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Determination of *Legionella* spp. prevalence in Spanish hotels in five years. Are tourists really at risk?

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ABSTRACT

Legionella spp. is the etiological agent of the serious respiratory pneumonia known as Legionnaires' disease. This respiratory illness is frequently associated with travel and tourist resorts. Spain is an important tourist destination, and one of the top European countries concerning Legionnaires' disease cases, both community and travel associated. Still, the colonization of *Legionella* in our hotels remains scarce. Here, we surveyed 204 hotels in the Canary Islands, Spain, for five years (2015–2019), to determine the *Legionella* prevalence. Samples were obtained and analysed following national and international guidelines. We detected the pathogen in 140 of 2,318 samples (6.0%). The water distribution systems (WDS) were more colonized (7.4%) than the whirlpools (4.7%). Contamination levels were minimal (<3 log CFU L^{-1}) in most of the cases, and only 3.6% of samples were highly contaminated minimal (>4 log CFU L^{-1}). We isolated *Legionella* in 4.3% and 8.5% of cold and hot water distribution systems, respectively. The *Legionella* prevalence in cold water systems samples was higher when free chlorine levels were below 0.2 mg L^{-1}, whereas in the hot water systems samples, the prevalence was higher at <50 °C. *Legionella pneumophila* was the most frequently isolated species, being the members of the serogroups 2–14 the most prevalent. The annual distribution showed a colonization pick in June, followed by the winter months. Regarding the geographical distribution, the presence of *Legionella* was more prevalent in the western islands. Our study concludes that *Legionella* contamination rates in samples from facilities of the Canary Islands is lower than most of the observed in other European studies. However, corrective measures are still needed to improve *Legionella* control.

1. Introduction

Legionella are fastidious rod-shaped aerobic Gram-negative bacteria. They are ubiquitous aquatic microorganisms found at low concentrations in the surface waters of rivers and lakes where they as free-living microorganism or intracellularly into algae or protozoa cells. There are about 60 known *Legionella* species isolated from aqueous environments, although new species continue to be described [1]. About 50% of species may infect people, mainly in the lower respiratory tract [2]. These air-borne pathogens transmit in tiny droplets known as aerosol, which allow the bacterium to reach the alveolus. Commonly (about 95%), the pathogen causes a self-limiting illness with influenza-like symptoms known as Pontiac fever. However, a second type of legionellosis called Legionnaires' disease (LD) may cause a severe and even deathly illness. In this case, the mortality rate is 10–15%. LD consists of atypical pneumonia with symptoms ranging from mild illness to severe pneumonia. The incubation period is 2–14 days, and the severity of symptoms depends on different risk factors, including age, immunodeficiencies, male sex and smoking abuse, among others. Although any *Legionella* species are able to cause LD pneumonia, *L. pneumophila* is, by far, the most frequently associated to this disease [2]. This species comprises different serogroups, the serogroup 1 being the major pathogen for humans causing around 70–90% of infections [3].

Legionella pass from the environment to man-made facilities like water distribution systems (WDS) of buildings, cooling towers,

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whirlpools spas and industrial equipment located in hospitals, hotels and other public buildings. All these installations have been associated with LD [4–7]. LD cases are mostly community-acquired (67%), although travel-related (24%) and healthcare-related cases (5%) are also described [2,8,9]. In Europe, 11,298 LD cases were informed by 28 countries in 2019 [8]. That is the highest notification rate ever observed for the EU/EEA, doubling the values in only five years. Four countries, France, Germany, Italy and Spain, whose combined populations only represent approximately 50% of the EU/EEA population, accounted for 71% of all notified cases [8].

Some travellers acquire the infection in the country of visit but, due to the long incubation period, the symptoms appear and are diagnosed at home. Those LD cases are known as travel-associated Legionnaires' disease (TALD). Italy, France and Spain are the most visited countries by the affected travellers [8]. The number of TALD cases raised to 1,657 in 2019, the higher records ever detected. These data urge the necessity in understanding the epidemiology of LD to improve risk evaluation, detection of pathogen niches and investigation and control of cases and outbreaks, particularly in hotels [2].

The prevalence of *Legionella* in hotels of tourist destinations as Italy, Greece or Turkey has been widely investigated [10–15]. However, the information of the hotels from Spain, a major tourist destination, is still limited.

The aim of this investigation was to survey the *Legionella* prevalence in the hotels located in the Canary Islands, Spain during a 5 years period (2015–2019), in order to get a representative picture of the present situation. Our results will facilitate the design of appropriate improvement procedures to minimize the risk associated with tourist accommodations.

2. Methods

2.1. Tourist facilities

The tourist facilities surveyed in the present investigation included hotels and resorts located in the Canary Islands, Spain. A total of 204 facilities were visited without prior notice from 9th January 2015 to 25th September 2019.

2.2. Samples collection

The sampling points were selected based on the characteristics of the facilities, following the recommendations of the Spanish Ministry of Health [16,17]. They included hot and cold water distribution systems (WDS) and pools with jets, waterfalls and/or air bubblers (like whirlpools or hot tubs). Water samples were collected from bathroom outlets (showerheads or bath taps) without flaming the outlet point and by the pre-flush technique, i.e., without a previous run of the water. That represents the best simulation for common use conditions and user exposure. Following national and international recommendations [16,18], 1 L samples were collected into sterile bottles containing 20 mg sodium thiosulphate (Sharlab, Spain), able to neutralize up to 5 mg L^{-1} free and combined chlorine. The temperature was determined in situ by a calibrated digital Testo 104 thermometer (Testo, Spain) 1 min after flushing. Cold water-free chlorine levels were also determined in situ with the Lovibond® portable MD100 instrument (Lovibond, Germany) by the colorimetric method described in Ref. [19]. Cold samples were transported in refrigeration whereas hot samples were transported at room temperature. All samples were kept at 4–8 °C in darkness until their filtration 24–48 h post-collection [20].

2.3. Laboratory investigation

The procedure for *Legionella* detection and enumeration in the water samples was based on international standards [20]. One liter of the sample was concentrated using a 47-mm nitrocellulose membrane with 0.22-μm pores (Sartorius SA, Spain). After filtration, the membrane was aseptically placed into one screw-capped sterile tube containing 10 mL sample. Bacterial cells were dislodged from the membrane by vortex for at least 2 min. Two 0.5 mL aliquots were directly plated onto GVPC (glycine, vancomycin, polymyxin and colimicyn) medium plates (Oxoid, Spain). To reduce the number of interfering bacteria, 1 mL from the tube was acid-treated with 0.2 mol 1−1 HCl–KCl at pH 2.2 for 5 min. Another mL was heat-treated at 50 °C in a water bath for 30 min. After the treatments, 0.1 mL were inoculated onto GVPC plates. All plates were incubated at 36 ± 1 °C for 10 days in anaerobic jars containing CO_2Gen Compact sachets (Oxoid, Spain) to generate a 2.5–5% CO_2 atmosphere. Readings were performed on days 4, 7 and 10. Colonies with characteristic morphological features compatible with *Legionella* detected in any GVPC plate were considered as presumptive *Legionella*. For confirmation, at least three of them were selected and subcultured onto buffered charcoal yeast extract (BCYE) and BCYE without cysteine (BCYE-cys) media (Oxoid, Spain). We considered *Legionella* members the isolates growing on BCYE but not on BCYE-cys. The commercially available *Legionella* latex agglutination test (Oxoid, Spain) was used for serotyping, identifying the isolates as *L. pneumophila* serogroup 1, *L. pneumophila* serogroup 2–14 and *Legionella* non-*pneumophila* species. The detection limit of the procedure was 10 colony forming units (CFU) L^{-1}.

2.4. Data analysis

We imported the collected data from the study into a Microsoft Excel® 2016 file from the Laboratory Integrated Management System (LIMS). Data was curated to eliminate duplicates and inconsistencies. Finally, values for 2,318 samples were considered. The CFU L^{-1} values were converted into log CFU L^{-1} values before analysis. A two-tailed χ^2 test was used for the qualitative data analysis and quantitative data was analysed by the *t*-Test. Results were considered statistically significant when the P value was <0.05. All the statistical analyses were also performed in Microsoft Excel® 2016.

3. Results

3.1. Legionella isolation from water samples

The results and characteristics of *Legionella* recovery from water samples in tourist facilities are shown in Table 1. We analysed a total of 2,318 samples, 1,155 from the WDS and 1,163 from whirlpools. Overall, the pathogen was isolated in 140 samples (6.0%), whereas in 2,178 (94.0%) the presence of the microorganism was not detected (Table 1). The analysis of the results showed significant differences between the isolation of *Legionella* in the WDS and the whirlpools ($p < 0.05$). The WDS were more often colonized by the bacteria (7.4%) than the whirlpools (4.7%).

The samples from WDS included 832 samples from the hot WDS (672 from the circuit and 160 from the storage tank) and 323 samples from the cold WDS (203 from the circuit and 120 from the tank). *Legionella* presence in hot WDS was higher than the observed in cold WDS (8.5% vs 4.3%, $p < 0.02$). Differences in the colonization rate between circuits and tanks were not detected in any case. However, the storage tanks of the hot WDS were more often colonized (8.5%) than the cold WDS tanks (3.3%) ($p < 0.03$).

3.2. Legionella serogroups present in the different water samples

Pure cultures obtained from the 140 positive samples were further characterized. *L. pneumophila* was the most prevalent species isolated from the facilities (Table 2), being identified in 124 samples (88.6%). The members of the *L. pneumophila* serogroups 2–14 were the most frequently isolated, constituting 59.7% of the *L. pneumophila* isolates and 52.9% of the total positives. *Legionella* non-pneumophila represented

Table 1
Legionella presence in water samples from tourist facilities.

	Water system							Whirlpools	TOTAL
	Hot water			Cold water			Total		
	Circuit	Tank	Total	Circuit	Tank	Total			
Positive samples n (%)	54 (8.0)	17 (10.6)	71 (8.5)	10 (4.9)	4 (3.3)	14 (4.3)	85 (7.4)	55 (4.7)	140 (6)
Negative samples n (%)	618 (92.0)	143 (89.4)	761 (91.5)	193 (95.1)	116 (96.7)	309 (95.7)	1070 (92.6)	1108 (95.3)	2178 (94)
TOTAL n (%)	672 (100)	160 (100)	832 (100)	203 (100)	120 (100)	323 (100)	1155 (100)	1163 (100)	2318 (100)

Table 2
Legionella species recovery from 140 positive water samples.

	Water system							Whirlpools	TOTAL
	Hot water			Cold water			Total		
	Circuit	Tank	Total	Circuit	Tank	Total			
L. pneumophila sg 1	14 (25.9)	1 (5.9)	15 (21.1)	4 (40.0)	2 (50.0)	6 (42.9)	21 (24.7)	29 (52.7)	50 (35.7)
L. pneumophila sg 2-14	33 (61.1)	12 (70.6)	45 (63.4)	5 (50.0)	2 (50.0)	7 (50.0)	52 (61.2)	22 (40.0)	74 (52.9)
L. non-pneumophila	7 (13.0)	4 (23.5)	11 (15.5)	1 (10.0)	0 (0.0)	1 (7.1)	12 (14.1)	4 (7.3)	16 (11.4)
Legionella spp.	54 (100)	17 (100)	71 (100)	10 (100)	4 (100)	14 (100)	85 (100)	55 (100)	140 (100)

only 11.4% of the positive isolates.

The predominant presence of *L. pneumophila* serogroups 2–14, followed by *L. pneumophila* serogroups 1, and *Legionella* non-pneumophila was also observed when only WDS samples were investigated. That was true for all the WDS samples, independently if the samples were from hot or cold WDS (Table 2). However, some differences arose when the tank samples were analysed. In hot WDS tanks, the prevalence of *Legionella* non-pneumophila (23.5%) was higher than that of *L. pneumophila* serogroup 1 (5.9%). In cold WDS tanks, *Legionella* non-pneumophila was not detected in any sample, whereas the isolation frequencies of *L. pneumophila* serogroup 1 and *L. pneumophila* serogroups 2–14 shown no differences.

Finally, *L. pneumophila* serogroup 1 was detected more often than *L. pneumophila* serogroups 2–14 in the whirlpools.

3.3. Quantitation of Legionella levels in water samples

The levels of *Legionella* in water samples were calculated by the culture method indicated in the Methods section. The bacterial levels quantified in the analysed facilities are shown in Table 3. Following international standards, the results were classified into three groups: minimal contamination (<3 log CFU L^{-1}), moderate contamination (3–4 log CFU L^{-1}) and high contamination (>4 log CFU L^{-1}) [20]. The average level of *Legionella* in the 140 positive samples from the tourist facilities was 2.26 ± 0.86. This level indicates that, overall, the contamination in the installations can be considered minimal. As a whole, all the species and serogroups investigated shown similar bacterial loads, with no significant differences. However, when *Legionella* non-pneumophila was detected, the contamination was moderate in 31.3% of cases. The few highly contaminated samples (3.6%) were always colonized by *L. pneumophila* species.

3.4. Seasonal distribution of Legionella in tourist facilities

The tourist facilities investigated in the present study work on an all-year opening regimen, opposite to other seasonal opening tourist destinations. Then, no bias was introduced and the distribution of the sampling was regular throughout the study. The seasonal distribution of the positive samples is shown in Fig. 1 as a black line. June was the month with the greatest number of positive samples (9.8%), followed by the winter period covering December–February (7.7–7.9% range).

We decided to determine if there were differences between *Legionella* species and serogroups isolated through the year. For this, the *Legionella* isolates were represented as stacked bars by the month of sampling (Fig. 1). *Legionella pneumophila* was the most frequently detected species throughout the year except in November when L. *pneumophila* sg 1 was not detected and 50% of isolates were non-pneumophila. *L. pneumophila* sg 2–14 was more frequent than *L. pneumophila* sg 1 throughout the year, particularly in February, June, November and December that was unique. Conversely, *L. pneumophila* sg 1 was more frequent in January, July and October.

3.5. Geographical distribution of Legionella in tourist facilities located in the Canary Islands

The water samples investigated in the present study were collected from four different islands of the archipelago: Fuerteventura, Gran Canaria, Lanzarote and Tenerife. We obtained positive samples from all the locations. The average bacterial loads for each island are shown in Table 4. Statistical differences were only found between the values from Gran Canaria and Tenerife (3.39 vs 2.18 log CFU L^{-1}).

The analysis of the colonization rate detected differences between the western islands (Gran Canaria and Tenerife) and the eastern ones (Lanzarote and Fuerteventura) where the presence of *Legionella* was lower. No differences were observed between the western islands,

Table 3
Legionella quantitation in the 140 positive water samples.

	Positives geometric mean count (logCFU L^{-1}) ± SD	Total positive samples	Minimally contaminated (<3 logCFU L^{-1})	Moderately contaminated (3–4 logCFU L^{-1})	Highly contaminated (<4 logCFU L^{-1})
L. pneumophila sg 1	2.20 ± 0.90	50	37 (74.0%)	12 (24.0%)	1 (2.0%)
L. pneumophila sg 2-14	2.30 ± 0.81	74	60 (81.1%)	10 (13.5%)	4 (5.4%)
L. non-pneumophila	2.41 ± 0.79	16	11 (68.8%)	5 (31.3%)	0 (0%)
Legionella spp.	2.26 ± 0.86	140	108 (77.1%)	27 (19.3%)	5 (3.6%)

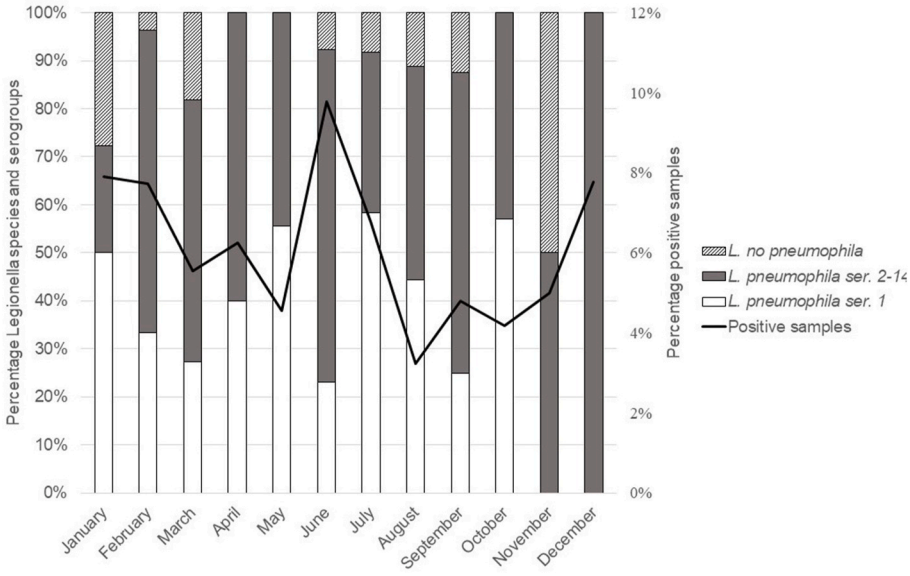

Fig. 1. Seasonal distribution of *Legionella* in tourist facilities. The monthly percentages of positive water samples from hotels located in the Canary Islands are shown by a black line, with values on the right axis. The percentages of the different *Legionella* species and serogroups isolated in those positive samples are represented as stacked bars by the month of sampling, with values on the left axis. The members of *L. pneumoniae* serogroup 1 are represented by white bars, those for *L. pneumoniae* serogroups 2–14 are represented by grey bars, and the non pneumophila species are shown by hatched bars.

Table 4
Geographical distribution of *Legionella* positive water samples.

	Positives geometric mean count (logCFU L^{-1}) ± SD	Total samples[a]	*L. pneumophila* sg 1[a]	*L. pneumophila* sg 2–14[a]	L. non-pneumophila[a]
Fuerteventura	2.12 ± 0.28	5 (1.2%)	5 (1.2%)	0 (0%)	0 (0%)
Gran Canaria	3.39 ± 1.40	4 (11.4%)	1 (2.3%)	3 (8.6%)	0 (0%)
Lanzarote	2.52 ± 0.94	20 (3.5%)	9 (1.6%)	10 (1.8%)	1 (0.2%)
Tenerife	2.18 ± 0.81	111 (8.5%)	35 (2.7%)	61 (4.7%)	15 (1.2%)

[a] Total positive samples (% positive samples in the location).

whereas the *Legionella* colonization rate was lower in Fuerteventura than in Lanzarote ($p < 0.05$). *L. pneumophila* serogroup 1 was detected in all islands. Moreover, all the isolates obtained from Fuerteventura samples belonged to this serogroup. Conversely, *Legionella* non-pneumophila was only detected in Tenerife.

3.6. Relationship between temperature and disinfectant levels in water and Legionella contamination

Treatment of water with disinfectants constitute the main strategy for *Legionella* control in cold WDS. In this study, the free chlorine levels were determined, as the investigated facilities used chlorine derivates in all cases. Spanish legislation establishes that chlorine levels should not drop below 0.2 mg L^{-1} in the WDS [16]. Therefore, we set this value as the reference for the study of the relationship between *Legionella* presence and disinfectant levels (Fig. 2). Our results demonstrate that, overall, *Legionella* colonization was lower when free chlorine levels exceeded 0.2 mg L^{-1}. This fact was even more apparent in the circuit samples. However, no significant differences were detected in the tank samples.

Another factor applied to *Legionella* prevention is the temperature [21]. The chlorine compounds mentioned above are volatile. Therefore, temperature becomes the main tool for *Legionella* control in hot WDS. In that case, temperature should not drop below 50 °C in any part of the system at any time, whereas storing water at 60 °C or higher is recommended [16,17]. We established three ranges of temperature to analyse the relationship between temperature and presence of *Legionella* in hot water: <50 °C, 50–60 °C and >60 °C. The results are shown in Fig. 3. The analysis of the complete set of hot water samples indicated that *Legionella* was more prevalent when the water temperature was lower than 50 °C. This temperature effect continues at higher temperatures, and *Legionella* contamination significantly decreased above 60 °C. The effectiveness of *Legionella* control by temperature was more apparent for

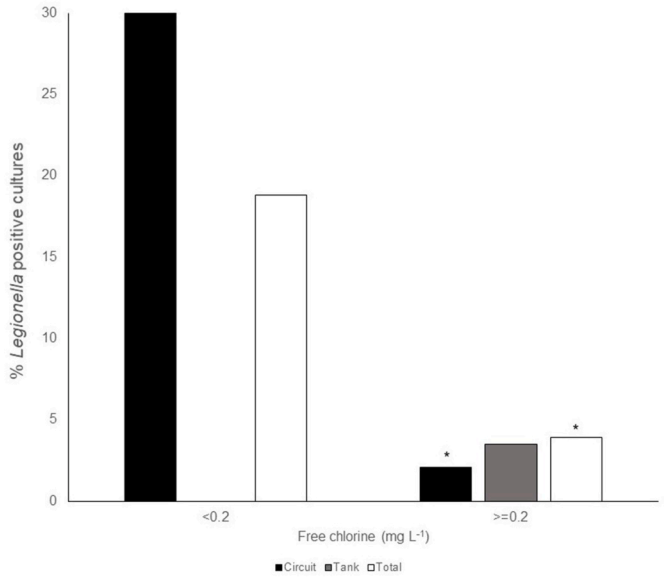

Fig. 2. Relationship between the free chlorine levels in water temperature and *Legionella* contamination. Samples were classified into two groups based on Spanish legislation: <0.2 and ≥ 0.2 mg L^{-1} free chlorine. The results of *Legionella* detection in samples from the circuits are shown in black bars as the percentage of positive cultures; those for the storage compartments or tanks, in grey bars; and the results for the complete set of samples are shown in white bars. The * symbol denotes significant differences ($p < 0.05$) between free chlorine levels.

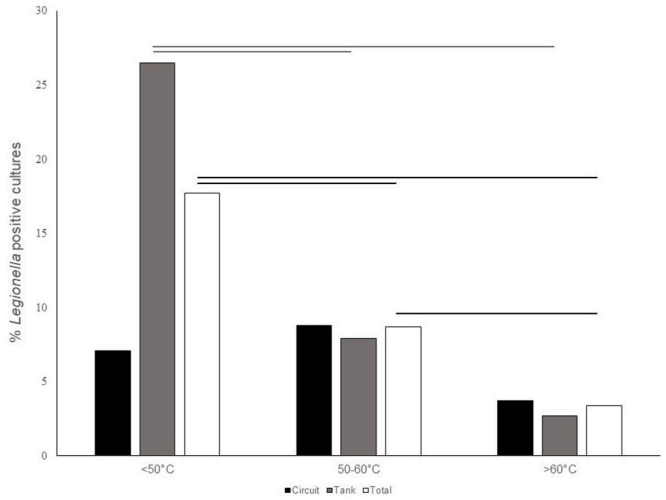

Fig. 3. Relationship between the water temperature and *Legionella* contamination. Samples were classified into three temperature ranges: <50 °C, 50–60 °C and ≥60 °C. The results of *Legionella* detection in samples from the circuits are shown in black bars as the percentage of positive cultures; those for the storage compartments or tanks, in grey bars; and the results for the complete set of samples are shown in white bars. The solid lines denote significant differences ($p < 0.05$) between temperature ranges. The grey lines correspond to tank samples, whereas the black lines correspond to the complete set of samples.

tank samples. However, no effects were observed for circuit samples.

In the case of the cold WDS temperature, temperature must remain under 20 °C to avoid the growth of the pathogen. However, all the samples analysed in the present work were at temperatures higher than 20 °C. In fact, the Spanish legislation changed the mandatory <20 °C level to a recommendation in our legislation due to the impossibility of maintaining it during some periods of the year. Nevertheless, *Legionella* growth rate rises at temperatures above 25 °C, constituting a risk. Thus, like other investigators [14], we also compared the temperature ranges 20–25 °C and >25 °C. However, we did not detect significant differences between installations (data not shown).

4. Discussion

The annual epidemiological reports of the European Centre for Disease Prevention and Control confirmed an important increase in Legionellosis cases in the last years. This fact directly affects Spain, always in the top four of the affected countries both in community and TALD cases. Yet, specific investigations in Spanish hotels remains scarce [22]. In the present investigation, we analysed the presence of this bacterium 204 hotels located in the Canary Islands, an important Spanish tourist destination. A total of 2,318 samples were collected and investigated for *Legionella* contamination, detecting the pathogen in 140 (6.0%) samples (Table 1). This contamination rate is clearly lower than the previously observed in hotels located in Spain (28%) [22] and other European countries like Italy (20–60%) [6,12,23]; the Netherlands (40%) [24]; Croatia (17–30%) [25,26]; or Greece (25–28%) [14,27]. The differences between studies and countries might be explained by some nonexclusive factors, like the number of hotels and samples analysed, the period covered and the evaluated installations mainly focused on hot water facilities. The present investigation includes a large number of samples, a five-year period and includes hot, cold WDS and whirlpools to reinforce our results. Moreover, the hotels were randomly selected, opposite to other studies that introduced a bias by selecting those with previous LD episodes. Therefore, our results constitute a highly consistent picture of the *Legionella* colonization of hotels, at least in our country. A recent study reported a 9.0% *Legionella* contamination when 78 samples from state-owned centres located in the Canary Islands were analysed [28]. Our investigation, with more than 2,000 samples, is clearly more representative.

As stated above, hot WDS constitutes the most investigated facility because of the high risk for *Legionella* dissemination. Our study detected *Legionella* in 8.5% of the hot water samples (Table 1). This contamination level is clearly lower than those reported in Hungary (72%) [29], Italy (42%) [11] or Greece (41%) [14]. The circuits and tanks showed similar values. *Legionella* prevalence in cold water samples was 4.3%, significantly lower than the hot water levels ($p < 0.05$). This observation agrees with the fact that cold water supplies are classified as low-risk installations [5]. Nevertheless, routinely evaluation of these facilities should be performed, as they have been linked to deaths caused by the pathogen. Special care should be taken when risk factors favouring *Legionella* propagation arise. That includes the typical high environmental temperatures in our country. Consequently, our guidelines include routine evaluations (*Guía técnica para la prevención y control de la legionelosis en instalaciones*, 2005), but not those of some temperate climate European countries like Germany [30]. This feature should be kept in mind from now on due to the increase in temperatures by global warming. Finally, the 4.3% *Legionella* contamination in cold WDS is evidently lower than in European hotels (21.4%) [14] and health care facilities (36.3%) [30].

The storing tanks from the hot WDS represent a higher risk than cold water tanks because of their colonization rate (10.6 vs 3.3%, $p < 0.05$). Then, here we recommend specific interventions like temperature increasing (see below) and performing the cleaning and disinfection more frequently. Nowadays, following current guidelines, this maintenance is performed in a yearly basis (*Guía técnica para la prevención y control de la legionelosis en instalaciones*, 2005).

We also analysed whirlpools for *Legionella* presence. To mention, these installations are present in several hotels. However, they are usually not included in surveys. Unexpectedly, the contamination rate in our samples was 4.7%, undoubtedly lower than the 50%, 75% or even 85% previously described [14,23,31]. Therefore, our results indicate that these pools may be considered low-risk in the hotels located in the Canary Islands.

The diversity of *Legionella* species and serogroups recovered in our survey depended on the installations, but in all cases, *L. pneumophila* was the most prevalent species (Table 2). Overall, the serogroups 2–14 were the most frequently isolated, as described in other European surveys [6,11,13,14,23,32,33] and state-owned buildings in the Canary Islands [28]. However, *L. pneumophila* serogroup 1 was predominant in one study in Turkey [34]. Curiously, we detected this serogroup as the predominant in the whirlpools from hotels in the Canary Islands. We also compared the bacterial loads in water samples for the different species and serogroups, and similar levels were detected. Most of the samples were minimally contaminated, and high contamination was only detected in a few cases and only for *L. pneumophila*.

The analysis of the seasonal distribution of *Legionella* prevalence detected a clear peak in June, followed by the winter months. Previous studies reported peaks usually at the end of the summer [23,35]. The distribution of species and serogroups also differed. According to their higher isolation, *L. pneumophila* sg 1 and 2–14 were the more frequently recovered in all months. However, an unexpected 50% of non-pneumophila isolates were obtained in November, when no serogroup 1 isolates were obtained. That particular case, i.e., an important increase of *Legionella* non-pneumophila prevalence associated with a significant decrease of *L. pneumoniae* sg 1, was also observed in a recent study in March isolates [28]. Interestingly, in our study, all December isolates belong to the *L. pneumophila* sg 2–14. As for the geographical distribution, bacterial loads were overall similar in the four analysed islands. However, the colonization was clearly higher in the western islands. *L. pneumophila* sg 1 was the only group detected in all islands, whereas *Legionella* non-pneumophila was only detected in Tenerife.

We decided to investigate the role of free chlorine and temperature on *Legionella* prevalence, as they are well known related factors. Our results reveal a higher prevalence in samples $\leq$0.2 free chlorine mg L^{-1} (the legal reference). Similarly, the study of Kyritsi et al. found that samples with free chlorine <0.375 mg L^{-1} were more often contaminated by the pathogen [14]. As for the temperature, all the samples were above the 20 °C limit, and no comparison could be made with higher temperatures. To avoid that, interventions like proper isolation avoiding the cold water heating by the high environmental temperatures or hot WDS proximity should be implemented to improve *Legionella* control. In the case of hot water samples, the prevalence of *Legionella* was higher for temperatures <50 °C. As *Legionella* resists these temperatures, this is an expected risk. Diverse studies have analysed the temperature effects in hot WDS [36], with some differences. Depending on the study, protective effects are set either at $\geq$55 °C or $\geq$60 °C. In any case, we have seen that *Legionella* colonizes the hot water installations even at $\geq$60 °C temperatures, suggesting that higher temperatures are required to eradicate it from these installations.

5. Conclusions

We characterized the presence of *Legionella* in hotels from the Canary Islands, Spain. Different installations were broadly analysed, and the levels of colonization were clearly lower than previously described in other European countries. Moreover, they generally constitute minimal contaminations. Nevertheless, permanent monitoring is still needed to ensure safe installations and the risk assessment should progress. Based on our results, we recommend specific measures to progress in *Legionella* control in particular installations, like increasing the temperature of hot WDS and the frequency of their cleaning and disinfection, together with cold WDS circuits isolation.

Credit authorship contribution statement

Antonio Doménech-Sánchez: Conceptualization, Methodology, Investigation, Writing - original draft, Supervision, Project administration, Funding acquisition. Elena Laso: Investigation, Writing - review & editing. Sebastián Albertí: Conceptualization, Methodology, Writing - review & editing, Supervision.

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