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Environmental surveillance of *Legionella pneumophila* in two Italian hospitals

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Summary. The aim of this study was to identify the most effective disinfection protocol to reduce the presence of *Legionella pneumophila* in the water system of two Italian hospitals. From 2004 to 2009, 271 samplings of hot water were carried out in 11 hospital units to detect the presence of *L. pneumophila*. Additionally, water samples collected from one boiler outlet and the hot water recirculation were tested. From 2004 to 2009, *L. pneumophila* was present in 37% of the samples. Of these, 68.3% and 18.8% were positive for serogroups 2-14 and 1, respectively. Furthermore, 12.9% of the samples were positive for both serogroups. Finally, a maximal count of 10^4 CFU/L was measured in the most distal sites. To reduce *L. pneumophila* colonization, a two-year long hyperchlorination (2004-2006) was carried out. Moreover, from June 2005 until now, continuous maintenance of boilers and tanks, substitution of the shower heads and increase of the boiler outlet temperature to 60 °C were performed. All these treatments led to a marked reduction of *L. pneumophila* colonization in the short but not in the medium-long term. Only the use of chlorine dioxide led, after four years, to a reduction of the loads of *L. pneumophila* to values below 100 CFU/L. However, in the distal sites a persistent degree of colonization (maximum value 700 CFU/L, average 600 CFU/L) was observed probably due to the presence of *L. pneumophila* in the stagnant water in dead legs. In conclusion, data show that long-term chlorination of hot water sources together with carefully aimed maintenance of water pipes can lead to an effective reduction of *L. pneumophila* concentration in hospital water systems.

Key words: Legionella pneumophila, environmental surveillance, hospitals, disinfection, chlorine dioxide.

Riassunto *(Sorveglianza ambientale di* Legionella pneumophila *in due ospedali italiani)*. In questo studio si è cercato di identificare il protocollo di disinfezione più efficace per il contenimento della presenza di *Legionella pneumophila* nel sistema idrico di due ospedali italiani. Dal 2004 al 2009 abbiamo eseguito 271 campionamenti d'acqua calda da 11 reparti per verificare la presenza di *L. pneumophila*. Inoltre abbiamo controllato l'acqua in mandata della caldaia e quella proveniente dal ricircolo. Il 37% dei campioni è risultato positivo a *L. pneumophila* (18.8% sierogruppo 1, 68.3% sierogruppi 2-14, 12.9% entrambi), con valori anche di 10^4 UFC/L nei punti più distali. Al fine di contenere la carica di *Legionella* sono stati fatti diversi interventi, quali iperclorazione shock (2004-2006), pulizia e manutenzione di caldaie e serbatoi (in continuo), sostituzione delle bocchette delle docce, aumento della temperatura a 60 °C in mandata (da giugno 2005). Tutti questi interventi hanno dato buon esito a breve ma non nel medio-lungo termine. Solo l'utilizzo del biossido di cloro ha portato, a distanza di quattro anni, a un contenimento delle cariche a valori inferiori a 100 UFC/L, seppure sia necessario evidenziare ancora una colonizzazione (valore massimo 700 UFC/L, average 600 UFC/L) nei reparti più distali, probabilmente a causa della presenza di acqua stagnante nei rami morti del circuito idrico.

Parole chiave: Legionella pneumophila, sorveglianza ambientale, ospedali, disinfezione, biossido di cloro.

INTRODUCTION

Legionella pneumophila is a Gram-negative bacterium that is normally found in water. It is known that *L. pneumophila* can persist for long periods of time in water and biofilms commonly found in man-made water systems, such as plumbing systems, air conditioning equipments or whirlpool spas.

It is widely accepted that biofilms play a critical role in the persistence of these bacteria within water systems, providing shelter and nutrients and preventing disinfectants from gaining access to the bacteria through the exopolysaccharide matrix [1].

The strategies of *L. pneumophila* to adapt and resist to stressful environmental conditions include interaction with amoeba and biofilm localization and the ability to enter in a viable but nonculturable (VBNC) state [2]. Since 1977, it has been well documented that *L. pneumophila* is the etiological agent

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of severe pneumonia in humans commonly defined as Legionnaire's disease (LD) [1-3]. Infection is normally caused by inhalation or aspiration of organisms from contaminated aerosol droplets. This process can then lead to sporadic cases as well as to severe outbreaks.

In recent years, the increasing incidence of both nosocomial and community-acquired *L. pneumophila* infections has been a major public health concern. In Italy LD infections increased from 100 cases/year before 1998 to 869 cases in 2005, and in 2008 reached a total of 1189 cases. *L. pneumophila* serogroup (Sg) 1 was found to be responsible for 94.5% of these cases. Interestingly, 7.1% of those infections were acquired in healthcare settings in 2008 [4].

The increasing reports of LD cases probably depends on the greater awareness of clinicians and on improved diagnosis rather than on an overall increased incidence of the disease [5]. Furthermore, the fatality rate of hospital-acquired LD patients affected by chronic degenerative diseases, tumors, immunocompromised patients, or those undergone to organ transplantation, is much higher than the one observed in community-acquired LDs (33.3% *vs* 7.5%, respectively) [4]. Indeed, the degree of *L. pneumophila* colonization in hospital water supplies has been correlated with the incidence of nosocomial LD [6, 7]. While US Centers for Disease Control and Prevention (CDC) reports [8, 9], and Italian [10] and local [11] guidelines establish which type of intervention is best needed to reduce *L. pneumophila* colonization from hospital water supplies, there is conflicting evidence about the precise concentration of *L. pneumophila* that constitutes a risk factor for nosocomial LD. In addition, further studies are urgently needed to determine new guidelines for the prevention of *L. pneumophila* colonization in hospital water supplies based on the characteristics and complexity of the water system.

From 2004 to 2009 an environmental surveillance was performed in two private hospitals in Milan (Italy), housing more than 900 patients with physical and mental disabilities. In these hospitals: 1) the concentration of *L. pneumophila* was measured in the water system; 2) the extent of colonization after different methods of intervention was determined in order to identify an effective protocol to reduce the concentration of *L. pneumophila* in nosocomial water supplies.

MATERIALS AND METHODS

The two private hospitals under study are adjacent and located in the Milan area (Italy). The two structures have different buildings, reached by a municipal water supply and supported by two private wells. Hot water is produced from a series of boilers, collected in tanks and then distributed by a unique ring pipeline to both the structures. In recently built wards, pipes are made of polyethylene, whereas the old ones are made of galvanised steel material.

In 2002 *L. pneumophila* concentration was measured in 21 sites to monitor the entire water system of the two hospitals. However, from 2004 11 sites, located in medium-high risk wards, were selected. In these sites, every 3 months, *L. pneumophila* concentration was measured in hot water samples from 11 showers, one boiler outlet and water recirculation. In the same time, water temperature and residual free chlorine content were measured. In March 2009, since new local guidelines [11] were published, also cold water was sampled in one site. The samples were subsequently analyzed in laboratory, following Italian guidelines [10]. On positive samples, serogrouping, was performed using the *Legionella* latex test (Oxoid).

Cold water from municipal water supply and wells was analyzed according to Italian Decree [12], detecting *Escherichia coli*, enterococci, coliforms at 37 °C, heterotrophic plate count at 22 °C and 36 °C, and *Pseudomonas aeruginosa* and *Aeromonas hydrophila* as additional parameters.

RESULTS

To detect the presence of *L. pneumophila* in nosocomial water, from 2004 to 2009, a total of 271 samples of hot water from 11 units of two separate hospitals was analyzed as described in the "Materials and methods" section.

In 2004-2009 *L. pneumophila* was present in 37% (99/271) of the samples and had a concentration equal or greater than 100 CFU/L, thus demonstrating that all units were colonized *(Table 1)*. Serogroup 2-14 was the most frequent contaminant and, therefore, isolated from 68.3% of the positive samples. In contrast, serogroup 1 was found in 18.8% of the positive samples. Both serogroups were detected in 12.9% of the positive samples. In particular, hospital 1 was positive for *L. pneumophila* in 41% of the samples. Similarly, *L. pneumophila* was present in 37% of the samples taken from hospital 2. Lastly, 13% positive samples in the boiler outlet and 33% in the recirculation water were found. Both hospitals had similar levels of *L. pneumophila* concentration ranging from < 100 CFU/L (detection limit) to 10^4 CFU/L. The highest values were observed in the most distal points, indicated with R2 and R10, in both hospitals *(Figure 1)*.

Since these sites were found to be highly contaminated by *L. pneumophila* (10^3 to 10^4 CFU/L), the sanitation of the entire water system was performed and after a week the measurements repeated to observe the efficacy of the treatment.

Hyperchlorination was performed five times from September 2004 to February 2006. During this process, free chlorine residual medium concentration was kept between 30 and 40 mg/L.

To reduce *L. pneumophila* colonization, from June 2005 the hospital administrators decided to increase the water temperature from 50 °C to 60 °C at the boiler outlet. Furthermore, they improved the over-

Table 1 | *Detection of Legionella pneumophila concentrations and percentage of positive samples in two Italian hospitals from 2004 to 2009*

	Min value	Max value	Positive/Total samples	Positive samples (%)							
	CFU/L	CFU/L		2004-2009	Oct 2004-May 2005	Jul 2005-Apr 2006	May 2006-Mar 2007	Jul 2007-Jun 2008	Sep 2008-Jun 2009	pre dioxide	post dioxide
Boiler out	< 100	1200	3/24	13	50	0	0	0	0	27	0
Boiler recirculation	< 100	2200	8/24	33	50	100	0	0	0	73	0
Hospital 1	< 100	10 000	56/135	41	48	87	4	10	8	68	8
Hospital 2	< 100	17 000	32/86	37	80	75	31	5	0	77	12
Cold water	< 100	< 100	0/2	0					0		

all maintenance of boilers and showers as described in the materials and method section. Moreover, they had dead end pipes localized and removed. All these measures are indicated as "continuous maintenance" in *Figure 1*.

Despite these improvements to the water system, the overall levels of *L. pneumophila* colonization increased instead of diminishing. Indeed, from 2005 to 2006, 87% of the samples of hospital 1 were positive, compared to the 48% of the previous year *(Table 1)*. Only the boiler outlet usually positive in 50% of cases, became negative when the temperature was raised to 60 °C. Lastly, while in year 2004-2005 water recirculation was found to be positive for *L. pneumophila* in 50% of the samples, in 2005-2006 it was positive in every measurement.

Because of these discouraging results, another disinfection method was proposed: continuous exposure to chlorine dioxide. Following water treatment with 0.70 mg/L chlorine dioxide (April 2006), which was then gradually decreased to 0.2 mg/L, a dramatic reduction of *L. pneumophila* positive samples was observed in both hospitals (2% for values greater than 10^3 CFU/L). Moreover, a reduction of the count to low-range 100 CFU/L was noticed in all sampling sites, except in three units located in distal areas (maximum value of 700 CFU/L, average 600 CFU/L) with values always below the limits indicated in the new local guidelines [11]. Positive samples in the boiler outlet and recirculation water system that were respectively 27% and 73% before dioxide treatment reached 0% following dioxide exposure. For hospital 1, the colonization decreased from 68% to 8% and from 77% to 12% for hospital 2. Above all, *L. pneumophila* colonization has remained to acceptable levels up to present times. In this regard, in 2006-2007 hospital 1 and hospital 2 showed respectively 4% and 31% of positive samples, in 2007-2008 10% and 5% and in 2008-2009 8% and 0%.

According to local guidelines (March 2009), *L. pneumophila* was searched in cold water in the last two samples and it was always found to be absent. The other values from the analysis on cold water from municipal water system and wells were always below the limits of Italian law [12].

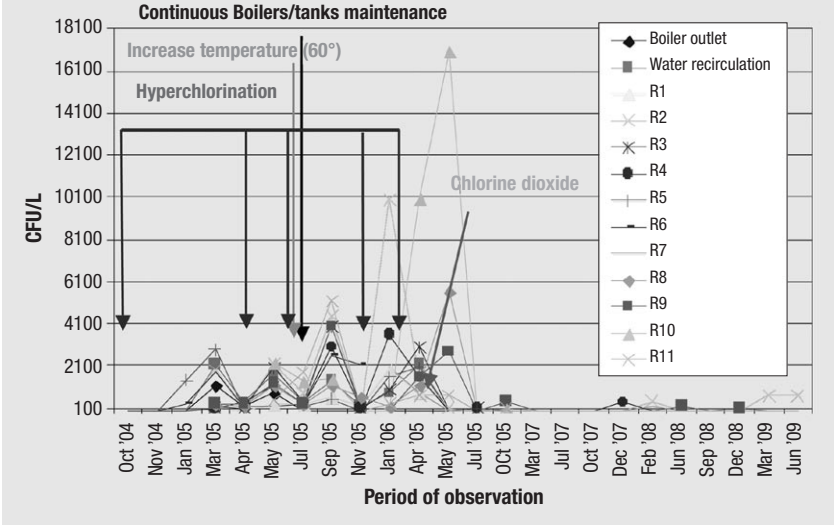

Fig. 1 | *Concentration of* Legionella pneumophila *and sanitations performed from 2004 to 2009 in nosocomial settings. 11 sites (indicated with R1-R11), water from boiler outlet and hot water recirculation were analyzed.*

DISCUSSION

LD is one of the emerging public health issues. *Legionella*-related illnesses are increasing in number but probably they are still underestimated because of lack of awareness from the clinicians and difficulties in the diagnosis. Therefore, an improved *L. pneumophila* surveillance is necessary, especially in the healthcare system.

In this study, 271 samplings of hot water were performed in two adjacent hospitals in Milan (Italy), housing more than 900 patients with different degree of physical and mental disabilities. At the beginning of the study, all wards, situated in different buildings, were colonized with different concentrations of bacteria. In 2002 two cases of LD, probably coming from other hospitals, were reported. For this reason, an environmental monitoring was performed in addition to clinical surveillance.

The surveillance plan consisted in: 1) monitoring of the water system in 11 end-points, from one boiler outlet, and recirculation water every three months; 2) standard operating procedures of maintenance of water system (tanks and boilers, taps and showers substitutions); 3) pilot study of clinical surveillance (with retrospective analysis of medical records); 4) sensitization of physicians on these issues.

To limit *L. pneumophila* colonization, hyperchlorination was performed five times from September 2004 to February 2006, in addition temperature was raised from 50 °C to 60 °C in the boiler outlet from June 2005. Immediately after disinfection, the count was lowered considerably and thus acceptable, but after one or two months it went back to higher levels.

Only upon persistent treatment with chlorine dioxide, starting April 2006, using concentrations initially of 0.70 mg/L, which were then gradually decreased to 0.2 mg/L (present time) to limit by-products and corrosive effect on pipe, a dramatic reduction of the plate count to values lower than 100 UFC/L was obtained in all sampling points, except in three units located in distal areas where a maximum value of 700 CFU/L and an average of 600 CFU/L were measured.

No clinical cases were reported from 2004 to 2009. Thus, reclamations performed in this period, especially chlorine dioxide treatment, succeeded in limiting the colonization of *L. pneumophila* to acceptable values.

In parallel to this study, a clinical retrospective survey (2002-09) aimed to analyze hospital and community acquired pneumonia, with particular attention to LD in institutionalized patients in health facilities, was carried out. The surveillance data were analyzed by Epinfo, crossed with results of environmental monitoring for the detection of areas most at risk and compared with data from national and international literature (unpublished data). Moreover, during environmental surveillance, several isolates were stored in a collection of *L. pneumophila* strains. Some of them were genotyped using sequence based typing and analyzed with the international EWGLI database to characterize the evolution in space and time of different genotypes circulating in the water system, taking into account the possible virulence of these strains and the sensitivity of these patients [13-17].

In conclusion, data show that at the beginning of the environmental surveillance *L. pneumophila* colonization was present in the water supply of two Italian hospitals. All the protocols of intervention, with the exception of continuous chlorine dioxide treatment, succeeded in limiting *L. pneumophila* colonization in the short but not in the medium-long term. Only the use of chlorine dioxide in continuum (0.2 mg/L) led, after four years, to a reduction of the plate counts to values below 100 UFC/L. Thus, data clearly demonstrate that prolonged water treatment with chlorine dioxide is an effective procedure to reduce *L. pneumophila* colonization of nosocomial water sources.

Intriguingly, in the distal units there was still a low degree of colonization although no cases of LD have been reported so far. This current colonization is probably due to the presence water circuit stagnation in dead legs. Thus, further studies are needed to closely monitor levels of *L. pneumophila* in hot water of distal units. Furthermore, undergoing studies will determine chlorine susceptibility in *L. pneumophila* strains collected in medium-high risk wards.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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