

TITLE: Control of *Legionella* Contamination and Risk of Corrosion in Hospital Water Networks following Various Disinfection Procedures

Isabella Marchesi, Greta Ferranti, Antonella Mansi, Anna M. Marcelloni, Anna R. Proietto, Navneet Saini, Paola Borella, Annalisa Bargellini – May 2016

INTRODUCTION

This study, originally published in the *American Society for Microbiology Journal*, Applied and Environmental Microbiology, the researchers evaluated several common chemical water treatment modalities for their control of *Legionella*, as well as their risk of corrosion.

PURPOSE

The purpose of this study was to assess the long-term effectiveness of various disinfection procedures in order to reduce *Legionella* in hospital water networks treated with superheat and flush, chlorine dioxide, monochloramine, and hydrogen peroxide.

METHOD

- The study was conducted in two hospitals; three different parallel networks in hospital 1 (A, B, and C); four individual buildings, each with their own hot water networks of hospital 1 (D, E, F, and G); and the water network in hospital 2 (H). In both hospitals, the water systems were contaminated with *Legionella* prior to installation.
- Two chlorine dioxide devices were installed on water network A and B in 2005.
- A monochloramine device was installed on water network C in 2009.
- A hydrogen peroxide device was installed on water network D in 2012.
- Hyperthermia (superheat and flush) was routinely used on water network H since 2012.
- Rectangular corrosion-testing coupons were used to evaluate corrosion at each site.
- Each site was routinely tested for adequate disinfectant levels as well as *Legionella* levels.

RESULTS

- In total, 237 of the 662 samples (35.8%) were contaminated by *Legionella* after 3 years of treatment. The researchers concluded that none of the treatments tested (monochloramine, chlorine dioxide, hydrogen peroxide, and superheat and flush) eradicate *Legionella* from water distribution systems.
- The monochloramine-treated water network showed 9.5% positivity for *Legionella*.
- The chlorine dioxide-treated water network showed 29.8% positivity for *Legionella*.
- The hydrogen peroxide-treated water network showed 38.5% positivity for *Legionella*.
- The superheat and flush-treated water network showed 54.5% positivity for *Legionella*.
- The researchers saw an increase in corrosion across all chemically-treated networks. The mean corrosion rate was 0.17 mm/year for pipes exposed to hydrogen peroxide, 0.15 mm/year for hyperthermia, 0.14 mm/year for the untreated network, 0.14 mm/year for chlorine dioxide, and 0.11 mm/year for monochloramine.

NOTEWORTHY

- The monochloramine-treated water network showed the lowest percentage of *Legionella*-positive samples – 9.5%, which still poses a threat after 3 years of treatment.
- The network treated with superheat and flush (hyperthermia) was more contaminated than the other treatments both in terms of their positive sites as well as its bacterial load.

Control of *Legionella* Contamination and Risk of Corrosion in Hospital Water Networks following Various Disinfection Procedures

Isabella Marchesi,^a Greta Ferranti,^a Antonella Mansi,^b Anna M. Marcelloni,^b Anna R. Proietto,^b Navneet Saini,^a Paola Borella,^a Annalisa Bargellini^a

University of Modena and Reggio Emilia, Department of Biomedical, Metabolic and Neurosciences, Section of Public Health, Modena, Italy^a; INAIL Research Area, Department of Occupational and Environmental Medicine, Epidemiology and Hygiene, Monte Porzio Catone, Rome, Italy^b

Physical and chemical disinfection methods have been proposed with the aim of controlling *Legionella* water contamination. To date, the most effective procedures for reducing bacterial contamination have not yet been defined. The aim of this study was to assess the long-term effectiveness of various disinfection procedures in order to reduce both culturable and nonculturable (NC) legionellae in different hospital water networks treated with heat, chlorine dioxide, monochloramine, and hydrogen peroxide. The temperature levels and biocide concentrations that proved to give reliable results were analyzed. In order to study the possible effects on the water pipes, we verified the extent of corrosion on experimental coupons after applying each method for 6 months. The percentage of positive points was at its lowest after treatment with monochloramine, followed by chlorine dioxide, hydrogen peroxide, and hyperthermia. Different selections of *Legionella* spp. were observed, as networks treated with chlorine-based disinfectants were contaminated mainly by *Legionella pneumophila* serogroup 1, hyperthermia was associated with serogroups 2 to 14, and hydrogen peroxide treatment was associated mainly with non-*pneumophila* species. NC cells were detected only in heat-treated waters, and also when the temperature was approximately 60°C. The corrosion rates of the coupons were within a satisfactory limit for water networks, but the morphologies differed. We confirm here that chemical disinfection controls *Legionella* colonization more effectively than hyperthermia does. Monochloramine was the most effective treatment, while hydrogen peroxide may be a promising alternative to chlorine-based disinfectants due to its ability to select for other, less virulent or nonpathogenic species.

The number of cases of Legionnaires' disease has increased steadily over the years, especially in Italy and Europe (1, 2). In 2013 in Italy, most cases were community acquired (83.4%), followed by travel-associated (9.8%) and health care-associated (4.6%) (3) cases. Despite the lower percentage of nosocomial cases, the control of *Legionella* sp. contamination is essential in health care settings, where patients, in particular those with compromised immune systems, are at high risk of contracting the disease, with a possible fatal outcome (4). For this reason, national and international guidelines recommend using preventive measures to control *Legionella* water contamination, with particular reference to health care structures (3, 5). A range of physical and chemical disinfection methods have been proposed, but to date, the most effective procedures for controlling contamination have not been defined (6). Moreover, their impacts on pipe deterioration/corrosion have not been documented extensively and are studied mainly in model water distribution systems (7–10).

Molecular investigation tools have been used together with conventional culture for monitoring corrective actions (11–13). Culture is essential for identifying and typing bacterial strains; however, it is time-consuming and can give false-negative results due to the possible presence of cells in a viable but nonculturable (VBNC) state (14). In the VBNC state, pathogens are not generally able to initiate disease but virulence is retained, and infection can follow their resuscitation to the actively metabolizing state (15). Quantitative PCR (qPCR) techniques have proved to have several advantages, including high sensitivity and accuracy and rapid evaluation of germ contamination. The main disadvantage of these techniques is that they cannot distinguish viable and nonviable cells, which is an important factor to take into account in evaluating the effectiveness of corrective actions (16, 17). In order

to overcome this problem, new PCR-based strategies, collectively called molecular viability analyses, have been developed (18). Among these, methods based on DNA-intercalating dyes, such as ethidium monoazide (EMA), have been proposed in combination with qPCR (19–21). EMA selectively binds the DNA of cells with compromised membranes, while intact cell membranes represent a barrier for the dye. In the bound state, the DNA cannot be amplified by qPCR, while DNA from cells with an intact membrane can be amplified and quantified (21). The only insurmountable limitation of this method is the inability to detect bacteria inactivated by conditions that do not alter membrane permeability, such as short-wave UV and low-temperature pasteurization (18).

The main aim of the present study was to assess the long-term effectiveness of monochloramine, chlorine dioxide, hydrogen peroxide, and heat in reducing/eliminating both culturable and nonculturable (NC) legionellae in various hospital water networks. For this purpose, traditional culture was used with qPCR alone and in combination with EMA. In our study, the EMA-qPCR method proved to be suitable for measuring bacterial via-

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Address correspondence to Isabella Marchesi, isabella.marchesi@unimore.it.

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bility because oxidative disinfectants and heat rapidly act against bacterial cells, causing damage to cellular components, including the cytoplasmic membrane (18). In order to verify the possible corrosive action of each disinfection procedure, carbon steel coupons were inserted along the water distribution systems and were periodically examined for weight loss and morphology.

MATERIALS AND METHODS

Hospital setting. This study was carried out in two hospitals situated in Modena, northern Italy: a private hospital built in the 1980s and a university hospital consisting of a central block, called building 1, and four separate blocks built between the 1970s and the 1990s. The same municipality provides the incoming cold groundwater in both hospitals. Hot water is produced *in situ* using heat exchangers and reaches the peripheral points through a water recirculation system. Three different water networks (A, B, and C) distribute hot water in parallel in building 1, while the other four buildings of the university hospital have their own hot water networks (D, E, F, and G), as previously described (22). The private hospital has a unique hot water distribution network (H).

Since 2000, sampling plans have been implemented in order to assess *Legionella* sp. contamination in the water distribution systems. In both hospitals, the samples collected prior to intervention were contaminated mainly by *Legionella pneumophila* serogroups 2 to 14, at concentrations of $>10^4$ CFU liter⁻¹, which led to the implementation of a wide range of control strategies (23). The disinfection strategies still operating in both hospitals are described below.

(i) **University hospital, building 1 (nine floors, 40 years old).** A monochloramine device operating since March 2009 is used on water network C; monochloramine is produced *in situ* from the chemical reaction between a stabilized chlorine-based precursor and an ammonium salt (Sanipur s.r.l., Brescia, Italy). The monochloramine generator is set to maintain a concentration of biocide in the recirculation loop between 2.0 and 4.0 mg liter⁻¹. Residual levels are in line with the guideline value of 3 mg liter⁻¹ and the maximum contaminant level of 4 mg liter⁻¹, established by WHO and the U.S. Environmental Protection Agency (EPA), respectively (24, 25).

Two chlorine dioxide devices (Sanipur s.r.l., Brescia, Italy) operating since January 2005 are used on water network A and, since November 2005, on water network B. Chlorine dioxide is produced *in situ* by injecting hydrochloric acid and sodium chlorite into the recirculating hot water. The system is set up to ensure concentrations of at least 0.30 mg liter⁻¹ at distal points, as previously reported (22), without exceeding the EPA maximum residual disinfectant level of 0.80 mg liter⁻¹ (25).

(ii) **University hospital, building D (four floors, 40 years old).** An experimental hydrogen peroxide device operating since January 2012 (O2 s.r.l., Bergamo, Italy) is used for building D. A 48% hydrogen peroxide solution is injected continuously into the recirculating hot water by a dosing pump in order to ensure concentrations of 15 to 20 mg liter⁻¹ at distal outlets.

(iii) **Private hospital H (four floors, 35 years old).** Hyperthermia has been used since April 2012. The hot water is produced at a temperature of at least 60°C and distributed at temperatures constantly at $>50^\circ\text{C}$.

Sample collection. Over a 3-year period (January 2012 to December 2014), hot water samples ($n = 662$) were taken from heaters, return loops, and distal outlets (showers and/or taps) of the water networks treated with the disinfection strategies listed above. In both hospitals, the protocols anticipated sampling from at least one remote point every 50 beds, with repeat sampling at the same sites every 3 or 4 months. The network that was experimentally treated with hydrogen peroxide was monitored more frequently, i.e., every week for the first 3 months, every month until the end of the second year, and every 4 months during the last year. Water was collected in sterile plastic bottles without flaming and after flushing for 1 min. Sodium thiosulfate (10 mg liter⁻¹) was added (final concentration, 1 ml liter⁻¹) to neutralize residual free chlorine. At sampling, water temperature (digital thermometer), chlorine dioxide (Microquant DPD

method; Merck, Darmstadt, Germany), monochloramine (indophenol method; Hach Lange, Milan, Italy), and hydrogen peroxide (RQflex 2 reflectometer; Merck, Darmstadt, Germany) were measured. The samples were returned to the laboratory immediately after collection and analyzed within 24 h as described elsewhere (26).

Laboratory methods. Culture and identification of *Legionella* spp. were carried out according to the ISO 11731:1998 method (27) as previously described (28). The results were expressed as numbers of CFU per liter, and the limit of detection (LOD) of the procedure was 25 CFU liter⁻¹.

DNA was extracted by use of a QIAamp DNA minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, as reported elsewhere (19). The extracted DNA was stored at -20°C until use.

The minimum number of samples to be analyzed by molecular methods in order to have statistical power was determined by carrying out a power analysis based on the results of a previous pilot study (29). For each treatment, the first 22 negative samples with culture (total of 88 samples) were analyzed by qPCR, with and without the EMA pretreatment. The water samples were treated with EMA (Sigma Chemical Co., St. Louis, MO) at a final concentration of 6 μM (2.53 $\mu\text{g ml}^{-1}$) prior to DNA extraction, as reported by Mansi et al. (19).

DNA amplification was carried out with a Rotor-Gene Q 2plex instrument (Qiagen, Hilden, Germany) and using a commercial kit (new *Legionella* quantitative kit; Diatheva, Fano, Italy) validated to be in agreement with the ISO/TS 12869:2012 method (30, 31). The results were expressed as numbers of genome units (GU) per liter. Under the experimental conditions used in this study, the LOD and the limit of quantification of the qPCR method were estimated to be 100 GU liter⁻¹ and 500 GU liter⁻¹, respectively.

Corrosion study. Rectangular coupons (area, 21.81 cm²; density, 7.87 g cm⁻³) of carbon steel C1010 foils with frosted surfaces were used to evaluate the type and extent of corrosion according to the standard practices ASTM G1-03:2011 and ASTM G4-01:2014 (32, 33). Nonalloy steel with a carbon content of up to 0.22% is suitable for the conveyance of aqueous liquids, including water for human consumption (34). We selected C1010 steel with a maximum carbon content of 0.13% because it is easily found on the market as coupons proper for our experimental conditions. The coupons were inserted into five separate racks made from polytetrafluoroethylene (Fig. 1). The racks were connected to the return loops of the treated networks (A, B, D, and H) and an untreated network (F). Before the beginning of the study, four coupons were inserted into each rack. After 2 and 4 months, one coupon from each rack was removed and a new coupon was added. After 6 months, all the coupons were removed. In line with this practice, we analyzed six coupons for each rack, i.e., two for each exposure time (2, 4, and 6 months). After collection of the coupons for corrosion analysis, they were immediately dried with dimethyl ketone and placed in vials containing silica gel for transportation to the laboratory. The weight loss method was used in order to determine the corrosion rate. The coupons were scraped with a brass brush to remove surface deposits, washed in an ultrasonic bath for 6 min, and then weighed. The cleaning cycles were suspended as soon as the weight value was stabilized. With the aim of determining the mass loss of the base metal during removal of the corrosion products, an uncorroded control coupon was cleaned using the same procedure performed on the test coupons. The average corrosion rates were calculated by means of the following formula: corrosion rate (in millimeters per year) = $(K \times W)/(A \times T \times D)$, where K is a constant (3.65×10^4), W is the mass loss (in grams), T is the time of exposure (in days), A is the area of the carbon steel coupon (in square centimeters), and D is the density (carbon steel) (in grams per cubic centimeter).

An optical microscope (Carl Zeiss, Milan, Italy) equipped with a system for automatic digitization of the images was used in order to characterize the corrosive phenomena.

Statistical analysis. All statistical analyses were performed with PASW Statistics, version 21.0 (SPSS Inc., Chicago, IL). Logarithmic transforma-



FIG 1 Example of a rack used to support coupons for the test of corrosion.

tions were used to normalize the bacteriological data, and the results are presented as geometric mean values. The chi-square test, paired *t* test, and one-way analysis of variance (ANOVA) with the Bonferroni test were applied whenever necessary.

RESULTS

In total, 237 of 662 samples (35.8%) were contaminated by *Legionella* spp. The disinfection treatments significantly affected both the percentage of positive samples ($\chi^2 = 104.385$; $P < 0.001$) and

the bacterial load of positive samples, expressed as the geometric mean ($F = 26.007$; $P < 0.001$). Monochloramine showed the lowest percentage of positive results (9/95 samples [9.5%]), followed by chlorine dioxide (60/201 samples [29.8%]), hydrogen peroxide (80/208 samples [38.5%]), and hyperthermia (36/66 samples [54.5%]). Regarding *Legionella* concentrations, no differences in the geometric mean were observed according to chemical treatments (2.2×10^2 CFU liter⁻¹ for monochloramine, 3.0×10^2 CFU liter⁻¹ for chlorine dioxide, and 1.3×10^2 CFU liter⁻¹ for hydrogen peroxide), while a significantly higher geometric mean (1.7×10^3 CFU liter⁻¹) was observed for the heat-treated positive samples than for the samples treated with all biocides ($P < 0.05$ by the Bonferroni test).

Table 1 shows the numbers and percentages of positive samples and their geometric means according to the biocides and temperatures examined. No significant difference in the percentage of positives relating to the concentrations of monochloramine was observed, and 3 mg liter⁻¹ was required in order to obtain legionella levels of $< 10^2$ CFU liter⁻¹. Levels of chlorine dioxide of ≥ 0.50 mg liter⁻¹ significantly reduced the percentage of positive points, to below 30% ($\chi^2 = 3.930$; $P < 0.05$). Hydrogen peroxide at concentrations between 15 and 19.9 mg liter⁻¹ was associated with a significant reduction in positive points ($\chi^2 = 3.823$; $P < 0.05$), yet levels of ≥ 20 mg liter⁻¹ were required to obtain fewer than 30% positive distal points. A significant reduction of positive results was observed by increasing the temperature to 55 to 59.9°C ($\chi^2 = 7.796$; $P < 0.010$), but no positive sample was observed when the temperature reached 60°C. For all treatments, the bacterial load did not differ according to the biocide/temperature level, and only a limited number of samples exceeded 1.0×10^4 CFU liter⁻¹ (one each with monochloramine and hyperthermia and two with chlorine dioxide).

Table 2 shows that waters treated with chlorine-based systems were mainly contaminated with *L. pneumophila* serogroup 1, hyperthermia was strictly associated with *L. pneumophila* serogroups 2 to 14, and hydrogen peroxide treatment was mainly associated

TABLE 1 Numbers and percentages of positive samples and their geometric means determined by culture

Treatment	No. of positive samples/ total no. of samples (%)	χ^2 value, <i>P</i> value ^a	Geometric mean CFU liter ⁻¹ for positive samples (range)	<i>F</i> value, <i>P</i> value ^a
Monochloramine		4.131, NS		1.963, NS
<2.0 mg liter ⁻¹	2/8 (25.0)		2.0×10^3 (1.2×10^2 – 3.1×10^4)	
2.0–2.9 mg liter ⁻¹	4/29 (13.8)		3.7×10^2 (25 – 5.7×10^3)	
≥ 3.0 mg liter ⁻¹	3/58 (5.2)		25 (25)	
Chlorine dioxide		6.928, <0.05		2.058, NS
<0.30 mg liter ⁻¹	31/84 (36.9)		4.0×10^2 (25 – 2.5×10^4)	
0.30–0.49 mg liter ⁻¹	18/54 (33.3)		3.5×10^2 (25 – 4.1×10^4)	
≥ 0.50 mg liter ⁻¹	11/63 (17.5)		1.0×10^2 (25 – 5.5×10^3)	
Hydrogen peroxide		10.339, <0.001		2.468, NS
<15.0 mg liter ⁻¹	46/91 (50.5)		1.1×10^2 (25 – 1.4×10^3)	
15–19.9 mg liter ⁻¹	14/43 (32.6)		2.6×10^2 (25 – 5.0×10^3)	
≥ 20.0 mg liter ⁻¹	20/74 (27.0)		1.1×10^2 (25 – 2.3×10^3)	
Hyperthermia		33.31, <0.001		0.481, NS
50–54.9°C	19/21 (90.5)		2.5×10^3 (25 – 1.2×10^4)	
55–59.9°C	17/27 (63.0)		1.2×10^3 (25 – 7.9×10^3)	
$\geq 60^\circ\text{C}$	0/18 (0)			

^a NS, not significant.

TABLE 2 Numbers and percentages of species and serogroups of *Legionella* according to treatment ($\chi^2 = 270.042$; $P < 0.001$)

Treatment	No. (%) of positive samples		
	<i>L. pneumophila</i> serogroup 1 alone or with others	<i>L. pneumophila</i> serogroups 2–14	<i>Legionella</i> spp.
Monochloramine	7 (77.8)	0 (0.0)	2 (22.2)
Chlorine dioxide	51 (85.0)	4 (6.7)	5 (8.3)
Hydrogen peroxide	21 (26.3)	5 (6.2)	54 (67.5)
Hyperthermia	0 (0.0)	36 (100.0)	0 (0.0)

with non-*pneumophila* species (54.5% *L. jamestowniensis*, 36.4% *L. anisa*, and 9.1% both).

Among the 394 water samples whose cultures were negative, 88 were analyzed by molecular methods (Table 3). Chemical biocides showed similar percentages of positive results by qPCR but no positive sample by EMA-qPCR. Over 95% of the heat-treated samples were positive by qPCR, and 50% were positive by EMA-qPCR. Positive results for EMA-qPCR were also associated with samples at temperatures over 60°C (6/11 [54.5%]).

The average losses of thickness of the coupons exposed to treated and untreated waters did not differ significantly according to the type of treatment and time of exposure. The mean corrosion rate was 0.17 ± 0.03 mm/year for the coupons exposed to hydrogen peroxide, 0.15 ± 0.03 mm/year for hyperthermia, 0.14 ± 0.04 mm/year for the untreated network, 0.14 ± 0.03 mm/year for chlorine dioxide, and 0.11 ± 0.05 mm/year for monochloramine.

The morphology of corrosion did not change over time but differed according to treatment. As an example, the morphologies of corrosion according to treatment following 6 months of water exposure are shown in Fig. 2. The coupons exposed to chlorine dioxide presented a uniform corrosion with pitting and ulcerations (Fig. 2A), those exposed to monochloramine showed a uniform corrosion as well as rare pitting (Fig. 2B), and those exposed to hydrogen peroxide were characterized by pitting, with pit sizes ranging from a few micrometers to several millimeters (Fig. 2C). Finally, coupons from the heat-treated waters (Fig. 2D) showed a uniform corrosion with some ulcerations similar to those observed on untreated samples (data not shown).

DISCUSSION

In this study, we followed the trend of contamination by *Legionella* spp. in hospital hot water networks treated by different disinfection procedures. The effectiveness of these procedures was evaluated using traditional culture, qPCR, and EMA-qPCR in order to detect culturable and NC *Legionella* cells. Among the studied disinfection strategies, we included monochloramine, an innovative method which proved to be effective for controlling *Legionella* contamination in a hospital water network (22, 35). We also studied hydrogen peroxide, which has not yet been used extensively to control *Legionella* in hospital water distribution systems (36, 37). A comparison was carried out between these new procedures and two popular methods, chlorine dioxide and hyperthermia, which have widely been reported to be effective (38–42).

Our study confirms the effectiveness of continuous chemical disinfection, but we emphasize that all systems must be monitored continuously, since none of them eradicates legionellae from water distribution systems (6, 36, 43). On comparing the three dis-

TABLE 3 Molecular analysis of culture-negative samples

Treatment	No. of positive samples/total no. of samples (%) ^a	
	qPCR	EMA-qPCR
Monochloramine	11/22 (50.0)	0/22 (0.0)
Chlorine dioxide	9/22 (40.9)	0/22 (0.0)
Hydrogen peroxide	10/22 (45.4)	0/22 (0.0)
Hyperthermia	21/22 (95.4)	11/22 (50.0)

^a For qPCR, $\chi^2 = 15.246$ and $P < 0.005$; and for EMA-qPCR, $\chi^2 = 27.957$ and $P < 0.001$.

infectants, monochloramine proved to be the most effective approach, as it gave the best results in reducing the percentage of positive points by culture, followed by chlorine dioxide and hydrogen peroxide. Moreover, for all biocides, approximately 50% of the culture-negative samples analyzed by molecular methods were positive by qPCR but negative by EMA-qPCR. This confirms that qPCR can give false-positive results when the biocides are applied in a contaminated system, as previously reported (17), and that continuous injection of chemicals that are capable of killing the circulating microbes avoids the induction of VBNC forms of legionellae.

In contrast, the network treated with hyperthermia was more contaminated in terms of both percentage of positive sites and bacterial load. The Italian and European guidelines recommend constantly maintaining the water temperature between 55 and 60°C in order to prevent *Legionella* contamination (44, 45), yet in our study this range proved to be ineffective, as over 60% of the samples remained positive. Interestingly, the presence of NC legionellae was also observed at temperatures of around 60°C, which is considered to be a safe value for preventing *Legionella* contamination (46). Recent studies demonstrated that VBNC legionellae are again culturable upon resuscitation within amoebae and that infection can be initiated following their resuscitation (14). In this respect, the NC cells generated following the thermal treatment used in our hospital constitute a potential public health hazard. For all these reasons, we do not advise using hyperthermia as the only method for controlling *Legionella* contamination in hospital water networks.

We stress the importance of finding an adequate level of biocide for controlling *Legionella* contamination, as our long-term experience suggests that the effectiveness of chlorine-based chemicals changes over time. After 1 year of disinfection applications, we proposed a level of chlorine dioxide of 0.30 to 0.40 mg liter⁻¹ and a level of monochloramine of 2.0 mg liter⁻¹ to lower contamination to below 10² CFU liter⁻¹ (35). In the following 3 years, the levels required to obtain the same reduction were 0.50 to 0.70 mg liter⁻¹ for chlorine dioxide and 2 to 3 mg liter⁻¹ for monochloramine (22). In the present study, chlorine dioxide levels of ≥ 0.50 mg liter⁻¹ were associated with 10³ CFU liter⁻¹, but the percentage of positives was below 30%, a value reported as being an indicator of low risk for disease transmission (47). Similarly, a monochloramine level of ≥ 3 mg liter⁻¹ was required to maintain *Legionella* levels below 10² CFU liter⁻¹, in accordance with other studies (48–50), but the percentage of positive sites was less than 30% independently of the biocide levels, thus confirming the satisfactory results obtained with this disinfectant.

Hydrogen peroxide produced satisfactory results in reducing

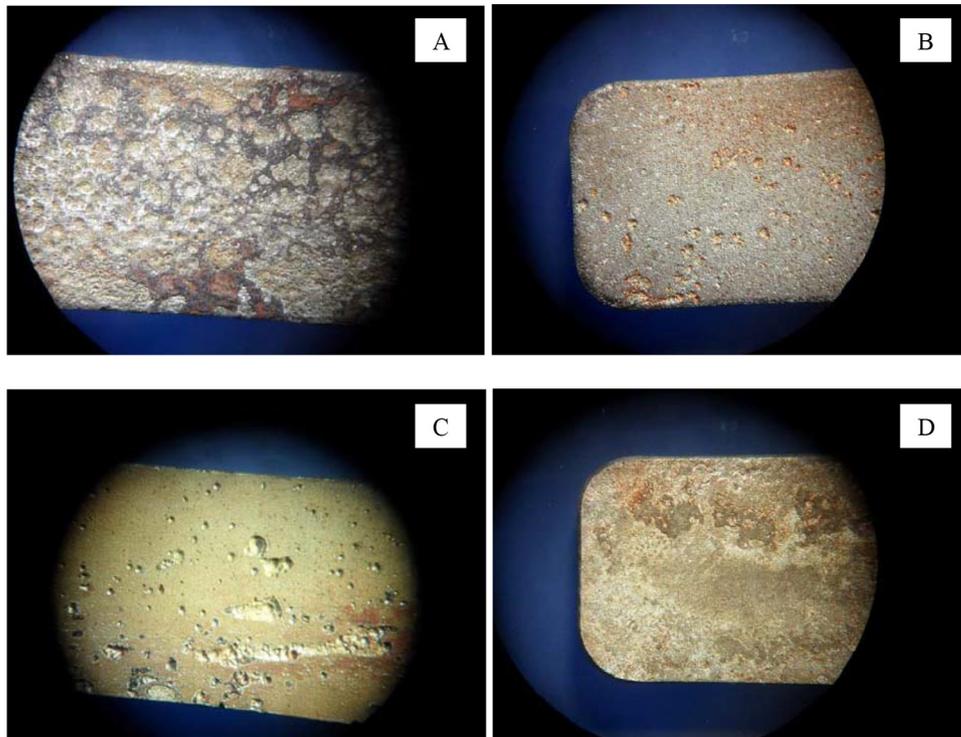


FIG 2 Digitalized images showing one of the two coupons removed after 6 months of water exposure for each treatment. Magnification, $\times 3$. (A) Coupon treated with chlorine dioxide; (B) coupon treated with monochloramine; (C) coupon treated with hydrogen peroxide; (D) coupon treated with hyperthermia (similar to untreated carbon steel coupons [not shown]).

Legionella contamination only when the biocide level was ≥ 20 mg liter⁻¹, in agreement with other studies (36, 37). For this procedure, the high percentage of positive points, although at low levels, was due to the difficulty in regulating the disinfectant concentration, probably because the building was under renovation and many of the outlets were seldom used. Hydrogen peroxide appears to be a promising alternative for decreasing *Legionella* colonization; however, further field studies in other health care and community settings are required to confirm its effectiveness.

The chlorine-based biocides caused a shift from *L. pneumophila* serogroups 2 to 14 to *L. pneumophila* serogroup 1, while hydrogen peroxide favored a switch from *L. pneumophila* to other species, mainly *L. jamestowniensis*, which has not yet been associated with human disease (51, 52). Contrastingly, no shift was observed with hyperthermia, which is in line with its ineffectiveness at reducing *Legionella* colonization. The continuous injection of chlorine-based biocides evidently selects the most resistant *Legionella* spp., in our experience *L. pneumophila* serogroup 1, which is also the most virulent (1). In order to support this hypothesis, other authors reported the persistence of serogroup 1 in hospital water systems despite the adoption of chlorine-based disinfection strategies (53, 54). Duda et al. (50) reported a shift from *L. pneumophila* serogroup 1 to *L. bozemanii*, both of which are associated with human pathologies, following 24 months of monochloramine applications.

It is well known that disinfection can speed up corrosion and cause plumbing leaks, even though contradictory results are reported in the literature concerning the impact of disinfection on corrosion (7–10). To complete the study, we studied the appearance of a favorable environment for corrosion within the water

networks according to the disinfection methods applied. The loss of thickness of the carbon steel foils which were used to evaluate the extent of corrosion over a 6-month period did not exceed the average value of 0.50 mm/year that is considered satisfactory for water networks (55), and no significant differences were observed between treated and untreated networks. On the other hand, differences were observed regarding the morphology of corrosion. Hydrogen peroxide and chlorine dioxide caused pitting, which is a type of corrosion that can create holes in tubes (9). Monochloramine and hyperthermia appeared to be less aggressive, since monochloramine caused a uniform corrosion with rare formation of pitting and hyperthermia showed a morphology of corrosion similar to that observed on the untreated coupons. The results encouraged us to continue the corrosion study; therefore, we are now analyzing the long-term corrosive effects of these four disinfection procedures on commonly used plumbing materials, such as copper, stainless steel, galvanized steel, and polyvinyl chloride, the last two of which are also used in our hospitals.

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