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Continuous Disinfection by Monochloramine on Domestic Hot Water System of Health-care Facilities for the Control of *Legionella* Contamination in Italy

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Abstract: Backgroud*:* The prevention of *Legionella* spp. colonization of water distribution systems is a critical issue in healthcare settings and only an effective disinfection of water systems and appropriate environmental surveillance strategies allow to prevent nosocomial legionellosis. Methods*:* Due to the temporary effectiveness (increase of the temperature of water in boilers and shock hyperchlorination), the high costs (point-of-use water filters) or the ineffectiveness (hydrogen peroxide, H_2O_2) of the previous control procedures, 3 devices (one for each hot water loop) continuously injecting monochloramine have been installed in two different Italian hospitals heavily contaminated by *Legionella pneumophila* SG3 and SG6. Aim*:* To evaluate the efficacy of continuous disinfection by monochloramine for control of *Legionella* on domestic hot water (DHW) distribution system of health-care facilities. Findings*:* One month after the disinfection of DHW with monochloramine, the load of *L. pneumophila* SG3 and SG6 (previous mean count ranging from 10^3 to 10^5 CFU/L), as well as the Heterotrophic Plate Count (HPC) (previous mean count ranging from > 10^2 to > 10^4 CFU/mL), decreased at undetectable levels in 100% of the sampling points in the two hospitals. Conclusion*:* The results suggest that continuous injection of monochloramine on DHW systems can fully control *L. pneumophila* and HPC in contaminated hospitals since the beginning of its application.

Keywords: Hospitals, *Legionella*, monochloramine.

1. Introduction

Legionella is an organism of public health interest for its ubiquitous presence in water distribution systems (WDS) and for its ability to cause infection in susceptible humans.

It is well known that factors that most enhance colonization of a WDS include the water temperature, piping obstruction and stagnation, biofilm formation and the presence of protozoa, in which legionellae multiply. One of the key issues for controlling the colonization of a WDS is to recommend an effective disinfection method. At present, chlorination is the most commonly used treatment for *Legionella* control in WDS, but it has been shown that lapses in chlorination or discontinuous chlorination with chlorine or chlorine dioxide can lead to an increased resistance of *Legionella* to the disinfectant [1].

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Copper-silver ionization is currently used for *Legionella* control in WDS in the US, while in Europe copper based products for drinking water disinfection have not been allowed since 1st February 2013 [2].

Among the antimicrobial agents of relatively most recent application in the disinfection of water, monochloramine seems to be more effective for *Legionella* remediation in hospital plumbing systems [3-5]. The present study shows the results obtained by the continuous injection of monochloramine for 1 year on domestic hot water (DHW) in two Italian hospitals heavily contaminated by *Legionella*. The effectiveness of monochloramine in controlling *Legionella* has been compared with other control systems previously adopted.

2. Methods

2.1 Background and Setting

In 2012, a sampling plan was implemented to assess *Legionella* contamination of the water systems in two Italian hospitals: the hospital "Umberto I" (Siracusa) and the hospital "Nuovo Ospedale di Lentini" (Lentini, Siracusa).

The hospital "Umberto I" is a very old hospital, built during the period 1930-1960, which comprises two different 5-floors buildings with a total of 350 beds. The "Nuovo Ospedale di Lentini" is a 5-floors building, 150-bed facility built in the 1990s but opened in 2012, thus with a WDS that remained unused for a long period of time. For each hospital incoming cold groundwater, disinfected with chlorine, is provided by two different local Municipalities. DHW is produced and distributed in three different loops: two loops (one for each building with two 2,000 L boilers) at the hospital "Umberto I" and one loop (with three 5,000 L boilers) at the hospital "Nuovo Ospedale di Lentini".

Between 2012 and 2013, 148 hot water samples (96 at the hospital "Umberto I" and 52 at the hospital "Nuovo Ospedale di Lentini") were collected from the boilers, return loops and outlets (faucets and showers selected on the basis of distance from the boilers and exposure risk) of the two hospital WDS. The sampling strategy included DHW loops and at least one ward for each floor, reiterating the same sites every 4 months. In total, 3 boilers with their respective return loops were sampled: 2 at the hospital "Umberto I" (one for each building) and 1 at the "Nuovo Ospedale di Lentini".

High levels of *L. pneumophila* detected in the two hospitals led to the adoption of a combined control strategy that included the raise of the water temperature in boilers, the performance of shock hyperchlorinations, the installation of point-of-use water filters in the most at risk wards and, in 2013, only at the hospital "Nuovo Ospedale di Lentini", continuous disinfection of the DHW loop with hydrogen peroxide (H_2O_2).

Eventually, due to the persistence of a strong contamination of the DHW loops, experimental equipments—2 at the hospital "Umberto I" (one for each building) and 1 at the hospital "Nuovo Ospedale di Lentini"—continuously injecting monochloramine (Sanikill patented system, Sanipur Srl, Brescia, Italy) at 2.0-2.5 mg/L were installed at the beginning of 2014. Monochloramine efficacy for *Legionella* disinfection was evaluated over a 12-month period.

2.2 Sample Collection

After the start of hot water monochloramination, between January 2014 and January 2015, a total of 387 hot water samples (244 at the hospital "Umberto I" and 143 at the hospital "Nuovo Ospedale di Lentini") were monthly collected from the same sites within the DHW networks of the two hospitals. The water samples were collected measuring water temperature and chlorine levels (free chlorine and monochloramine; DPD Free and Monochlor-F methods, HACH Company, USA). Boilers and return loops were collected after a 5 min flushing and flaming while outlets (taps and showers) were collected without flushing and flaming in accordance with the Italian Guidelines for Legionellosis Prevention [6].

One-liter sterile bottles, added with 1 mL of sodium thiosulphate in order to neutralize residual free chlorine or monochloramine were used for bacteriological analyses. According to ISO 19458 [7], procedures for sample collection, transport and storage were established. Bottles were returned to the laboratory immediately after sampling for bacteriological analyses.

2.3 Microbiological Analyses

The water samples were analyzed within 12 h from collection for the detection of *Legionella*.

The Heterotrophic Plate Count (HPC) at 22 °C was detected in PCA (Plate Count Agar-Oxoid) in accordance with ISO 6222 [8] to evaluate the relationship between total microbial concentration and *Legionella* positivity.

Isolation of *Legionella* was performed in accordance with standards procedures ISO 11731 [9]. One liter of water from each sampling point was concentrated using 0.2 μm filter polyamide membranes (Sartorious). Each membrane was shaken for 15 min in 10 mL of the correspondent water sample to detach bacteria. For each water sample, an aliquot of 5 mL of the concentrated sample underwent immediate cultural examination while the remaining 5 mL was treated with heat by exposure to 50 °C for 30 min. From both the samples (concentrated and heat-treated sample), aliquots of 0.1 mL were inoculated onto one plate of BCYE agar (CYE agar with added BCYE*α* growth supplement, Oxoid) and 0.1 mL onto one plate of GVPC agar (BCYE agar with added GVPC selective supplement, Oxoid). After 4, 8 and 14 days of incubation at 37 °C in 2.5% CO_2, colonies suggestive for *Legionella* grown on BCYE and GVPC were confirmed on the basis of cultural testing (lack of growth on CYE agar) and serogrouped by slide agglutination using commercial antisera (Oxoid and Biogenetics).

Results were expressed in CFU/L and the counts referred to water samples concentrated 100 times (1 L in 10 mL of the water sample). The detection limit of the culture procedure was 10 CFU/L (1 CFU/mL of inoculums corresponds to 1 CFU/100 mL of the untreated sample).

2.4 Chemical and Physical Analyses

For each water sample the water temperature (in °C) was registered at the time of the sampling using a calibrated thermometer placed in the middle of the water stream. pH and ORP were measured with portable instruments. Total hardness was measured with a titration test kit (HACH). The chemical analyses (free Cl_2, total Cl_2, NH_2Cl, NH_4^+, NO_3^-, NO_2^-) were conducted on site with a HACH DR/900 portable photometer. All the chemical parameters above were determined before the injection of the monochloramine (baseline) and successively.

3. Results

At the beginning of the monitoring programme, in 2012, two years before the installation of the 3 monochlorammine generator devices, a systemic colonization of the water networks was demonstrated. *L. pneumophila* SG3 and SG6 was isolated from 100% of the sampling points in the two hospitals (SG3 at the hospital "Umberto I" and SG6 and the hospital "Nuovo Ospedale di Lentini"), with a mean count ranging from 10^3 to 10^5 CFU/L and HPC at 22 °C ranging from more than 10^2 to more than 10^4 CFU/mL.

Due to these levels of colonization, first of all the temperature of water in boilers in the two hospitals was raised from 55-60 °C to 65-70 °C. Following the temperature increase the number of positive supply points for *L. pneumophila* and the mean bacterial loads decreased immediately but remained stable only for 1 month. For this reason, shock hyperchlorinations (sodium hypochlorite, 50 ppm of free chlorine at distal points for 1 h) were performed. After each shock hyperchlorination the number of positive supply points and the mean bacterial loads decreased immediately. Nonetheless, after 3 months *L. pneumophila* appeared again in all the sampling points. In total, shock hyperchlorinations were performed 4 times, from 2012 to 2013, in the two hospitals. In order to control the level of colonization, point-of-use water filters (0.2 m sterile filters) were installed in high risk areas, such as haematology, oncology and intensive care units to insure complete protection toward legionellosis to high risk patients.

After the installation of the point-of-use water filters positive sites rate was reduced by 100%, but the filters had to be replaced every 30 days according to the manufacturer's specifications and the entire procedure was extremely expensive. Finally, taking into consideration that the point-of-use water filers had been installed only in selected areas and that 3 months after each shock hyperchlorination *Legionella* appeared again, at the beginning of 2013 a continuous stabilized H_2O_2 system was installed in the DHW system of the hospital "Nuovo Ospedale di Lentini", assuring a minimum concentration, within the limits set by the European Standard EN902: 2009 [10], of 17 mg/L of H_2O_2. Nonetheless, after 1 year the levels of colonization remained stable. In fact, apart from the distal outlets in which point-of-use water filters had been installed, 3 months after each shock hyperchlorination and during the continuous addition of H_2O_2 *Legionella* returned to the previous concentrations.

The disinfection of the DHW with chlorine dioxide was taken into consideration but it was rejected after the evaluation of its potential corrosive effects on water pipes [11-15].

Moreover, the possibility of using alternative methods like, for example, peracetic acid or U.V. lights were rejected for their documented ineffectiveness in controlling strong levels of colonization [16-18]. Thus, due to the limited effectiveness (increase of the temperature of water in boilers, shock hyperchlorination) or the high costs (point-of-use water filters) or the ineffectiveness (H_2O_2) of the controlling strategies adopted, at the beginning of 2014 a new control strategy had to be chosen. A scientific literature survey [3-5] and the risk assessment of the two hospitals directed the choice to the monochloramine.

Three monochloramine generator devices were installed in the DHW systems of the two hospitals: 2 at the hospital "Umberto I" (one for each building), and 1 at the hospital "Nuovo Ospedale di Lentini". At the beginning of the disinfection program monochloramine was injected into the DHW at an average concentration of 3 mg/L for 30 days in order to disinfect the entire water systems. Subsequently, the dosage was regulated to obtain a continuous monochloramination within the range 2.0-2.5 mg/L. Continuous monitoring of oxidation reduction potential (ORP) in the hot water return line and flow rate of supply water to the hot water system were used to control dosage.

One month after the continuous injection of monochloramine, the load of *Legionella* (Fig. 1), as well as the HPC at 22 °C, decreased at undetectable levels. In particular, at the hospital "Nuovo Ospedale di Lentini", only 1 week after the start of the new disinfection system, the percentages of positive sample points decreased at 8%, with a mean *L. pneumophila* load lower than 10^3 CFU/L. In the same hospital, however, as a consequence of a stop of the monochloramine generator device, during which the release of disinfectant was interrupted for around 15 days, *Legionella* was isolated in all the sampling sites with counts > 10^5 CFU/L.

Anyway, all samples became negative as soon as the system got back to operation (Fig. 1).

Chemical analyses did not detect changes in the water chemical and physical properties and no specific disinfection by product (DBP) was detected. In particular, as shown on Table 1, ammonium, nitrite and nitrate concentrations did not exceed their limits during the study.

Observed pH values ranged from 7.8-8.5, while ammonium concentrations ranged from 0.00-0.55 ppm, while nitrite concentrations ranged from 0.002-0.040 ppm, and nitrate concentrations ranged from 4.6-25.6 ppm.

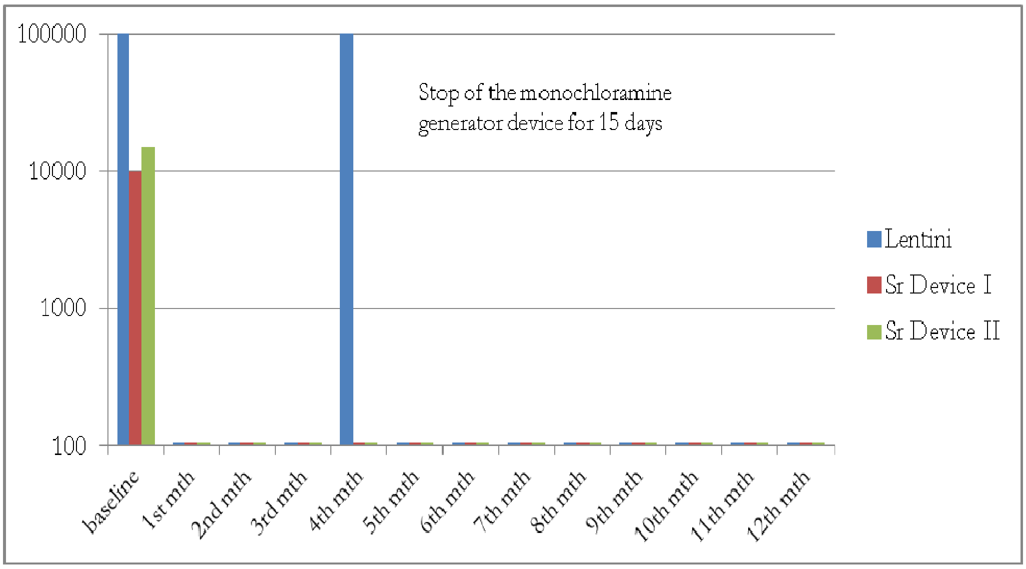

Fig. 1 Mean concentrations of *L. pneumophila* before and after the disinfection with monochloramine.

Table 1 Levels of ammonium, nitrates and nitrites before (baseline) and after the treatment of the domestic hot water with monochloramine.

	Ammonium NH_4^+ (limit 0.50 ppm)									
Hospital	Feb (baseline)	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Hospital "Nuovo Ospedale di Lentini"	0.05	0.48	0.32	0.29	0.25	0.21	0.44	0.50	0.11	0.15
Hospital "Umberto I"										
Block 1	0.00	0.05	0.43	0.22	0.14	0.08	0.50	0.22	n.d.	0.35
Block 2	0.05	0.00	0.50	0.36	0.38	0.13	0.55	0.44	0.50	0.49
	Nitrites NO_2- (limit 0.50 ppm)									
Hospital	Feb (baseline)	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Hospital "Nuovo Ospedale di Lentini"	0.025	n.d.	0.002	n.d.	0.040	n.d.	0.008	0.027	n.d.	0.019
Hospital "Umberto I"										
Block 1	0.025	n.d.	0.019	n.d.	0.017	n.d.	0.019	0.007	n.d.	0.003
Block 2	0.019	n.d.	0.024	n.d.	0.009	n.d.	0.021	0.011	0.003	0.009
	Nitrates NO_3- (limit 50 ppm)									
Hospital	Feb (baseline)	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Hospital "Nuovo Ospedale di Lentini"	17.0	n.d.	9.6	n.d.	4.6	n.d.	13.7	9.2	n.d.	5.7
Hospital "Umberto I"										
Block 1	29.0	n.d.	13.2	n.d.	9.0	n.d.	15.9	21.4	n.d.	25.6
Block 2	30.0	n.d.	10.6	n.d.	8.7	n.d.	13.7	10.4	14.4	12.7

4. Discussion

Twelve-months application of monochloramine in the DHW systems of the two hospitals heavily contaminated by *L. pneumophila* gave satisfactory results. The previous control procedures showed to be insufficient (increase of the temperature of water in boilers and shock hyperchlorination), effective but too expensive (point-of-use water filters) or absolutely ineffective (H_2O_2) to control contamination. On the contrary, a significant decrease in *Legionella* percent positivity was observed in the two hospitals DHW following monochloramine application. In particular, in one of the two hospitals (“Nuovo Ospedale di Lentini”), only 1 week after the start of disinfection of the DHW with monochloramine, the percentages of positive sample points decreased at 8% with *L. pneumophila* lower than 10^3 CFU/L, suggesting that monochloramine is fully active since the beginning of its application.

The results are consistent with previous studies conducted both in Italy and the US. In particular, Marchesi *et al.* [4] observed a significant reduction of *L. pneumophila* from 97% positivity to 13.3% positivity after one year of monochloramine injection into a hospital WDS. Casini *et al.* [5] found that at the initial monitoring phase before the start of the monochloramine disinfection, all the sampling sites resulted positive for *L. pneumophila*, while no sample resulted positive after treatment. In the US, at the end of 1 year of a monochloramine injecting system evaluation Kandiah *et al.* [3] observed a reduction in water sample culture positivity rate from 53% to 0.35% for *Legionella*.

In the present study, at the hospital “Nuovo Ospedale di Lentini” the temporary stop of the monochloramine generator device increased the levels of *L. pneumophila* (ranging from more than 10^2 to 10^3 CFU/L), suggesting that keeping a stable concentration of monochloramine is mandatory to reduce the risk of hospital acquired legionellosis. This is consistent with Casini *et al.* [5], which found that all the samples became positive for *L. pneumophila* when the release of monochloramine was interrupted for around 24 h as a consequence of a failure of the disinfectant generator device. Anyway, the authors found that all samples became negative as soon as the system got back to operation.

Moreover, although HPC bacteria are not pathogenic and are not considered predictive of the presence or the absence of *Legionella*, they can be used for monitoring the disinfection system performance. Previous studies reported elevated HPC concentrations following several months of continuous monochloramine application [19]. The authors did not observe this trend during our investigation. In fact, average HPC concentrations decreased at undetectable levels following monochloramine application. Recently, Duda S. *et al.* [20] reported HPC reduction after DHW system chloramination of a US hospital which is consistent with our results.

Finally, although the aim of the authors’ study was not to calculate cost-effectiveness, we noticed that the disinfection of DHW with monochloramine demonstrated to be a cost-saving procedure. In fact, only thanks to the decrease of the temperature in water boilers from 65-70 °C to 60 °C it was calculated a money saving of around 4.164,00 € (4.747,00 $) per year for the hospital “Nuovo Ospedale di Lentini” and of 7.988,00 € (9.107,00 $) per year for the hospital “Umberto I”. These savings should be sufficient to at least compensate for the post-sale service and maintenance of the three monochloramine generators. It was not possible to evaluate the cost-saving of the applied technology in reducing the risk of infection because no nosocomial cases was detected, probably because *L. pneumophila* SG3 and SG6 are less frequently associated with legionellosis than SG1 [21].

In conclusion, the present study shows that the injection of monochloramine only on DHW at a concentration of 2.0-2.5 mg/L can be sufficient in controlling heavily contaminated hospitals WDS since the beginning of its application. Obviously, the method requires careful management to reach the right concentration of the disinfectant and to avoid changes in water chemical composition, in particular regarding the content of nitrite, nitrate and free ammonia and the production of DBPs.

5. Conflict of Interest Statement

Dr Stefano Melada, responsible for the Research & Development department of Sanipur Srl, contributed with a scientific and technical support necessary for the development of the management plan. He did not participate to the samplings of the water, isolation and

identification of legionellae. No financial support was given to the authors.

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