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Combination of two methods for *Legionella* disinfection of water systems (cooling towers and potable water) in a Spanish hospital

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Abstract

This field study assesses the effectiveness of the installation of the Pastormaster system in potable water systems and a photo-catalyst in the cooling system as strategy for *Legionella* control in a Spanish hospital. Monthly, water samples were collected from taps in the Haematological Unit and the hospital cooling tower from 30th March 2005 to 31st December 2010. In the pre-installation period, all *Legionella* isolates from taps belonged to serogroups 2-14, and the isolation rate was 100% reduced after the Pastormaster system installation. In the cooling system, *L. pneumophila* serogroup 1 accounted for 80.0% of all *Legionella* isolates in the pre-installation period. After the photo-catalyst installation, isolation of *L. pneumophila* serogroup 1 was 57.2% reduced, but there was a significant increase in the isolation of *Legionella* isolates belonging to other serogroups or species. This study shows that the strategy implemented was highly effective in reducing serogroup 2-14 *Legionella* isolation from taps, thus potentially preventing nosocomial legionellosis, but not in reducing overall *Legionella* isolation in cooling towers.

Keywords: Water purification; Water microbiology; *Legionella pneumophila*; Hospitals; Disinfection

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Introduction

Many environmental investigations have linked outbreaks of Legionnaires' disease with contaminated water in cooling systems, especially with those poorly maintained. Based on this the WHO recommends regular maintenance of cooling systems, including microbiological verifications as part of surveillance systems.[1]

Nosocomial clusters of *Legionella* infection have been associated with contaminated cooling towers and potable water supplies, but since 1985 virtually all cases of nosocomial legionellosis have been linked to potable water.[2] Nosocomial infection is underestimated because the most widely used test (the *Legionella* urinary antigen test) is specific for serogroup 1, thus not all nosocomial pneumonias are aetiologically assigned.[2-4] Because patients can be exposed by inhaling, aspirating or ingesting contaminated water,[2] the percentage of distal sites positive for *Legionella* has been correlated with the incidence of nosocomial Legionnaires' disease.[4] Previous studies have shown that the number of disseminating points (and subsequently the number of potentially exposed individuals) is more important than the infectious dose.[5] However, since not all exposed patients develop the disease, policies for control of nosocomial legionellosis should take into account the size of the hospital together with the number of susceptible patients exposed to disseminating points.[5] Nowadays, most European countries and U.S. state health departments mandate routine culturing of hospital potable water despite the lack of support by the CDC.[3] As result, there has been a proliferation of disinfection methods both for cooling systems and hospital potable water systems.

Background

Bioseguridad Ambiental SL (Madrid, Spain) is responsible for sampling and analysing water systems for *Legionella* at least once a year in different Spanish hospitals, in compliance with Spanish regulations (R.D. 865/2003) and to implement strategies for disinfection.

The aim of this field study was to assess through microbiological surveillance the practical effectiveness of the commercial disinfection devices installed in a Spanish hospital as strategy for *Legionella* control in the hospital water systems.

Material and methods

Data on *Legionella* isolation obtained from 30 March 2005 to 31 December 2010 through the routinely monthly surveillance performed by Bioseguridad Ambiental SL (Madrid, Spain) in the Hospital Ruber of Madrid, Spain, was analysed. As control measures at *Legionella*-link risk points, on March 2009 the Pastormaster system[6] was connected to the system's boiler for disinfection of the potable water system, and a photo-catalytic method[7] was installed in the returning line to the hospital cooling tower. The Pastormaster system consists of heating the water of hospital distribution systems at a specific point at sufficient temperature for a minimum time.[6] In the pasteurization unit, the controlled temperature is 70ºC, and the water is maintained inside the unit at least two minutes at maximum flow volume and very low speed in order to ensure proper pasteurization. The photo-catalytic method consists of a device containing a fibrous photocatalyst incorporating a high-strength titania (TiO_2) fibre with a nanoscale surface structure that decomposes organic materials that come into contact with its surface when exposed to light.[7]

Sampling

Monthly, at least one sample of one litre was collected from the water returning to the hospital cooling tower and 100mL from taps (immediately after opening the valve) located in the Haematological Unit, also inserting a sterile swab into the faucet that was further introduced into a sterile vessel that was made up to 1L using water.

Measuring temperature and chlorine levels

Immediately after collection, the water temperature was measured using a thermometer testo 106 (range: from -50ºC to +275ºC) (Testo AG, Germany), and chlorine levels were measured with ISM Hanna HI 93734 (Hanna Instruments, USA).

Microbiological processing of water samples

Water samples were concentrated 100-fold immediately on arrival at the laboratory. Three 1mL aliquots were used: one untreated, one heat-treated (50ºC for 30 min.), and one acid-treated (in 9mL of HCl-KCl acid buffer at pH 2.2 for 5 min.). Of each aliquot, 0.1 mL was plated onto GVPC (glycine, vancomycin, polymyxin B, cyclohexamide) selective agar medium

(Oxoid Ltd., Basingstoke, Hampshire, UK). Plates were incubated at 36ºC for 10 days and examined for growth every 48h. Colonies morphologically consistent with *Legionella* species were plated onto buffered charcoal yeast extract (BCYE) agar (Oxoid) and blood agar (Oxoid), and incubated for 48h. Colonies growing on BCYE agar but not on blood agar were definitively identified as *Legionella* using a commercially available latex agglutination test (Oxoid, DR0800) that distinguishes *Legionella pneumophila* serogroup 1, *L. pneumophila* serogroups 2-14, and other *Legionella* species (including *L. longbeacheae*, *L. bozemannii*, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. micdadei*, and *L. anisa*).

Statistical analysis

Data were described by mean and standard deviation for quantitative variables and by percentages for qualitative variables. Comparison of percentages was performed by the X^2 test or the Fisher's exact test when necessary. Comparisons of quantitative variables were performed using the Mann-Whitney test due to the non-normal data distribution. SPSS v.14 (SPSS Inc, Chicago IL) was used for statistical analysis.

Results

A total of 490 samples were collected: 102 from the cooling system and 388 from taps. Table I shows number of samples collected pre- and post- installation of devices and number and percentage of samples from taps and cooling towers yielding *Legionella* growth.

In potable water systems all isolates from taps samples in the pre-installation period were *L. pneumophila* serogroup 2-14, and a significant (p=0.006) decrease in the isolation rate was found after the installation of the Pastormaster (7.2% vs. 0.0%) linked to a significantly higher temperature (ºC) of water (48.1 ± 26.0 pre-installation vs. 58.5 ± 1.8 post-installation, p<0.001) with similar chlorine levels (ppm; 0.55 ± 0.37 pre- vs. 0.46 ± 0.14 post-installation, p=0.867).

In the cooling system, *L. pneumophila* serogroup 1 accounted for 80.0% (12 out of 15 isolates) of all *Legionella* isolates in the pre-installation period. After the installation of the photo-catalyst there was a non-significant decrease in *L. pneumophila* serogroup 1 (from 15.2% pre-installation to 8.7% post-installation, p=0.731), together with a significant increase in *L. pneumophila* serogroups 2-14 (from 0% to 13%, p=0.010) and in *Legionella* belonging to species other than *pneumophila* (3.8% vs. 17.4%, p=0.044). No significant differences between pre- and post-installation periods were found in temperature (ºC; 19.4 ± 5.3 vs. 21.5 ± 3.5, p=0.090) or in chlorine levels (ppm; 0.86 ± 0.85 vs. 0.34 ± 0.19, p=0.294).

Discussion

Ecology of *Legionella* differs between cooling systems and potable water. In a previous study by our group, *L. pneumophila* was the most frequent species isolated but while in cooling towers serogroup 1 was the most frequent (88.3%) in potable water two-third of isolates belonged to serogroups other than serogroup 1.[8] In the present study species and serogroups distribution was confirmed since 84.8% of all isolates belonged to the species *L. pneumophila* (100% isolates from potable water and 70.8% from the cooling tower), with 100% isolates from taps belonging to serogroups 2-14 and 82.4% isolates from the cooling tower to serogroup 1. From these data it can be deduced that strategies for *Legionella* disinfection of hospital's water systems should be directed to *L. pneumophila* serogroup 1 in cooling systems and to non-serogroup 1 in potable water systems. Data from surveillances match the epidemiological spectrum of Legionnaires' disease, with most community outbreaks linked to serogroup 1 (without documentation of outbreaks linked to other serogroups) contrasting with nosocomial legionellosis among immunocompromised patients linked to other serogroups and other *Legionella* species.[1]

It has been suggested that strategies for disinfection in a specific hospital should be implemented by infection control practitioners rather than healthcare facilities personnel.[3] The strategy implemented in Hospital Ruber of Madrid consisting of the Pastormaster method for potable water and the photo-catalyst for the cooling system showed 100% reduction in *Legionella* isolation from taps, thus preventing nosocomial legionellosis, and 57.2% reduction in *L. pneumophila* serogroup 1 in the cooling system. However this reduction in the cooling system was accompanied by a significant increase in the isolation of *Legionella* isolates belonging to other serogroups or species, not usually linked to outbreaks.

Table I. Number (percentage) of samples from taps and cooling towers yielding *Legionella* spp. pre- and post- installation of the Pastormaster system (potable water) and the catalytic device (cooling towers). Colony counts (cfu/ml) in positive samples expressed in median (interquartilic range)

	Taps			Cooling towers		
	Pre-	Post-	p	Pre-	Post-	p
Number of samples	304	84	-	79	23	-
L. pneumophila						
Serogroup 1	0 (0.0)	0 (0.0)	-	12 (15.2)	2 (8.7)	0.731
Serogroups 2-14	22 (7.2)	0 (0.0)	0.006	0 (0.0)	3 (13.0)	0.010
Colony counts (cfu/ml) ($x10^3$)	9.8 (1.9-16.0)	0 (0-0)		0.5 (0.1-6.3)	0.8 (0.4-4.0)	
Other *Legionella* species	0 (0.0)	0 (0.0)	-	3 (3.8)	4 (17.4)	0.044
Colony counts (cfu/ml) ($x10^3$)	0 (0-0)	0 (0-0)		21 (0.3-30)	4.0 (0.9-12.8)	
Total *Legionella* spp.	22 (7.2)	0 (0.0)	0.006	15 (19.0)	7 (30.4)*	0.240
Colony counts (cfu/ml) ($x10^3$)	9.8 (1.9-16.0)	0 (0-0)		0.4 (0.2-14.0)	2.5 (0.8-6.5)	

*Two samples yielding growth of *L. pneumophila* serogroup 1 and other *Legionella* species

Considering that a previous study showed that about half of the cooling towers examined were contaminated with *Legionella* spp.[9] and *L. pneumophila* isolation occurs even in well maintained towers,[10] routine environmental cultures should be performed at regular intervals to avoid failures despite installation of adequate disinfection systems. Given the proliferation of commercial methods offered for disinfection of water systems, publication of control results become important to have evidence-based medicine as criteria for selection of strategies for global disinfection of hospital's water systems.

In conclusion, the strategy implemented for *Legionella* disinfection was highly effective in reducing serogroup 2-14 *Legionella* isolation from taps, thus preventing nosocomial legionellosis, but not in reducing overall *Legionella* isolation in cooling towers since there was an increase in *L. pneumophila* serogroups 2-14 and non- *L. pneumophila* species.

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