DOI:10.24850/j-tyca-2018-03-02

Article

Water disinfection methods and their affect on legionellosis

Métodos de desinfección del agua y su implicación en la legionelosis

Enrique Gea-Izquierdo[1,2]

[1]Facultad de Medicina, Pontificia Universidad Católica del Ecuador, Quito, Ecuador, enriquegea@yahoo.es

[2]Cátedra de Seguridad y Salud en el Trabajo, Universidad de Málaga, Málaga, Spain

Corresponding author: Enrique Gea-Izquierdo, enriquegea@yahoo.es

Abstract

Legionellosis is a respiratory disease caused by the bacterium *Legionella* spp., which is primarily found in water distribution systems. The objective was to describe whether water disinfection methods used in cooling systems are related to hotel compliance with Spanish health legislation for the prevention of legionellosis. Principal components and correspondence analyses were done on samples from air-water cooling systems (cooling towers, evaporative and adiabatic condensers) at hotels (n = 26) located in the province of Malaga (Spain), in relation to water disinfection methods (chlorination, ultraviolet radiation, ozonation, bromination and copper-silver ionization). The period studied was between 2004 and 2009. A positive association was found between the different water disinfection methods and facilities as well as between those factors and certain hotel categories. Chlorination corresponded to two-, three- and five-star hotels and cooling towers and evaporative condensers. The four-star hotels showed a relation to ozonation and copper-silver ionization, identifying the latter with adiabatic condensers. Luxury hotels corresponded with ultraviolet radiation, and this was not associated with specific risk facilities. The degree of correspondence suggests a specific relationship between the prevention of legionellosis at high-risk hotel facilities and water disinfection systems.

Keywords: *Legionella pneumophila*, legionellosis, disinfection, air-water cooling systems, hotels.

Resumen

La legionelosis es una enfermedad respiratoria causada por la bacteria *Legionella* spp. y de origen preferente en sistemas de dispersión de agua. El objetivo fue determinar si los métodos de desinfección del agua empleados en los sistemas de enfriamiento pueden estar relacionados con el cumplimiento hotelero de la legislación sanitaria española para la prevención de la legionelosis. Se realizó un análisis de componentes principales y correspondencias aplicado sobre una muestra de equipos de intercambio aire-agua (torres de refrigeración, condensadores evaporativos y adiabáticos) correspondientes a hoteles (n = 26) ubicados en la provincia de Málaga, España, y métodos de desinfección vinculados (cloración, radiación ultravioleta, ozonización, bromación e ionización cobre-plata). El periodo de estudio comprendió los años 2004-2009. Se presentó una asociación positiva entre diferentes métodos e instalaciones, así como una correspondencia de los primeros con ciertas categorías de hoteles. La cloración se relacionó con los hoteles de dos, tres y cinco estrellas, en torres de refrigeración y condensadores evaporativos; los de cuatro estrellas presentaron una relación con la ozonización e ionización cobre-plata, identificándose esta última con los condensadores adiabáticos; y los de gran lujo con la radiación ultravioleta, sin asociación con instalaciones específicas. El grado de correspondencia sugiere que existe una relación específica entre la prevención de la legionelosis en instalaciones de alto riesgo del sector hotelero y los sistemas de desinfección del agua.

Palabras clave: *Legionella pneumophila*, legionelosis, desinfección, equipos de intercambio aire-agua, hoteles.

Received: 14/10/2015

Accepted: 11/12/2017

Introduction

Legionellosis is a disease caused by the *Legionella* spp. bacterium, which is found worldwide, throughout North America, South America, Asia, Africa, Australia, and Europe. The incidence of this disease in Spain has shown a growing trend from 1997 to 2002, with a yearly growth rate of 52.5% during this period. From 2002 until 2009, the incidence rate of legionellosis maintained a conservative trend (Gea-Izquierdo, Mezones-Holguin, & Haro-García, 2012).

Legionella is able to grow in both natural and artificial settings, and can survive in a wide range of environmental conditions (Hilbi, Hoffmann, & Harrison, 2011).

In some cases, legionellae is able to colonize water resources in low concentrations, and subsequently contaminate the supply network.

In optimal physicochemical water conditions, the microorganism's ability to grow increases and it becomes able to spread through air-water cooling facilities. The optimal pH range for its survival is between 6 and 8, and its metabolic activity can be sustained for prolonged periods of time, even in settings with high salt concentrations. The fact that temperatures between 32 and 42°C persist within these systems contributes to a favorable setting for the bacterium during its development and survival stages (Rhoads, Ji, Pruden, & Edwards, 2015).

In order to prevent *Legionella* infections, certain quality criteria are needed in terms of physicochemical and microbiological water parameters, in addition to temperature controls.

Legionella pneumophila often associates itself with other microorganisms from which it sometimes obtains organic nutrients or protection (Richards, Von Dwingelo, Price, & Abu-Kwaik, 2013; Dietersdorfer, Cervero-Aragó, Sommer, Kirschner, & Walochnik, 2016), and therefore microbiological control is a key element in legionellosis prevention (Iervolino, Mancini, & Cristino, 2017; Ohno, Kato, Yamada, & Yamaguchi, 2003).

Thus, interactions between aquatic bacteria and (potential) pathogens in habitats as diverse as free water and biofilms are essential for the survival or growth of organisms relevant to sanitation (Szewzyk, Szewzyk, Manz, & Schleifer, 2000). The intracellular invasion of *Legionella pneumophila* and its replication in environmental protozoa plays a key role in the transmission of legionellosis. These protozoa provide a habitat for environmental survival and the reproduction of *Legionella* species.

While several physical and chemical disinfection methods have been proposed in order to eliminate the bacterium from infection sources, to completely control *Legionella* in environmental waters, where it is protected from disinfection through intracellular protozoan growth and "biofilms," suggests the need for new approaches so as to control sporadic outbreaks of this disease (Gea-Izquierdo, 2016). Therefore, controlling environmental factors influences the development of the bacteria. The simple and unipolar flagellum expression is modulated by temperature, viscosity, and the osmolarity of the environment, as well as by amino acids (Heuner, Brand, & Hacker, 1999). Thus, the persistence of *Legionella pneumophila* in biofilms that have been treated with disinfectants suggests that regular testing practices underestimate the risk of legionellosis infection (Williams & Braun-Howland, 2003).

The use of disinfection methods (either physical, chemical, or physicochemical) constitutes a barrier in water sanitation quality control (Real Decreto 865/2003, 2003). These methods must be proven effective against *Legionella*, and should not pose any risk to the facilities or the health and safety of operators or other persons that may be exposed to it. Physicochemical systems are used to destroy the bacterial load of the water through the application of electrochemical procedures, and must be registered in accordance with the regulatory norms for biocides. Since the application of a disinfection method to the detriment of another is usually based on water quality criteria (turbidity, microbial flora, etc.), specific disinfection systems in an at-risk facility can be identified. In other cases, a combined action improves control over the bacteria and prevents its possible spread.

Air-water cooling systems (cooling towers, evaporative and adiabatic condensers) constitute one of the facilities with the highest probability of developing and spreading *Legionella pneumophila*, and are seen as high-risk facilities (Fields, Benson, & Besser, 2002). For the sanitary upkeep of these facilities, disinfection systems for treating water are considered to be indispensable to the control of risks. The objective of this study was to determine whether the water disinfection methods used by the facilities studied are related to hotels complying with Spanish health legislation for the prevention of legionellosis.

Methods

Air-water exchange equipment (cooling towers, evaporative and adiabatic condensers) were analyzed in hotels (n=26) located in both inland and coastal communities of Malaga (Andalusia, Spain), comparing the results obtained during the 2004-2009 period with Spanish regulations that establish sanitary criteria standards for the prevention and control of legionellosis (Real Decreto 865/2003, 2003).

The study design involved gathering information through a self-administered survey (Gea-Izquierdo, 2008) given to directors and persons in charge of buildings with at-risk facilities, as well as drawing water samples from the ponds of air-water exchange equipment for a later analysis by laboratories authorized by the Spanish Sanitation Authority, in accordance with standard regulations (UNE-ISO 11731:2007, 2007).

For the sanitary control of health risks related to bacteria development, 41 variables were selected (Gea-Izquierdo, 2008): hotel category, the existence of cooling towers, evaporative condensers, adiabatic condensers, the seasonality of the aforementioned equipment, daytime operation, origin of water, equipment notification to sanitation authorities, equipment improvements, water control, certified laboratory for sample analysis, at-risk facilityexposed person distance (< 20 meters), at-risk facility-air conditioning outlet distance (< 20 meters), circuit draining of the cooling system, droplet separator (water slug flow > 0.05% circulating water flow), the existence of biocide, chlorination, ultraviolet radiation, ozonation, bromination, copper-silver disinfection, physical-chemical and microbiological water controls, frequency of control types (physicochemical > monthly and microbiological: *Legionella pneumophila* > quarterly and total aerobes > monthly), program and records of preventive sanitary maintenance, qualified personnel training in compliance to regulations, sanitation company, company registry, water temperature control, water filtering, equipment access, pipeline structure, chemical products applied, and safety data sheet of those products.

An analytical study and a principal components analysis were carried out to explain the observations (hotel types), *n*, and the original variables (cooling towers, evaporative and adiabatic condensers), *p,* according to a biplot graphic display. To this end, their scores were represented on the coordinate axes of the first two principal components that characterize the *p* variables. To obtain factorial solutions, the principal component analysis considered the total variance, estimating the factors with low proportions of unique variance, and in specific cases, the error variance. The study's interest focused on the prediction or the minimum number of factors required to explain the largest proportion of the variance represented in the original series of variables. As an objective means of interpretation, a vector model was used to capture the disinfection systems' position scores on the perceptual map. The bacterium removal capacity was considered to be a function of the disinfection method used and of the physicochemical and microbiological water controls, in addition to the frequency of those controls, and in particular, of *Legionella* spp. and total aerobes (Gea-Izquierdo, 2012).

The observations were organized by hotel category (two [2H], three [3H], four [4H], five stars [5H] and grand luxury [GL]), and each different disinfection system was analyzed: chlorination (Cl), ultraviolet radiation (RU), ozonation (Oz), bromination (Br), and copper-silver ionization (CP).

In addition, the relation (correspondence) between "water disinfection systems" and "hotel types" was analyzed according to the observations corresponding to the number of hotels that included facilities that were at-risk for bacteria. To this end, a χ^2 independence test was carried out, where the null hypothesis was the independence between both variables. For the correspondence analysis, χ^2 was used to standardize the frequency values and generate the association baselines. A measurement of metric distance and orthogonal dimensions was created, based on which categories were specified to determine the strength of the association represented by the χ^2 distances. The analysis attempted to simultaneously satisfy the relations by producing dimensions representing the χ^2 distances.

Once the metric distances were obtained, solutions for reduced dimensions were defined, and self-values were calculated for each dimension, which indicated the relative contribution of each one to explaining the variations in the categories. The interdependence was then presented with the dimensional reduction and perceptual map. The map was based on the association between hotel category and disinfection systems. This expresses the correspondence of the variable category with a unique capacity to represent rows and columns in a single multidimensional space, and provides a multivariant interdependence expression for non-metric data, which would not be possible to obtain otherwise.

The computer programs used for gathering and analyzing the data were: SPSS (Copyright SPSS Inc., 1 989-2 006. Windows. Version 15.0.1. Nov 22, 2006) and R Development Core Team (2010), R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria.

Results

The description of the distribution of the variables according to hotel categories was based on related studies by other authors (Gea-Izquierdo, 2008). Figure 1 presents the results of applying classification data to the perceptual compositional map, which shows two distinct types of attribute dimensions pointing in the same direction: 1) adiabatic condensers and 2) cooling towers and evaporative condensers. The first type is almost perpendicular when compared to the other two dimensions, suggesting a separate and distinct dimension of its own. This representation is expressed on the biplot graphic display, which shows the initials corresponding to the different disinfection methods and the original variables. With regard to cooling towers and evaporative and adiabatic condensers, the variance for both variables was very similar, since they are associated with vectors of similar lengths. A correlation was also found, since the angle that separates the corresponding vectors was small, whereas it was larger between cooling towers and adiabatic condensers. The direction of the axis corresponding to the first principal component arranges the data, and based on the scores obtained by the first two principal components in the observations, chlorination (Cl) was found to be the best disinfection system in terms of prevention compliance (with a coordinate near 1 with respect to the first principal component). Meanwhile, the worst disinfection systems in regard to sanitation criteria (Real Decreto 865/2003, 2003) were ultraviolet radiation and ozonation (RU and Oz, respectively), always with respect to what the first principal component represents, which is the most important one and which holds the largest data matrix variability. The approximation order is the same for cooling towers and evaporative condensers (Cl > Br > CP > Oz/ RU), while the adiabatic condensers varied (CP > Br > Cl > Oz/ RU). The χ^2 independence test shows a Pearson λ value = 32.129 and a *p* value = 0.001.

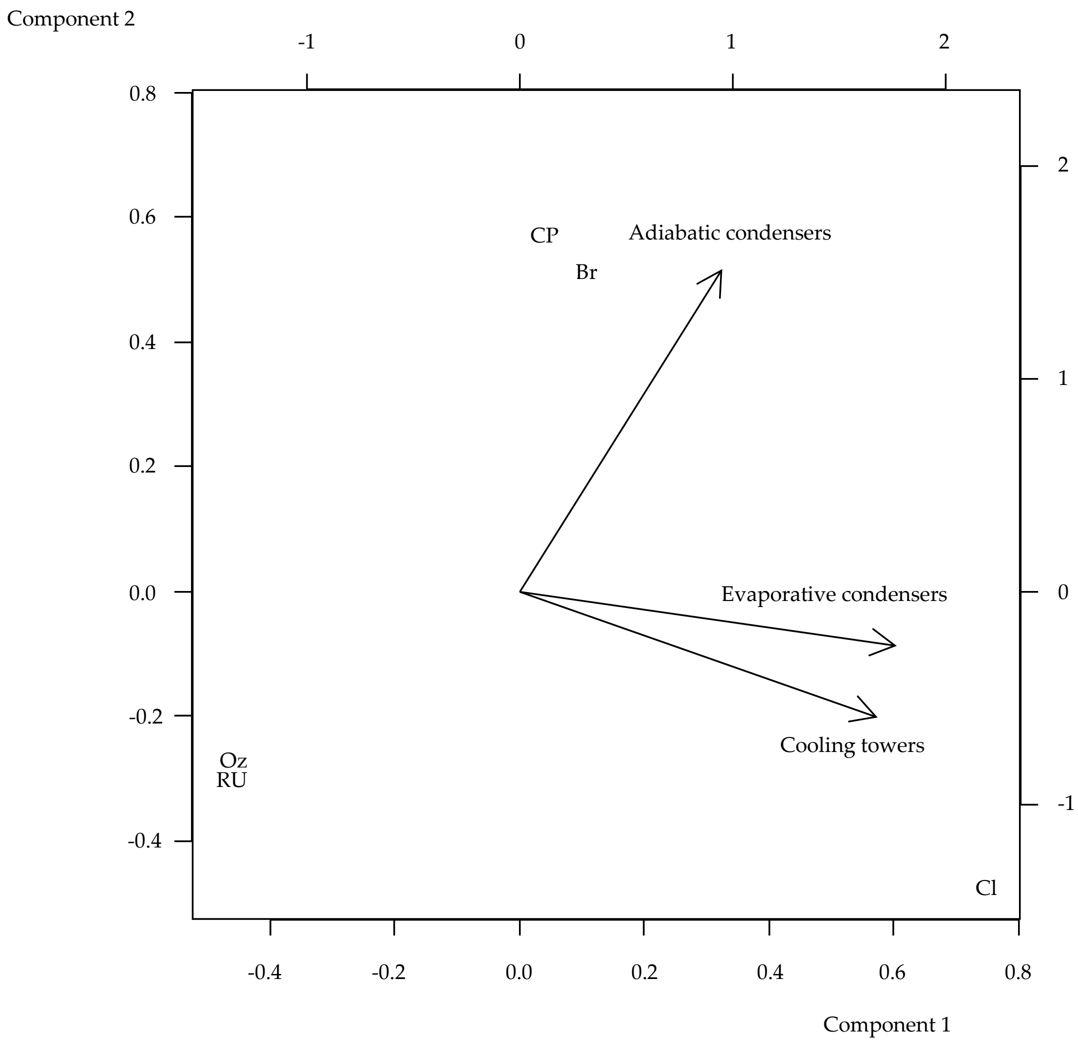

Figure 1. Analysis of disinfection systems and at-risk facilities.

By analyzing the two coordinates, the first canonical correlations were obtained (1 and 0.696), as well as the correspondence graph (Figure 2). In order to analyze whether the use of the first two coordinates was adequate, the total inertia value (1.504) was obtained. With an absolute value of 1.485, the two-dimensional representation holds at 98.7%, which is enough to conclude that the two-dimensional representation is adequate. This means that the total inertia value was held at a 98.7% rate by the two-dimensional representation, which leads to the conclusion that this representation is valid.

It is worth noting that the perceptual map obtained during the correspondence analysis was weighted by the existing inherent interdependences and the potential biases from the possibility of omitting an attribute in a given disinfection system, or from a single inappropriate attribute (or system). In Figure 2, disinfection systems are shown near the hotel category with which they are highly related, and are further from categories with lower correspondences.

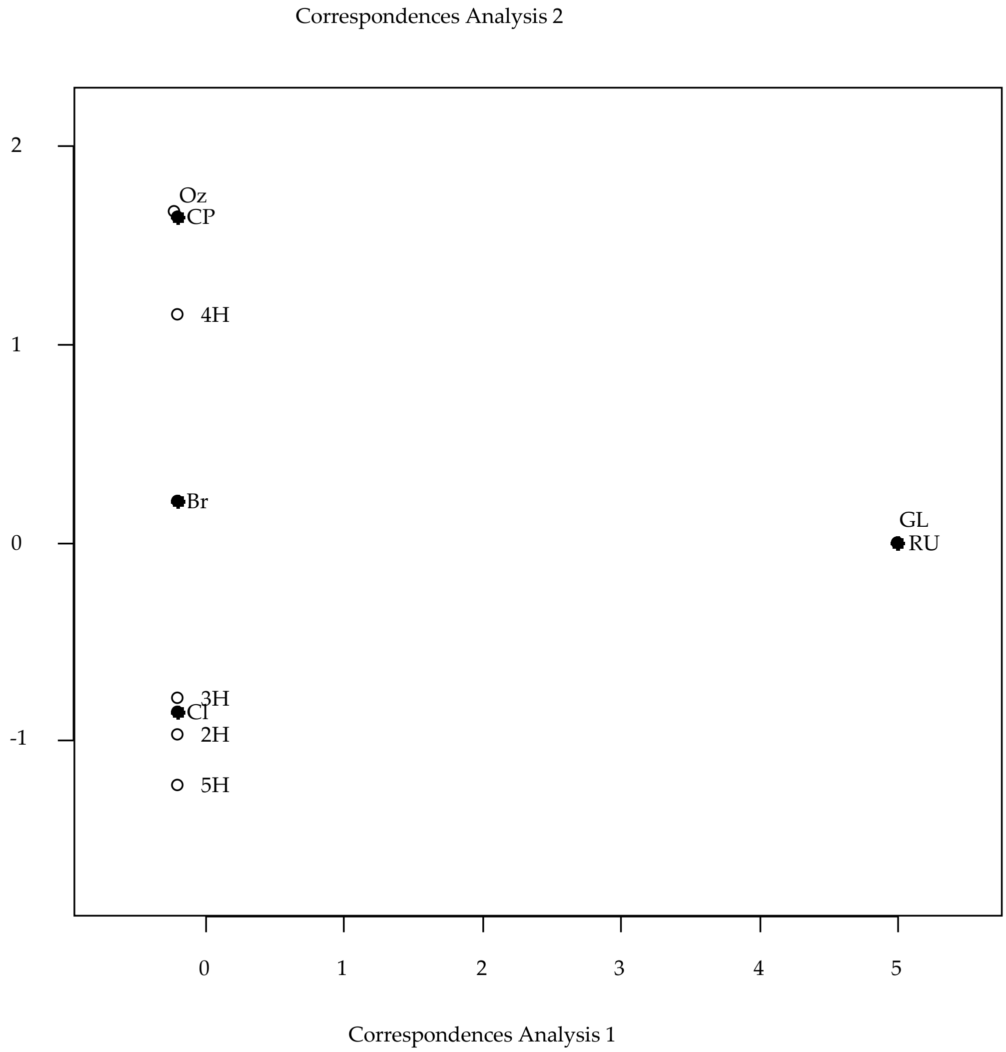

Figure 2. Analysis of disinfection systems and hotel categories.

Discussion

This study shows a positive association between certain water disinfection systems used for *Legionella* spp. and hotel categories, and between these systems and certain air-water exchange systems. Therefore, it is feasible to determine the risk of bacterial presence and which facilities are likely to develop the agent.

Therefore, the use of bacterial control methods for the sanitary management of water quality to prevent legionellosis is required. The best known disinfection systems include chemical methods that use disinfectants such as metallic ions (silver and copper), oxidizing agents (halogen compounds, as chlorine, bromine, iodine, chlorine dioxide, chloramines and halogenated hydantoins; ozone, and hydrogen peroxide), and non-oxidizing agents (heterocyclic ketones, guanidine, thiocarbamates, aldehydes, amines, thiocyanates, halogenated amides, and halogenated glycols). Generally speaking, oxidizing disinfectants are more effective than their non-oxidizing counterparts.

Chlorine is an especially well-known and widely-used oxidizing agent, and in terms of non-oxidizing agents, 2,2-dibromo-3-nitrilopropionamide seems to be the most effective, followed by glutaraldehyde, isothiazolinone, polyhexamethylene biguanide, and 2-bromo-2-nitrilopropionamide (Kim, Anderson, Mueller, Gaines, & Kendall, 2002).

The assessment of the effects of the different methods for controlling *Legionella* has been widely proven.

While in a silver solution of 50 µg/l certain *Legionella* strains are destroyed within a 6-hour period, others are only destroyed by ultraviolet radiation at 90 mW x s/cm^2, and when exposed to an aqueous chlorine solution of 2 mg/l they are destroyed within a 3 minutes (Miyamoto, Yamaguchi, & Sasatsu, 2000).

Thus, a significant difference has been detected in the physiological behavior of biofilm populations depending on the disinfection technique used.

Compared to with chlorine dioxide disinfection (0.12-0.16 mg/l), the microorganisms' respiratory activities increase in all materials when using ultraviolet radiation disinfection (254 nm, 400 J/m^2).

Because of this, chlorine dioxide may be a promising solution to the *Legionella* water contamination problem (Srinivasan *et al*., 2003), although drinking water supplies containing free chlorine as a residual disinfectant show a higher propensity for legionellosis outbreaks than a supplies containing monochloramines as a residual disinfectant.

It is estimated that 90% of drinking water-related outbreaks would not occur if monochloramines were used for disinfection purposes instead of free chlorine, with chloramination of drinking water possibly being a cost-effective method to control legionellosis at municipal levels

(Heffelfinger *et al*., 2003). Consequently, its generalized use may prevent thousands of legionellosis cases (Kandiah, Yassin, & Stout, 2013).

In some systems, zinc levels < 20 mg/l and copper > 50 mg/l seem to protect against *Legionella* colonization, although the distributions of certain species and serogroups in at-risk facilities varies, as does water temperature and free chlorine. This suggests that the sensitivity and resistance of *Legionella* strains to environmental factors may differ, and they may have different ecological niches (Borella *et al*., 2004).

With copper ions, some authors reported a 1-log decrease (in the number of *Legionella* over 24 hours) when the pH rises to 9, compared to a 6-log decrease at pH 7. Meanwhile, with silver ions a 6-log decrease over 24 hours can be obtained for all water quality parameters, while the precipitation of insoluble copper compounds is observed at a pH near 6. Therefore, the pH in water systems may be an important factor for the effectiveness of using copper-silver ionization to control *Legionella* (Lin, Vidic, Stout, & Yu, 2002; Dziewulski, Ingles, Codru, Strepelis, & Schoonmaker-Bopp, 2015).

Other studies suggest that copper-silver ionization is not entirely effective against the development of the bacteria (Triantafyllidou, Lytle, Muhlen, & Swertfeger, 2016), since a tolerance to silver ions exists. Or a silver ion centration of 3 μg/l is enough to control the growth of legionellae in circulating hot water, but not at certain critical points in facilities (Kusnetsov, Iivanainen, Elomaa, Zacheus, & Martikainen, 2001). Copper-silver ionization is another disinfection method with several positive assessment criteria (Stout & Yu, 2003).

The advantages of ionization include a relatively low cost, simple installation and maintenance, "nontoxic products," and the presence of residual disinfectant. Positively-charged copper ions form electrostatic bonds with negatively-charged areas in the cell wall. The cellular membrane is distorted, allowing the influx of silver ions that attack the cell through by attaching to specific sites in DNA, RNA, respiratory enzymes, and cellular proteins, thereby causing the death of the bacterium.

In this manner, silver and copper ion concentrations of 40 and 400 μg/l, respectively, are efficient effective against planktonic legionellae in cold and hot water systems with soft water.

In hard water, ionization is ineffective due to the inability to control the silver ion concentrations. A high concentration of suspended solids may create dysfunction in the electrodes and a silver ion compound.

The use of free chlorine combined with copper and silver ions has a synergic effect on ionization, with a higher inactivation of amoebas (a

Legionella pneumophila reservoir) (Hambidge, 2001), which suggests that combining a copper silver ionization system with a continuous chlorination system may decrease the number of "points" colonized by *Legionella pneumophila* by 41.6% over a 5-month period (Biurrun, Caballero, Pelaz, Leon, & Gago, 1999).

Legionella pneumophila has been documented to be sensitive to ultraviolet radiation doses of 2 500 to 7 000 $\mu W/cm^2$. Continuous ultraviolet radiation causes a 5-log reduction in *Legionella pneumophila* concentrations in circulating water systems.

In laboratory buffer solutions, exposure to $1\mu W/cm^2$ of ultraviolet light destroys 50% of *Legionella pneumophila* within 17 minutes. The exposure time required for a 99% *Legionella pneumophila* destruction rate is 48 minutes.

Continuous ultraviolet disinfection has the advantage of not altering taste, odor, or producing direct harmful residuals in the water, and requires minimum maintenance. However, it does not disinfect biofilm systems due to its low penetration into the microbial mat. In cooling systems with ultraviolet radiation, most of the bacterial growth occurs in the biofilm and the sediment, which are not exposed to the radiation. While its use with other systems may result in a lack of bacterial identification, and the prevention of diseases over many years (Hall, Giannetta, Getchell-White, Durbin, & Farr, 2003).

When it comes to control techniques, germicide ultraviolet radiation and titanium dioxide photocatalysis have been considered promising methods for the inactivation of *Legionella pneumophila* (Li, Tseng, Lai, & Chang, 2003). Ultraviolet radiation is useful to protect water systems in small areas, but since it lacks residual activity it should be combined with other disinfection methods. Water system need to be maintained in order to reduce the formation of biofilms and *Legionella* recolonization (Franzin, Cabodi, & Fantino, 2002), and to increase the effectiveness of ultraviolet radiation.

Legionella pneumophila is a bacterium that is considered to be highly resistant to other methods, such as ozonation treatment (Hambidge, 2001), since 1-2 mg/l of continuous ozone in water reduces the bacteria (5 log) after 6 hours of contact. On the other hand, this treatment shows a relevant capacity to inactivate chlorine-resistant pathogenic microorganisms without generating toxic compounds.

Thus, in order to control bacterial development, a χ^2 study was undertaken to determine whether disinfection systems were related to the hotel category. The result, according to the Pearson's λ statistic, and because the test's p value was very small, was the rejection of the null hypothesis of independence between disinfection system and hotel type. Likewise, Figure 2 shows that a particular disinfection system is more likely to be related to hotel type, or that these approaches may affect bacterial growth and development. The principal conclusions were thereby obtained based on proximity and not on the representation of the variables.

It is worth noting that ultraviolet radiation disinfection systems present a large correspondence with grand luxury hotels. Chloration systems are identified in two-, three- and five-star hotels, as well as in cooling towers and evaporative condensers, while four-star hotels present a correlation with ozonation and copper-silver ionization. Bearing in mind that four-star hotels are at the highest risk of not complying with the Spanish sanitation regulations for the prevention of legionellosis (Gea-Izquierdo, 2008), and that these hotel types present an overwhelming correspondence with ozonation and copper-silver ionization disinfection systems, it would be necessary to improve the effectiveness of those systems when used in air-water cooling facilities belonging to this category, with special attention to the effectiveness of disinfection against *Legionella pneumophila*.

Bromation, which is most commonly used by three- and four-star hotels, may strengthen other disinfection methods in certain cases, and it was identified in 3 at-risk facilities. The lowest risk, that is, completely complying with sanitation regulations, was identified in two-, three-, and five-star hotels as well as grand luxury hotels. The former three used chlorination as a preventive maintenance treatment, which in principle guarantees a residual value as long as there are other water quality control methods in place (Gea-Izquierdo *et al*., 2012), such as halogenated compounds (chlorine and bromine), which could optimize compliance with sanitation criteria (Real Decreto 865/2003, 2003).

It is suggested that the chlorination of water systems may help limit the development of *Legionella pneumophila*, keeping the bacterial concentration at a low threshold range. Copper-silver ionization was identified mainly in adiabatic condensers. And no clear association was found between ozonation or ultraviolet radiation and the three aforementioned air-water exchange systems.

In certain cases, it is difficult to identify the presence of *Legionella pneumophila* in facilities that have adequate water disinfection methods and a preventive sanitary maintenance protocol. However, the existence of different biofilm types, along with the presence of certain organism phenotypes that are adapted to these biofilms, indicate that the application of certain biocides to water systems may be less effective with biofilms than with planktonic microorganisms. Since microorganisms attach themselves to surfaces and phenotype development in biofilms occurs quickly, it is almost impossible to completely eradicate biofilm formation, although it can be prevented beforehand with periodic disinfections. The destruction and eradication of established biofilms require intensive treatments, mostly using oxidizing biocides. Given the nature of the biofilm in question, different biocides may be useful, and the best one for a specific biofilm must be determined based on practical conditions, as long as the water features remain constant.

The correspondence analysis provides a method to directly compare the similarities and differences among disinfection systems and associated features. It is not possible to always assume that the relations found are independent or linear, or that they are maintained over time. The technique’s solution depends on the set of characteristics present, assuming that all of them are appropriate for all systems, and that the same dimension is applied to each. Therefore, it is important to note that the resulting perceptual map was created solely based on the context of the systems and hotel types included in this analysis. This provides a useful tool to gain an optimal perspective of the direction of the relative position of disinfection systems and the hotel types associated with these positions. Because of the high degree of correspondence, it is concluded that the results reflect the problem according to the applied design, but this type of concordance does not guarantee their generalization to other population samples.

Conclusion

Legionellosis was prevented and controlled by adopting sanitary measures within the industrial facilities where *Legionella* spp. were able to proliferate and spread. These measures were applied to facilities that used water in their operations, to produce aerosols, and were located either inside or outside hotels that could be at risk to becoming focal points for disease outbreaks during their operations, service testing, or maintenance.

The results showed that the hotels did not comply with the Spanish sanitary legislation related to legionellosis prevention. Therefore, the lack of specification or absence of water disinfection methods may seriously affect exposed populations, especially depending on the hotel category.

These results should be considered as a first step towards determining perceptual information and for improving bacterial control, especially given the inexistence of previous studies in southern Spain. Thus, it will be possible to determine the potential risk for each type of facility as a function of its location and the water disinfection method used. This will make it possible to adopt specific preventive measures against the development and spread of the bacterium, as well as improve the epidemiological surveillance of legionellosis and the actions that need to be taken in terms of public health.

References

Biurrun, A., Caballero, L., Pelaz, C., Leon, E., & Gago, A. (1999). Treatment of a *Legionella pneumophila*-colonized water distribution system using copper-silver ionization and continuous chlorination. *Infection Control* & *Hospital Epidemiology*, 20(6), 426-428.

Borella, P., Montagna, M. T., Romano-Spica, V., Stampi, S., Stancanelli, G., Triassi, M., Neglia, R., Marchesi, I., Fantuzzi, G., Tatò, D., Napoli, C., Quaranta, G., Laurenti, P., Leoni, E., De Luca, G., Ossi, C., Moro, M., & Ribera-D'Alcalà, G. (2004). *Legionella* infection risk from domestic hot water. *Emerging Infectious Diseases*, 10(3), 457-464.

Dietersdorfer, E., Cervero-Aragó, S., Sommer, R., Kirschner, A. K., & Walochnik, J. (2016). Optimized methods for *Legionella pneumophila* release from its *Acanthamoeba* hosts. *BMC Microbiology*, 16, 74.

Dziewulski, D. M., Ingles, E., Codru, N., Strepelis, J., & Schoonmaker-Bopp, D. (2015). Use of copper-silver ionization for the control of legionellae in alkaline environments at health care facilities. *American Journal of Infection Control*, 43(9), 971-976.

Fields, B. S., Benson, R. F., & Besser, R. E. (2002). *Legionella* and Legionnaires' disease: 25 years of investigation. *Clinical Microbiology Reviews*, 15(3), 506-526.

Franzin, L., Cabodi, D., & Fantino, C. (2002). Evaluation of the efficacy of ultraviolet irradiation for disinfection of hospital water contaminated by *Legionella*. *Journal of Hospital Infection*, 51(4), 269.

Gea-Izquierdo, E. (2008). *Influencia del mantenimiento higiénico-preventivo de las instalaciones con riesgo de desarrollo de* Legionella pneumophila *en la provincia de Málaga*. Málaga, España: Universidad de Málaga, Servicio de Publicaciones e Intercambio Científico.

Gea-Izquierdo, E. (2012). Enfoque al riesgo de *Legionella* sp. en centros sanitarios del sur de España. *Boletín de Malariología y Salud Ambiental*, 52(2), 269-274.

Gea-Izquierdo, E. (2016). *Legionellosis*: Epidemiology, clinical features and control strategies. In: Berhardt, L. V. (ed.), *Advances in Medicine and Biology* (pp. 103-113). New York, USA: Nova Science Publishers, Inc.

Gea-Izquierdo, E., Mezones-Holguin, E., & Haro-García, L. (2012). Acciones de prevención y control de la legionelosis: un reto para la salud pública española**.** *Revista Peruana de Medicina Experimental y Salud Pública*, 29(2), 272-276.

Hall, K. K., Giannetta, E. T., Getchell-White, S. I., Durbin, L. J., & Farr, B. M. (2003). Ultraviolet light disinfection of hospital water for preventing nosocomial *Legionella* infection: A 13-year follow-up. *Infection Control* & *Hospital Epidemiology*, 24(8), 580-583.

Hambidge, A. (2001). Reviewing efficacy of alternative water treatment techniques. *Health Estate*, 55(6), 23-25.

Heffelfinger, J. D., Kool, J. L., Fridkin, S., Fraser, V. J., Hageman, J., Carpenter, J., & Whitney, C. G. (2003). Risk of hospital-acquired Legionnaires' disease in cities using monochloramine versus other water disinfectants. *Infection Control* & *Hospital Epidemiology*, 24(8), 569-574.

Heuner, K., Brand, B. C., & Hacker, J. (1999). The expression of the flagellum of *Legionella pneumophila* is modulated by different environmental factors. *FEMS Microbiology Letters*, 175(1), 69-77.

Hilbi, H., Hoffmann, C., & Harrison, C.F. (2011). *Legionella* spp. outdoors: Colonization, communication and persistence. *Environmental Microbiology Reports*, 3(3), 286-296.

Iervolino, M., Mancini, B., & Cristino, S. (2017). Industrial cooling tower disinfection treatment to prevent *Legionella* spp. *International Journal of Environmental Research and Public Health*, 14(10), 1125. DOI: 10.3390/ijerph14101125

Kandiah, S., Yassin, M. H., & Stout, J. (2013). Monochloramine use for prevention of *Legionella* in hospital water systems. *Infectious Disorders Drug Targets*, 13(3), 184-190.

Kim, B. R., Anderson, J. E., Mueller, S. A., Gaines, W. A., & Kendall, A. M. (2002). Literature review efficacy of various disinfectants against *Legionella* in water systems. *Water Research*, 36(18), 4433-4444.

Kusnetsov, J., Iivanainen, E., Elomaa, N., Zacheus, O., & Martikainen, P.J. (2001). Copper and silver ions more effective against legionellae than against mycobacteria in a hospital warm water system. *Water Research*, 35(17), 4217-4225.

Li, C. S., Tseng, C. C., Lai, H. H., & Chang, C. W. (2003). Ultraviolet germicidal irradiation and titanium dioxide photocatalyst for controlling *Legionella pneumophila*. *Aerosol Science and Technology*, 37(12), 961-966.

Lin, Y. S., Vidic, R. D., Stout, J. E., & Yu, V. L. (2002). Negative effect of high pH on biocidal efficacy of copper and silver ions in controlling *Legionella pneumophila*. *Applied and Environmental Microbiology*, 68(6), 2711-2715.

Miyamoto, M., Yamaguchi, Y., & Sasatsu, M. (2000). Disinfectant effects of hot water, ultraviolet light, silver ions and chlorine on strains of *Legionella* and nontuberculous mycobacteria. *Microbios*, 101(398), 7-13.

Ohno, A., Kato, N., Yamada, K., & Yamaguchi, K. (2003). Factors influencing survival of *Legionella pneumophila* serotype 1 in hot spring water and tap water. *Applied and Environmental Microbiology*, 69(5), 2540-2547.

Real Decreto 865/2003, de 4 de julio, por el que se establecen los criterios higiénico-sanitarios para la prevención y control de la legionelosis, número 171, BOE (18 de julio de 2003).

Rhoads, W. J., Ji, P., Pruden, A., & Edwards, M. A. (2015). Water heater temperature set point and water use patterns influence *Legionella pneumophila* and associated microorganisms at the tap. *Microbiome*, 3, 67.

Richards, A. M., Von Dwingelo, J. E., Price, C. T., & Abu-Kwaik, Y. (2013). Cellular microbiology and molecular ecology of *Legionella*-amoeba interaction. *Virulence*, 4(4), 307-314.

Srinivasan, A., Bova, G., Ross, T., Mackie, K., Paquette, N., Merz, W., & Perl, T. M. (2003). A 17-month evaluation of a chlorine dioxide water treatment system to control *Legionella* species in a hospital water supply. *Infection Control* & *Hospital Epidemiology*, 24(8), 575-579.

Stout, J. E., & Yu, V. L. (2003). Experiences of the first 16 hospitals using copper-silver ionization for *Legionella* control: Implications for the evaluation of other disinfection modalities. *Infection Control* & *Hospital Epidemiology*, 24(8), 563-568.

Szewzyk, U., Szewzyk, R., Manz, W., & Schleifer, K. H. (2000). Microbiological safety of drinking water. *Annual Review of Microbiology*, 54, 81-127.

Triantafyllidou, S., Lytle, D., Muhlen, C., & Swertfeger, J. (2016). Copper-silver ionization at a US hospital: Interaction of treated drinking water with plumbing materials, aesthetics and other considerations. *Water Research*, 102, 1-10.

UNE-ISO 11731:2007. Calidad del agua. Detección y recuento de *Legionella*, ISO 11731:1998 (2007).

Williams, M. M., & Braun-Howland, E. B. (2003). Growth of *Escherichia coli* in model distribution system biofilms exposed to hypochlorous acid or monochloramine. *Applied and Environmental Microbiology*, 69(9), 5463-5471.