions and Emergency Response (CESER), 26 W. Martin Luther King Drive, Cincinnati, Ohio 45268

[2] U.S. Environmental Protection Agency, ORD,
Center for Environmental Measurement and Modelling
(CEMM), 26 W. Martin Luther King Drive, Cincinnati, Ohio 45268

[3] Ohio Environmental Protection Agency, Emerging Contaminants Section,
Division of Drinking and Ground Waters, 50 West Town Street, Suite 700
Columbus, OH 43215

Abstract

Opportunistic pathogens such as *Legionella* are of significant public health concern in hospitals. Microbiological and water chemistry parameters in hot water throughout an Ohio hospital were monitored monthly before and after the installation of a monochloramine disinfection system over 16 months. Water samples from fifteen hot water sampling sites as well as the municipal water supply entering the hospital were analyzed using both culture and qPCR assays for specific microbial pathogens including *Legionella, Pseudomonas* spp., nontuberculous *Mycobacteria* [NTM], as well as for heterotrophic bacteria. *Legionella* culture assays decreased from 68% of all sites being positive prior to monochloramine addition to 6% positive after monochloramine addition, and these trends were parallel to qPCR results. Considering all samples, NTMs by culture were significantly reduced from 61% to 14% positivity ($p<0.001$) after monochloramine treatment. *Mycobacterium* genus-specific qPCR positivity was reduced from 92% to 65%, but the change was not significant. Heterotrophic bacteria (heterotrophic bacteria plate counts [HPCs]) exhibited large variability which skewed statistical results on a per room basis. However, when all samples were considered, a significant decrease in HPCs was observed after monochloramine addition. Lastly, *Pseudomonas aeruginosa* and *Vermamoeba vermiformis* demonstrated large and significant decrease of qPCR signals post-chloramination. General water chemistry parameters including monochloramine residual, nitrate, nitrite, pH,

temperature, metals and total trihalomethanes (TTHMs) were also measured. Significant monochloramine residuals were consistently observed at all sampling sites with very little free ammonia present and no water quality indications of nitrification (e.g., pH decrease, elevated nitrite or nitrate). The addition of monochloramine had no obvious impact on metals (lead, copper and iron) and disinfection by-products.

Keywords: *Legionella*, drinking water, hospital, monochloramine

*Correspondence – Darren Lytle, 26 W. Martin Luther King Dr., Cincinnati, OH 45268, USA.
Email: lytle.darren@epa.gov

1. INTRODUCTION

Opportunistic pathogens such as *Legionella* are of public health concern especially in buildings where sensitive populations are present (Mancini et al., 2015; Demirjian et al., 2015; Baron et al., 2014; Marchesi et al., 2013; Flannery et al., 2006). Such pathogens have been reported to cause disease in hospital patients, who are often immunocompromised from illness or treatments and are thus more susceptible to agents of disease (Anaissie et al., 2002; Squier et al., 2000; Kool et al., 1999).

Many factors in building water supplies and plumbing systems have been reported to influence the growth of *Legionella* and other opportunistic pathogens including water temperature, disinfectant type and residual, iron, copper, distal sites and others (Rakic et al., 2012; Wang et al, 2012; Serrano-Suarez et al., 2013; Lu et al., 2015; Baron et al., 2015; Kyritsi et al., 2018; LeChevalier, 2019). Some hospitals install disinfection systems to boost disinfectant levels in their building's drinking water supply to overcome disinfectant residual demand and provide protection against opportunistic pathogens. Treatment options include free chlorine, chlorine dioxide, monochloramine and copper-silver ionization (Baron et al. 2014; Duda, et al., 2014; Marchesi et al., 2013; Lin et al., 2011; USEPA, 2016).

Monochloramine is an attractive disinfectant option because it does not react readily with natural organic matter to form regulated disinfection byproducts (DBPs) (total trihalomethanes [TTHM] and haloacetic acids [HAA5]) like free chlorine does. Monochloramine has a more persistent and stable disinfectant residual than chlorine because of its lower reactivity (White, 1999). As a result, it is effective for controlling bacterial regrowth and biofilms

due to its ability to penetrate the biofilm, although excess ammonia can cause biofilm growth (LeChevallier et al., 1988a; 1988b; Pressman et al., 2012).

An extensive review of monochloramine disinfection and *Legionella* can be found elsewhere (USEPA, 2016) but relevant information is summarized. Controlled laboratory studies have demonstrated the effectiveness of monochloramine to kill *Legionella* under a variety of conditions (e.g., Jakubek et al., 2013; Dupuy et al., 2011; Jacangelo et al., 2002; Donlan et al., 2002; Türetgen, 2008). The effectiveness of monochloramine to control *Legionella* within biofilm on surfaces has been studied as well (Wang et al., 2012; Loret et al., 2005). Several studies have documented monochloramine addition in building hot water systems to varying degrees of benefit and comprehensiveness (Baron et al., 2015; Baron et al., 2014a; Duda et al., 2014; Casini et al., 2014; Marchesi et al., 2013; Marchesi et al., 2012). Others (Weintraub et al., 2008; Flannery et al., 2006; Moore et al., 2006; Heffelfinger et al., 2003; Kool et al., 2000; Kool et al., 1999) have assessed *Legionella* control in buildings supplied with chloraminated municipal water.

Monochloramine (NH_2Cl) addition has potential adverse implications that include the possibility of excess ammonia, biological nitrification (formation of nitrite and nitrate), and bacterial ecological shifts (Kirmeyer et al., 2004; Baron et al., 2015; Revetta et al., 2013), and the formation disinfection byproducts such as N-nitrosodimethylamine (NDMA) (Choi and Valentine, 2002). The application of monochloramine has also been associated with an increase in *Mycobacterium* and total coliform bacteria (Pryor et al., 2004; Moore et al., 2006; Baron et al., 2014). In the US, monochloramine disinfectant is regulated with a maximum

residual disinfectant level (MRDL) of 4.0 mg Cl_2/L. Lastly, monochloramine can attack rubber and plastic components (Kirmeyer et al., 2004).

Despite the efficacy of monochloramine for *Legionella* control, few reports are available that have comprehensively examined the efficacy and practicality of applying disinfection approaches for controlling other pathogenic microorganisms in building drinking water systems. Furthermore, very few case studies are available that consider the potential unintended consequences of installing disinfection treatment in large buildings such as hospitals (Triantafyllidou et al., 2016). Therefore, the objective of this work was to monitor microbiological and water chemistry parameters in the drinking water of a medium-sized hospital at 16 sampling sites for 16 months before and after the addition of monochloramine disinfection. The three main goals were to better understand the effectiveness of monochloramine disinfection in reducing opportunistic pathogens (e.g., *Legionella pneumophila, Pseudomonas spp.,* nontuberculous Mycobacteria [NTM]); to monitor for evidence of nitrification (e.g., nitrate, nitrite); and to monitor for changes in other important drinking water quality parameters (e.g., total chlorine, monochloramine, pH, temperature, lead, copper, and other metals).

2. METHODS AND MATERIALS

2.1. Hospital. The study facility is a medium-sized (317 beds, six floors) urban teaching hospital in Ohio, with most patient rooms having overnight capabilities including full restrooms. The hospital receives chlorinated drinking water from the local water utility that uses lime softening to treat its source water that is designated as surface water, and practices pH and alkalinity

adjustment for corrosion control. After passing through the community's distribution system, drinking water enters the hospital at two locations.

The hospital performed sampling of hot and cold water for *Legionella* by culture between 2006-2013. In April 2013, they observed positive samples in the domestic hot water systems for *Legionella pneumophila* which triggered a more extensive monitoring effort. The hospital contracted with a consultant to survey hot and blended water faucets for the presence of *Legionella* bacteria prior to initiation of this work. Culture results from 2014 revealed that hot water was positive for *L. pneumophila* serogroup 1 (sg1) at 71% of sampled faucets, although no cases of legionellosis had been reported. In response, hospital staff systematically flushed every tap in the facility with superheated water once every two weeks. While effective at first, analysis indicated that *Legionella* reappeared shortly after treatment. Furthermore, patient scalding risk concerns eliminated the possibility of a consistent regular building-wide increase in hot water temperature. The hospital decided to proactively install on-site monochloramine disinfection to the facility's hot water.

At the time of the study, the Ohio EPA (OEPA) considered installation of monochloramine disinfection to the hospital's hot water as a treatment process which voided the exemption of 40 CFR Section 141.3(a). For the monochloramine system to be approved, the OEPA required the hospital to submit detailed plans. Upon approval of detail plans the hospital was designated a Class 1, non-transient non-community public water system (PWS), subjected to regular water quality monitoring, reporting and operator staffing (White et al., 2016). The OEPA did not impose all Safe Drinking Water Act (SDWA) standards on the hospital, in accordance with 40 CFR Section 141.29, as they were a purchased water system. However,

operational monitoring was required as special conditions of plan approval and was limited to analytes that could be impacted by monochloramine (Table 1).

2.2. Monochloramine System. The monochloramine system was added to the recirculating hot water system in mid-June 2015. The patented system (Sanipur Sanikill, Brescia, Italy) produces monochloramine on-site by combining ammonium ions, supplied as ammonium salt, with sodium hypochlorite (Figure 1a). An electronic control system was used to maintain an initial desired monochloramine residual of 2-3.5 mg Cl_2/L that was eventually adjusted to a target concentration range 1-1.5 mg Cl_2/L at all sampling sites in the facility. The system limited the accumulation of excess ammonium ions based on redox potential. The disinfection system was connected to the tempered hot water system that served the two building loops. These two loops were supplied by a single set of hot water tanks and heat exchangers.

2.3. Water Sampling. Water sampling was performed over sixteen months between December 2014 and April 2016. "Baseline" (no treatment) sampling was performed monthly between December 2014 and June 2015 for water chemistry parameters, and sampling in March, April and May of 2015 included microbiological samples. Post monochloramine sampling for chemical and microbiological parameters was performed in seven months between June 2015 and April 2016 based in hospital staff availability and hospital activities.

Water samples were collected from 16 locations or sites throughout the hospital. Thirteen hot water samples (cold water was not sampled) were collected throughout the hospital from patient and medical rooms, a rehabilitation facility, and bathrooms (total of eleven sites) (Table 2). The sites were selected to capture differences in water usage, facility age, plumbing material, water age and distance from monochloramine treatment system. Hot

water was also collected from shower heads at two of the sites (i.e., a faucet and shower head were collected from the same room). Because the sampling sites were in actual active hospital patient rooms, there were instances where gaining location access during a sampling event was not possible for various reasons (e.g., medical exams or procedures were occurring, potentially infectious patients were in the room, water was in use). The hospital campus and associated plumbing systems were split by age. The "old" section (constructed before 1971) consisted of copper and galvanized materials and was represented by sampling sites 1-5 (Table 2). The "new" section (built after 1983) was plumbed with copper and was represented by sampling sites 6-11. Hot water samples were also collected from two locations in the hot water recirculation loop: the hot water entering the loop (after the boiler) and the hot water return location (before returning to the boiler). Lastly, municipal drinking water entering the hospital was sampled the main entry point. Four different types of taps were represented at the locations: traditional double lever, double lever push button, spigot taps, and shower heads (Figure 1, Table 2).

Water samples were collected after random and uncertain stagnation times because locations were actively used by medical staff and patients. This study limitation was unavoidable and should be recognized when evaluating unintended consequences. A series of hot water samples were sequentially collected from each location without pre-flushing as follows: 500 mL (on-site analysis of temperature, dissolved oxygen, pH, free ammonia, nitrite, free chlorine, and monochloramine; with the remaining volume sent to U.S. Environmental Protection Agency (USEPA) (Cincinnati, Ohio) for metals, organic carbon, total ammonia, nitrite, nitrate, orthophosphate, TTHMs, and total alkalinity analyses), out of which 15 mL were poured

off and analyzed on-site for oxidation-reduction potential (ORP), pH, temperature and conductivity; 1000 mL (microbial culture of *Legionella*, heterotrophic plate counts [HPCs], and *Pseudomonas*); 1000 mL (microbial culture of NTMs); two 1000 mL (pooled for microbial DNA analysis); and lastly, 15 mL (repeat on-site analysis of ORP, pH, temperature and conductivity). Bottles designated for microbiological analyses were sterile and contained sodium thiosulfate (0.1 mL of 3% sodium thiosulfate per 120 mL). The NTM culture sample bottle did not contain sodium thiosulfate based on the work of Thomson et al., (2008).

2.3. Chemical and Microbiological Analyses. Details regarding the analysis of water sample for chemical and microbiological parameters are provided in the SI. Specifically, field (on-site) measurements (section S1), laboratory water quality analyses (section S2), microbiological (qPCR) analyses (section S3), microbiological (culture-based) analyses (section S4), and statistical analyses (section S5) are fully described.

3. RESULTS

3.1. General Water Quality. The pH of incoming water ranged between 8.45 and 8.78, and the temperature ranged between 12.3-22.9^{o}C, with warmer and colder temperatures observed in the summer and winter, respectively (Supporting Information [SI] Table S1) over the entire study period. The calcium, magnesium and total organic carbon (TOC) concentrations averaged 17.3 mg/L, 26.2 mg/L and 0.75 mg C/L, respectively, and total hardness averaged 151 mg $CaCO_3$/L. Iron levels entering the hospital were negligible averaging 0.02 mg/L. Total alkalinity, chloride, nitrate and sulfate averaged 89 mg $CaCO_3$/L, 62 mg/L, 1.1 mg N/L and 44 mg/L, respectively, over the entire study. Nitrite and ammonia concentrations were negligible.

Seasonal water quality changes, except for temperature and free chlorine (section 3.2), were not apparent.

Water quality in the hot water outlet and return locations prior to monochloramine addition were similar to the source water with respect to major anion concentrations. Calcium, magnesium, sodium and other cations reflected the impact of ion exchange softening used to treat a fraction (approximately 50% removed) of hot water to minimize scaling issues throughout the study (Table S1). Iron levels in the hot water entry and return locations were elevated, averaging 0.17 mg/L and 0.67 mg/L, respectively, although it should be noted that the return sampling location had elevated individual samples (2.89 and 0.89 mg/L). Hot water temperature in the recirculation loop ranged between 40.2^{o}C and 50.2^{o}C over the entire study.

On-site measurements of pH, temperature and ORP at all sampling sites collected at the beginning and end (after approximately 4.5 L) of the sample series were compared pre- and post-chloramination treatment (Table S2). The pH remained relatively consistent between the first and last samples whereas temperature increased from the first to last sample as water was pulled in from the hot water recirculating hot loop (sites 13 and 14). ORP reflects the presence of a disinfectant residual and did not appear to change from the first and last sample. The ORP did, however, increase following treatment reflecting the presence of monochloramine. ORP levels in the building sites were considerably lower than the water entering the hospital reflecting the differences in ORP of a chlorinated versus a chloraminated drinking water (Copeland and Lytle, 2014).

3.2. Chlorine and Monochloramine. The free and total chlorine residual entering the hospital over the entire study period averaged 0.81 and 0.78 mg Cl_2/L, respectively (Table S1). Free

chlorine levels were as high as 1.1 mg Cl_2/L in winter months to as low as 0.64 mg Cl_2/L during summer months. Prior to monochloramine addition, the free (and total) chlorine residual in the hot water loop averaged 0.04 mg Cl_2/L (0.04 mg Cl_2/L) and 0.04 mg Cl_2/L (0.04 mg Cl_2/L) in the outlet and return loop locations, respectively. Enhanced chlorine decay in hot water, extended water age in the large premise plumbing system, and other factors contributed to the chlorine demand.

Following the installation of the monochloramine treatment system, monochloramine levels entering the hot water loop and at the hot loop return averaged 2.01±0.66 mg Cl_2/L and 1.96±0.90 mg Cl_2/L, respectively (Table S1). Although there was some variability in monochloramine residuals, they were reliably within the initial target dose range of 2-3.5 mg Cl_2/L, remained relatively unchanged as the water moved through the loop, and were enough to reach the sampling sites. Total chlorine levels were slightly greater than monochloramine averaging 2.18±0.82 mg Cl_2/L (Table S1).

Monochloramine levels water at sites immediately (within 48 hours) after treatment start-up varied widely and ranged between undetectable (<0.03 mg Cl_2/L) to 3.9 mg Cl_2/L (Figure 2), while the hot water entering the loop and return locations had 4.01 mg Cl_2/L and 3.5 mg Cl_2/L, respectively. During this period, monochloramine feed adjustments were being optimized and system demand was likely greatest since previous oxidation reduction potential (ORP) was low. After the initial sampling event, monochloramine levels in sampling sites averaged between 0.38-2.08 mg Cl_2/L (Figure 2, Table S3) and were lower than the hot water loop that fed them. Differences in concentration between the hot water loop and sampling locations reflected differences in monochloramine degredation rates, plumbing material,

stagnation time, and location between sampling taps. Despite the demand, monochloramine residuals were always present in the first 500 mL water draw at all sampling locations including showers during the study. Total chlorine levels were similar to monochloramine levels at all locations throughout the study (Table S2) indicating low free chlorine levels from the treatment plan, and low monochloramine conversion to other chloramine species.

3.3. Ammonia, Nitrite, and Nitrate. Monitoring of nitrogen species was required by OEPA (Table 1) and the results can also be used as an indicator of nitrification. Decomposition products of monochloramine include ammonia, nitrate, nitrogen gas, and chloride, whereas biological nitrification can produce nitrite and nitrate. Nitrite and nitrate have acute health-based drinking water maximum contaminant level (MCL) standards of 1 and 10 mg N/L, respectively (USEPA. 1991d).

Free ammonia levels based on on-site measurements post-monochloramine treatment were very low, averaging 0.01±0.01 mg N/L and 0.05±0.05 mg N/L at the hot water loop entry and return, respectively (Table S1), closely matching the levels entering the hospital (0.03 mg N/L). Total ammonia (free ammonia and combined ammonia included ammonia associated with monochloramine) based on laboratory analyses averaged 0.41±0.12 mg N/L and 0.40±0.12 mg N/L entering the hot water loop and at the hot loop return (includes first sampling day after treatment start-up), respectively (Table S1). Total ammonia concentrations in the hot water loop corresponded to an average calculated monochloramine dose of approximately 2.08 mg Cl_2/L which was consistent with measured values (Table S1). Nitrite and nitrate levels in the hot water loop were essentially unchanged from the source water entering the building. Nitrite levels (based on laboratory analyses) averaged 0.01±0.00 mg N/L and 0.01±0.00 mg N/L

entering the hot water loop and at the hot loop return, respectively (Table S1). Nitrate levels averaged 1.24±0.29 mg N/L and 1.11±0.56 mg N/L entering the hot water loop and at the hot loop return, respectively (Table S1), which were nearly the same to nitrate levels entering the hospital. Field on-site screening measurements of nitrite were consistent with laboratory results (Table S1). Hot water loop analysis results indicated that the monochloramine feed consistently met desired target levels without degradation after an initial adjustment period. No indication of nitrification (e.g., increase in nitrite and/or nitrate) was noted throughout the hot water loop that fed the hospital wings.

Average free ammonia levels measured at hospital hot water sampling sites after treatment over the entire study ranged between 0.05-0.33 mg N/L (Table S3), and the highest measured value was 0.71 mg N/L (site 7). Free ammonia levels immediately after treatment start-up were considerably greater than the respective average levels following extended treatment at half of the locations as the treatment system was being adjusted (Figure 3a). The presence of free ammonia could have resulted from the decay of monochloramine and/or monochloramine reactions with organics, biofilm, pipe metals and other plumbing materials. Total ammonia levels were considerably greater than average levels at nearly every location during the first sampling event collected within 48 hours after monochloramine treatment start-up before the chemical feed system was adjusted and optimized. During this adjustment period, total ammonia levels ranged from 0.14-0.89 mg N/L at sampling locations and the hot water loop was 0.76 mg N/L (Figure 3b). The range of total ammonia levels was consistent with corresponding total chlorine and monochloramine levels (Figure 2). Except for sampling site 6, average total ammonia concentrations after the initial post treatment sampling event were

near or below the average total ammonia concentration in the hot water recirculation loop (0.4 mg N/L) and ranged between 0.21-0.45 mg N/L (Figure 3b). Differences in total ammonia concentration decrease were associated with sampling site-specific differences in monochloramine decomposition pathways and product distributions and/or nitrification.

Field measured nitrite levels pre- and post- monochloramine treatment averaged low, ranging between 0.010-0.10 mg N/L (Figure 4a, Table S3). Site 4 had the greatest average nitrite concentration. Laboratory analysis results were consistent with field measurements. The highest recorded nitrite level recorded post- monochloramine addition was 0.20 mg N/L at site 4. Low nitrite levels could indicate nitrification was not a concern, capable of oxidizing nitrite.

Average nitrate levels at sampling sites prior to and post monochloramine treatment ranged between 1.09-1.26 mg N/L and 1.01-1.19 mg N/L, respectively (Figure 4b, Table S3), which were both well within the bounds of the city water entering the hospital prior to and post monochloramine treatment 1.21±0.28 mg N/L and 1.13±0.20 mg N/L, respectively. The absence of increase in nitrate (or nitrite) concentration indicates an environment not conducive to nitrification over the study period.

3.4. Disinfection Byproducts. In this study, chlorinated disinfection byproducts, total trihalomethanes (TTHMs), in drinking water entering the hospital and at hot water locations were monitored during three sampling events prior to and three sampling events post-monochloramine treatment (Figure S1). TTHMs in the cold water entering the facility were 27.6 µg/L and 34.1 µg/L in the successive sampling dates prior to monochloramine addition (Figure S1a). TTHMs increased to as high as 53.6 µg/L (94%) (site 10) and 42.8 µg/L (26%) (site 1) on corresponding dates between the hospital entry point sampling location and the hot water

recirculation loop as chlorine was consumed through reactions with organic matter in the water and plumbing surfaces (Figure S1a). Additional increase in TTHM levels in plumbing between the hot water recirculation loop and site sampling locations was generally minimal (Figure S1a) likely because little to no free chlorine residual was present (i.e., much of TTHM formation was assumed to have occurred in the cold water). Chloroform was the specific TTHM that observed the greatest increase as water moved through the facility, increasing from 7 µg/L in the water entering the hospital to a maximum of 28.8 at site 10 in April, 2015 (Figure S1b), for example, at the hot water sampling taps throughout the hospital. The addition of monochloramine did not increase TTHM formation (Figure S1a).

Nitrosamine compounds including N-Nitroso-dimethyline (NDMA) have been linked to monochloramine disinfection byproducts. One set of NDMA samples were analyzed from all sampling locations post-monochloramine treatment installation. NDMA was not detected (< 2.5 ng/L) in any water sample.

3.5. Lead, Copper and Iron. Although a complete suite of metals was routinely analyzed at all sampling locations, no regulated metals were found above the respective MCLs and therefore, except for lead, copper, and iron, are not presented. Lead and copper have non health-based "action levels" (ALs) of 0.015 mg/L and 1.3 mg/L, respectively, in a 1 L water sample collected from faucets after more than 6 hours of stagnation as described under the USEPA's Lead and Copper Rule (LCR) (USEPA, 1991a; 1991b; 1991c). Although water samples collected under the study plan were not performed in accordance with the LCR protocol (i.e., samples were collected from hot water and, in some cases, showers, and were not 1 L first draw samples collected after 6 hours of stagnation), examination of the data is worthwhile. Monochloramine

addition did not impact lead and copper concentrations in drinking water through the hospital (Figure S2). Prior to the initiation of monochloramine, the mean and standard error for lead and copper were 0.003±0.006 mg/L and 0.071±0.055 mg/L, respectively. The median lead and copper levels were 0.002 mg/L and 0.071 mg/L, respectively. Following the addition of monochloramine, the mean and standard error for lead and copper were 0.003±0.004 mg/L and 0.056±0.024 mg/L, respectively. The median lead and copper levels were 0.003 mg/L and 0.054 mg/L, respectively. Considering all samples, lead ranged from the analytical method detection limit of 0.002- 0.043 mg/L. Only three water samples (all shower samples) out of 218 samples (1.4%) (pre- and post-treatment) had lead levels above the 0.015 mg/L AL. Copper ranged from the analytical method detection limit of 0.001-0.388 mg/L and all levels were all well below the AL.

LCR sampling of cold-water taps at twenty locations in the hospital were required given the small shared temperature volume in some faucets. The facilities staff selected taps based on distance of the location from drinking entry point and plumbing age. Since the initiation of monochloramine treatment, three LCR sampling events have been performed. Only five compliance samples (out of sixty total samples or 8%) had lead levels above the reporting limit (2 µg/L), the highest of which was 5.6 µg/L. Copper compliance samples ranged between 0.021-0.219 mg/L across all events.

There was no apparent impact of monochloramine addition on iron levels (Figure S3). Prior to the initiation of monochloramine treatment, the mean and standard error for iron for all sites was 0.17±0.41 mg/L (median concentration was 0.02 mg/L). Following the addition of monochloramine, the iron concentration was 0.14±0.37 mg/L (median of 0.01 mg/L). There

were individual sites that had relatively high average iron levels before and/or after monochloramine treatment, although they could generally be attributed to one iron spike (likely particulate in nature). For example, site 3 (post-treatment) and site 5 (pre-treatment) had iron spikes of 2.45 mg/L and 1.59 mg/L, respectively. Elevated iron spike observances appeared to be more prevalent in the old section of the hospital where galvanized materials had been used.

3.6. *Legionella.* Cold water entering the hospital contained no detectable culturable *Legionella.* *Legionella* culture results prior to the addition of monochloramine (5-7 monthly sampling events per location) showed that the frequency of positive *Legionella* detects in hot water taps ranged from 0-100% per room (faucet and showerhead) (Table 3), and 9 of the 13 sampling (room) sites had detects >50% of the time. Sampling Site 7 never had a positive *Legionella* detect and Sites 9 and 6 only had one and two detects, respectively, prior to monochloramine addition. There was no apparent relationship between *Legionella* detects, faucet type (traditional double lever versus double lever push button) and observed water usage trends. Furthermore, *Legionella* positive samples in showerhead water samples were only more frequent than the faucet sampled from the same location (Table 3, Site 8a and 8b, and 11a and 11b) in one of the two locations. However, there was a much greater frequency of detects in the old section of the building (sites 1-5), where 97% (30/31) of samples collected were *Legionella* positive before monochloramine treatment, and presumptive *Legionella* colonies ranged from 10,000 to nearly 100,000 CFU/L. By contrast, 47% (24/51) of all samples or 39% (15/38) of samples excluding shower sites in the new building section were positive. Interestingly, positive *Legionella* detects were not predictable as they varied within sites from

month to month (not shown). *Legionella* was detected in the hot water recirculation loop outlet and return 86% and 71% of the time, respectively (Table 3). Considering all 96 hot water samples collected in the hospital prior to monochloramine addition, 68% were *Legionella* positive, which was consistent with the 2014 independent building investigation. The positivity rate was well above the empirically derived 30% positivity threshold above which it has been suggested action should be taken to lower risk for disease transmission (Stout et al., 2007; VHA 2008).

After monochloramine start-up, *Legionella* was never detected in the hot water recirculation outlet (site 13) and return loop (site 14), and in the water entering the hospital (site 12) (Table 3). In initial sampling performed six days after the onset of chloramination, all 15 sites (excluding cold water entering the hospital) were negative for *Legionella* whereas 10 of 15 sites were positive during the last sampling event prior to treatment start-up (data not shown). *Legionella* detects in hot water following the addition of monochloramine (5-7 monthly sampling events per sampling location) ranged from 0-50% per site and detect frequency was reduced at all sites (Table 3). Nine sampling sites had no detects and three sites had only 1 *Legionella* detect after monochloramine addition. The shower head sampled in location 11 (site 11b) had *Legionella* detected in 50% of the samples. Considering all one hundred hot water samples collected in the hospital after monochloramine addition, only 6% (6/100) were positive with *Legionella*, far less frequent than before treatment. The improvement was noteworthy in the old building section where the number of *Legionella* positives decreased to 6% (2/34) after monochloramine addition with the highest colony count of 750 CFU/L.

Legionella spp. were not detected in the water entering the hospital at any stage of the study based on qPCR analysis. A summary of qPCR testing for *Legionella* (16S) in water samples from the rooms showed that there were significant differences of average levels between pre- (7040 cell equivalents [CE]/L) and post-monochloramine treatment (200 CE/L) (Figure 5a). Sample site 6 was an anomaly in that *Legionella* was never detected in water samples despite being detected by culture method (albeit relatively infrequently). Although *Legionella* spp. levels appeared to be higher in shower heads (pretreat: 200 CE/L) as compared to adjacent faucets in the same room (room 8 and 11), the differences were not statistically different (p_8=0.123 and p_{11}=0.280). This was consistent with culture results as well. *Legionella* in the hot water outlet (site 13) and return loop (site 14) averaged 1350 CE/L and 638 CE/L, respectively, showed no significant difference ($p_{13\text{-}14}$=0.162) and were in most cases lower than the average levels in room sampling sites, suggesting *Legionella* was replicating between the hot water loop and the plumbing lines to the faucets. There were also no significant differences between traditional double lever faucets and shower head ($p_{8A\text{-}8B}$=0.121, $p_{11A\text{-}11B}$=0.199). The further detection showed that 100% of *Legionella* occurrence and copy number prior to treatment were *L. pneumophila* (Figure 5b), and the occurrence and copy numbers of *L. pneumophila* sg 1 accounted for 99% and 62% of the *L. pneumophila*, respectively (Figure 5c). In general, *Legionella* numbers were higher as a group in the old section of the hospital (sites 1-5).

3.7. NTMs, HPCs and *Pseudomonas* by Culture Methods. NTM were never detected by culture (< 5 CFU/200 mL) before or after monochloramine treatment at 27% of sampling sites (Sites 6, 7, 10, 11a) (Table 4, Figure 6a) and the cold water entry site. Presumptive NTM levels in the old section of the building (sites 1-5) were greater than the new section of the building (sites 6-11)

throughout the study. Five sites including the hot water recirculation outlet (site 13) and return loop (site 14) had detections higher on average before monochloramine treatment but not after (Sites 4, 8a, 8b, 13, and 14). NTM were detected continuously at one site, site 2, during the entire study and at relatively high levels (>400 CFU/200 mL water sampled).

Examining the data more closely, an interesting observation was that NTM were not detected at any location except site 2 (26±37 CFU/200 mL) during the June 2015 sampling period collected within six days after treatment start-up (Figure 6a). In sampling performed after the initial monochloramine start-up samples, seven sites showed statistically significant decreases in average NTM concentrations, including sites 1, 3, 4, 5, 8b, 11b and 14 (Figure 6a, Table 4). Except for site 2, monochloramine decreased average NTM levels in the old section of the hospital. It is also noteworthy that although statistical NTM differences in pre- and post-treatment samples were not identified in sites 8a and 13 (hot loop entry location), average post-treatment levels were below detection on average. Statistical analyses were not performed on the four sites that had average NTM levels below detection.

Of 139 isolates submitted for 16S rRNA gene sequencing, 118 isolates produced high quality sequences. Of those, 51 were putative NTM species. Thirty *Mycobacterium* isolates were obtained prior to addition of monochloramine treatment from seven sites (sites 1, 2, 5, 6, 9, 11a, 14) and 21 isolates were obtained after treatment was added from three sites (sites 2, 3, 9). A breakdown of *Mycobacterium* species recovered before and after treatment are in Table 5. The growth medium for mycobacteria is not genus selective, and seven other isolates identified as potential pathogens were obtained, all of which were isolated after addition of monochloramine treatment: *Neiserria subflava* (site 10), *Pseudomonas mendocina* (sites 3, 4,

5), *Pseudomonas stutzeri* (site 2), and *Stenotrophomonas maltophila* (site 1). Other isolates were not known to be pathogenic (e.g., *Bradyrhizobia* species, *Rhodopseudomonas* species, *Brevibacillus* species).

Average HPC measurements were consistently higher in the pre-chloramination samples when compared to post-chloramination (Figure 7, Table 4). As a point of reference, before treatment, only four sites had average HPC levels below the recommended upper limit of 500 CFU/mL (USEPA 1989) whereas after treatment, ten sites were below, although the limit was not intended to be applied to buildings. However, due to high temporal variability for most sampling sites before monochloramine treatment (see calculated standard deviations, Table 4), statistical testing at the 0.05 significance level indicated that only three sites (1, 3 and 8a) showed significant HPC differences after treatment.

Methods for testing of *P. aeruginosa*, which provide results within 24 hours, produced largely negative results in both pre- and post-chloramination. Overall, only six of 102 samples (6%) tested positive in the pre-chloraminated testing and nine out of 85 samples were positive post-chloramination. In seven of the 15 samples that were positive by the assay, the MPN value was greater than the largest possible value (>2419.6 MPN/100 mL).

3.8. *Mycobacterium*, *Vermamoeba vermiformis* and *Pseudomonas aeruginosa* by qPCR Methods. *Mycobacterium* results using genus-specific qPCR primers showed that average cell equivalents (CEs) for the pre-chloramination samples were consistently higher than in the post-chloramine treated samples except sites 2 and 9 (Figure 6b) and there were not detections in cold water entering the hospital. Site 2 was also identified as a problematic site with respect to NTM levels by culture as well (Figure 6a). Although averages were lower at all other sites

following treatment, the decreases were not statistically significant (P>0.05) at five sites (sites 2, 4, 5, 9, and 14). Decreases in *Mycobacterium* levels were generally observed at most sites shortly after treatment start-up (June 2015) when compared to mean levels before treatment at most sites; however, the reductions were not as evident as the culture results. *M. avium* were not detected by qPCR at any location during the study. *M. intracellulare*/*chimera* were detected by qPCR in six samples: five samples in April 2015, prior to chloramination addition (sites 3, 5, 11B, 12, and 14) and one sample after addition of chloramination in June 2015 (site 1). All detections were below the limit of quantification (<10 CE).

The qPCR results for *Pseudomonas aeruginosa* and *Vermamoeba vermiformis* showed measurable CE levels in most of the pre-chloramination samples, with rare detections in the post-chloramination samples (Figure 8) and no detections in cold water entering the hospital. For these two assays, except in rare cases, the CE levels dropped to non-detection or very low in the sampling event immediately after chloramination was initiated (June 2015 sampling event). For the *Pseudomonas aeruginosa* ecfx gene primer sets, 19 of 45 samples had measurable CE levels pre-chloramination, ranging from 0 to 62,112 CE/L, while in the post-chloramination samples only one of 90 samples had a detection (excluding June 2015 sampling event). For the *Vermamoeba vermiformis* assay, 40 of 45 samples tested were positive pre-chloramination with CE values ranging from 0 to 8,424 CE/L and for post chloramination samples, 16 of 86 samples had CEs above the detection limit (excluding the June 2015 sampling event). In general, both results prior to treatment were higher as a group in the old section of the hospital (sites 1-5).

4. DISCUSSION AND CONCLUSIONS

The on-site monochloramine generation system performed within the parameters recommended by the manufacturer and maintained detectable concentrations of monochloramine without exceeding the respective MRDL at any hot water site monitored during the 16-month study period. Given the random nature of sampling and uncertain stagnation period before sampling, it was impressive that significant monochloramine residuals were always present. Free ammonia levels were minimal at building sample taps and there were no water quality indications that nitrification was occurring in the hospital during the study following the introduction of monochloramine. The role of hot water in inhibiting nitrification could be important in this observation. There were no other water quality changes or known unintended consequences after monochloramine addition including increases in lead and copper, iron and disinfection by-products including NDMA, although these conclusions were based on a single sampling event.

Reduction in culturable *Legionella* was the most notable of the microbiological evaluations. The number of taps that tested culture positive decreased from 68% prior to chloramination to 6% after chloramination, which is well below the 30% positivity threshold for action to lower risk for disease transmission (Stout et al., 2007; VHA 2008). It was also notable that culturable *Legionella* decreased below the limit of detection at all 15 sampling sites (excluding cold water entering the hospital) six days following the onset of chloramination. Sites from the old section of the hospital (sites 1-5) exhibited high presumptive *Legionella* colonies prior to chloramination but were reduced after monochloramine addition. Considering all pre- and post-treatment samples, *Legionella* based on culture were significantly reduced ($p<0.001$)

after monochloramine treatment (Table 6). *Legionella* qPCR trends generally followed culture trends. *Legionella* culture and qPCR results differed with respect to the first sampling event performed six days after chloramination treatment was initiated. *Legionella* culture results exhibited an immediate conversion to no growth on the plates whereas the qPCR signal remained high before dropping off after subsequent sampling events. This potentially corresponds to a time period where *Legionella* cells may have entered a viable but non-culturable (VBNC) state but were subsequently inactivated after prolonged exposure to monochloramine. Alternatively, the large number of positive qPCR CE values may be from the sloughing off as dead or dying cells since the first sampling event was just six days after chloramination was started. Considering all pre- and post-treatment samples, *Legionella pneumophila* sg1 based on qPCR results were significantly reduced ($p=0.006$) after monochloramine treatment (Table 6).

Heterotrophic bacteria culture results exhibited large variability between sampling events. Although average HPC values were consistently lower after chloramination, variability (i.e., large standard errors) between sampling events, particularly before treatment, contributed to the outcome that only three sites significantly decreased after treatment. However, considering all pre- and post-treatment samples, HPCs were significantly reduced ($p<0.001$) by 83% after monochloramine treatment (Table 6). The results were consistent with the observations of Duda et al., (2014). HPCs did not statistically correlate to *Legionella* indicating that they were not a reliable indicator of *Legionella* but results generally reflected disinfection system operations.

NTM culture results showed that 64% (7/11) of sampling sites with detection before treatment had a significant reduction in NTM CFUs after treatment. Again, sites 1-5 and the shower sites had the highest levels of NTM colonies. NTMs were significantly reduced from 61% to 14% positivity ($p<0.001$) after monochloramine treatment (Table 6). The *Mycobacterium* genus-specific qPCR results were highly variable. When comparing culture and qPCR results over the entire testing period, seven of 15 sites were positive 100% of the time for qPCR testing whereas only one site was 100% positive for culture testing. Site 2 was positive 100% of the time for both culture and qPCR. On average, *Mycobacterium* levels indicated by qPCR generally decreased after monochloramine treatment although the difference was not as apparent as culture results. Considering all pre- and post-treatment samples, *Mycobacterium* positivity was reduced from 92% to 65%; however, the difference was not statistically significant ($p<0.11$) (Table 6). Although biofilm analyses were not performed, one possible explanation for differences between culture and qPCR results could be NTM survived in the biofilm.

The qPCR testing for *Pseudomonas aeruginosa* demonstrated a significant decrease ($p=0.011$) of signal post-chloramination (Table 65). Considering all samples, 40% positivity pre-monochloramine treatment decreased to 1% post-treatment.

The qPCR testing for the amoeba *Vermamoeba vermiformis* demonstrated a significant decrease ($p<0.001$) of signal post-chloramination. Considering all samples, 88% positivity pre-monochloramine treatment decreased to 17% post-treatment. If chloramination truly inactivates a broad range of grazing biofilm protozoans, this would eliminate a vector for NTMs and *Legionella* as they are known to be associated with intracellular infection and transmission.

Lastly, the most apparent association with poor microbiological water quality in the hospital was the age of plumbing and the shower. Old plumbing and the presence of galvanized plumbing appeared to more likely to harbor *Legionella* and other microorganisms prior to treatment perhaps due to factors including time to colonize building plumbing and the protective nature of iron corrosion by-products. Shower locations may be more susceptible to *Legionella* and biofilm growth because of the relative lack of use.

5. ACKNOWLEDGEMENTS

The authors acknowledge the hospital's management and staff for facilitating this sampling effort and the Ohio EPA for support. We would also like to thank Maily Pham and Dan Williams for assistance with analyses of water samples, and Ken Rotert and Dave Wahman for providing manuscript reviews. At the time of this work, Colin White was supported by a graduate research traineeship through Cooperative Agreement Grant CR 83558601 from the USEPA to the University of Cincinnati.

Notice

The information in this article has been reviewed in accordance with the U.S. Environmental Protection Agency's (USEPA's) policy and approved for publication. The views expressed in this article are those of the authors and do not necessarily represent the views or the policies of USEPA. Any mention of trade names, manufacturers, or products does not imply an endorsement

by the U.S. Government or USEPA; USEPA and its employees do not endorse any commercial products, services, or enterprises. This project was supported in part by an appointment to the Research Participation Program at the Office of Research and Development, USEPA, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and USEPA.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

6. REFERENCES

American Public Health Association, American Water Works Association, Water Environment Federation, 2012. In: Rice EW, Baird RB, Eaton AD, Clesceri LS, editors. Microbiological Examination. 22nd ed. Washington: Standard methods for the examination of water and wastewater, 9.49–9.52.

Anaissie, E.J., Penzak, S.R. and Dignani, M.C. (2002) The hospital water supply as a source of nosocomial infections. Arch Intern Med 162, 1483-1492.

Baron, J.L., Kirk Harris, J., Holinger, E.P., Duda, S., Stevens, M.J., Robertson, C.E., Ross, K.A., Pace, N.R. and Stout, J.E. (2015) Effect of monochloramine treatment on the microbial ecology of *Legionella* and associated bacterial populations in a hospital hot water system. Syst Appl Microbiol 38, 198-205.

Baron, J.L., Vikram, A., Duda, S., Stout, J.E. and Bibby, K. (2014) Shift in the microbial ecology of a hospital hot water system following the introduction of an on-site monochloramine disinfection system. PloS ONE 9, e102679.

Beumer, A., King, D., Donohue, M., Mistry, J., Covert, and Pfaller, S. (2010) Detection of *Mycobacterium avium* subsp. paratuberculosis in Drinking Water and Biofilms by Quantitative PCR. Applied and Environmental Microbiology, 76:21:7367–7370.

Casini, B., A. Buzzigoli, M. L. Cristina, A. M. Spagnolo, P. DelGiudice, S. Brusaferro, A. Poscia, U. Moscato, P. Valentini, A. Baggiani, and G. Privitera. 2014. Long-term effects of hospital water network disinfection on *Legionella* and other waterborne bacteria in an Italian University Hospital. Infection Control and Hospital Epidemiology, 35(3): 293-299.

Choi, J. and Valentine, R.L. (2002) Formation of N-nitrosodimethylamine (NDMA) from reaction of monochloramine: a new disinfection byproduct. Water Res 26, 817-824.

Copeland, A. and Lytle, D.A. (2014) Measuring the oxidation-reduction potential of important oxidants in drinking water. Journal - American Water Works Association 106(1), E10-E20.

Demirjian, A., Lucas, C.E., Garrison, L.E., Kozak-Muiznieks, N.A., States, S., Brown, E.W., Wortham, J.M., Beaudoin, A., Casey, M.L., Marriott, C., Ludwig, A.M., Sonel, A.F., Muder, R.R. and Hicks, L.A. (2015) The importance of clinical surveillance in detecting Legionnaires' disease outbreaks: a large outbreak in a hospital with a *Legionella* disinfection system – Pennsylvania, 2011-2012. Clin Infect Dis 60, 1596-1602.

Donlan, R., R. Murga, J. Carpenter, E. Brown, R. Besser, and B. Fields (2002). Monochloramine disinfection of biofilm-associated *Legionella pneumophila* in a potable water model system. In: *Legionella*, R. Marre, Y. A. Kwaik, and C. Bartlett, (eds.). p.406-410. Washington, DC: American Society for Microbiology

Duda, S., Kandiah, S., Stout, J.E., Baron, J.L., Yassin, M., Fabrizio, M., Ferrelli, J., Hariri, R., Wagener, M.W., Goepfert, J., Bond, J., Hannigan, J. and Rogers, D. (2014) Evaluation of a new monochloramine generation system for controlling *Legionella* in building hot water systems. Infect Control Hosp Epidemiol 4, 1356-1363.

Dupuy, M., S. Mazoua, F. Berne, C. Bodet, N. Garrec, P. Herbelin, F. Menard-Szczebara, S. Oberti, M. H. Rodier, S. Soreau, F. Wallet, and Y. Héchard (2011). Efficiency of water disinfectants against *Legionella pneumophila* and *Acanthamoeba*. Water Research. 45: 1087-1094.

Flannery, B., Gelling, L.B., Vugia, D.J., Weintraub, J.M., Salerno, J.J., Conroy, M.J., Stevens, V.A., Rose, C.E., Moore, M.R., Fields, B.S. and Besser, R.E. (2006) Reducing *Legionella* colonization of water systems with monochloramine. Emerg Infect Dis 12, 588-596.

Jacangelo, J. G., N. L. Patania, R. R. Trussel, C. N. Haas, and C. Gerba (2002). Inactivation of Waterborne Emerging Pathogens by Selected Disinfectants. Denver, Colo.: AWWA Research Foundation and AWWA.

Heffelfinger, J. D., J. L. Kool, S. Fridkin, V. J. Fraser, J. Hageman, J. Carpenter, and C. G. Whitney (2003). Risk of hospital-acquired Legionnaires' disease in cities using monochloramine versus other water disinfectants. Infection Control and Hospital Epidemiology. 24(8): 569-574.

Jakubek, D., C. Guillaume, M. Binet, G. Leblon, M. Dubow, and M. Le Brun (2013). Susceptibility of Legionella strains to the chlorinated biocide, monochloramine. Microbes and Environments. 28(3): 336-345.

Kirmeyer, G.J., M. LeChevallier, H. Barbeau, K., Martel, G. Thompson, et al.: Optimizing Chloramine Treatment, 2nd ed. Denver, CO: Prepared for the Water Research Foundation, 2004.

Kool, J.L., Carpenter, J.C. and Fields, B.S. (1999) Effect of monochloramine disinfection of municipal drinking water on risk of nosocomial *Legionnaires*' disease. Lancet 353, 272-277.

Kool, J. L., J. C. Carpenter, and B. S. Fields (2000). Monochloramine and Legionnaires' disease. American Water Works Association. 92(9): 88-96.

Kyritsi, M.A., Mouchtouri, V.A., Katsioulis, A., Kostara, E., Nakoulas, V., Hatzinikou, M. and Hadjichristodoulou, C. (2018) Legionella Colonization of Hotel Water Systems in Touristic Places of Greece: Association with System Characteristics and Physicochemical Parameters. Int J Environ Res Public Health 15(12).

LeChevallier, M. W., Cawthon, C. D., and Lee, R. G. (1988a). Factors promoting survival of bacteria in chlorinated water supplies. Applied and Environmental Microbiology, 54(3), 649-654.

LeChevallier, M. W., Cawthon, C. D., and Lee, R. G. (1988b). Inactivation of biofilm bacteria. Applied and Environmental Microbiology, 54(10), 2492-2499.

LeChevallier, M.W. (2019) Occurrence of culturable Legionella pneumophila in drinking water distribution systems. AWWA Water Science 1(3).

Lin, Y.E., Stout, J.E. and Yu, V.L. (2011) Controlling *Legionella* in hospital drinking water: an evidence-based review of disinfection methods. Infect Control Hosp Epidemiol 32, 166-173.

Loret, J. F., S. Robert, V. Thomas, A. J. Cooper, W. F. McCoy, and Y. Lévi (2005). Comparison of disinfectants for biofilm, protozoa and *Legionella* control. IWA Journal of Water and Health, 3(4): 423-433.

Lu, J., Struewing, I., Yelton, S. and Ashbolt, N. (2015) Molecular survey of occurrence and quantity of *Legionella* spp., *Mycobacterium* spp., *Pseudomonas aeruginosa* and amoeba hosts in municipal drinking water storage tank sediments. J Appl Microbiol 119, 278-288.

Mancini, B., Scurti, M., Dormi, A., Grottola, A., Zanotti, A. and Cristino, S. (2015) Effect of monochloramine treatment on colonization of a hospital water distribution system by *Legionella* spp.: a 1 year experience study. Environ Sci Technol 49, 4551-4558.

Marchesi, I., S. Cencetti, P. Marchegiano, G. Frezza, P. Borella, and A. Bargellini (2012). Control of *Legionella* contamination in a hospital water distribution system by monochloramine. American Journal of Infection Control. 40: 279-281.

Marchesi, I., Ferranti, G., Bargellini, A., Marchegiano, P., Predieri, G., Stout, J.E. and Borella, P. (2013) Monochloramine and chlorine dioxide for controlling *Legionella pneumophila* contamination: biocide levels and disinfection byproduct formation in hospital water networks. J Water Health 11, 738-747.

Moore, M.R., M. Pryor, B. Fields, C. Lucas, M. Phelan, and R.E. Besser: Introduction of monochloramine into a municipal water system: Impact on colonization of buildings by *Legionella* spp. Appl Environ. Microbio. 72(1):378–383 (2006).

Pressman, J.G., Lee, W.H., Bishop, P.L., and Wahman, D.G. (2012) Effect of free ammonia concentration on monochloramine penetration within a nitrifying biofilm and its effect on activity, viability, and recovery. Water Research. 46:882-894.

Pryor, M., S. Springthorpe, S. Riffard, T. Brooks, T.,Huo, et al.: Investigation of opportunistic pathogens in municipal drinking water under different supply and treatment regimes. Water Sci. Technol. 50(1):83–90(2004).

Rakic, A., Peric, J., and Foglar, L. (2012) Influence of temperature, chlorine residual and heavy metals on the presence of *Legionella pneumophila* in hot water distribution systems. Annals of Agricultural and Environmental Medicine. 19 (3), 431-436.

Revetta, R.P., Gomez-Alvarez, V., Gerke, T.L., Curioso, C., Santo Domingo, J.W. and Ashbolt, N.J. (2013) Establishment and early succession of bacterial communities in monochloramine-treated drinking water biofilms. Microbiol Ecol 86, 404-414.

Serrano-Suárez, A., Dellundé, J., Salvadó, H., Cervero-Aragó, S., Méndez, J., Canals, O., Blanco, S., Arcas, A. and Araujo, R. (2013) Microbial and physicochemical parameters associated with Legionella contamination in hot water recirculation systems. Environmental Science and Pollution Research 20(8), 5534-5544.

Squier, C., Yu, V.L. and Stout, J.E. (2000) Waterborne nosocomial infections. Curr Infect Dis Reports 2, 490-496.

Stout, J.E., Muder, R.R., Mietzner, S, et al. (2007) Role of environmental surveillance in determining the risk of hospital-acquired legionellosis: A national surveillance study with clinical correlations. Infect Control Hosp Epidemiol 28(7):818–824.

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.

Triantafyllidou, S., Lytle, D., Muhlen, C (2016) Copper-Silver Ionization at a US Hospital: Interaction of Treated Drinking Water with Plumbing Materials, Aesthetics and Other Considerations. Water Research 02:1-10.

Türetgen, I. (2004). Comparison of the efficacy of free residual chlorine and monochloramine against biofilms in model and full-scale cooling towers. Biofouling. 20(2): 81-85.

USEPA, 1989. National primary drinking water regulations; surface water treatment rule. Federal Register, 40 CFR 141.70-141.75. USEPA. 54 FR 27527.

USEPA, 1991a. Drinking water regulations; maximum contaminant level goals and national primary drinking water regulations for lead and copper. Federal Register, 40 CRF Parts 141 and 142. USEPA. 56:32112.

USEPA, 1991b. Maximum contaminant level goals and national primary drinking water regulations for lead and copper. Federal Register, 40 CFR Part 141, Subpart I. 56:26460.

USEPA, 1991c. Final regulatory impact analysis of national primary drinking water regulations for lead and copper (1991.W.91.E.A). EPA, Washington.

USEPA, 1991d. National primary drinking water regulations: Final rule. Federal Register, 40 CFR Parts 141, 142, and 143. USEPA 56(20):3526-3507.

USEPA (U. S. Environmental Protection Agency), (1983. Nitrogen, Nitrate-Nitrite. Method 353.2 (Colorimetric, Automated, Cadmium Reduction). In Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020, 353-2.1 – 353-2.5.

USEPA, 1995. Method 551.1: Determination of Chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogenated Pesticides/Herbicides in Drinking Water by Liquid-Liquid Extraction and Gas Chromatography with Electron-Capture Detection. Revision 1.0.

USEPA, 1994a. Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma – Mass Spectrometry, Revision 5.4.

USEPA, 1994b. Method 200.7: Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry, Revision 4.4.

USEPA, 2016. Technologies for *Legionella* Control in Premise Plumbing Systems: Scientific Literature Review. Office of Water (4607M) EPA 810-R-16-001. Accessed at https://www.epa.gov/ground-water-and-drinking-water/technologies-legionella-control-premise-plumbing-systems.

Veterans Health Administration (VHA) (2008). VHA directive 2008-010: prevention of *Legionella* disease. Washington, DC: Department of Veterans Affairs.

Wang, H., S. Masters, Y. Hong, J. Stallings, J. O. Falkinham III, M. A. Edwards, and A. Pruden (2012). Effect of disinfectant, water age, and pipe material on occurrence and persistence of *Legionella*, *mycobacteria*, *Pseudomonas*, *aeruginosa,* and two amoebas. Environmental Science and Technology. 46: 11566-11574.

Weintraub, J. M., B. Flannery, D. J. Vugia, L. B. Gelling, J. J. Salerno, V. A. Stevens, C. E. Rose, R. E. Besser, B. S. Fields, M. R. Moore, and M. J. Conroy (2008). *Legionella* reduction after conversion to monochloramine for residual disinfection. American Water Works Association. 100(4): 129-139.

White, C., Lytle, D, Jingrang, Lu, 2016. Reducing *Legionella* in Hospital Hot Water Systems Using Monochloramine. 13th Annual USEPA Drinking Water Workshop. Cincinnati, OH, August 23-25.

White, G. C. 1999. Handbook of chlorination and alternative disinfectants, 4th ed. John Wiley & Sons, Inc., New York, NY.

Table 1. Ohio EPA (OEPA) sampling requirements of the hospital following monochloramine treatment during this study. OEPA requirements may have changed since this work. Hospital monitoring results will not be presented here. The table does not include the EPA study sampling plan.

.

Analyte	Frequency	Sampling Location
Required OEPA Regulatory Sampling		
Monochloramine	Weekly	Entry Point to DS
HPCs	1 per month then reduced to quarterly	Distribution Point
Free ammonia	Daily	Entry Point to DS
Nitrate	Daily	Entry Point to DS
Nitrite	Daily	Entry Point to DS
Lead and copper	Per US EPA LCR	Distribution Cold Water
TTHM and HAA5	2 sites, 1 per year	Distribution Point
Self-Study Sampling		
Monochloramine	Daily	5 Distribution Points
Free ammonia	Daily	5 Distribution Points
Nitrate	Daily	5 Distribution Points
Nitrite	Daily	5 Distribution Points
Legionella	3 sites, quarterly	Distribution Point

Table 2. Description of EPA study sampling site locations including tap type and water usage. Water usage was based on system operator's observations and knowledge of building operations. Sampling sites 1-5 were in the "old" section of the building (constructed before 1971) and was plumbed with copper and galvanized plumbing materials. Sampling sites 6-11 were in the "new" section (built after 1983) and was plumbed with copper.

Sampling site ID	Description	Tap type	Building Section	Usage
1	Physical therapy wound care sink, 2nd floor	traditional double lever	Old	Frequent
2	Women's bathroom sink, 2300	double lever push button	Old	Frequent
3	Nuclear medicine west sink, 1st floor	traditional double lever	Old	Seldom
4	Medical room sink, 6600	traditional double lever	Old	Seldom
5	Staff bathroom sink, 6600	traditional double lever	Old	Seldom
6	ICU patient room, sink, 3214	traditional double lever	New	Frequent
7	Patient surgical recovery room, sink 3903	traditional double lever	New	Moderate
8A	Patient room, sink, 6102	traditional double lever	New	Frequent
8B	Patient room, shower, 6102	shower head	New	Moderate
9	Adult behavior room, sink, 5128	double lever push button	New	Seldom
10	MICU patient room, sink, 4205	traditional double lever	New	Frequent
11A	Patient room, sink, 4102	traditional double lever	New	Frequent
11B	Patient room, shower, 4102	shower head	New	Moderate
12	Influent water from city supply	spigot	--	--
13	Hot water loop (CUP 2nd floor)	spigot	--	--
14	Hot water loop (Return CUP, 2nd floor)	spigot	--	--

Table 3. *Legionella* culture monitoring results pre- and post-monochloramine treatment. Results are presented by indicating presence (positive).

	Legionella by Culture	
Sampling Site ID	Positives Pre-monochloramine	Positives Post-monochloramine
1	6/6 (100%)	0/7 (0%)
2	5/5 (100%)	1/6 (17%)
3	7/7 (100%)	0/7 (0%)
4	6/6 (100%)	1/7 (14%)
5	6/7 (86%)	0/7 (0%)
6	2/7 (29%)	0/6 (0%)
7	0/6 (0%)	0/7 (0%)
8A	4/7 (57%)	0/7 (0%)
8B	3/7 (43%)	0/7 (0%)
9	1/5 (20%)	1/7 (14%)
10	5/7 (71%)	0/5 (0%)
11A	3/6 (50%)	0/7 (0%)
11B	6/6 (100%)	3/6 (50%)
13	6/7 (86%)	0/7 (0%)
14	5/7 (71%)	0/7 (0%)
Totals	65/96 (68%)	6/100 (6%)

Table 4. Heterotrophic bacteria and *Mycobacterium* culture monitoring results pre- and post-monochloramine treatment (* data was normally distributed).

		Heterotrophic bacteria CFU/mL				*Mycobacterium* CFU/200 mL			
		n	avg	std	Signif at 0.05	n	avg	std	Signif at 0.05
Site 1	Pre	6	10452.8	16435.1	Y	4	400	0	Y
	Post	4	2.8	2.3	P=0.01	7	5.9	10.2	P=0.006
Site 2	Pre	5	14310.0	16356.7	N*	3	268.7	227.5	N
	Post	3	107.3	82.8	P=0.124	5	391.9	18.1	P=0.786
Site 3	Pre	7	3914.2	3926.9	Y	3	400	0	Y
	Post	4	12.1	21.6	P=0.006	7	9	15.3	P=0.017
Site 4	Pre	6	6275.7	3671.1	N*	3	400	0	Y
	Post	2	2833.3	2572.8	P=0.420	7	1.3	2.4	P=0.017
Site 5	Pre	6	8690.075	8271.9	N	3	400	0	Y
	Post	3	2845	4594.8	P=0.262	7	6.6	13.9	P=0.017
Site 6	Pre	7	6829.8	17148.5	N	3	3	1.5	N*
	Post	3	3.3	5.8	P=.067	6	0.4	0.8	P=0.081
Site 7	Pre	6	2022.3	4765.3	N	3	1.7	1.2	N
	Post	3	30.3	51.7	P=0.167	7	0.7	1.9	P=0.117
Site 8A	Pre	7	386.9	887.7	Y	4	52.2	103.5	N
	Post	4	0.3	0.5	P=0.024	7	0.7	1.9	P=0.412
Site 8B	Pre	5	4714.9	5861.0	N*	4	257.8	100.1	Y
	Post	3	1741.3	1508.7	P=0.286	7	0.9	2.5	P=0.006
Site 9	Pre	5	34587.0	43883.0	N*	4	45.9	54.5	N
	Post	4	1613.1	1779.9	P=0.125	7	15.9	32.1	P=0.369
Site 10	Pre	5	325.0	704.5	N	4	1.8	0.6	Y*
	Post	4	2.3	2.3	P=0.556	5	0.2	0.3	P=0.012
Site 11A	Pre	6	134.7	282.8	N	4	4.9	5.2	Y
	Post	4	52.5	101.7	P=0.914	7	0.29	0.4	P=0.006
Site 11B	Pre	5	14086.1	28270.2	N	4	234.8	193.5	Y
	Post	2	722.5	1021.8	P=0.571	7	57.1	151.2	P=0.042
Site 13	Pre	6	1138.2	2754.6	N	4	65.0	97.4	N
	Post	4	2.5	4.4	P=0.352	7	0.1	0.2	P=0.412
Site 14	Pre	7	61.2	134.7	N	4	118.8	189.8	Y
	Post	4	4.3	7.2	0.527	7	0.7	1.5	P=0.012

Table 5. Number and species of mycobacteria isolated before and after addition of monochloramine.

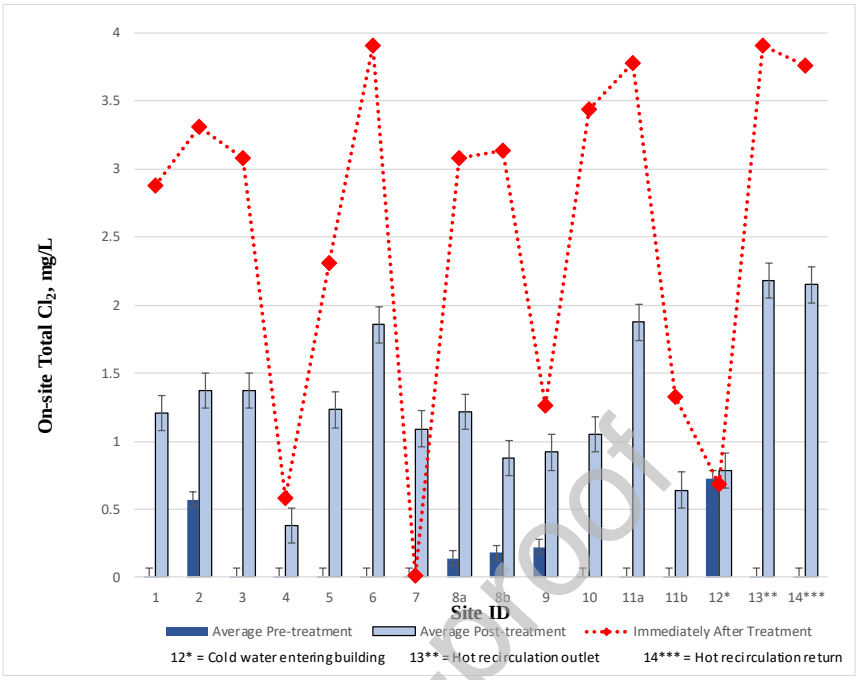
Site	Faucet type	Room type	Isolate ID (no. of isolates) **Before monochloramine**	Isolate ID (no. of isolates) **After monochloramine**
1	Double lever	Physical therapy wound care	*M. gordonae* (1)	
2	Push button	Psychiatric patient room	*M. phocaicum* (1)	*M. phocaicum* (8) *M. liatzerense* (1) *Mycobacterium* spp. (1)
3	Double lever	Nuclear medicine lab		*M. gordonae* (1)
5	Double lever	Staff bathroom sink	*M. gordonae* (2) *M. mucogenicum* (3) *M. phocaicum* (3)	
6	Double lever	ICU patient room	*M. kansasii* (3)	
9	Push button	Adult behavior room	*M. phocaicum* (3)	*M. phocaicum* (9) *M. mucogenicum* (1)
11A	Double lever	Patient room	*M. kansasii* (3) *M. mucogenicum* (2)	
14	spigot	Hot water return loop	*M. kansasii* (9)	

Table 6. Summary of all microbiological parameters before and after monochloramine treatment. Results and statistical comparisons consider all samples collected.

Microbiological Parameter	Units	Pre-Treatment	Post-Treatment	Significance
HPC (culture)	CFU/mL	6952 CFU/mL	1204 CFU/mL	Yes, $p<0.05$ ($p<0.001$)
Mycobacterium (culture)	positivity	33/54 (61%)	14/101 (14%)	Yes, $p<0.05$ ($p<0.001$)
Legionella (culture)	positivity	65/96 (68%)	6/100 (6%)	Yes, $p<0.05$ ($p<0.001$)
Legionella pneumophila sg1 (qPCR)	positivity	40/48 (83%)	31/91 (34%)	Yes, $p<0.05$ ($p=0.006$)
Mycobacterium (qPCR)	positivity	44/48 (92%)	59/91 (65%)	No, $p>0.05$ ($p=0.11$)
Pseudomonas aeruginosa (qPCR)	positivity	19/48 (40%)	1/96 (1%)	Yes, $p<0.05$ ($p=0.011$)
Vermamoeba vermiformis (qPCR)	positivity	42/48 (88%)	16/92 (17%)	Yes, $p<0.05$ ($p<0.001$)

Figure 1. Photographs of (a) monochloramine on-site generator, (b) sampling site taps with double lever push button, (c) taps with traditional double lever, and (d) showerheads.

(a)



(b)

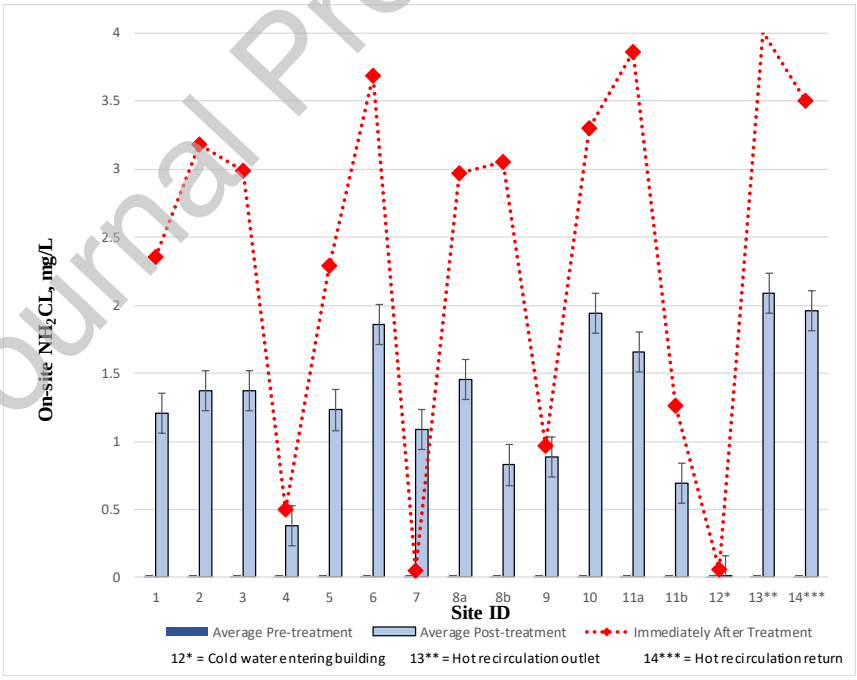
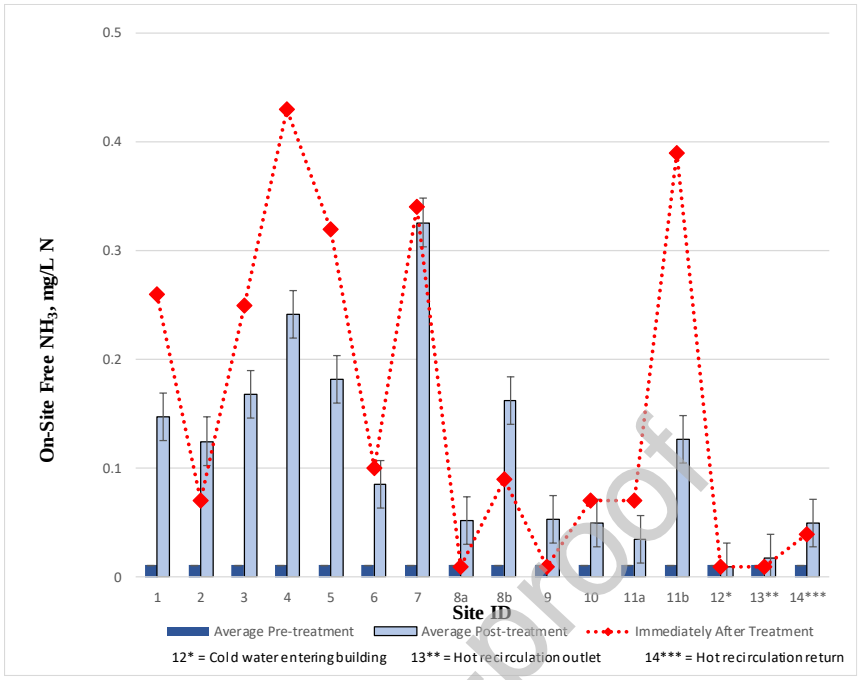


Figure 2. The concentrations of (a) total chlorine, and (b) monochloramine, before and after the installation of the monochloramine feed. Immediately after treatment refers to within 48 hours after monochloramine startup.

(a)



(b)

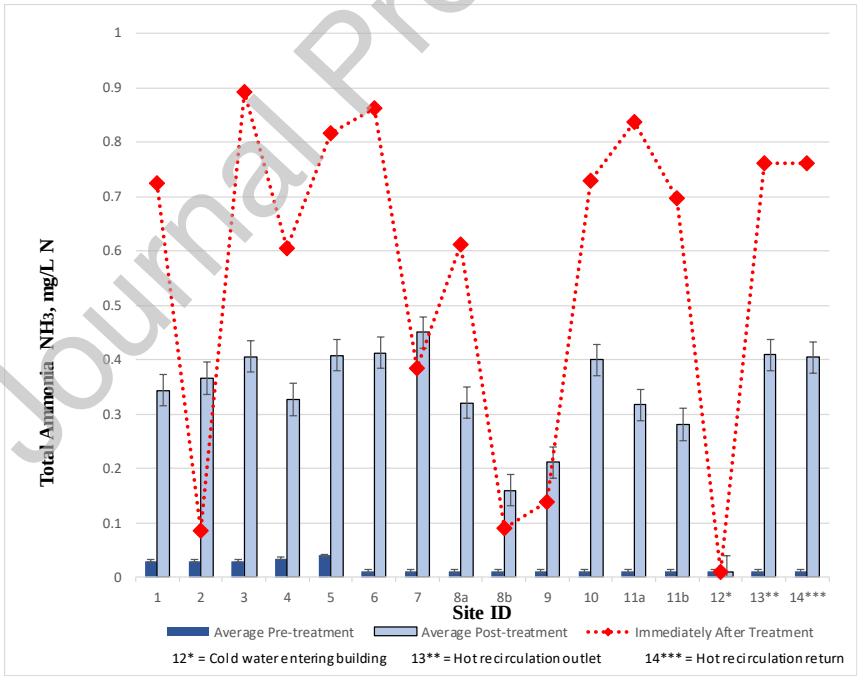
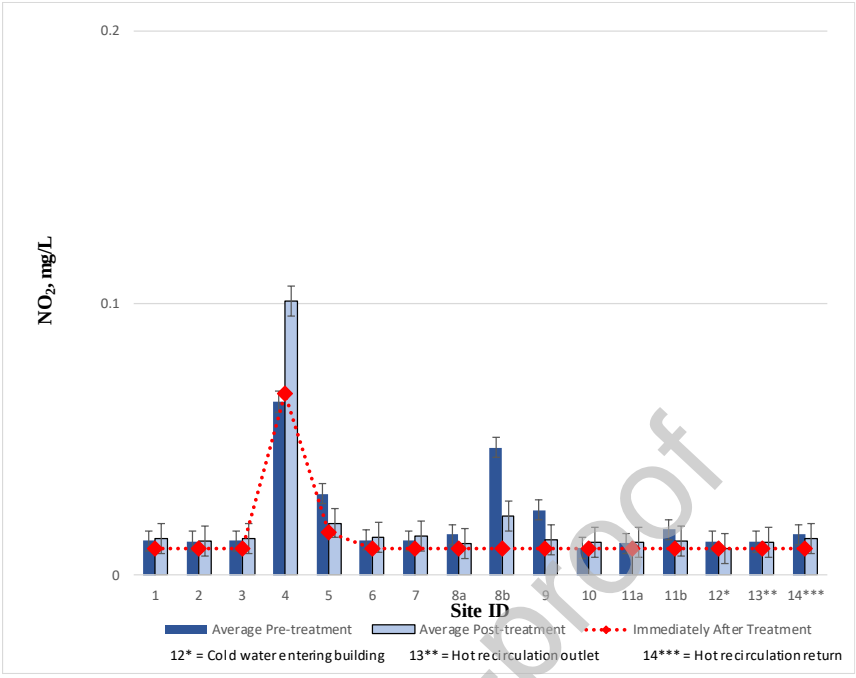


Figure 3. The concentrations of (a) free ammonia, and (b) total ammonia, before and after the installation of the monochloramine feed. Immediately after treatment refers to within 48 hours after monochloramine startup.

(a)



(b)

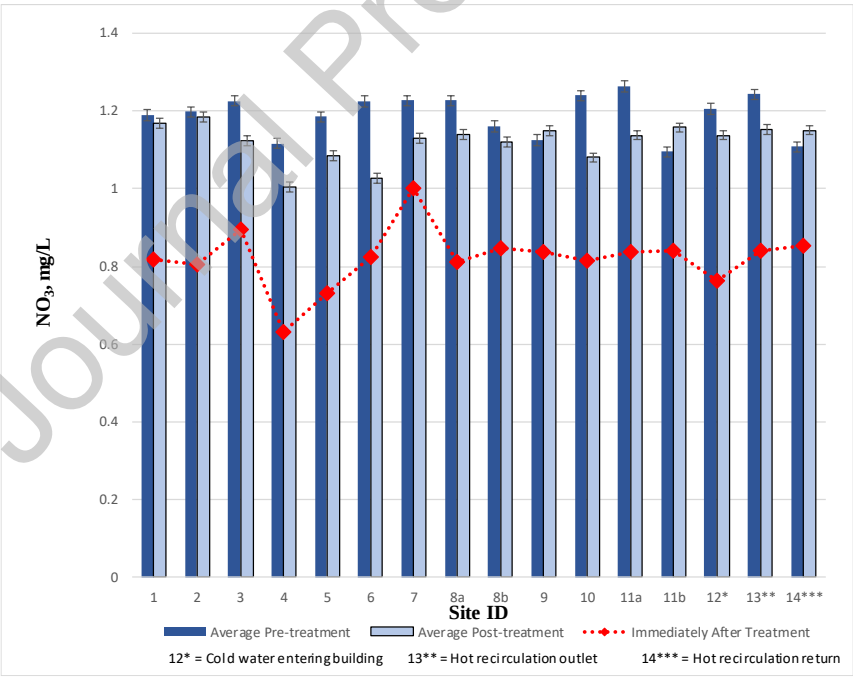


Figure 4. The concentrations of (a) nitrite, and (b) nitrate, before and after the installation of the monochloramine feed. Immediately after treatment refers to within 48 hours after monochloramine startup.

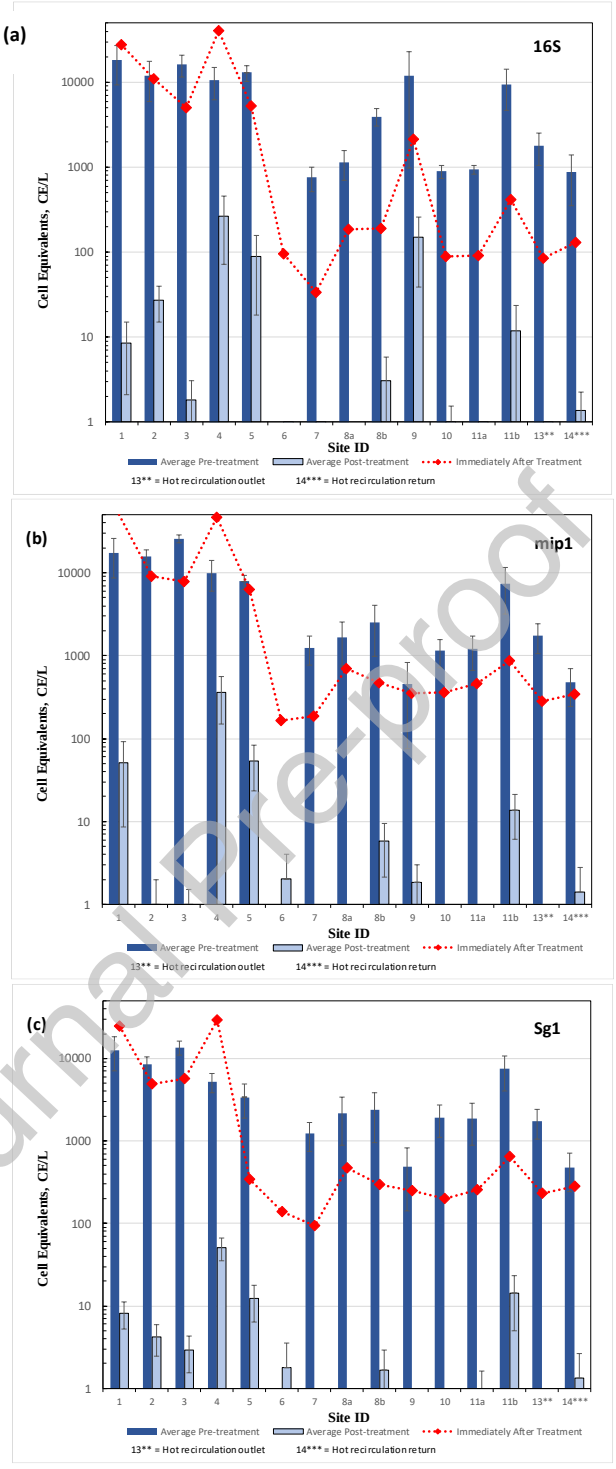


Figure 5. Results from qPCR analysis of water samples at all locations before and after monochloramine treatment (a) to *Legionella spp*, (b) *Legionella pneumophila*, and (c) *Legionella pneumophila* sg 1. Immediately after treatment refers to within 48 hours after monochloramine startup. Site 12, municipal drinking water entering the hospital, did not have detections and is not shown. Non-detect data points below 1 CE/L are not shown.

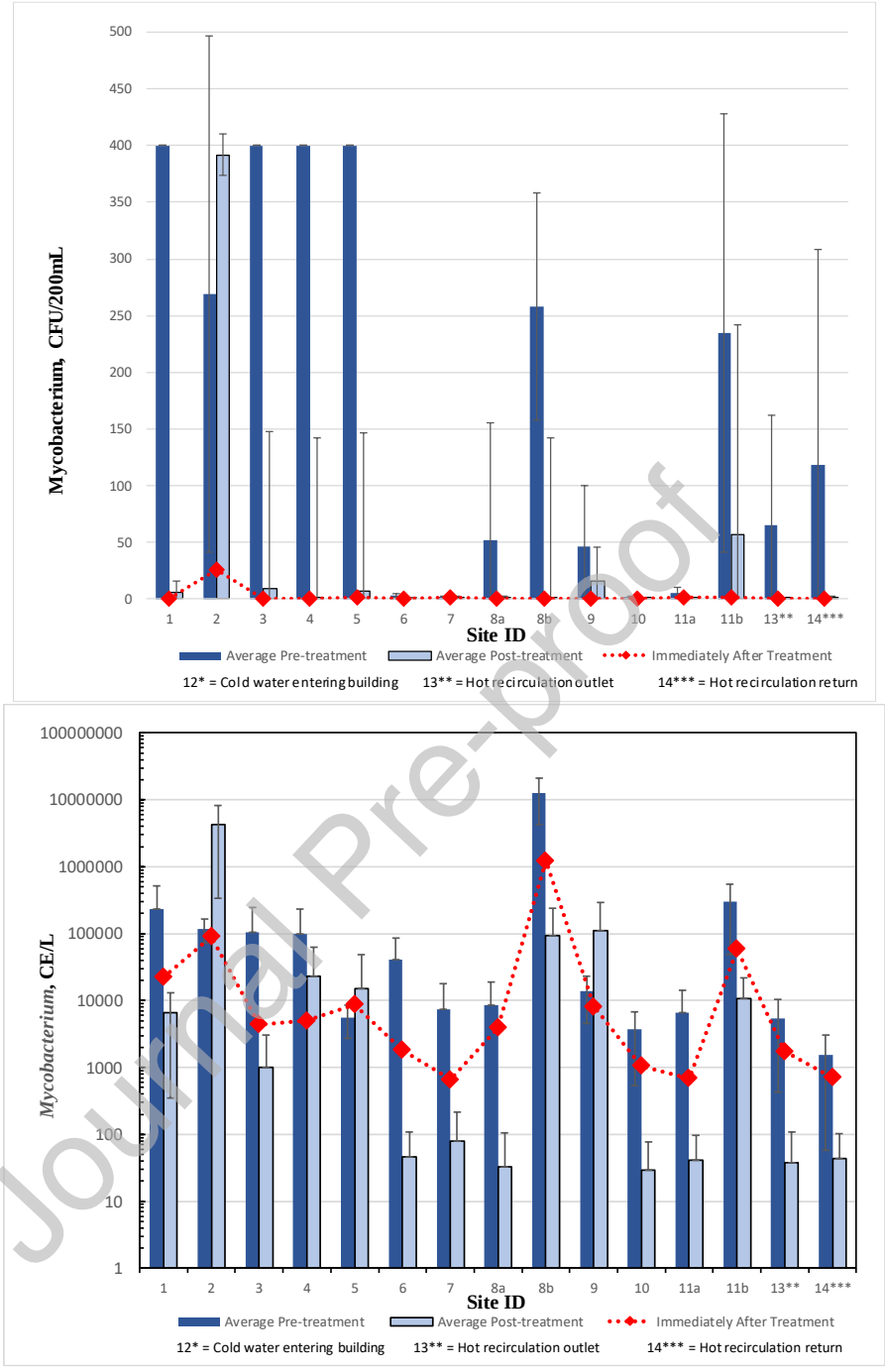


Figure 6. *Mycobacterium* analysis of water samples at all locations before and after monochloramine treatment (a) by culture method, and (b) by qPCR. Immediately after treatment refers to within 48 hours after monochloramine startup. Site 12, municipal drinking water entering the hospital, did not have detections and is not shown.

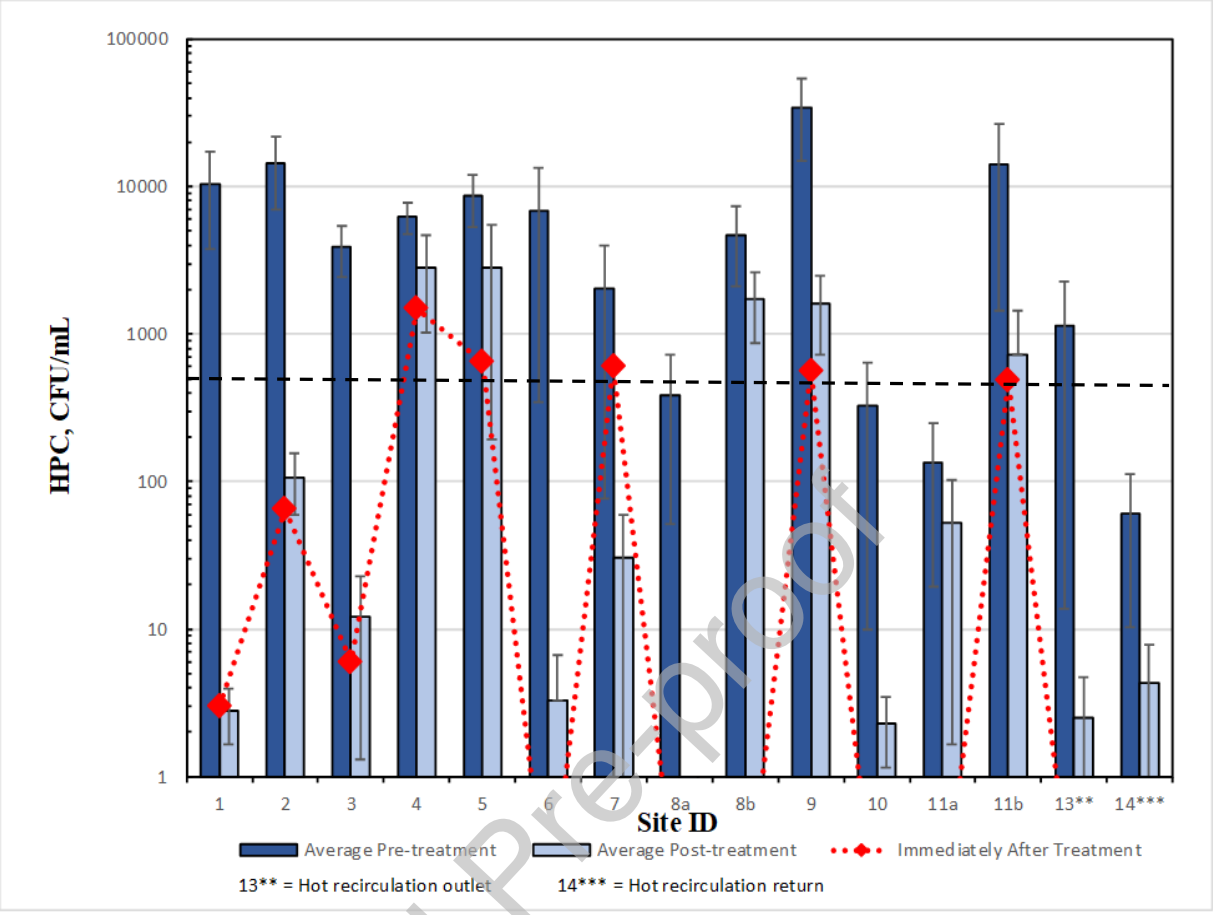
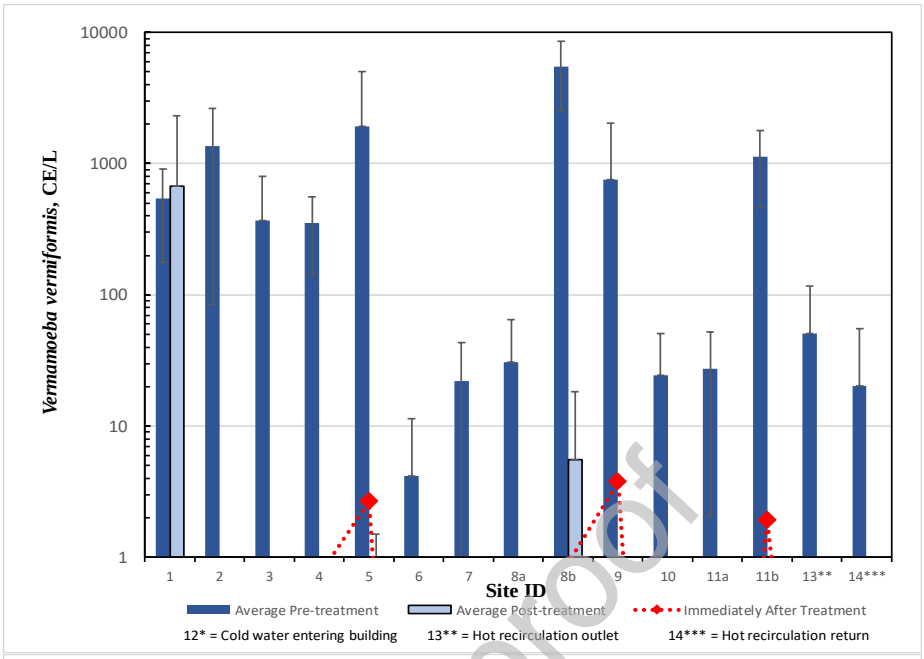


Figure 7. Heterotrophic bacteria analysis of water samples at all locations before and after monochloramine treatment. Immediately after treatment refers to within 48 hours after monochloramine startup. Site 12, municipal drinking water entering the hospital, did not have detections and is not shown. Dashed line represents 500 CFU/mL. Non-detect data points below 1 CFU/mL are not shown.

(a)



(b)

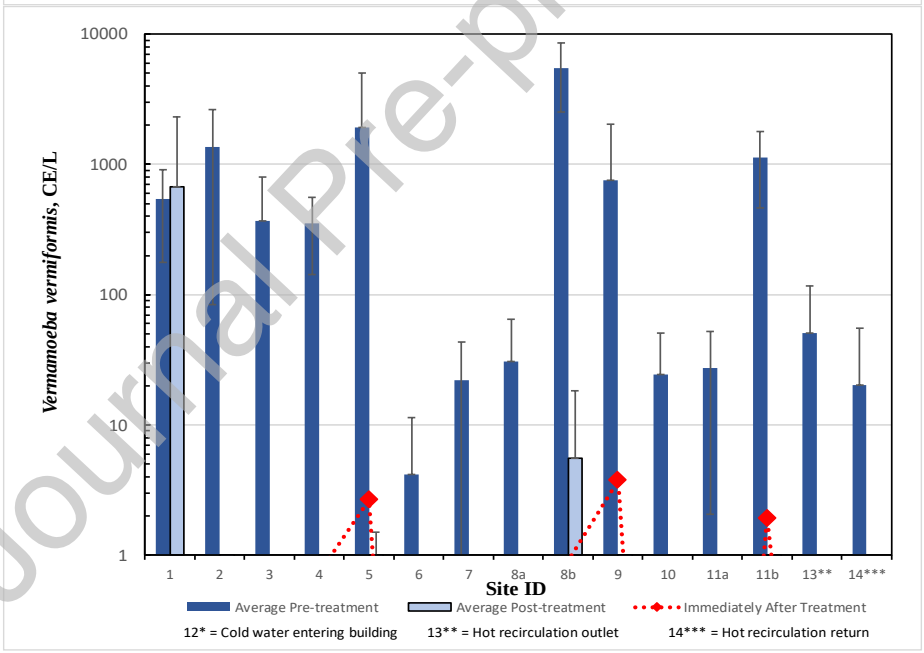


Figure 8. Results from qPCR analysis of water samples at locations before and after monochloramine treatment (a) *Pseudomonas aeruginosa,* and (b) *Vermamoeba vermiformis*. Immediately after treatment refers to within 48 hours after monochloramine startup. Site 12, municipal drinking water entering the hospital, did not have detections and is not shown. Non-detect data points below 1 CE/L are not shown.