Water quality supply in a Portuguese teaching hospital: monitoring and studies on detection of critical points

Pedro Norton[a,b], Joana Amaro[a,c], Natália Martins[b] and Manuela Vieira da Silva[d]

[a]EPIUnit – Institute of Public Health, University of Porto, Porto, Portugal; [b]Occupational Health Service of the Hospital Epidemiology Centre, São João Hospital Centre, Porto, Portugal; [c]Department of Clinical Epidemiology, Predictive Medicine and Public Health, University of Porto Medical School, Porto, Portugal; [d]Environmental Health Department, School of Allied Health Technologies of Polytechnic Institute of Porto (ESTSP.IPP), Vila Nova de Gaia, Portugal

ABSTRACT

Surveillance of drinking water quality is extremely important to human health, assuming greater relevance in hospital environments, especially to those individuals who are immunocompromised. This study is aimed to determine the effect of increasing free chlorine (Cl) concentration in a hospital water network in regard to water quality monitoring and microbial growth control, between 2010 and 2013 in Porto. The average of free Cl concentration in the period under analysis showed some heterogeneity per floor, varying between 0.84 and 1.25 mg/L. In addition, there was a rise in proportion of samples that exceeded WHO guidelines (free Cl $\geq$ 0.5 mg/L), particularly in the last two years of the same period. With respect to microbial analysis, 22.4% of the samples were positive for *Legionella* spp., 6.4% for *Pseudomonas aeruginosa*, 15% and 30.4% for aerobic plate counts at 36 and 22 °C, respectively. The proportion of positive samples decreased throughout the period under analysis, in particular for *Legionella* spp. (41.7% in 2010 vs. non-detectable in 2013) and *P. aeruginosa* (10.8% in 2010 vs. 3.3% in 2013). These results are in accordance with the gradual rise in free Cl concentration (0.78 $\pm$ 0.94 mg/L in 2010 vs. 1.16 $\pm$ 0.51 mg/L in 2013). In conclusion, a suitable plan for drinking water quality was instituted which resulted in reducing microbiological growth in the waterwork network, improving public health protection. However, the detection of critical points associated with lower levels of free Cl were found on certain floors/points-of-use, requiring the need to improve the monitoring water treatment system and/or implementation of additional technologies.

KEYWORDS

Water quality; hospitals; chlorine; *Legionella* spp.; *Pseudomonas aeruginosa*

Introduction

Hospital water networks are one of the main sources of nosocomial infections (Department of Health 2006; HPSC 2015). Two of the most common bacterial agents are *Legionella* spp. and *Pseudomonas aeruginosa* (Leprat et al. 2003; Sehulster et al. 2003) –

water persistent gram-negative bacteria. Colonization of the respiratory tract by these bacteria may progress to pneumonia and/or other severe infections (Sehulster et al. 2003).

Legionella spp. is a waterborne pathogen that is normally transmitted through aerosols and a common species found in hospital water supplies (Rafiee et al. 2014). The prevalence of *Legionella*-related diseases in a hospital environment is considerably high when compared to the general community (33.3% vs. 7.5%) (Tesauro et al. 2010). The presence of Legionnaires' disease in hospitals globally reached 47% (Joly et al. 2006; Yu 2008; Rafiee et al. 2014). However, this may be an underestimation due to advances in identification techniques required for detection, which were not available in the past and also the unspecific nature of *Legionella*-related diseases' clinical manifestations (Fields, Benson, and Besser 2002; Rafiee et al. 2014; HPSC 2015).

The Hospital studied in Porto is supplied by the public water network whose quality is guaranteed by the city council. However, since it has a 55 km network with more than 50 years at some points, it is difficult to assure water quality in all its extension. On the other hand, hospitalized patients consume this water on a daily basis (bottled water is not provided), including immunocompromised patients and children (through infant's formula milk). It should be noted that the lethality rate associated with *Legionella* spp. in immunocompromised patients is considerably high (Flannery et al. 2006), as well as in patients undergoing hemodialysis (Vorbeck-Meister et al. 1999). Patients suffering from burns and neutropenia have higher morbidity and mortality rates associated with *Pseudomonas* (Sehulster et al. 2003). Therefore, health care facilities have a special responsibility controlling disinfection and water quality consumed by patients or used in their personal hygiene, to prevent nosocomial infections namely *Legionella* and *Pseudomonas*.

In Portugal, *Legionella* spp. water analysis is mandatory in a context of inner air quality assessment, but there is no specific legislation concerning water used for human consumption and for hemodialysis. Even considering the implementation of an effective drinking water disinfection control system, the hospital needs to have the practice of *Legionella* monitoring. WHO recommends, for hospitals that use systematic water disinfection, that the water cultures of *Legionella* be verified every three months for efficacy of disinfection (Bartram et al. 2007).

Different factors are required to be studied when selecting the most suitable water disinfection method in health care facilities in order to guarantee water quality (Zhang et al. 2007). No method can guarantee absolute total disinfection and when colonization occurs, it is extremely difficult to eliminate it (Scaturro et al. 2007; Lin, Stout, and Yu 2011; Cristino, Legnani, and Leoni 2012; Orsi et al. 2014). Chlorine (Cl) is widely used as a disinfectant in Portuguese water systems (Diegues 2013) and it is considered efficient. However, this method encompasses several disadvantages, including (1) corrosion (necessary to add other anti-corrosive chemical products and silicate material), (2) difficulty in penetrating the biofilm, (3) high levels of *Legionella* resistance, and (4) potential of production of disinfection by-products (DBPs), as trihalometanes (THM) (Lin, Stout, and Yu 2011; Diegues 2013; Hrudey et al. 2015). Further, it is well known that the biofilms in drinking water systems may become transient or long-term habitats for particular micro-organisms, such as *P. aeruginosa* (Wingender and Flemming 2011).

In 2005, a Drinking Water Quality Control Working Group was created in a university hospital which included microbiologists, hygiene and safety technicians, and infectious

disease specialists. The initial goal was to keep the free Cl reference range between 0.2 and 0.6 mg/L, according to the national legislation (Decree-Law 306/2007). However, considering the type of patients treated, the structural dimension of the building (with a 55 km water network), and previous history of microbial growth (including *P. aeruginosa* and *Legionella* spp.), it was decided in 2012 to increase the reference range according to WHO recommendations.

Thus, the aim of this study was to determine the effect of increasing free Cl concentration in a hospital water network in regard to microbial growth control, namely *P. aeruginosa and Legionella* spp.

Material and methods

Hospital characteristics

The hospital under analysis is one of the largest in Portugal with approximately 5600 workers. It is a university hospital constituted by a main building with over 50 years of existence. The hospital is divided into 11 floors, 2 of which are underground, and has five satellite buildings. The hospital has approximately 1100 beds and more than 50 medical and surgical specialties, as well as a variety of complementary means of diagnosis and therapeutic support.

The hospital has a water network of more than 55 km that is supplied by the public water network with seven water reservoirs and a sodium hypochlorite injection system used for disinfestation in three of these reservoirs. There is a booster injection in a technical area located on the fourth floor. The distribution network is mixed – it is constituted by both new and outdated pipes of different types of materials such as cross-linked polyethylene (PEX) and iron.

Water monitoring plan and strategy

With regard to the parameters under analysis, free Cl and temperature levels were assessed daily in at least 22 different collection points including point-of-use outlets of the supply network such as taps, sinks, showers, reservoirs, and water fountains distributed and covering the entire hospital. Microbiological parameters such as aerobic plate count (APC) at 22 and 36 °C as well as *P. aeruginosa* were analyzed monthly at 21 collection points. *Legionella* analysis was performed once a month.

Chlorine and temperature monitoring

Free Cl concentrations were measured in a separate sample bottle. Temperature was measured with a handheld thermometer (Hanna-Instruments brand) directly at the point-of-use outlet. Residual free Cl was determined by using the N,N-diethyl-p-phenylenediamine method with a colorimeter and test kit (HACH LANGE brand, the Pocket Colorimeter model). For determination of the Cl_2 concentration, 5 mL water samples were collected and after assessment of the white portion, the sample was diluted and added to a conductor, DPD-Free Chlorine. The results were expressed in mg/L of Cl_2.

Sample collection and microbiological analysis

The collection and processing of the samples were carried out by an accredited lab (Sagilab®). For this purpose, each sample was collected into a sterile 1 L bottle containing 0.5 mL 0.1 N sodium thiosulfate to neutralize free Cl, and then conditioned at control temperature.

The microbiological analyses were performed according to the following standards and technical instructions: (1) APC, quantification at 36 °C (ISO 6222:1999); (2) APC, quantification at 22 °C (ISO 6222:1999); (3) *P. aeruginosa* survey and quantification (ISO 16266:2006); (4) intestinal *Enterococcus* spp. survey and quantification (ISO 7899-2:2000); (5) *Legionella* spp. survey and identification (ISO 11731:1998); (6) total coliforms survey and quantification (pema028, 2012-03); (7) *Escherichia coli* survey and quantification (pema028, 2012-03); (8) *Staphylococcus aureus* survey and quantification (XP T 90-412, 2014-05); and (9) *Clostridium perfringens* survey and quantification (pema008, 2012-10).

According to the Portuguese legal law for drinking water quality (Decree-Law 306/2007), the following microbiological references were used: APC at 36 °C, 20 colony forming units/mL (CFU/mL); APC at 22 °C, 100 UFC/mL; 100 CFU/L for *Legionella* spp., and for cooling towers, 1000 CFU/L. *P. aeruginosa* reference level was internally established as 0 CFU/mL.

Statistical analysis

Statistical analyses were performed with IBM SPSS statistics 21.0. Chi-Square test or Fisher's exact test was used to analyze microbial qualitative data. Residual Cl qualitative data was analyzed by one-way analysis of variance (ANOVA).The criterion for significance was set at $p < 0.05$.

Results

Between 2010 and 2013, the mean free Cl concentration per floor showed some differences varying between 0.84 and 1.25 mg/L (Table 1), exceeding the national recommendations, but is in accordance with WHO guidelines. A proportion of samples with a non-detectable free Cl concentration (0 mg/L) was found on the eighth and second floors (3.5% and 3.3%, respectively), and on the first and sixth floor, 0.2% of the water samples reaching to 11 mg Cl/L (Table 1). In addition, there was a rise in proportion of samples that exceeded WHO guidelines (free Cl $\geq$ 0.5 mg/L), particularly in the last two years of the same period (Figure 1). With respect to microbial analysis, 22.4% samples were positive for *Legionella* spp., 6.4% for *P. aeruginosa*, and 15% and 30.4% for APC at 36 and 22 °C, respectively (Figure 2). The proportion of positive samples decreased throughout the period under analysis, in particular for *Legionella* spp. (41.7% in 2010 vs. non-detectable percentage in 2013) and *P. aeruginosa* (10.8% in 2010 vs. 3.3% in 2013). These results are in accordance with gradual elevation in free Cl concentration (0.78 $\pm$ 0.94 mg/L in 2010 vs. 1.16 $\pm$ 0.51 mg/L in 2013) (Table 2).

P. aeruginosa was detected in 7 of 11 floors, with 4 floors showing more than 10% positive samples, namely 2nd basement, 1st, 3rd, and 8th floors. In two of these floors (first and third), positive samples for *Legionella* were also detected (above 100 CFU/mL) (Table 3). These results were consistent with APC results at 22 and 36 °C, which also demonstrated

Table 1. Free chlorine concentration (mg Cl_2/L) per floor (2010−2013).

Floor building	No. of samples (*N*)	Free chlorine (mg Cl_2/L)				
		Mean ± SD	Min	No. of samples corresponding to min concentration (%)	Max	No. of samples corresponding to max concentration (%)
02	1223	1.02 ± 0.96	0.00	16 (1.3)	11.00	1 (0.1)
01	1653	1.00 ± 0.88	0.00	25 (1.5)	11.00	1 (0.1)
1	3894	1.02 ± 0.94	0.00	79 (2.0)	11.00	9 (0.2)
2	4480	0.84 ± 0.83	0.00	148 (3.3)	11.00	4 (0.1)
3	1878	0.86 ± 0.68	0.00	28 (1.5)	11.00	2 (0.1)
4	1894	0.98 ± 0.89	0.00	21 (1.1)	11.00	2 (0.1)
5	2504	0.92 ± 0.67	0.00	50 (2.0)	7.00	1 (0.0)
6	1962	1.25 ± 1.10	0.00	8 (0.4)	11.00	3 (0.2)
7	2838	1.09 ± 1.04	0.00	51 (1.8)	11.00	4 (0.1)
8	2628	1.03 ± 0.95	0.00	94 (3.5)	11.00	1 (0.0)
9	1651	1.05 ± 0.84	0.00	21 (1.3)	11.00	2 (0.1)
Roofing	nd	nd	nd	nd	nd	nd

Note: nd − not determined.
SD − standard deviation.

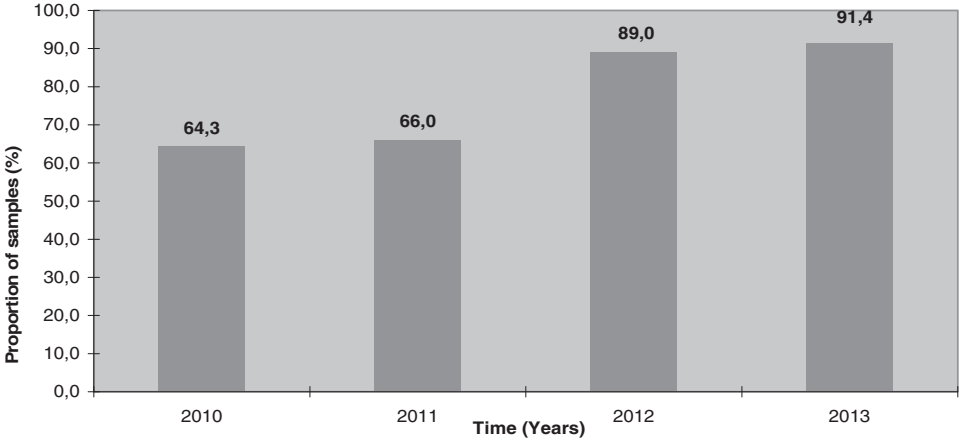

Figure 1. Proportion of water samples that met goals (≥0.5 mg/L Cl_2) in the period 2010−2013.

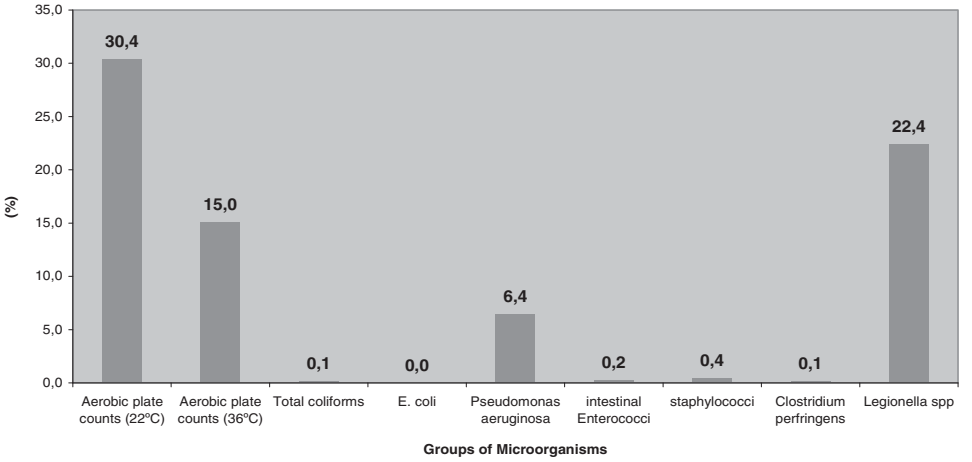

Figure 2. Proportion (%) of samples above reference for microbial growth in the period 2010−2013.

Table 2. Average concentration of annual free chlorine and positive samples of different groups of micro-organisms in the period 2010−2013.

Year of sampling	No. of samples (*N*) chlorine determination	Free chlorine (mg Cl_2/L) Mean ± SD	Aerobic plate counts (22 °C)		Aerobic plate counts (36 °C)		*Pseudomonas aeruginosa*		*Legionella* spp.	
			No. of positive samples (%)	No. of positive samples above reference (%)	No. of positive samples (%)	No. of positive samples above reference (%)	No. of positive samples (%)	No. of positive samples above reference (%)	No. of positive samples (%)	No. of positive samples above reference (%)
2010	7778	0.78 ± 0.94	173 (69.5)	65 (26.1)	156 (62.7)	78 (31.3)	27 (10.8)	27 (10.8)	5 (41.7)	5 (41.7)
2011	6809	0.97 ± 1.22	119 (47.4)	32 (12.7)	109 (43.4)	51 (20.3)	26 (10.4)	26 (10.4)	6 (37.5)	6 (37.5)
2012	6082	1.12 ± 0.64	105 (43.9)	16 (6.7)	129 (54.0)	54 (22.6)	2 (0.8)	2 (0.8)	2 (11.1)	2 (11.1)
2013	5941	1.16 ± 0.51	157 (60.9)	37 (14.3)	120 (46.5)	54 (20.9)	8 (3.3)	8 (3.3)	0 (0.0)	0 (0.0)
	p(*)	<0.001	<0.001	<0.001	<0.001	0.013	<0.001	<0.001	0.024	0.024

Table 3. Number of positive samples per floor of different groups of microorganisms in the period 2010−2013.

Floor	Aerobic plate counts (22 °C)		Aerobic plate counts (36 °C)		*Pseudomonas aeruginosa*		*Legionella* spp.	
	No. of positive samples (%)	No. of positive samples above reference (%)	No. of positive samples (%)	No. of positive samples above reference (%)	No. of positive samples (%)*	No. of positive samples above reference (%)	No. of positive samples (%)	No. positive samples above reference (%)
02	23 (48.9)	4 (8.5)	24 (51.1)	8 (17.0)	6 (12.8)	6 (12.8)	nd	nd
01	39 (45.3)	12 (14.0)	37 (43.0)	24 (27.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
1	89 (58.9)	37 (24.5)	82 (54.3)	56 (37.1)	26 (17.2)	26 (17.2)	6 (37.5)	6 (37.5)
2	49 (53.8)	13 (14.3)	49 (53.8)	22 (24.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
3	32 (54.2)	11 (18.6)	30 (50.8)	16 (27.1)	6 (11.3)	6 (11.3)	2 (50.0)	2 (50.0)
4	34 (55.7)	9 (14.8)	32 (52.5)	12 (19.7)	5 (9.1)	5 (9.1)	0 (0.0)	0 (0.0)
5	71 (63.4)	12 (10.7)	70 (62.5)	30 (26.8)	6 (5.7)	6 (5.7)	nd	nd
6	23 (44.2)	7 (13.5)	27 (51.9)	12 (23.1)	3 (5.8)	3 (5.8)	0 (0.0)	0 (0.0)
7	20 (47.6)	6 (14.3)	20 (47.6)	9 (21.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
8	77 (72.6)	33 (31.1)	76 (71.7)	40 (37.7)	11 (10.4)	11 (10.4)	nd	nd
9	25 (47.2)	3 (5.7)	30 (56.6)	2 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Roofing	72 (52.6)	3 (2.2)	37 (27.0)	6 (4.4)	0 (0.0)	0 (0.0)	5 (35.7)	5 (35.7)

Note: nd − not determined.
SD − standard deviation.
* The number of positive samples match the number of positive samples above reference since the cut off point is 0 CFU/mL.

a higher proportion of samples above the reference limit in most of the same floors (first, third, and eighth).

Discussion

Hospital water networks are one of the main sources of nosocomial infections (Department of Health 2006; HPSC 2015). Knowledge of water distribution, identification of critical areas, and selection of a disinfection method are particularly the important measures to control microbiological growth and prevent nosocomial infections. Knowledge of water distribution may be a difficult task in old hospitals with extensive distribution networks, constituted by new and outdated pipes of different types of materials.

Water networks of considerable size (Ozerol et al. 2006; Ghotaslou et al. 2013), old and complex plumbing, as well as the use of reduced-temperature water are characteristics that correlated with *Legionella* spp. exposure (Fields, Benson, and Besser 2002; Ozerol et al. 2006; Pancer, Rabczenko, and Stypulkowska-Misiurewicz 2006; Koziol-Montewka et al. 2008), and other bacteria, thus, representing a public health issue, as the increase in the amount of this bacteria in hospitals is proportional to the frequency of Legionnaires' disease (Kohler et al. 1999; Ghotaslou et al. 2013). In this particular case, the distribution system was comprised of a variety of materials used for piping, fitting, coating, valves, and appurtenances that promoted microbial growth. Pipe materials were found to influence biofilm accumulation through corrosion, release of compounds that support biological growth, and other surface characteristics (Stewart, McFeters, and Huang 2000). There is some evidence that pipe materials such as PVC and cross-linked polyethylene (PEX) may affect *Legionella* growth in water systems (van der Kooij, Veenendaal, and Scheffer 2005). Disturbances in water pressures or inadequate levels of chemical biocides create conditions that disrupt biofilms or allow *Legionella* and other waterborne pathogens to multiply. Lin, Stout, and Yu (2011) suggested that the use of the electronic faucets poses a potential risk for nosocomial infection-risk areas of hospital, due to the water-saving function of electronic faucets, since there was insufficient amount of water to flush and clean them.

In this study, several areas were identified as critical, namely the first floor, where the water network is obsolete and the collecting points were in most parts located at the louvers in the operating rooms. At these points, the water temperature is tepid, promoting bacteriological growth. With respect to microbial analysis between 2010 and 2013, 22.4% of the samples were positive for *Legionella* spp., 6.4% for *P. aeruginosa*, 15% and 30.4% for APC at 36 and 22 °C, respectively. The high prevalence of *Legionella* and *Pseudomonas* may be due to relapses.

The total count of viable micro-organisms at 36 and 22 °C is an indicator of the disinfection status of the distribution network and drinking water. A high number of colonies presumably indicate the presence of biofilm. In some instances (namely to control *Legionella* spp. and *P. aeruginosa* growth), there was the need to promote chemical disinfection of the supply circuit, increasing free Cl above recommended values. On the other hand, in drinking water systems, all surfaces in contact with water may be colonized by micro-organisms. Wingender and Flemming (2011) estimated that about 95% of all microbial cells present in drinking water distribution systems exist as biofilms on pipe surfaces and only 5% occur in water phase. These micro-organisms might be opportunistic pathogens which harm humans, especially immunocompromised individuals (Wingender and

Flemming 2011). After biofilm creation, micro-organisms grow in a matrix and are protected from external stressors, such as disinfection action and physical removal. Our study showed that Cl, a low-cost widely used disinfectant, might be an effective method to control microbial growth. In fact, the gradual increase in Cl concentration was associated with an improvement in microbiological control of drinking water, even with respect to known Cl-resistant species such as *Legionella* and *Pseudomonas*.

Adverse health effects that may result from chronic exposure to mixtures of DBPs present in drinking waters may be linked to both the types and concentrations of DBPs involved (Hrudey et al. 2015). Depending on the characteristics of the source water and treatment processes used, both types and concentrations of DBP found in drinking waters vary substantially (Miltner et al. 2008; Schenck, Sivaganesan, and Rice 2009). However, the WHO states that the small amount of Cl typically used to disinfect water does not pose risks to human health, and has established a guideline value of 5 mg/L for Cl in drinking water, a value that is 'conservative', since no adverse effects at that concentration in drinking water were reported in studies reviewed by WHO (WCC 2008).

In conclusion, the increase of free Cl concentration resulted in a decrease in microbiological growth in the water network, potentially improving public health protection and reducing economic costs by streamlining current management practices.

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