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Persistence of viable but nonculturable *Legionella pneumophila* state in hospital water systems: A hidden enemy?

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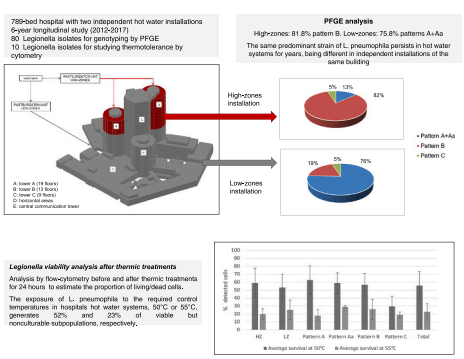
HIGHLIGHTS

- The same strain of L. *pneumophila* persists in hospital hot water systems for years.
- Viable but nonculturable L. *pneumophila* are detected at temperatures required by law.
- After being exposed to 50 °C 24 h, 52.0 % of *L. pneumophila* were still alive.
- After an exposition to 55 °C 24 h, 23.1 % of *L. pneumophila* were still alive.
- *Legionella* surveillance through the standard culture underestimates its presence.

GRAPHICAL ABSTRACT



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ABSTRACT

There is little evidence of the long-term consequences of maintaining sanitary hot water at high temperatures on the persistence of Legionella in the plumbing system. The aims of this study were to describe the persistence and genotypic variability of L. *pneumophila* in a hospital building with two entirely independent hot water distribution systems, and to estimate the thermotolerance of the genotypic variants by studying the quantity of VBNC L. *pneumophila*. Eighty isolates from 55 water samples obtained between the years 2012–2017 were analyzed. All isolates correspond to L. *pneumophila* serogroup 6. The isolates were discriminated in four restriction patterns by pulsed-field gel electrophoresis. In one installation, pattern A + Aa predominated, accounting for 75.8 % of samples, while the other installation exhibited pattern B as the most frequent (81.8 % of samples; $p < 0.001$). The

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mean temperature of the isolates was: 52.6 °C (pattern A + Aa) and 55.0 °C (pattern B), being significantly different. Nine strains were selected as representative among patterns to study their thermotolerance by flow-cytometry after 24 h of thermic treatment. VBNC bacteria were detected in all samples. After thermic treatment at 50 °C, 52.0 % of bacteria had an intact membrane, and after 55 °C this percentage decreased to 23.1 %. Each pattern exhibited varying levels of thermotolerance. These findings indicate that the same hospital building can be colonized with different predominant types of *Legionella* if it has independent hot water installations. Maintaining a minimum temperature of 50 °C at distal points of the system would allow the survival of replicative L. *pneumophila*. However, the presence of Legionella in hospital water networks is underestimated if culture is considered as the standard method for Legionella detection, because VBNC do not grow on culture plates. This phenomenon can carry implications for the Legionella risk management plans in hospitals that adjust their control measures based on the microbiological surveillance of water.

1. Introduction

Several reports have described the long-term colonization of hospital water systems with L. *pneumophila*, often with persistence of the same strain, as being the cause of nosocomial legionellosis (Sabria et al., 2001, Garcia-Nunez et al., 2008, Oberdorfer et al., 2008, Iatta et al., 2013, Alexandropoulou et al., 2015, Pancer et al., 2013). The reasons why a strain with a particular molecular pattern is selected in a water system are not well known. It has been described that in buildings with the same water supply network, certain factors such as the system design and the materials of the pipes can determine the composition and diversity of the water microbiome (Pan Ji et al., 2015). In the case of hospital buildings, the disinfection methods used in hot water systems can play an important role, too. However, the long-term consequences of using continuous disinfection methods in hot water hospital networks remain uncertain (Casini et al., 2014).

Most European countries' regulations require water environmental surveillance in hospitals (Van Kenhove et al., 2019). Till the moment, the culture plate is still being the method of choice, and in the Spanish regulations updated in 2022 has approved more techniques in the control of *Legionella* but culture plate remains the mandatory minimum. The main drawback of this technique is not only the long period to obtain results, but also that viable and culturable bacteria are the only subpopulation detected. The pathogen can change its metabolic state to viable but not culturable (VBNC) under environmental stress due to an increase/decrease in temperature, variation of the medium pH or presence of bactericidal compounds, among others. Under these conditions, bacteria can settle in biofilms or parasite amoebas to obtain more protection against these threats. In the VBNC state, *Legionella* has a low metabolic activity and does not grow in culture media, but the bacteria retain the features of viable cells such as their membrane integrity and virulence (Oliver, 2010). Starved VBNC *Legionella*, which likely occurs in oligotrophic biofilms, are able to infect amoebas even after months on this state. Unlike the replicative state, the pathogen will need more time and requires higher multiplicity of infection in order to internalize the amoeba (Dietersdorfer et al., 2018). VBNC *Legionella* is more difficult to detect, as this subpopulation does not grow in culture plate so its eradication is even more difficult.

The first objective of this study is to describe the persistence and genotypic variability of L. *pneumophila* in a hospital building with two entirely independent hot water distribution systems, where no additional treatments have ever been used for continuous disinfection purposes. The second objective is to estimate the thermotolerance of the different genotypic variants by studying the quantity of VBNC L. *pneumophila* after thermic treatments.

2. Methods

2.1. Setting

This is a six-year longitudinal study performed from 2012 to 2017 at the Bellvitge University Hospital, a 789-bed teaching hospital located in the southern metropolitan area of Barcelona, Catalonia. The centre accepts referrals for more than two million people requiring high-complexity procedures, except for hematological, pediatric, and obstetric patients.

2.2. Hospital building and hot water system

The hospital building has a vertical disposition with three towers of different heights (tower A: 19 floors, tower B: 13 floors; and tower C: 9 floors) and a central tower of communication between them. The lowest four floors have annexed facilities to the towers with a horizontal design. The building has two fully independent hot water installations. An installation corresponds to the higher areas of towers A and B and feeds the floors 9 to 19 and 9 to 13, respectively (high-zones installation). The other installation corresponds to the rest of the building (low-zones installation) (Fig. 1). The number of faucets and showers is much smaller in the high-zones installation areas than in the low-zones installation areas.

Water is heated in two independent pasteurization units, where it reaches a temperature of 70 °C for a minimum of 3 min. The water temperature flowing out from the pasteurization units is maintained at a minimum of 60 °C, suitable to keep the circulating hot water temperature in peripheral outlets at a minimum of 55 °C. This minimum temperature level was established in June 2007 because it was found that below this threshold, *Legionella* control was suboptimal (Gavaldà et al., 2019). However, temperature in the low-zones installation areas is more difficult to control due to its larger size. Additionally, the return temperatures to the pasteurization are repeatedly lower for low-zones installation when comparing to high-zones installation, indicating a greater low of temperature within this circuit. Super-heat and flush treatments and continuous chemical treatments of hot water have never been applied for disinfection purposes in this hospital building.

2.3. Hot water sampling

Hot water is sampled and cultured monthly as part of the Risk Management Plan for *Legionella* Control. The sampling criteria and the sampling method were adapted according to the Spanish legislation in effect at the time of the study (Real Decreto 865/, 2003). To obtain the samples, a sterile Dracon swab was used for the recovery of the biofilm and the swab was introduced in a sterile polyethylene vessel containing 1000 mL of hot water sample. The temperature was immediately measured after filling the container. A total of 477 hot water samples were obtained from June 2007 (when it was established that the minimum required temperature in peripheral outlets was 55 °C) to December 2017. The temperature of those from high-zones was 56.8 °C (SD = 2.02; minimum = 50.0 °C, maximum = 62.0 °C), whereas those from low-zones was 54.5 °C (SD = 3.89; minimum = 42.0 °C, maximum = 61.5 °C). This difference was statistically significant ($p < 0.05$). By analyzing the temperatures by the studied periods, we obtained that during the period of 2012–2014 the average temperature in high zones was 56.8 °C ± 2.24 °C while in the low zones was 54.9 °C ± 3.56 °C. In the period of 2015–2017, the average temperature in high zones was 55.3 °C ± 2.07 °C while in the low zones was 54.4 °C ± 3.89 °C.

2.4. Bacterial strains and culture conditions

All strains were grown on buffered charcoal yeast extract agar (BCYEα agar) (Thermofisher, Massachusetts, USA) at 37 °C and 5 % CO_2 for 72 h. BCYEα agar is a selective growth medium used to culture or grow certain types of bacteria, particularly the Gram-negative species L. *pneumophila* with nutritional needs as cysteine supplementation. *Legionella* strains were cryopreserved for long-term storage in brain heart infusion broth with glycerol.

2.5. Pulsed-field gel electrophoresis of L. pneumophila

We selected a representative sample of cryopreserved L. *pneumophila* isolates obtained from outlets and showers between 2012 and 2017. Due to the different size of the two water systems, we carried out the identification of the samples by means of a cluster sampling, considering high-zones and low-zones separately. We have used pulsed-field gel electrophoresis (PFGE) in order to classify all strains in different genotypic variants. For PFGE, genomic DNA was prepared as described previously (Garcia-Nunez et al., 2008). Briefly, bacterial DNA extraction was performed with lysozyme and proteinase K, bacterial suspensions adjusted to an OD_{620} (Ultrospec 3100 Pro. Artisan Technology Group, Champaign, Illinois, USA) of 0.3 were mixed with 2 % Incert-agarose (FMC Bioproducts, Rockand, MD, USA). Restriction digestion of genomic DNA was performed with 50 U *SfiI* (New England Biolabs, Ipswich, Massachusetts, USA) according to the manufacturer's recommendations. The DNA fragments generated in the digestion were separated on a 1 % agarose gel. The gel was run in 0.5 M Trisborate- EDTA buffer (pH 8.3) in a CHEFDR II system (Bio-Rad, Ivry sur Seine, France) with a constant voltage of 5 V and increasing pulse times (5.6–50.6 s) at 14 °C for 24 h. The lambda ladder (New England Biolabs) was included as a molecular weight PFGE marker. PFGE patterns that differed by one or more bands were considered to belong to different genotypes and were designated with different capital letters.

2.6. Thermic treatments

Thermic treatments were performed in at least 3 strains of each pattern if there was enough representation. A total of 9 high and low-zone representative strains were selected to perform the thermotolerance study at 50, 55 and 60 °C using constant agitation at 300 rpm (Bioer, Hangzhou, China). An initial suspension was adjusted to an OD_{620} of 0.3 to obtain a value close to 10^8 cfu/mL. All the suspensions and incubations were done with autoclaved tap water as it mimics a more realistic natural physicochemical environment. This approach is based on the isothermal experiment approximation of Papagianeli et al. (2021). Treatments were performed using 1 mL of 10^6 cfu/mL suspensions in autoclaved tap water. After 24 h of thermic treatment, samples were kept at 35 °C baths for temperature adjustment and 100 μL of each suspension were plated in BCYEα agar and incubated at 37 °C and 5 % CO_2 for 72 h to quantify viable culturable microorganisms.

2.7. L. pneumophila viability analysis by cytometry

We used the cytometer BD FACSCanto™ II (New Jersey, USA) to quantify dead and live bacteria after thermic treatments. After 24 h of thermic treatment, samples were kept at 35 °C baths for temperature adjustment and 500 μl of the sample were used to perform the viability analyses by using two specific dyes. The green fluorescent staining of nucleic acids *SYTO™9* (ThermoFisher) has high affinity for DNA and

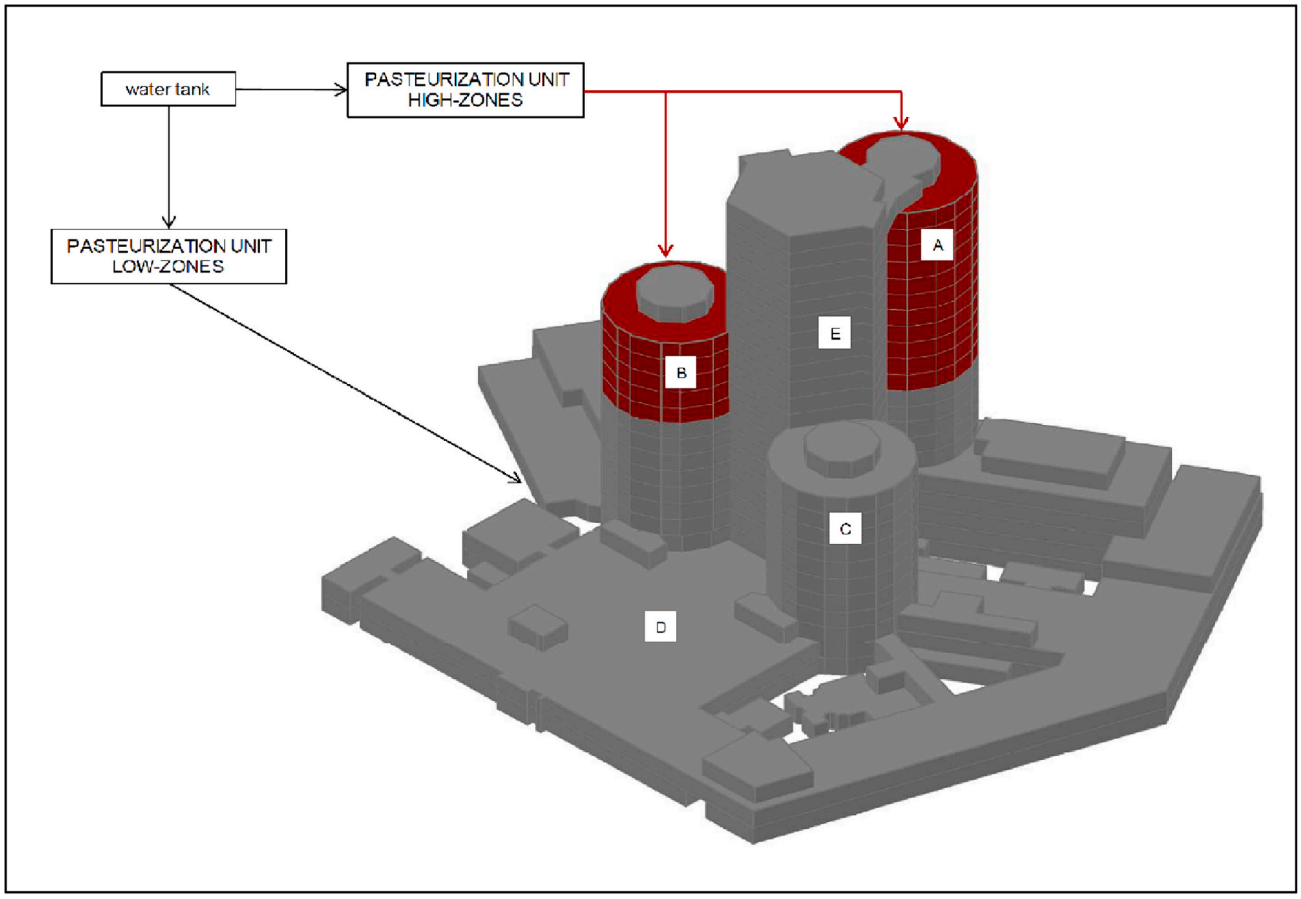

Fig. 1. Schematic drawing of the Bellvitge University Hospital building.
Red: high zones; grey: low zones; A: tower A (19 floors); B: tower B (13 floors); C: tower C (9 floors); D: horizontal areas; E: central communication tower.

exhibits enhanced fluorescence upon binding. This stain dyes both live and dead bacteria, so we used to dye total bacterial load to distinguish bacterial cells from the debris. Additionally, *Live-or-Dye*™ (LoD, Biotium, Fremont, California, USA) staining allows discrimination between live and dead cells as it is a cell membrane impermeable amine-reactive dye, so it can only enter into dead cells that have compromised membrane integrity. To test LoD staining, a control of dead cells was performed by heating 1 mL of 10^6 cfu/mL suspension during 15 min at 95 °C and shaking at 300 rpm.

We analyzed each suspension by flow-cytometry before and after thermic treatment to avoid the underestimation of the proportion of living/dead cells. At some temperatures, bacteria can suffer membrane disintegration losing these counts in the post-treated (PostTre) samples, so thermic effect was assessed by comparing PostTre against pre-treated (PreTre). This differential quantification between PosTre and PreTre is the subpopulation disintegrated by thermic treatment. Bacterial subpopulation with double dye (SYTO +, LoD +) are dead bacteria due to the thermic treatment, while Syto+ LoD- counts are the bacteria that still have intact membrane after the thermic treatment.

One limitation of the study was the preparation of the suspensions with tap water that could affect the viability of the cells, could represent a great stress and therefore overestimate the effect of the thermic treatment. In order to reduce this effect, we used in all experiments a cell suspension as a control without any thermic treatment but incubated 24 h in tap water to analyse the effect of changing from growing in BCYEα agar to tap water.

All experiments were analyzed by triplicates.

2.8. Statistical analysis

The Chi Square test was used to compare the proportional distributions among groups and the Student's *t*-test to compare the differences between group means. A p value of 0.05 or less was considered statistically significant. The free distribution software "R" was used for statistical analysis (R core team, 2021).

3. Results

3.1. Persistence and genotypic variability of L. pneumophila

We analyzed 80 isolates from 51 water samples obtained between the years 2012 and 2017. The distribution according to the water system was 58 isolates from low-zones installation and 22 isolates from high-zones installation. All isolates corresponded to L. *pneumophila* serogroup 6. The 80 isolates were discriminated in four restriction patterns by PFGE analysis: pattern A (41 isolates), pattern Aa (6 isolates), pattern B (29 isolates) and pattern C (4 isolates). Pattern Aa was a variant of pattern A, only one different band. Patterns A, B and C were clearly different from each other.

In each hot water system, a certain pattern prevailed: in the low-zones 75,8 % of the isolates corresponded to A + Aa pattern, whereas in the high-zones pattern B accounted for 81,8 %, and pattern A + Aa was minority ($p < 0.001$). Pattern C isolates were about 5 % in both installations. Table 1 shows that the percentage of samples with pattern B was higher in the later years of the study, at the expense of patterns A + Aa. Thus, 15.8 % of strains obtained in the low zones during the first three years of the study exhibited pattern B, with this percentage increasing to 25.0 % in the later years ($p < 0.001$). Similarly, in the high zones, the percentage of strains with pattern B increased from 75.0 % to 90.0 % ($p = 0.005$).

The samples temperature of the isolates was different between the predominant patterns (Table 1). Globally, the average temperature of the isolates with pattern A + Aa was 52.6 °C (SD = 3.42) and that of those with pattern B was 55.0 °C (SD = 1.85) ($p < 0.05$). The maximum observed temperature was 58.2 °C and corresponded to a pattern B isolate. The minimum observed temperature was 44.0 °C and corresponded to a pattern A isolate.

Table 1
Number of L. *pneumophila* samples analyzed in the periods 2012–2014 and 2015–2017 according to PFGE patterns, pattern distribution in each period, and average temperature of each pattern.

	N° (%) of isolates		Temperature, mean (SD)
	2012–2014	2015–2017	
Low zones			
Pattern A + Aa	31 (81.6 %)	13 (65.0 %)	52.5° (3.45)
Pattern B	6 (15.8 %)	5 (25.0 %)	55.5° (1.93)
Pattern C	1 (2.6 %)	2 (10.0 %)	54.3° (0.23)
High zones			
Pattern A + Aa	2 (16.7 %)	1 (10.0 %)	54.5° (2.60)
Pattern B	9 (75.0 %)	9 (90.0 %)	54.7° (1.99)
Pattern C	1 (8.3 %)	–	[a]

[a] Isolate temperature: 53.7 °C.

3.2. L. pneumophila viability analysis after thermic treatment by cytometry

We selected nine strains as representative of all patterns to study their thermotolerance by flow-cytometry. After 24 h incubations at 50, at 55 °C and at 60 °C, we did not obtain any colony in culture plates, although VBNC bacteria were detected in all cases. Fig. 2 shows membrane integrity of L. *pneumophila* after 24 h of thermic treatment at 50 °C *versus* 55 °C. After being exposed to 50 °C, intact membrane bacteria were 61.77 % in pattern A + Aa, 56.82 % in pattern B and 29 % in pattern C. After being exposed to 55 °C, intact membrane bacteria were 20.7 % in pattern A + Aa, 26.02 % in pattern B and 19.3 % in pattern C. After 24 h incubation at 60 °C, we obtained <5 % of intact membrane bacteria in all samples. All patterns showed similar results (pattern A + Aa 3.76 %, pattern B 3.86 % and pattern C 3.85 %).

When analyzing the thermotolerance of the strains within the same water system, the percentages of L. *pneumophila* membrane integrity in high zones were 59.0 % at 50 °C, 20.2 % at 55 °C and 2.87 % at 60 °C while in low zones were 52.0 % at 50 °C, 25.1 % at 55 °C and 4.57 % at 60 °C.

We analyzed the control of membrane integrity without thermic treatment, only incubated with tap water, to normalize the results obtained after thermal treatments. (data not shown). Hence, it can be inferred that the mortality described after the normalization is exclusively attributed to the thermic treatments.

4. Discussion

Our study revealed that the same L. *pneumophila* strains have persisted in the hot water distribution network of a hospital for at least six years. Furthermore, as the building has two independent installations, a significant finding was that a different strain has predominated in each of them. Although it is well known that the same *Legionella* strain can persist in hospital water systems for long periods of time (Garcia-Nunez et al., 2008, Oberdorfer et al., 2008, Iatta et al., 2013, Alexandropoulou et al., 2015, Pancer et al., 2013), studies analyzing the causes of the genetic variability in the same building are scarce. To our knowledge, only two previous studies have shown that different parts of the same hospital building can harbor different predominant *Legionella* subtypes (Allegra et al., 2011; Bartley et al., 2016). The reasons why a certain *Legionella* pattern persists as the main colonizer of a particular water network are not well known. It is assumed that *Legionella* arrives in the buildings from cold water network at concentrations undetectable by routine laboratory methods. Once in a building, the establishment of biofilm throughout the water distribution system facilitates the growth of the microorganism and interferes with the environmental stressful conditions. A change in the environment, such as may occur when applying disinfection measures, causes the ecological niche to

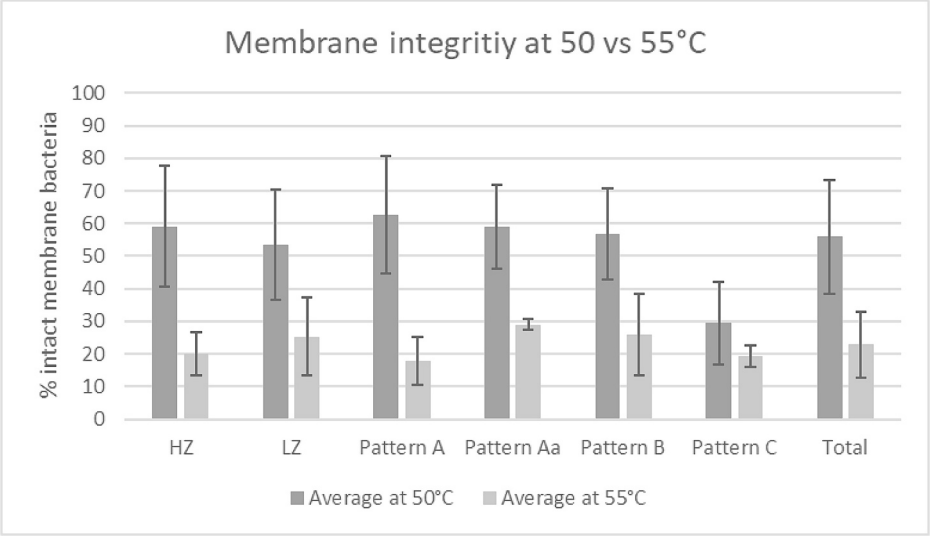

Fig. 2. Membrane integrity of L. *pneumophila* strains following 24-h heat treatments at 50 °C and 55 °C.
HZ = High zones. LZ = Low zones.
Error bars: standard deviation.

destabilize and the inoculum of less resistant subtypes to diminish, allowing other subtypes to overgrow (Thomas et al., 2004). Added to this is the capacity of *Legionella* for developing mechanisms of resistance to the disinfection methods because of its ability to grow within protozoa or enter in a VBNC status (Hwang et al., 2006; Chang et al., 2007; Cervero-Arago et al., 2014).

We postulate that, in our scenario, one of the reasons why different *Legionella* subtypes have colonized the two independent installations of the building is the difference in the hot water temperature maintained for prolonged periods of time. Several studies have demonstrated the ability of *Legionella* to persist in water systems despite routine super-heat and flush treatments (Perola et al., 2005; Scaturro et al., 2005; Allegra et al., 2011; Bédard et al., 2016). Moreover, these treatments select for thermic tolerant *Legionella* strains (Whiley et al., 2017). Super-heat and flush treatments have never been applied in our study. However, high-temperature levels have been maintained for years as the solely continuous disinfection method. In terms of *Legionella* control, this strategy provided good long-term results, although the microorganism was not completely eradicated (Gavaldà et al., 2019). Of particular concern is the growth of *Legionella* in water samples at temperatures up to 58 °C in the high-zones installation, which may lead to the suspicion that thermotolerant *Legionella* subtypes have been able to persist in the areas where higher temperatures have remained constant over the time. Cervero-Aragó et al. have recently described that L. *pneumophila* strains can infect amoebae for at least 85 days at 55 °C and 60 °C and for up to 8 days at 70 °C (Cervero-Aragó et al., 2019).

It is worth noting that Spain was one of the first European countries to legislate *Legionella* control measures in facilities, with the first law being published in 2003 (Real Decreto 865/, 2003). This law established that the minimum temperature of hot water in at-risk facilities should be 50 °C. This legislation was recently replaced by a new law which expanded and improved many of the technical specifications for control and allowed for an approach based on water risk assessment. (Real Decreto 487/, 2022). However, it still maintains the minimum temperature requirement of 50 °C, despite current scientific evidence indicating that it may be an insufficient threshold for optimal control.

To address the uncertainties regarding the role of high temperatures in the selection of specific *Legionella* strains, a study on thermotolerance was conducted using strains obtained from the hospital water network. Notably, the study was designed taking into consideration that in the two independent installations, from where the strains were recovered, the maintained temperature of the hot water had been different over the years. Considering that bactericidal effect of temperature is nonspecific, it is reasonable to see mortality differences at higher temperatures. In other words, the hypothetical resistance provided by pattern B (much more frequent in areas with higher temperatures) compared to pattern A was clearer at 55 °C than not so much at 50 °C. It should also be noted that this pattern (B) was less prevalent at the beginning of the study. A plausible explanation for this finding is that there may have been pressure due to temperature towards certain strains. One of the most surprising results was that we did not obtain any culturable strains after 24-h heat treatments in none of the experiments mentioned above (at 50 °C, at 55 °C and 60 °C). Nevertheless, results showed that these suspensions contain intact membrane bacteria that could revert its metabolic state to a more active (replicative or transmissive) or pathogenic. Both temperatures generate VBNC subpopulation not detectable by culture plate, at 50 °C there was an average of 52.0 % of VBNC, at 55 °C an average of 23.1 % of VBNC and at 60 °C an average of 3.75 % of VBNC.

Maintaining the temperature at 60 °C would reduce considerable *Legionella* risk proliferation as is the temperature that produced more *Legionella* mortality. Nevertheless, the use of temperature in hot water systems for *Legionella* control has certain limitations such as the risk of scalding injuries, being considered unsafe to have temperatures of 60 °C at the distal points. This temperature could be use as shock treatment in colonized facilities.

These experiments should also be carried out with previously starved *Legionella* to assess if the same results are obtained after incubations at these temperatures.

Our findings can yield practical implications for the management of *Legionella* risk in hospital settings. Firstly, it is necessary that the characteristics of the distribution network should be considered when sampling hot water in hospital buildings. The virulence of the subtypes of L. *pneumophila* present in the water networks has been described as an important factor for explaining the different occurrence of nosocomial legionellosis in colonized hospitals (Garcia-Nunez et al., 2009). It is thus plausible to find in the same building diverse genetic variants of *Legionella* with variable cytotoxicity that can differently affect patients. Secondly, microbiological surveillance of sanitary hot water through conventional culturing underestimates the presence of *Legionella* by failing to detect the VBNC states. Nevertheless, these forms could pose a genuine risk to hospitalized patients due to their ability to revert to

infective forms and infect them.

Further research is necessary to investigate the extent and implications of VBNC *Legionella* states within hospital water systems. This phenomenon can carry implications for the *Legionella* risk management plans in hospitals that assess and adjust their control measures based on the microbiological surveillance.

5. Conclusions

This study stands out for having analyzed both culturable and VBNC *Legionella* states obtained after temperature incubation of strains isolated from the hot water network of a hospital, thus estimating the actual risk of patient's exposure to this pathogen. In addition, the PFGE patterns derived from these strains have remained stable for years. According to the results obtained, we can conclude:

1. The same hospital building can be colonized for prolonged periods of time with different predominant types of *Legionella* if it has independent hot water installations, so risk management plans for *Legionella* control in hospitals should consider the characteristics of the distribution network.
2. Thermotolerance studies by culture plate may be insufficient as evidenced by the absence of culturable cells when comparing with viability flow-cytometry tests. Culture plate underestimates the presence of bacteria under stress factors that have switch into the VBNC state.
3. Keeping hot water systems temperature at 50 °C for 24 h in the most distal points would allow the survival of replicative L. *pneumophila*, although culturable bacteria would not be detected. The increase of five degrees in these points could reduce the presence of *Legionella* by >25 %. It is important to bear in mind that this study has been done with the replicative form of the bacteria, the most sensitive to environmental fluctuations. Survival in its transmissive form would be superior.
4. Analyzing the presence of *Legionella* according to the only mandatory method required by current Spanish legislation underestimates the presence of the pathogen in a facility. At this point, it could be argued that increasing five degrees the temperature in the most distal points of hot water circuits would help prevent cases of nosocomial legionellosis. Therefore, a revision of the present legislation would become an investment in favour of the citizens' health, especially for highly vulnerable individuals.

CRediT authorship contribution statement

Noemí Párraga-Niño: Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Roger Cortès-Tarragó:** Writing – original draft, Methodology, Formal analysis. **Sara Quero:** Writing – review & editing, Methodology, Formal analysis. **Marian Garcia-Núñez:** Writing – review & editing, Methodology, Data curation. **Elisenda Arqué:** Validation, Resources, Methodology. **Sara Sabaté:** Writing – review & editing, Resources. **Dolors Ramirez:** Writing – review & editing, Resources, Data curation. **Laura Gavaldà:** Writing – original draft, Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Corrigendum

Corrigendum to "Persistence of viable but nonculturable *Legionella pneumophila* state in hospital water systems: A hidden enemy?" [Sci. Total Environ. 2024. 927, 172410]

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