

Frequency of use of terminal points as an important risk factor for Legionella colonization in hospitals

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Research article

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Abstract

Background: Hospitals have a different structure than domestic buildings. *Legionella* is a bacterium that can colonize the hospital water causing many outbreaks. It's a Public Health problem and its prevention and control are essential to avoid nosocomial infections. The aims of this study are to evaluate several risk factors and parameters that can contribute to *Legionella* colonization in a hospital, thereby improving *Legionella* risk assessment process. **Methods**

A total of 136 water samples (hot water and cold water) was investigated from different points of a hospital water network. These samples were tested for *Legionella* sp by three laboratories using different diagnostic tests: culture, polymerase chain reaction (PCR) and a method based on immunomagnetic separation (IMS). The results for these three techniques were combined and interpreted by three microbiologists with the aim of defining a new standard index. A multivariate analysis was performed by logistic regression to estimate the adjusted risk associated with the type of water, length of the pipe, chlorine, temperature, type of terminal point, period of the year, type of health care (ambulatory or hospital) and the frequency of use of the terminal point.

Results: There was no statistically significant difference, on the basis of the new standard index, neither between laboratories nor between diagnostic tests. Positive results of this index were significantly correlated with the outpatient medical consultations, shower, frequency of use of the terminal points, and temperature. Logistic regression model revealed that outpatient medical consultations ($p=0.058$), shower ($p=0.007$) and the frequency of use of the terminal points ($p=0.001$) are predictors of *Legionella* colonization.

Conclusions: The inclusion of rapid techniques (IMS, PCR) in the development of a new standard index offer increased sensitivity of *Legionella* detection improving classical control strategies usually based on the culture method. The proportion of hospital water-system sites testing positive, according to the defined standard index, was well correlated with the frequency of use of terminal points and the temperature, suggesting that the control of these factors may help prevent legionnaires' disease in the hospital.

Background

Legionellosis is an atypical pneumonia that is acquired when inhaling water drops contaminated with pathogenic bacteria belonging genus *Legionella* and they reach the lungs and replicate in macrophages (1). *Legionellae* are ubiquitous water-borne gram-negative bacilli. Legionellosis is primarily associated with two clinically distinct syndromes: Legionnaires' disease (LD), a potentially fatal form of pneumonia, and Pontiac fever, a self-limited, non-pneumonic illness, which usually resolves on its own without hospitalization or antibiotic treatment, within 2–5 days, so that it is generally not diagnosed. LD is the pneumonic form of legionellosis, with a case-fatality rate of 10% (25% for healthcare-associated infections). Mortality is higher in admitted patients and immunosuppressed patients, reaching 80% ever

with proper treatment (2,3). In developed countries, the incidence has increased largely due to the introduction of clinical diagnostic tests, such as the test for detection of *Legionella pneumophila* serogroup 1 antigen in urine samples (2).

Legionellosis is a relevant pathology of increasing impact on human health. In 2016, in the 30 countries of the European Union, 6,560 cases were confirmed by the European *Legionella* Surveillance Network (European Legionnaires' Disease Surveillance Network - ELDSNet) (4). The exact incidence of legionellosis worldwide is difficult to measure and compare because countries have many differences in the methods of definition and notification of cases, having a low notification rate (2,5,6). *Legionella* is an important cause of both community-acquired and nosocomial pneumonia. The incidence of nosocomial infections is very low (7), but it represents a major public health concern mainly because nosocomial cases occur in a significant proportion in patients without any specific risk factor, and also due to the high lethality (50% in the Valencian Community, Spain). (8,9)

The presence of bacteria in water is considered a prerequisite for infection (10,11). The growth of *Legionella* depends on several factors, among others, pH and hardness of the water, presence of old pipes, standing water, difficulty in maintaining the right temperature, biofilm formation, and the pipe length as an indirect factor (12) (13). In particular, health structures, including hospitals, health centers, residences, and dialysis centers, may present a risk environment for the transmission of LD due to the use of an old water network and the commissioning of medical devices with immunocompromised patients (14,15). Most of the sanitary water networks of the hospitals have been colonized by *Legionella* and, over time, it can cause the appearance of cases of nosocomial legionellosis.

Guidelines and regulations for the primary prevention of legionellosis (ie, Legionnaires' disease and Pontiac fever) have been developed by various public health and other government agencies. These guidelines are similar in recommending maintenance of building water systems; federal and other guidelines differ in the population/institutions targeted, the extent of technical detail, and support of monitoring water systems for levels of *Legionella* contamination. However, LD outbreaks are not uncommon despite guidelines and regulations are widely known and implemented (16). In Spain, for example, the regulation is focused on the levels of chlorine and temperature as risk factors, which is clearly insufficient. On this last point, the correct management of water quality, by including both cleaning procedures and rapid methods of analyzing *Legionella* in water, is considered as a critical issue to prevent LD outbreaks (17,18). This is due, among other factors, to the inability to predict the changes of the concentrations of free and intact *Legionella* in the aqueous phase, from which bioaerosols are generated (19).

All the factors explaining both colonization and infection of *Legionella* in sanitary environments are not yet clear (20,21). Volker et al. have tried to study some of these factors such as temperature, water stagnation and the pipe length. However, it must be considered that this study was not carried out on a sanitary structure, which involves specific difficulties and characteristics regarding a building for domestic use, so that the factors to be studied may be different. In addition, the author demonstrates that

traditional culture-based methods are not always able to reveal the risk of *Legionella* contamination and water infection (13). For this reason, it is very important to study the factors that can be associated with an increase in the presence of *Legionella* in the water of a sanitary structure. Furthermore, a control of legionella colonization in a healthcare environment can improve health globally. In this context, the aim of this study is to evaluate the frequency of use of terminals points like a risk factor for *Legionella* colonization in a hospital (22).

Methods

Data and sample collection

Water samples were collected from different critical points distributed in the different floors and pavilions at the University Clinical Hospital of Valencia (Spain), according to UNE Standard, in January, May and October 2017.

A volume of five liters of water was collected for each sample and divided in four portions, which were distributed among three laboratories accredited by the norm ISO 17025, as follows: 1 liter-portion for LAB 1, 1 liter-portion for LAB2, and 2 liter-portion for LAB3. The remaining 1 liter-portion was discarded. The temperature of the samples was measured at the time of collection. The samples were transported directly to the different laboratories under controlled temperature of 5 ± 3 °C and simultaneously analyzed by all of them. The time between the collection of the samples and the execution of the *Legionella* assays was less than 24 hours.

Three type of assays were conducted by the laboratories: *Legionella* culture method on GVPC Agar (Biomerieux, Madrid, Spain), qPCR (IELAB, Alicante, Spain) and immunomagnetic separation (IMS) based method (Legipid® *Legionella* Fast Detection Test, Biótica, Spain). The samples were analyzed by culture and IMS methods for both LAB 1 and LAB2. The LAB 3 samples were analyzed by culture, IMS and PCR techniques. LAB3 used one liter of sample for conducting both the culture and IMS techniques, as the other laboratories, and used another liter of sample for conducting PCR technique. Each 1 liter-portion for each sample was mixed by shaking it manually and then it was filtered through a 0.4 µm-pore diameter polycarbonate membrane filter (Millipore). The membrane filter was then removed and placed with 15 ml of the diluent L0 (Biótica) in a 100 ml sterile container and then vigorously vortexed for 2 minutes. After that, nine milliliters of this concentrate were assayed by IMS based method (Legipid® *Legionella* Fast Detection Test, Biótica) and the rest of the volume was used to conduct the culture assay for *Legionella* species following the norm ISO 11731 (23).

For qPCR trial (LAB3) each 1-liter water sample was mixed by shaking it and filtered through a 0.2 µm-pore diameter polycarbonate membrane filter (Millipore). The membrane filter was then removed and placed with 10 ml of sterile water and a portion of this concentrated was assayed by qPCR. This kit includes internal controls to control sample inhibitions or non-optimal reactions.

A new type of standard was used for diagnosis through consensus among three microbiologists from the three laboratories as it is described in the state-of-the-art, considering both culture, PCR and Legipid® *Legionella* Fast Detection test results (24).

Among the three participating laboratories cited above, a total of 527 water samples were examined to develop the standard index. Of these, 252 (47.82%) were cold water and 275 (52.18%) were hot water. Samples from areas where patients could not be exposed were excluded for the analysis, as in the case of water reservoirs and accumulators in the basement of the hospital. Once the index was defined, 136 water samples assayed by the LAB3 using all the analytical techniques (culture, PCR and IMS) was considered for this study. All the variables were recorded in an Excel spreadsheet.

Variables

The variables to be studied were the type of water, the pipe length, the chlorine, the temperature, the type of terminal point, the period of the year, the type of health assistance (outpatient or hospital), and the frequency of use of the terminal point. A terminal point was considered as frequent if this point was opened for at least 5 minutes every day.

The temperature factor was divided into three categories (<25 °C, 25–50 °C, and > 50°C), given that *Legionella* multiply where temperatures are between 20°C and 50° C and that the effectiveness of maintaining sanitary hot water at a minimum temperature of 55°C is significantly better than at 50°C for *Legionella* environmental control (25,26).

Statistical analysis

Statistical data analysis was performed using the SPSS IBM Statistics V 21.0 software package. Univariate, bivariate and multivariate analysis were conducted. Simple statistical analysis was conducted by estimating the odds ratio (OR), bivariate analysis used the Chi square test and the Fisher's exact test, and the binary logistic regression was applied for conducting multivariate adjustment of the risk factors.

Results

There was no statistically significant difference between the three laboratories (LAB1, LAB2 and LAB3) and there was also no difference between the IMS based method and the culture method (Table 1) confirming that they are equivalent methods as it is described in the validation of IMS-based method (27).

The Floors and Pipe length (distance from the terminal point) were not statistically significant factors. These variables are interrelated so that Pipe length was considered as adjustment variable provided that it is the most objective summary measure and it is cited in the literature. Although it is likely that there are others interacting factors.

The risk is associated with an increase in temperature above 25°C and with hot water. The variable defined as temperature by ranges, which is best adapted to the theoretical behavior of *Legionella* growth, was included. The shower proved to be a terminal point of risk (OR = 2.05; $p < 0.05$). Ambulatory health assistance was found to be a risk factor for colonization by *Legionella* (OR = 2.9, $p < 0.05$).

Conversely chlorine (OR = 0.090) and frequency of use (OR = 0.155) were protective factors ($p < 0.05$) (table 2).

In the logistic regression model the parameters of date, type of health assistance, terminal point, frequency of use, pipe length, temperature range and chlorine were considered. The May date (OR = 0.454; $p < 0.05$) and Chlorine (OR = 0.032; $p < 0.05$) turned out to be a protective factor. The odds-ratio (OR) for infrequent use was 12.187 ($p < 0.05$). The increased risk is also confirmed for the shower point of sample OR = 8.587; $p < 0.05$). The ambulatory health assistance (OR = 12.248) was not, however, statistically significant (Table 3).

Usage frequency and temperature were taken into consideration. It was found that the hot water was the one that underwent the most changes. At terminal points of infrequent use (NFU) it had a greater percentage of temperatures less than 50 degrees. There was not any point of NFU showing a temperature higher than 55°C (which is present at points of frequent use - FU) (fig 1).

Discussion

Legionellosis was included in 1996 among notifiable diseases at the national level in Spain (Royal Decree 2210/95). In the Community of Valencia, the Basic Surveillance System, including legionellosis as a Notifiable Disease (OED), was developed by the Ordinance of March 4, 1997. Ten years later, in 2017, 236 cases of legionellosis were declared with an incidence rate of 4.7 cases per 100,000 inhabitants (9).

The national regulation currently in force is the Royal Decree 865/2003, which is an old text (28). Currently, this law notes the culture method described in ISO1173:1998 for the *Legionella* assay in water samples from cooling towers, but not explicitly for other risk installations. However, culture method is a slow method that can lead to a delay in taking timely steps on the facilities to prevent risk situations (23,28,29).

Although recovery and isolation of *Legionella* colonies by culture is recognized as the gold standard method, well-known limitations of this method could compromise their utility in preventative or rapid control action. Among these drawbacks, we can highlight: (a) long time to confirm results (from 2 to 28 days are required), (b) changes on environmental water samples have been identified during their transport to laboratory, which might take up to 1–2 days, (c) likely presence of viable and infective but non-culturable cells, (d) poor sensitivity, and (e) a rate of inconclusive results up to 20% by interfering microbiota (30,31).

The new standard UNE 100030: 2017 (29) tries to cover the lag of the Royal Decree by incorporating new technical requirements in the prevention and control of *Legionella* with the main objective of complementing it. One of the main contributions of the new standard is the explicit recognition of alternative not growth-based methods to the culture for the detection of *Legionella*, if they meet the specified technical requirements on validation and certification.

Nevertheless, although some technical recommendations are established in section 6.15 of this standard, it is not applicable to facilities of sanitary/therapeutic use, so the development of other complementary initiatives can be expected.

Given the importance of fast detecting the presence of *Legionella* and knowing the factors that determine the quickly facilities colonization by this bacterium, it is necessary to define a new standard of *Legionella* determination which is not based on culture method, and also to develop new diagnostic methods.

According to the Royal Decree 865/2003, regulation on the prevention and control of legionellosis in Spain, a temperature between 25°C and 50°C is considered a risk factor for the proliferation and colonization of *Legionella*. In fact, thermal disinfection (hot water overheating) was the first method used to control *Legionella* in water distribution systems in hospitals (32,33). Water at 70 °C kills *Legionella pneumophila* in 10 minutes, while at 60 °C it takes 25 minutes (34). The temperature rise above 60 °C for several days ensures the success and absence of legionellosis (35). Its advantage lies in its minimum cost. However, the loss of heat in some points of the hot water distribution network (heater, recirculation, representative points of use) may cause defective return valves in the faucets, providing generalized temperature losses due to the mixing of cold and hot water. Moreover, although thermal disinfection is the most common, economical and effective treatment for the *Legionella* control, it does not eliminate the bacterial biofilm, which protects *Legionella* (36).

In our study, the average temperature is higher in the points of frequent use than those of infrequent use. The importance of maintaining temperatures above 50 °C, and even better above 55 °C (26), is well known.

Furthermore, more *Legionella* positive results were found using the new standard index than using a single technique, whether it is culture or IMS, only for temperatures around 40–50 °C (data not shown). For this reason, it is important to consider not-growth based diagnostic methods, able of measuring *Legionella* properties other than its growth in an artificial medium on a plate.

The importance of pipe length and water stagnation as risk factors for *Legionella* colonization have also been studied. These studies were conducted in residential facilities (13). The structural complexity of a health facility suggests means that pipe length, floor and pavilion cannot be used as risk factors, while it is important to consider both a correct and frequent use of terminal points (26) to prevent colonization and formation of biofilms.

Limitations

A limitation in this work is the difficulty to have a standard for the *Legionella* assay. The culture method is not a reliable test for the detection of *Legionella* in water with a preventative value. There is no consensus to establish an environmental diagnostic standard. The criterion used in this study increased the sensitivity of *Legionella* detection in water. However, it could be necessary to define an internationally validated standard in the future.

Conclusions

Legionnaires' disease (LD) is recognized as an important hospital-acquired disease. Despite the several methods available, the optimal method to control hospital-acquired LD is not well established and their overall efficacy requires further evaluation. A combination of culture, PCR and IMS techniques offer the best index for detecting *Legionella* positivity in environmental samples collected at the hospital. This study suggests that this index is effective in detecting *Legionella* positivity in environmental samples at hospital. Based on this standard index, risk factors have been identified as relevant for the *Legionella* control in the hospital, mainly the frequency of the use of terminal points.

Differences in *Legionella* colonization between types of buildings are not due to a variation in water supply but to other factors, such as the specific structural complexity of the water network in a hospital. Besides the importance of pipe length or temperature, this study demonstrated the favorable action of low frequency of use of terminal points on the proliferation and colonization of *Legionella*. In fact, neighboring terminal points are used with different frequency.

The decrease in the usage frequency of the terminal point causes a temperature lowering in hot water. The frequency of use is related to temperature, the presence of *Legionella* and probably biofilm. In this sense, the frequency of use, representing a combination of other variables, could be a relevant factor for the control of *Legionella* in a health establishment.

List Of Abbreviations

LD: Legionnaires' disease

PCR: polymerase chain reaction

IMS: immunomagnetic separation

NFU: infrequent use

FU: frequent use

Declarations

Ethics approval and consent to participate

This manuscript was approved by the Hospital Clínico Universitario de Valencia (Spain).

Availability of data and material

Data are available from the authors upon reasonable request and with permission from the Hospital Clínico Universitario de Valencia.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Dr. Rafael Ortí is the principal investigator of the Public Health and Patient Safety Research Group at the Catholic University of Valencia. He directed the meetings and the preparation of this document. Eugenio Luciano is the Co-I of this working group. Both authors contributed to the writing of the text. All authors read and approved the final manuscript.

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Tables

Table 1. Difference between laboratories and methods

	n (%)	OR	95% CI		<i>p</i>
Laboratory					
LAB 1	56 (19)	1			
LAB 2	102 (34,7)	1,442	0,748	2,781	0,274
LAB 3	136 (46,3)	1,414	0,755	2,647	0,279
Method					
Culture	164 (55,8)	1			
IMS	130 (44,2)	1,000	0,631	1,585	1

Table 2. Bivariant analysis of different factors

	n (%)	OR	95% CI		<i>p</i>
Date					
Gen	26 (19,1)	1			
May	56 (41,2)	0,643	0,252	1,638	0,354
Oct	54 (39,7)	1,247	0,486	3,201	0,647
Type of assistance					
Hospital	108 (74,9)	1,000			
Ambulatory	28 (20,6)	2,900	1,176	7,154	0,021
Floors					
1	6 (4,4)	1			
2	20 (14,7)	0,750	0,110	5,109	0,769
3	16 (11,8)	0,833	0,115	6,013	0,857
4	26 (19,1)	0,800	0,123	5,202	0,815
5	30 (22,1)	0,438	0,069	2,762	0,379
6	10 (7,4)	0,333	0,040	2,769	0,309
7	20 (14,7)	0,214	0,031	1,504	0,121
8	8 (5,9)	0,500	0,056	4,473	0,535
Point of sample					
washbasin	54 (39,7)	1			
shower	82 (60,3)	2,053	1,021	4,128	0,043
Frecuency of Use					
not frequent	41(30,1)	1			
frequent	95 (69,9)	0,155	0,064	0,371	0,000
Type of water					
Hot water	68 (50)	1			
Cold water	68 (50)	0,111	0,051	0,241	0,000
Temperature Range (°C)					
<25	56 (41,2)	1			
25-50	48 (35,3)	3,500	1,548	7,913	0,003
>50	32 (23,5)	10,833	3,752	31,277	0,000
Pipe lenght (m)		0,997	0,986	1,008	0,562

Chlorine (mg/l)	0,090	0,038	0,214	0,000
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Table 3. Logistic regression considering all important factors

	OR	95% CI		<i>p</i>
Date				
Gen	1			
May	0,150	0,028	0,808	0,027
Oct	0,454	0,092	2,238	0,332
Type of health assistance				
Hospital	1			
Ambulatory	12,248	0,923	162,533	0,058
Point of sample				
washbasin	1			
Shower	8,587	1,781	41,387	0,007
Frequency of Use				
Frequent	1			
Unfrequent	12,187	2,650	56,044	0,001
Pipe lenght (m)	1,006	0,986	1,027	0,555
Temperature Range				
<25	1			
25-50	0,483	0,105	2,220	0,350
>50	0,626	0,087	4,522	0,642
Chlorine (mg/l)	0,032	0,005	0,203	0,0003

Figures

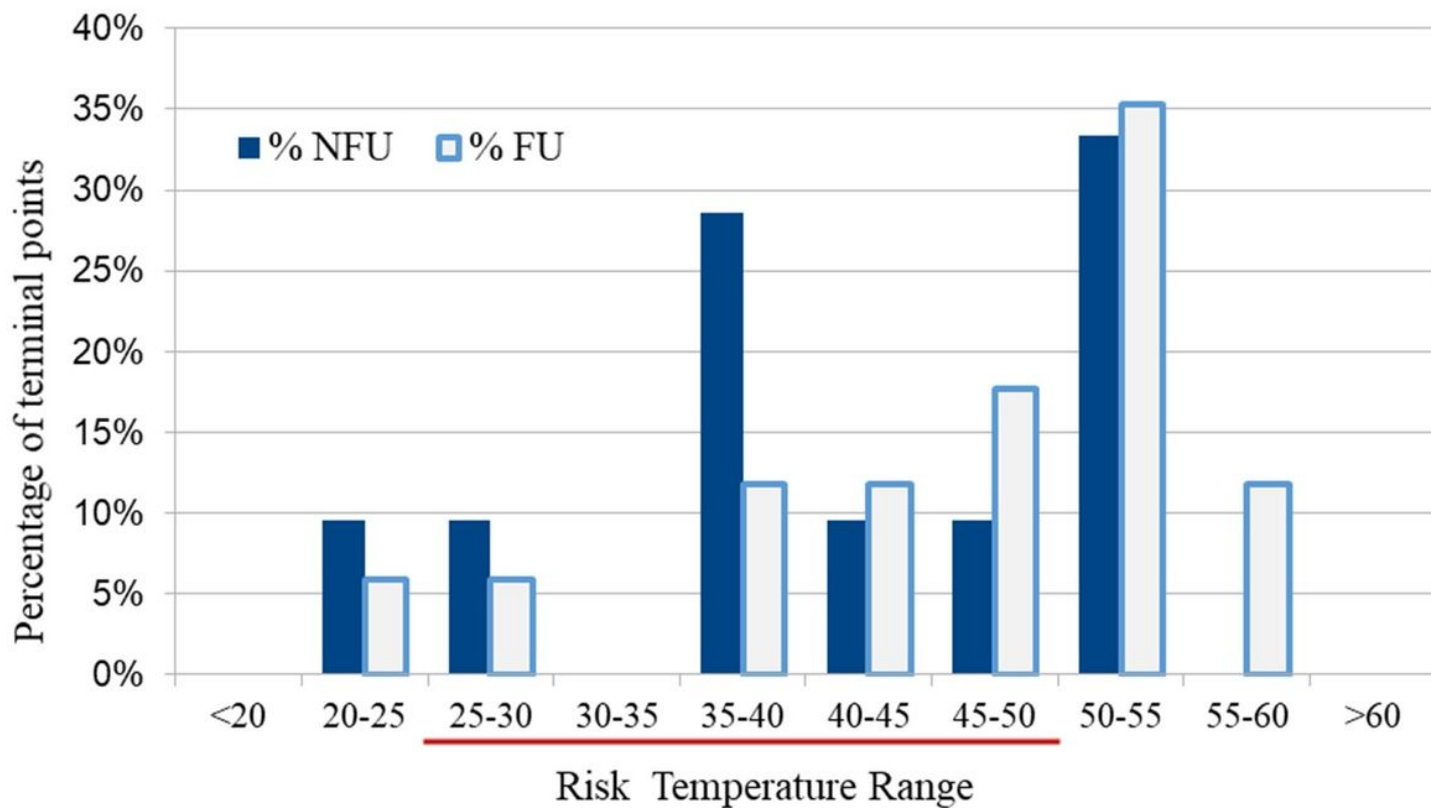


Figure 1

Percentage of terminal points with a given temperature range (differently colored). Comparison between points of infrequent use (NFU) and points of frequent use (FU).