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Environmental surveillance of *Legionella pneumophila* in hot water systems of hotels in Morocco

Abdelwahid Assaidi[a,b], Afaf Soummane[b], Mostafa Ellouali[a], Hassan Latrache[a], Mohammed Timinouni[b], Hafida Zahir[a] and El Mostafa Mliji[b,*]

[a] Laboratory of Bioprocess and Biointerfaces, Faculty of Sciences and Technics, Sultan Moulay Slimane University, Beni Mellal, Morocco
[b] Laboratory of Water Microbiology and Environmental Hygiene, Institut Pasteur du Maroc, Casablanca, Morocco
*Corresponding author. E-mail: mostafa.mliji@pasteur.ma

ABSTRACT

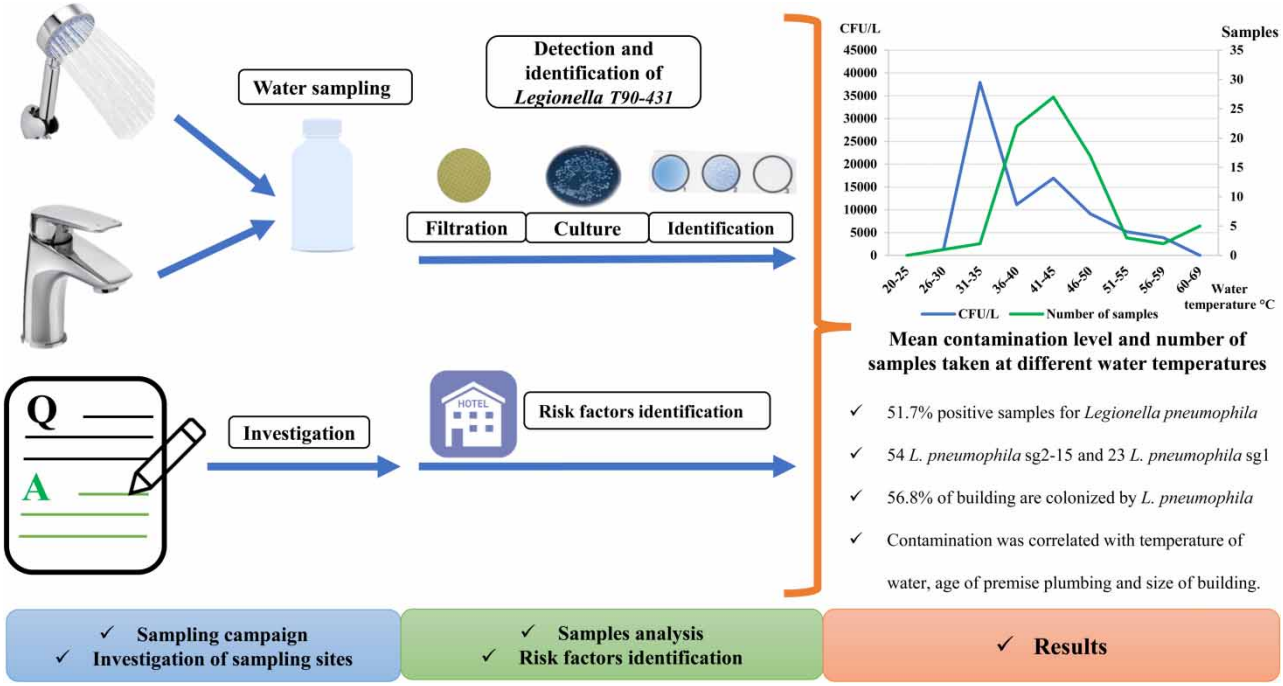
Objective: Environmental monitoring of *Legionella* in hot water systems of hotels in Morocco was performed during the period from January 2016 to April 2018. A total of 149 water samples from 118 different hotels were analyzed. *Methods*: A total of 149 water samples from 118 different hotels were analyzed. Possible risk factors were prospectively recorded, and data were analyzed in connection with building and plumbing systems characteristics. Data about building and risk factors were collected through a questionnaire survey. *Results*: Out of the 149 samples, 77(51.7%) were positive for *L. pneumophila*. Serological typing of the isolates revealed that 54 (70.1%) are *L. pneumophila* serogroup 2–15 and 23 (29.9%) are *L. pneumophila* serogroup 1. 56.8% of all buildings were colonized by *L. pneumophila*. Counts were over 1,000 CFU/L in 44%. Contamination was strongly correlated with temperature in the circulation, the age of the premise plumbing and the size of the building. *Conclusions*: The results showed a relevant exposure to *L. pneumophila* in the community and the identified risk factors can serve as indicators for risk assessment and relevant actions.

Key words: building, environmental monitoring, infectious diseases, *Legionella pneumophila*, water-related infection

HIGHLIGHTS

- Travel and stays in accommodation sites will continue to represent a significant cause of travel-associated LD, especially in a region of increasing travel to countries that may not have the expertise or resources to prevent or reduce the risk of exposure to *Legionella* species.
- *Legionella* in Moroccan hotels is a major concern still poorly investigated.
- *Legionella* found in 51.7% of analyzed samples and 56.8% of investigated buildings.
- Temperature in the circulation, age of the premise plumbing, and the size of building were positively associated with contamination.
- The results showed a relevant exposure to *L. pneumophila* in the community and the identified risk factors can serve as indicators for risk assessment and relevant actions.

GRAPHICAL ABSTRACT



INTRODUCTION

Legionnaires' disease (LD) is often associated with travel and with staying in hotels (Beauté 2017), as was the case in the 1976 outbreak in Philadelphia; the first report of an LD outbreak which occurred after the discovery of 182 cases including 29 fatal cases affecting a group of persons attending the 1976 American Legion convention in a hotel (Fraser *et al.* 1977). Soon after, the etiologic agent of LD was identified as a fastidious gram-negative bacillus and named *Legionella* in reference to the Philadelphia convention and legionnaire victims of this first outbreak (McDade *et al.* 1977). The infection is transmitted by inhalation of aerosols generated from water systems such as cooling towers, hot water systems, showers, spas and faucets, or by aspiration and direct instillation into the lung during respiratory tract manipulations (Fields *et al.* 2002). Person-to-person transmission has not been reported (Guyard & Low 2011). In fact, there are two distinct clinical syndromes: LD and Pontiac fever; LD accounts for 2–9% of cases of community-acquired pneumonia, it can be a severe pneumonia that may be accompanied by systemic symptoms such as fever, chills, headache, myalgia, and impaired renal and liver functions (Massoni *et al.* 2013). Pontiac fever is a self-limited, influenza-like illness of short duration, without pneumonia and no record of fatal cases (Guyard & Low 2011). There is no single clinical manifestation that distinguishes LD from other types of pneumonia. Host-related risk factors for LD include increasing age, smoking, male sex, chronic lung disease, diabetes, lung cancer, and immunosuppressive treatment (Fields *et al.* 2002; Farnham *et al.* 2014). The highest number of cases occur in older people (74–90% of patients $\geq$50 years) and predominantly in men (Phin *et al.* 2014). Most cases of LD are community-acquired, followed by travel-associated and nosocomial pneumonia (Vaccaro *et al.* 2016).

The genus *Legionella* consists of almost 53 species and 70 serogroups (Lück *et al.* 2010). *L. pneumophila* is the major cause of outbreaks (91.5%) and serogroup 1 (sg1) is the predominant serotype (84.2%) (Guo *et al.* 2015). *Legionella* species are found worldwide in natural and artificial water systems. They are frequently detected in water distribution systems of residentials facilities and institutions such as hotels, hospitals, and health resorts, to which they could adhere to various surfaces, survive within biofilms and free-living amoebae (Kuroki *et al.* 2017). These bacteria are present at the highest concentrations in biofilms within hot water systems and openings of water outlets; biofilms constitute a protective niche against water treatments procedures and stressful conditions. Stagnating warm water and water temperature between 20 and 45 °C provide an ideal habitat for massive growth of this bacterium (Assaidi *et al.* 2018a). Previous studies showed high isolation rates of *Legionella* spp. in building and accommodations sites worldwide, with 43.6% in Kuwait (Al-Matawah *et al.* 2012), 32.7%

in Germany (Kruse *et al.* 2016), 31.5% in Iran (Rafiee *et al.* 2014), 8.3% in South Korea (Hwang *et al.* 2016), 84.1% in Italy (Mazzotta *et al.* 2021), 74.77% in Poland (Sikora *et al.* 2015), and 34.1% in Japan (Kanatani *et al.* 2017). Several studies in European countries have reported a high prevalence of *Legionella* spp. in hot water systems in hotels, hospitals, and domestic buildings (Leoni *et al.* 2005). These studies demonstrate the importance of survey, monitoring, and control of *Legionella* colonization in hot water systems, while little is known about the occurrence of *Legionella* and contamination of hot water systems in Moroccan hotels.

Actually, in Morocco, there is no regulation for environmental surveillance of *Legionella* and most buildings are operated without the recognition and management of *Legionella* risk. Therefore, no national limit of intervention has been published (Mouchtouri *et al.* 2007; Bulletin 2017). However, the European Working of *Legionella* infections (EWGLI) define 1,000 colony forming units (CFU)/L as a limit value of public health concern, counts below 1,000 CFU/L indicate that the system is under control; if it exceeds this limit in over half of the samples, intervention is necessary. Immediate intervention is obligatory in case of *Legionella* counts over 10,000 CFU/L (EWGL Technical Guidelines, 2011).

The majority of recognized LD outbreaks are associated with travel and hotel stays. The European Working Group for *Legionella* Infections (EWGLI) and the USA Centers for Disease Control and Prevention (CDC) have identified numerous cases of travel-associated LD; the most common source of infection has been contaminated water in hotels (Beauté 2017; Shah *et al.* 2018). In the present study, we performed an environmental monitoring in Moroccan hotels; the purpose was dual: firstly, determine *Legionella* prevalence in hot water systems of hotels in Morocco and, secondly, investigate the risk factors associated with contamination which are suspected to be related to the presence and/or growth of *Legionella*, because those parameters can feed into an initial or preliminary risk assessment.

METHODS

Investigation of sampling sites: risk factors investigation

Our investigation includes the visual inspection of the boiler rooms, water networks conception, and hygiene. A detailed questionnaire survey was developed to collect the maximum data about the building (age, size), potable water source, hot water production (the heating systems, tanks and their volume, existence softening) and premise plumbing characteristics (plumbing materials, age of the systems, main disinfection, water operating temperature). The presence of additional risk sources (e.g., decorative fountain, cooling tower, air-conditioning, humidifiers) was also investigated.

Water sampling approach

In total, 149 water samples were taken from hot water systems from 118 hotels in the main tourist cities in Morocco during the period from January 2016 to April 2018. The samples were obtained from showerheads and faucets in rooms that were located furthest from the hot water source and the number of collected samples was determined by the size of each hotel. The samples were collected after the water had drained for 15–30 s in a sterile 1-litre plastic bottle containing 1 mL of 0.1 N sodium thiosulfate to neutralize residual free chlorine. The collection of samples was performed by trained staff according to standard methods.

Detection of *Legionella* by the standard culture method

Samples were analyzed in the laboratory using microbiological culture and identification methods according to T90-431 (November 2014) standard 'Detection and enumeration of *Legionella* spp. and *Legionella pneumophila* – Method by direct inoculation and after concentration by membrane filtration or centrifugation'. The isolates were serologically identified using a commercially available Latex agglutination (SLIDEX LEGIONELLA KIT, BioMérieux, France).

For all buildings, the level of *Legionella* contamination was determined. The different levels of contamination are presented in Table 1 (EWGLI Technical Guidelines 2011). Then, the proportions of positive samples and the implications of a number of factors suspected to be associated with contamination were investigated.

STATISTICAL ANALYSIS

Data analysis was performed by two-way analysis of variance (ANOVA). Tests were two-tailed, with $\alpha = 0.05$. All analyses were performed using SPSS 13.0.

Table 1 | Definition of levels of contamination with *Legionella* in potable water (Kruse *et al.* 2016)

Level of *Legionella* contamination	*Legionella* spp. concentration (CFU/100 mL)
No or low level of concentration	≤100
Medium level of concentration	>100 and ≤1,000
High level of contamination	>1,000 and ≤10,000
Extremely high level of concentration	>10,000

RESULTS

Colonization by *Legionella*: levels of contamination and species distribution

A total of 149 water samples taken from 118 different hot water systems of hotels were analyzed. Out of the 149 samples, 77 (51.7%) were positive for *L. pneumophila*. Serological typing of the 77 *L. pneumophila* isolate revealed that 54 (70.1%) are *L. pneumophila* serogroup 2–15 and 23 (29.9%) are *L. pneumophila* serogroup 1 (Table 2).

Among the 77 positive samples, 13 (16.88%) samples contained a bacterial load between 10 and 10^2 CFU/L, 36 (46.75%) samples were in the range of 10^2–10^3 CFU/L, and 28 (36.36%) samples contained between 10^3 and 10^4 CFU/L (Table 2). Therefore, 64 (83.11%) counts exceeded 1,000 CFU/L. The highest counts observed in the present study were over 7.5×10^4 CFU/L.

L. pneumophila was isolated from 77 of 149 hot water samples (51.7%) derived from 67/118 hotels (56.8%) (Tables 2 and 3). Regarding the contaminated hotels, the highest detected count exceeded the limit of public health concern (1,000 CFU/L) in 58/67 (86.5%) buildings. Moreover, 41/67 (61.19%) of hotels had a medium or higher level of contamination and 26/67 (38.8%) had an extremely high level of contamination (Table 3) requiring legally mandated disinfection measures. The hotel rating ranged from three to five stars, and it was negatively associated with the extent of *Legionella* occurrence.

Sampling sites characteristics

The majority of showers were manual mixers, from which 8% were supplied with hot water from the electric tank and instantaneous heater, 74% from fuels boiled with integrated tanks and 16% from fuels boiled with separate tanks (Table 4).

Coldwater was supplied from the mains for all showers. The mean age of the building was 14 years, but the water pipes of some of them had been partially renovated over the years. According to the data that were collected through the questionnaire interviews, 35% of the hotels were more than 15 years old, 25% were from 10 to 15 years old, and 40% had been constructed in the last 10 years. All the hotels had an independent water heating system. There was no recent mention of water leakage or disruption of the water supply.

Based on questionnaire results, seven plumbing materials were commonly used in water distribution systems as follows: polypropylene random copolymer (PPR), galvanized steel, polyvinyl chloride (PVC), cross-linked polyethylene (PEX-c), chlorinated polyvinyl chloride (CPVC) stainless steel and copper (stainless steel and copper are used partly in the pipes of some studied buildings).

Our investigation shows that the most implicated risk factor of contamination was the temperature of water in circulation (Figure 1). Mean contamination levels were highest for temperatures between 31 and 40 °C, and still twice as high for temperatures between 46 and 50 °C as for temperatures between 51 and 55 °C (Figure 1). The temperature was associated both with the level of contamination and the proportion of positive samples. Moreover, the size of the building (as measured in the numbers of floors) was correlated with the level of contamination.

Table 2 | Level of *Legionella pneumophila* contamination in analyzed samples ($n = 149$)

	Number of samples						
		Maximum *Legionella* count (x) in the sample (ranges as CFU/L)				Identification	
Samples	Negative $x < 10$	$10 \leq x < 10^2$	$10^2 \leq x < 10^3$	$10^3 \leq x < 10^5$	Total positive	Lpn sg1	Lpn sg2–15
Samples ($n = 149$)	72 (48.3%)	12	36	29	77 (51.7%)	23 (29.9%)	54 (70.1%)

Table 3 | Level of *L. pneumophila* contamination in the hot water system of the investigated hotels ($n = 118$)

	Number of facilities						
		Maximum *Legionella* count (x) in the facility (ranges as CFU/L)				Identification	
Building	Negative $x < 10$	$10 \leq x < 10^2$	$10^2 \leq x < 10^3$	$10^3 \leq x < 10^5$	Total positive	Lpn sg1	Lpn sg2–15
Hotels ($n = 118$)	51 (43.2%)	09	32	26	67 (56.8%)	21 (31.34%)	50 (74.62%)

Table 4 | The effect of environmental factors on *L. pneumophila* colonization in the investigated hot water systems

		Numbers of water samples (x = *Legionella* colony count CFU/L)			
Parameters	Variable	Negative	$10 \leq x < 1{,}000$	$x \geq 1{,}000$	Total
Class of the hotel	3 Star	12 (67%)	0 (0%)	6 (33%)	18 (17%)
	4 Star	32 (43%)	07 (9%)	35 (47%)	75 (64%)
	5 Star	12 (48%)	04 (16%)	09 (36%)	25 (19%)
Age of the premise plumbing	Older than 10 years	31 (52%)	05 (08%)	22 (37%)	60 (62%)
	10 years old or younger	25 (43%)	05 (09%)	28 (48%)	58 (38%)
Type of plumbing materials	Galvanized steel	17 (44%)	02 (5%)	20 (51%)	39 (33%)
	PVC	06 (50%)	00 (0%)	06 (50%)	12 (10%)
	PPR	27 (52%)	06 (12%)	16 (31%)	52 (44%)
	PEX-c	04 (37%)	01 (09%)	06 (55%)	11 (09%)
	CPVC	02 (50%)	00 (0%)	02 (50%)	04 (04%)
Type of production	Instantaneous heater without tank	02 (67%)	01 (33%)	00 (0%)	03 (0%)
	Heater with integrated tank	51 (49%)	09 (9%)	45 (43%)	105 (17%)
	Electric tank	03 (33%)	02 (22%)	04 (45%)	09 (75%)
Hot water temperature	Normal temperature (≥55 °C)	12 (86%)	01 (07%)	01 (07%)	14 (17%)
	Critical temperature (<55 °C)	59 (44%)	11 (8%)	65 (48%)	135 (83%)
Number of hot water storage tanks	Less than 3	20 (49%)	02 (5%)	19 (46%)	41 (35%)
	3 or more	40 (52%)	07 (9%)	30 (39%)	77 (65%)
Volume of the stored hot water	<3 m^3	32 (46%)	05 (7%)	33 (47%)	70 (59%)
	≥ 3 m^3	26 (54%)	04 (8%)	18 (38%)	48 (41%)
Orientation of the tank	Vertical	48 (59%)	9 (11%)	25 (30%)	82 (70%)
	Horizontal	15 (43%)	01 (3%)	19 (54%)	36 (30%)
Softener	Used	39 (44%)	11 (13%)	38 (43%)	88 (74%)
	Not used	18 (60%)	08 (27%)	04 (13%)	30 (26%)
Treatment	Yes	17 (40%)	05 (12%)	20 (48%)	42 (36%)
	No	40 (53%)	36 (47%)	06 (09%)	76 (64%)

Colonization is characterized as the number of samples with *L. pneumophila* count in the following categories: <10 CFU/L (negative), 10–1,000 CFU/L (low risk), and >1,000 CFU/L (high risk).

Regarding the time of the year when the sample was taken and the transport time to the laboratory, they were not associated with either the percentage of positive samples or the level of contamination. Neither was the place where the samples were taken (showers, outlets in the bathroom, or boilers). Plumbing networks in buildings are complex structures, where the distance between the boiler room and the distant taps can amount to hundreds of meters. In fact, the temperature can be decreased at farther points from the boiler room which allows *Legionella* colonization and proliferation.

DISCUSSION

In water distribution systems, *Legionella* represents a potential source of water contamination, resulting in an important number of infections with high mortality levels (Bartram 2007). Previous studies have reported a direct link between the

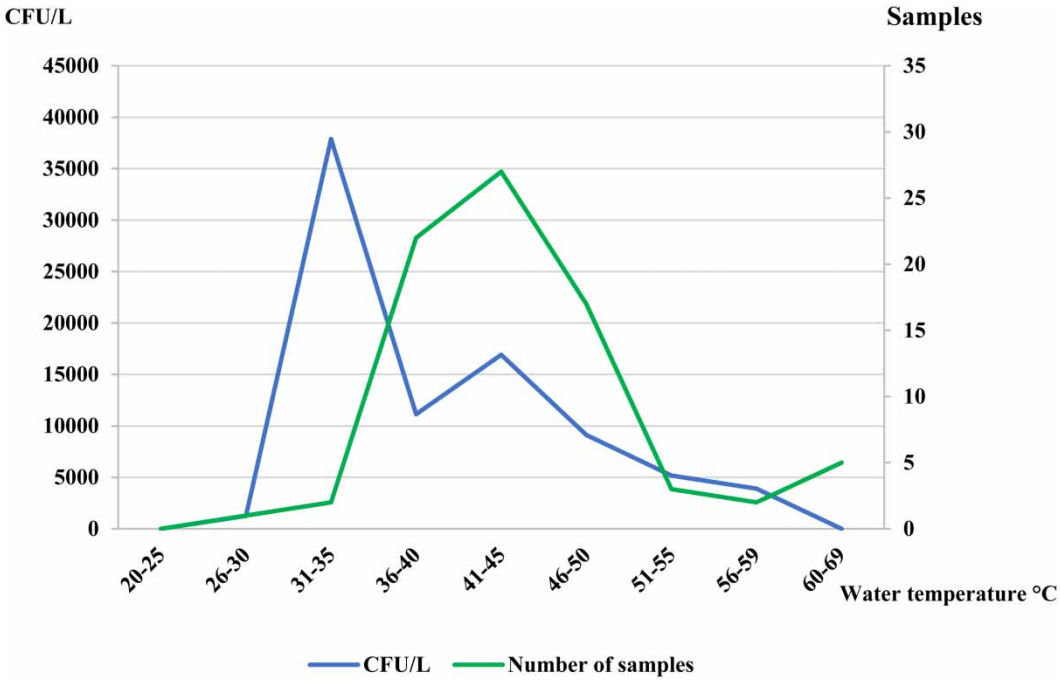


Figure 1 | Mean contamination level (colony forming units of *Legionella*/L.) (blue line) and the number of samples taken (green line) at different water temperatures. Please refer to the online version of this paper to see this figure in colour: https://doi.org/10.2166/wh.2021.175.

colonization of *Legionella* in building plumbing systems and the occurrence of infection (Fragou *et al.* 2012). Plumbing networks are among the recognized sources of infection and provide multiple favorable conditions for the development of biofilm and *Legionella* (Buse *et al.* 2012). Biofilm offers protection against disinfection and can harbor amoebas, a growth vector for *Legionella* (Cateau *et al.* 2014; Boppe *et al.* 2016). Moreover, some reconstruction works tend to provide optimal growth conditions for *Legionella* (Bargellini *et al.* 2011; Barna *et al.* 2016).

Nowadays, there is no regulation for environmental *Legionella* monitoring in Morocco. In fact, the majority of the Moroccan hot water systems are exploited without any awareness of *Legionella* risk. For this reason, we investigated the prevalence of *Legionella* isolated from hot water systems in Moroccan hotels. This work is the first baseline analysis in Morocco to identify those risk factors that are easily observed or measured by building operators and thus are associated with water contamination and *Legionella* proliferation.

L. pneumophila is widely distributed in hot water systems (outlets and showers). In our study, *L. pneumophila* was isolated in 51.7% of hot water samples, with a mean number of *L. pneumophila* in positive samples of 7.6×10^3 CFU/L; the highest number of viable *L. pneumophila* cells was 7.5×10^4 CFU/L.

Colonization rates were systematically higher than those observed in other parts of the world: 44.7% in Italy (Napoli *et al.* 2010), 43.6% in Kuwait (Al-Matawah *et al.* 2012), 32.7% in Germany (Kruse *et al.* 2016), 31.5% in Iran (Rafiee *et al.* 2014), 8.3% in South Korea (Hwang *et al.* 2016), and 7.8% in Turkey (Erdogan & Arslan 2007). According to several studies, *L. pneumophila* (predominantly serogroup 2–15) is the most abundant species in hot water samples (Barna *et al.* 2016; Kanatani *et al.* 2017). Based on these results, there is a risk of potential exposure to *Legionella*: in two of the five buildings tested, at least a medium level of contamination was detected, from this level additional measures must be implemented such as disinfection and/or renovation of the water system.

LD is severely underdiagnosed and underreported in Morocco as in other countries in Africa. The data about the rate of the disease are not available. Worldwide, the true incidence is largely unknown due to underdiagnosis and underreporting (Kruse *et al.* 2016), however, (Roysted *et al.* (2016) have reported that *Legionella* was identified from 6% of patients hospitalized for community-acquired pneumonia.

Furthermore, the infectious dose for *Legionella* remains uncertain (Whiley *et al.* 2014); most outbreaks have been reported for contamination levels high than 1,000 CFU/L, but it should be noted that is aerosol exposure that mainly constitutes the

risk for LD (Hines *et al.* 2014). In buildings, this would mostly derive from the use of showers, vapor from hot water and cooling towers. Moreover, the risk of infection depends also on several factors including population at risk, water system conception, differences of virulence among *Legionella* species, and the intensity of exposure (Erdogan & Arslan 2007).

Indeed, for LD and water contamination, a certain seasonality can be observed as previously reported (Rodriguez-Martinez *et al.* 2015). No relationship was found between the month or season when the sample was taken and the level of contamination. This may suggest that the influence of the season on disease incidence arises from factors other than level of contamination, like differences in water use patterns, population susceptibility, pathogen characteristics, or from other sources of exposure apart from the home (van Heijnsbergen *et al.* 2015). In contrast, (Borella *et al.* (2004) have reported that contamination was consistent throughout the year, both in terms of bacteria concentration and isolated *Legionella* species, suggesting that the occurrence of LD most often in the summer is not necessarily related to higher water contamination.

Thus far, no correlation was found between the hotel rating (number of stars) and *Legionella* prevalence. Also, the relevant influence of the transport time from the sample site to the laboratory has been discussed (Flanders *et al.* 2014); no such influence was found in our study. Exceedingly long transport times (>24 hours) did not occur, and transport times longer than 8 h were very rare.

Previous research has reported that the contamination of plumbing networks is mostly driven by water temperature, as the distance between the boiler room and the distant taps in some buildings can amount to hundreds of meters (Erdogan & Arslan 2007; Kruse *et al.* 2016). In accordance with these reports, the temperature in circulation in our study was associated both with the level of contamination and the proportion of positive samples; it was the primary risk factor of colonization with *Legionella* (Figure 1).

Several studies have reported that the growth and the proliferation of *L. pneumophila* are influenced by many factors, such as pipe materials, temperature, stagnation, flow circumstances, corrosion, and pipe roughness (Assaidi *et al.* 2018a, 2018b). In our study, the aggravating effect of the pipe materials on colonization was also confirmed. We have found that the contamination was also correlated with the type of plumbing materials (Table 4), which indicates that the choice of the most appropriate pipe materials is also fundamental.

Although water systems provide optimal conditions for *Legionella* growth and proliferation, only a few reports have found a correlation between the building characteristics (size and age of the building or the plumbing network) and *Legionella* occurrence (Borella *et al.* 2004; Mazzotta *et al.* 2021). According to previous investigations, the age of premise plumbing and the size of the building were positively correlated with the presence of *Legionella* (Table 4). All factors discussed above are preliminary indicators of the infection risk; however, the virulence of the *Legionella* strains present in the system, the potential means of exposure and the immune status of the exposed population should be taken into consideration.

To limit *Legionella* colonization, we suggest simple and general measures: maintaining high cleaning standards, periodically replacing components of the system which could favor presence or proliferation of bacteria, and increasing the temperature of water systems periodically above 50 °C.

Travel and stays in accommodation sites will continue to represent a significant cause of travel-associated LD, especially in a region of increasing travel to countries that may not have the expertise or resources to prevent or reduce the risk of exposure to *Legionella* species. The growing importance of national and international tourism and the significance of morbidity and mortality of hotel-associated LD justify the attention given to this issue by Moroccan tourism authorities and the medical community in recent years.

CONCLUSION

Our study is an initial overview of *Legionella* occurrence and colonization in plumbing systems and the associated risk factors. Any given system can harbor the bacteria, so routine and continuous control procedures should be implemented to minimize the risk of *L. pneumophila* colonization and infection. Our observations could serve as a basis for the regulatory recommendations for the monitoring and management of environmental *Legionella* risk in buildings.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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