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Comparative assessment of *Legionella pneumophila* prevalence among hospitals and hotels water systems

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ABSTRACT

Legionella pneumophila (*L. pneumophila*) is an opportunistic waterborne pathogen which can be transmitted to humans. Transmission to humans mainly happens through the inhalation of contaminated aerosols which can cause Legionellosis and public health concerns. The present cross-sectional study provides the first *L. pneumophila* contamination assessment in Jordan, aimed at estimating the presence as well as the extension of *L. pneumophila* contamination levels using a culturing method. In which, 183 samples of drinking water were collected from four hospitals and five hotels in Jordan. Statistical comparative assessment between the two sectors, tourism and healthcare, was conducted. *L. pneumophila* was detected in 64% of the collected water samples, of which the greatest level detected reached 1.26×10^5 colony forming units per liter (cfu/L). The contamination rate and concentration of *L. pneumophila* among the hotels sampled were significantly higher than the samples taken from the hospitals sector, 85% and 50%, respectively. The mean of colony-forming units among the samples that tested positive from hotels was 9.0×10^3 cfu/L where in hospitals – 6.5×10^3 cfu/L. Fifty-one percent of samples that tested positive also exceeded 1,000 cfu/L, the level conducive to infection. The seasonal effect on contamination level and frequency were found to be the strongest during spring, followed by autumn, 76% and 73%, respectively. The findings of the present study provided significant data to the water safety stakeholders, and confirmed the need to undertake microbiological surveillance of water systems in hospitals and hotels on regular basis.

Keywords: Legionella; Plate culture; Potable water; Water system; ISO 11731; Jordan

1. Introduction

Legionella are aerobic, non-spore-forming, unencapsulated, gram-negative, and small rods bacteria. They exist mainly in aquatic environments and are able to tolerate a temperature range of 0°C–63°C. Additionally, they are more resistant to traditional water treatments and disinfectants [1,2]. Even though 60 species of *Legionella* have been identified, *L. pneumophila* is considered the causative agent of *Legionellosis* (either pontiac fever with flu-like symptoms, or a pneumonic condition known as *Legionnaires*) [3]; therefore, it has been identified as a serious waterborne pathogen. Inhalation of aerosol particles contaminated with *L. pneumophila* is a significant source of public exposure and infection [2,4]. However, outbreaks due to *Legionella* spp. have not been reported in Jordan to date because the detection of the antigen in urine is not routinely performed in patients with pneumonia. Similarly, few published studies

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and data reported contamination, or prevalence thereof, of *Legionella* in the Jordanian water systems [5].

Jordan is ranked second in the world in water scarcity with demand rising rapidly due to a growing population with expectations of higher living standards [6–13], and with the overwhelming pressure on water and sanitation as a result of hosting over one million of refugees in Jordan since 2011 [14–19]. Additionally, observance of the quality and safety of potable water increases the economic burden and challenges of achieving a balance between the increasing demand and limited supply in this sector. Water chlorination is a common action of water disinfection in Jordan [20]. However, chlorine dissipates in the presence of organics and generates toxic by-products (i.e., trihalomethanes) in water distribution systems in Jordan [21–23]. Hence, chlorine effectiveness in controlling the biofilms-dispersing bacteria is reduced. However, many factors are reported to affect *Legionella* survival such as water temperature, pH, metal contents, presence of other microorganisms, and accumulation of sediment [2,3,24].

In this context, an accurate estimation of *Legionella*'s contamination level as a potential source of pollution through regular monitoring could, in turn, become a gateway for management and prevention. Conversely, overestimation and underestimation might increase the economic costs significantly due to unnecessary or ineffective treatments and/or public health consequences [24]. Several factors influence the sampling frequency and the number of samples in the local regulatory requirements. For instance, some of these factors are the share of the population that benefits from the water system, the risk level and presence of immunocompromising cases, and the condition of water system and facilities [25,26].

In the United States, between 2011 and 2012, *Legionella* was linked to 66% of outbreaks in addition to 26% of illness [27], however, the situation in developed countries differs from that of developing countries where the association between contamination level and Legionellosis could not be easily confirmed. However, other waterborne pathogen causing diarrhea and gastrointestinal disease still occupy the forefront of water quality control. Moreover, according to international and local guidelines [3,28–30], the colonization limit of public health concerns for *L. pneumophila* is 1,000 cfu/L, at which point interventions need to be taken.

Early in 2017, the Jordanian Ministry of Health has circulated a new instruction to control and manage *Legionella* contamination, as per these regulations, all hotels, tourist accommodation and health care facilities must monitor their water system every 3 months with respect to *L. pneumophila* contamination testing in a certified laboratory. Accordingly, in the event of any positive test results, the accredited laboratory must notify the Ministry of any results of positive cultured samples exceeding the maximum limit of 1,000 cfu/L, in accordance with the instructions in circulation. The Ministry of Health has not yet issued any formal regulation, law or law to formalize the above-mentioned instructions and place them in the operational management system. Notwithstanding, in October 2018, a hotel in the Dead Sea was evacuated over risks of contaminated water after detecting in its water system the *Legionella* bacteria [31].

In this study, we investigated the prevalence of *L. pneumophila* in drinking water samples collected from several hospitals and hotels in Jordan. Furthermore, the objective of the present study is to assess the differences between the tourism and health sectors (as represented by water samples taken from hotels and hospitals) regarding the prevalence of *L. pneumophila* infection, and explore the seasonal variations effect.

2. Materials and methods

2.1. Samples collection

Over a period of 12 months, from August 2017 to end of July 2018, 183 potable water samples were collected randomly from the facility's entire plumbing system (sampling at water wells (cisterns) and storage tanks, hot and cold water distribution systems, and outlets including showerheads and taps). Four hospitals and five hotels located in Amman were selected for the purpose of the present study. The temperature of water sources were measured on-site. All samples were collected in 1 L sterile Duran bottles containing 10% sodium thiosulfate that was used to neutralize up to 5 mg/L residual chlorine [32]. The samples' bottles were transported to the Microbiology Laboratory at the Water, Energy and Environment Center (WEEC) at the University of Jordan, which is internationally accredited in ISO 17025, and all samples were analyzed in duplicate (sample is filtered twice) within 6 h of collection.

2.2. Culturing and enumeration of L. pneumophila

L. pneumophila was isolated by membrane filtration according to ISO 11731–2017 (Water quality—Enumeration of *Legionella*). Under laminar flow cabinet, 200 mL of thermally treated sample (in water bath 50°C for 30 min) was concentrated by filtration through 0.22 μm cellulose nitrate filters (Sartorius, Germany) by funnel filtration unit (Millipore, France). Subsequently, 30 mL of acid (0.2 M HCl–KCl, pH 2.2) was added on the top of the membrane and removed 5 min later by washing with 20 mL sterilized normal saline solution. This treatment was employed to eliminate the *Legionella*'s micro competitors and enhance recovery [33,34]. In the next step, the membrane filter was placed on a plate of selective GVPC media (CM0655, SR0110, SR0152, OXOID, UK) and then incubated for 7–10 d at 36°C ± 2°C (the actual incubation reading reading according to the monitoring sheets ranged from 34°C to 36.5°C). The plates were monitored daily to observe the colonies' growth. Colonies of *Legionella* were counted after 7 and 10 d of incubation.

2.3. Legionella identification and calculation

Legionella latex test (DR0800M, OXOID, UK) has been used according to manufacturer's instruction to recognize the serogroup type of *Legionella* isolates. This agglutination test identifies *L. pneumophila* serogroup 1 (SG1), serogroup 2-14 (SG2-14), and other seven *Legionella* species. Consequently, the number of colony forming units of *Legionella* per Liter of sample was estimated by dividing the total number of confirmed *Legionella* colonies by the volume filtered in

milliliters, then multiply by the reference volume chosen in milliliters (which is 1,000 mL for 1 L), as shown in Eq. (1) [34]:

$$\text{Number of } \textit{Legionella} \text{ cfu/L} = \frac{\text{no. of } \textit{Legionella} \text{ confirmed colonies}}{\text{volume tested in millilitres}} \times 1{,}000\text{mL} \quad (1)$$

Positive sample stands for result >5 cfu/L. The limit of detection was 5 cfu/L, and plates with no growth presented as "not detected" (ND).

To calculate cfu > 1,000, one needs to count 200 colonies into the plate. However, anything more than 100 colonies is very difficult to count. In the present study, early and daily monitoring of plates (from day 4) was performed by taking picture and repeat this to day 10, the picture downloaded, enlarged for better resolution, and the count was done by at least two lab technicians. In addition, samples suspected to have higher count were tested using extra plates with lower volume in addition to the procedure volume (200 mL). Decision matrix have been used as described in the reference standard (ISO 11731:2017).

2.4. Reference materials and lab quality control

A certified reference material (CRM) for *L. pneumophila* (IFM, Australia) was analyzed on December 2017, and all the results were within the acceptable ranges. According to the CRM provider, the acceptable range was 1.14479–7.96781 (log converted value). The present study log converted results was 3.94951 ± 1.03713, and the *z*-score of the test result was <2. In addition, the microbiology Laboratory of WEEC participated and satisfactory passed the proficiency test (PT) scheme on June 2018 (PT-WT-417: 417, LGC, UK) to provide objective evidence on the Lab accuracy and reliability regarding *L. pneumophila* detection and enumeration in water.

2.5. Statistical analysis

Statistical analyses were conducted using SPSS, version 19 (SPSS, Inc., Chicago, IL, USA). The bacterial count was transformed to log10. Previously, 1.0 were added to convert zero (for ND results) to positive values (log10 for 1 = zero). Descriptive statistics were used to summarize continuous variables and are presented as mean ± standard deviation (SD), while categorical variables were expressed in count number and percentage. Student's *t*-test and one-way ANOVA were used to explore differences between means, whereas Chi-square test were used to compare qualitative data, when appropriate.

All statistical tests were 2-sided and applied at 95% confidence level, as well, results were considered statistically significant at $p \leq 0.05$.

3. Results and discussion

3.1. Descriptive data and statistical analysis

Over a period of 12 months, from August 2017 to end of July 2018, 183 potable water samples were collected randomly from hot water outlets (showers and taps) in different hotels and hospitals located in Amman. One-hundred and ten (60.1%) samples were taken from four hospitals, whereas rest of the samples 73 (39.9%) were gathered form five hotels (Table 1). The temperature of water sources were measured on-site. The average temperatures in the hot sources were between 42°C and 55°C, while in the cold sources were 19°C–24°C.

Sixty-four percent of the investigated samples (117 out of 183) were contaminated with *L. pneumophila*, however, the percentage of positive samples from total of each region, as well as, mean value for number of bacteria (cfu/L), both were significantly higher in hotel's sector in comparison with hospitals ($p < 0.001$). (Fig. 1). The present study's data are consistent with what was reported by Sikora et al. [30] in which *Legionella* was isolated from 166 hot water samples out of 222 (74.77%). According to their data, the total percentage of positive samples in hotels was higher compared with hospitals (86.66% and 78.57%, respectively). Yu et al. [35] investigated *Legionella* spp. in 16 hospitals in Taiwan. In their study, 63% of the water system was contaminated with *Legionella pneumophila*. In contrast, low isolation rate (8.1%) was published by Collins et al. [36] when they examined the occurrence of *Legionella* in UK household showers.

3.2. Prevalence in hotels and hospitals

In the present study, the highest observed *L. pneumophila* count was over 1.26×10^5 cfu/L in one of the hotels (Table 2), while the minimum isolated count was 7 cfu/L, which is around the method's limit of detection (LOD = 5 cfu/L). The mean number of *L. pneumophila* in log converted value was 2.925 ± 1.008 cfu/L in all positive samples, while, for those positives plates from hospitals and hotels were 2.512 ± 1.105 and 3.292 ± 0.748, respectively. In addition, as demonstrated

Table 1
Number and percentage of tested potable water samples

	Hospitals	Hotels	Total	*p*-value
Number of locations (*N*)	4	5		
Total number of water samples *n* (%)	110 (60%)	73 (40%)	183 (100%)	
Positive water samples from total, *n* (%)	55/183 (30%)	62/183 (34%)	117/183 (64%)	
Rate of incidence within location	55/110 (50%)	62/73 (85%)		<0.0001*

*Chi-square test for independence, χ^2 (1, n = 183) = 21.73, $p < 0.0001$, phi = 0.356.

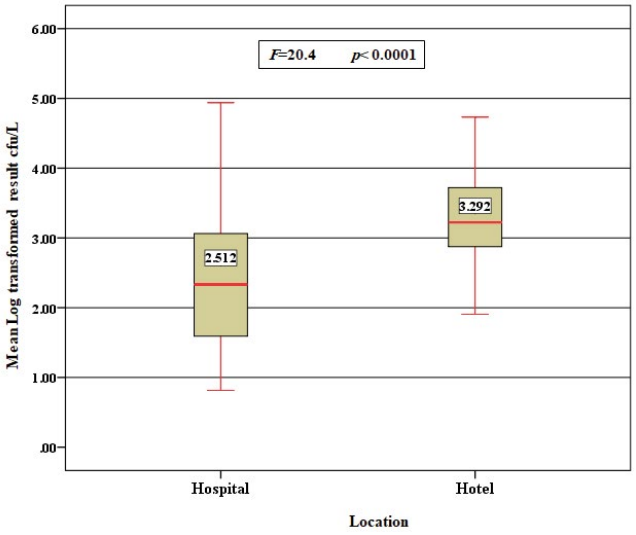

Fig. 1. *Legionella pneumophila* counts from positive plates only transformed into $\log_{10}$, box show the number of positive samples with the mean value as a red thick line, and error bars indicate the minimum and maximum values. Outliers excluded. Means compared with one-way between-groups ANOVA with planned comparisons, mean of positive samples from hotels was significantly higher than hospitals positives' mean, F (1,115) = 20.4, p = 0.0001.

in Table 2, this study showed that serogroup 2–14 (SG 2–14) was dominant over serogroup 1 (SG 1), with 80% counted from the whole positive samples. This can likely be reassuring positive point based on the fact that SG 1 is responsible for the majority of reported Legionnaires' disease [3]. Moreover, with the large colonization reported in the present study, more cases could be expected if the detection of the antigen in urine is routinely performed in patients with pneumonia. However, since the majority of colonization observed is by serogroup 2–14, then the rapid antigenuria test would not detect the cases caused by these colonized facilities.

The comparison between mean values of *L. pneumophila's* logarithmic counts was performed by one-way between-groups ANOVA with planned comparisons, and indicated significant differences among hospitals and hotels F (1,115) = 20.4, p = 0.0001, mean values were 2.512 ± 1.105 and 3.292 ± 0.748 cfu/L, as shown in Fig. 1.

The prevailing *L. pneumophila* serogroup is contradictory subjects among literatures. Whereas (SG 2–14), was the most prevalent culture detected in this study's results [29,37–40], Totaro et al. [41] and De Filippis et al. [42], in contrast, observed that SG1 is dominant.

The levels of contamination with *L. pneumophila* in potable water should not exceed 1,000 cfu/L [3,4]. Accordingly, the results under investigation were categorized into four groups with reference to bacterial concentration, (<10), (10 – <10^2), (10^2 – <10^3), (10^3 – <10^4), and (≥10^4) cfu/L (Table 3). In 60 of 117 positive samples (51%), *L. pneumophila* exceeded the level of 1,000 cfu/L, consequently, 49% (57/117) of the colonized samples contained safe level (<1,000) (Table 3). In this context, a statistical test was conducted to explore the variation between hospitals and hotels which indicated a significant difference between both categories

Table 2
Contamination rate of *Legionella* in each sector, and descriptive statistics for the detected counts related to total positives

	% of positives from total samples in same sector	% SG1 from total positive samples	% SG 2-14 from total positive samples	*Legionella pneumophila* in 1 L of water (cfu/L)				
				Mean of log10 count ± SD	Maximum detected count	Minimum positive detected count	Maximum log10 detected count	Minimum log10 detected count
Hospitals	55/110 (50%)	15/55 (27%)	40/55 (73%)	2.512 ± 1.105	86,875	7	4.939	0.813
Hotels	62/73 (85%)	8/62 (13%)	54/62 (87%)	3.292 ± 0.748	126,500	36	5.102	1.55
Total	117/183 (64%)	23/117 (20%)	94/117 (80%)	2.925 ± 1.008	126,500	7	5.102	0.813

Table 3
Number and percentage of positive tested water samples according to contamination level

	Count and percent of positive contaminated samples (ranges as cfu/L)					
	<10	10 – <10^2	10^2 – <10^3	10^3 – <10^4	>10^4	Total
Hospitals	2/117 (1.7%)	17/117 (14.5%)	18/117 (15.4%)	13/117 (11.1%)	5/117 (4.3%)	55/117 (47%)
Hotels	0 (0%)	2/117 (1.7%)	18/117 (15.4%)	31/117 (26.5%)	11/117 (9.4%)	62/117 (53%)
Total	2/117 (1.7%)	19/117 (16.2%)	53/117 (30.8%)	44/117 (37.6%)	16/117 (13.7%)	117/117 (100%)

regarding the extent of contamination, χ^2 (4, n = 117) = 23.120, p = 0.000, phi = 0.445 (Chi-square test for independence).

In the present study, 18 colonized samples with concentration >1,000 cfu/L detected in hospitals (forming 15.4% from total positive), on other hand, 42 samples from hotels (36%) belonged to the same previous unsatisfactory level.

In the present study, a high concentration (mean), prevalence (% of positive samples), and variation in *L. pneumophila* culture (range and significant difference) among the water samples was observed. This could be explained by many reasons such as the type and frequency of disinfections, age of plumping system, and pipes, temperature, and hardness of water [28,29]. The complexity of the water distribution system including branching and ended pipes, the presence of biofilms, protozoa and other microorganism that provided protection and transmission path, and the entrance of *Legionella* in the status of viable but not culturable (VBNC) [37], could also contribute to the high concentration, prevalence, and variation of *L. pneumophila* cultures found in the present study. It has been reported that, most water sources contain low levels of *Legionella*, but when the organism's preferred environmental conditions are met, most probably they can multiply to grow to a serious level [43–45]. However, all these variables and many others have not been investigated in the present study, but the provided data could form an important baseline for future further researches.

The lower frequency of *L. pneumophila* infection in Jordanian hospitals compared with hotels may reflect the higher awareness and commitment to routine testing in the health sector, in addition to the regular maintenance of water distribution systems and proper disinfection procedures. At the same time, however, a third of the total samples obtained from hospitals exceeded the level of 1,000 cfu/L (33%) as demonstrated in Fig. 2. This percent is much lower than what Montagna et al. [46] reported level from survey in Italian hospitals with 62% positive (>1,000 cfu/L), notwithstanding the control measures in the Italian hospitals for disinfection (i.e., thermal shock, chlorine dioxide, replacement or cleaning of faucets, and showerheads) were

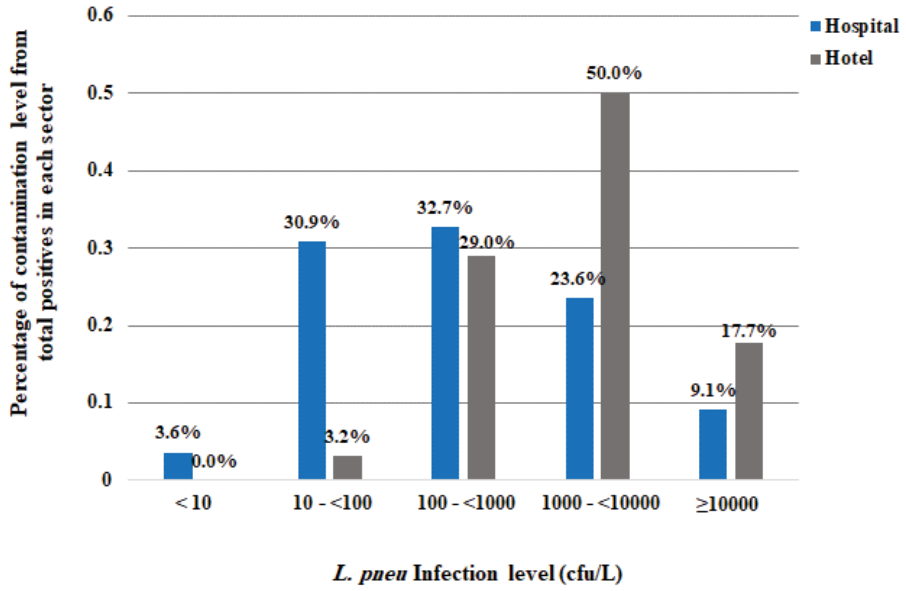

Fig. 2. *Legionella pneumophila* contamination levels in hospitals and hotels. Chi-square test for independence, χ^2 (4, n = 117) = 23.120, p = 0.000, and phi = 0.445.

much more effective and better than those employed in Jordan's hospitals and hotels.

The results also showed that the rate of *L. pneumophila* infection in hotels was double that in hospitals (67.7%:32.7%). Hospitals and other health service providers still signify a high-risk environment for the transmission of Legionellosis disease, as published by WHO [3], due to health practices and devices (e.g., ventilation, artificial respiration, naso-gastric intubation, and dental tools) and the presence of immune-compromised patients.

3.3. Seasonal variation and Legionella occurrence frequency

The climate in Jordan is usually subtropical arid, with quite cold winters due to the altitude, and sunny summers, which are hot but partly tempered by the altitude, as well. July and August are the hottest and driest months of the year. This climate variations shall be taken into consideration when *Legionella* occurrence frequency data are benchmarked with that in other countries. Seasonal discrepancy regarding *L. pneumophila* concentration in potable water and frequency rate showed rising contamination in spring and autumn (Table 4), with highest percent of positive samples from total positive samples received during November, April, and March were 11.5%, 9.8%, and 8.2%, respectively, in the same order (Fig. 3). However, the seasonal differences in the levels of *Legionella* in water samples could be due to the variability of hot water temperature during the year. The samples had a lower temperature in summer than the remaining seasons, as it has been reported in previous literatures [30,47].

However, the available data considered are not sufficient to predict patterns. In spite of the fact that certain seasonality could be observed for *Legionellosis* [2,3], seasonality in environmental contamination differ from country to other and could not be confirmed, moreover, association between season of disease and contamination season have not been proven statistically [38].

A one-way between-group analysis of variance was conducted to explore the impact of seasons on level of contamination as measured by (mean ± SD) for logarithmic *L. pneumophila* colonization (Table 4, Fig. 4).

There were statistically significant differences at the $p < 0.05$ level for the four seasons: F (3,113) = 10.078, P = 0.000. Post-hoc comparisons using the Tukey HSD test indicated that mean values for positive samples of winter (2.1204 ± 1.0307) was significantly lower than autumn and spring (3.209 ± 0.689 and 3.267 ± 1.010, respectively). Mean value for positive samples of summer has significantly not differed from other seasons (p-value > 0.05). This perhaps due to the low number of samples in that season in comparison with other seasons. The effect size, calculated using eta squared, was high (eta squared = 0.21) which unfortunately is considered as one of the study limitations. The climate variation, complexity of water distribution system, geographic differences, and the development of water monitoring and control system are variables that could explain the contradictory published results regarding *Legionella* seasonal contamination counts [29,30,38,48].

In that context, Kao et al. [49] found that the higher *L. pneumophila* detection rate in Taiwan was in fall, where the higher concentration was in spring. Whereas Pule et al. [48] published that the higher *L. pneumophila* occurrence in Latvia was in summer. In the current study, the positive rate obtained in winter was 73% (27/37) (Table 4) which was consistent with the finding of Sikora et al. [30], where they indicated that the positive rate in Poland winter was 71.7%, however, they could not detect a significant differences between seasons. Similar circumferences acquired by Kruse et al. [38] as they could not find an association between month or season of samples and level of contamination in Germany.

3.4. Limitations

Our study is limited in that it depends only on culture plate method for *L. pneumophila* recovery, which is the fundamental isolation method. It is unable to detect viable but not culturable bacteria which may lead to underestimation.

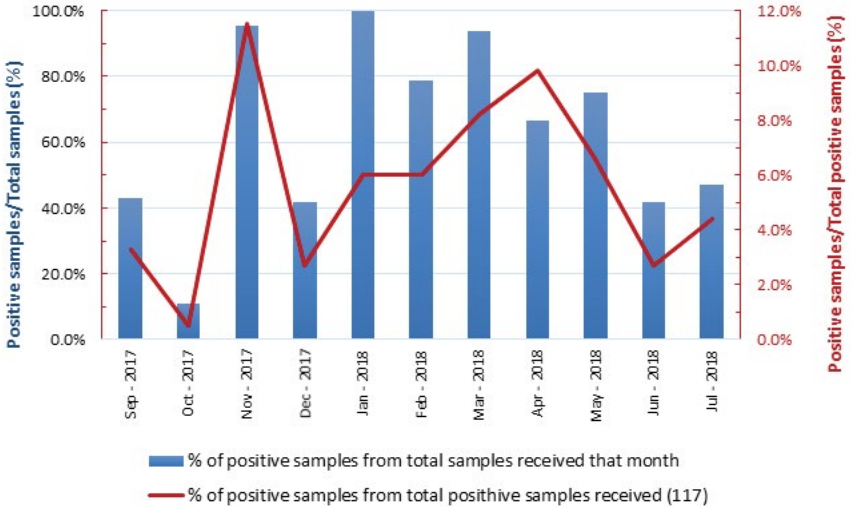

Fig. 3. *Legionella pneumophila* percent of contamination per months.

Table 4
Distribution of positive colonized samples on months and seasons

Season	Autumn			Winter			Spring			Summer		
Month	September – 2017	October – 2017	November – 2017	December – 2017	January – 2018	February – 2018	March – 2018	April – 2018	May – 2018	June – 2018	July – 2018	August – 2017
% of positive samples from total samples received that month	6/14 (42.9%)	1/9 (11.1%)	21/22 (95.5%)	5/12 41.7%	11/11 (100.0%)	11/14 (78.6%)	15/16 (93.8%)	18/27 (66.7%)	12/16 (75.0%)	5/12 (41.7%)	8/17 (47.1%)	4/13 (30.8%)
Total count (%)‡	28/45 (62.2%)			27/37 (73%)			45/59 (76.3%)			17/42 (23.6%)		
% of positive samples from total positive samples received (117)	3.3%	0.5%	11.5%	2.7%	6.0%	6.0%	8.2%	9.8%	6.6%	2.7%	4.4%	2.2%
Total count (%)	28/117 (23.9%)			27/117 (23.1%)			45/117 (38.5%)			17/117 (14.5%)		
Log10 for Legionella detection count Mean ± SD	3.209 ± 0.689**^{b}			2.120 ± 1.030**^{a}			3.267 ± 1.010**^{b}			2.768 ± 0.715^{a+b}		

‡Chi-square test for independence, χ^2 (3, n = 183) = 15.093, p = 0.002, and phi = 0.289.
Means compared with one-way between-groups ANOVA with post-hoc test using Tukey HSD, letters (*a* and *b*) represent the significant differences. Different letters indicate significant differences at $p < 0.05$ and the same letters indicates no significant differences.
**F (3,113) = 10.078, p = 0.000.

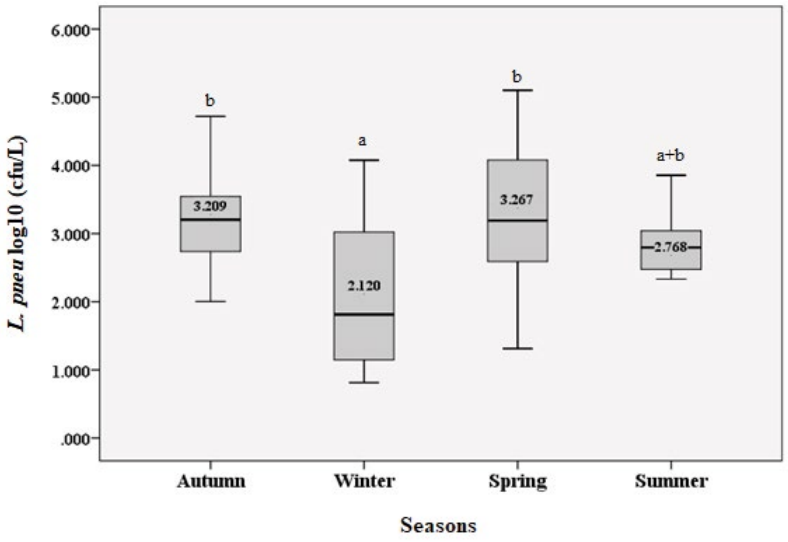

Fig. 4. *Legionella pneumophila* counts from positive plates only transformed into $\log_{10}$ grouped by season. Box show the mean value of positive samples for each category, and error bars indicate the minimum and maximum values. Outliers excluded. Means compared with one-way between-groups ANOVA with post-hoc test using Tukey HSD, the letters above figures (a and b) represent the significant differences. Different letters on the top of bars indicate significant differences at $p < 0.05$ and the same letters indicates no significant differences. Mean of positive samples during winter was significantly lower than both, autumn and spring means, F (3,113) = 10.078, p = 0.000.

Furthermore, number of hotels (5) and hospitals (4) included in the sample size were limited and was not able to sufficiently represent the whole city of Amman. This is due to the fact that the sampling scheme was following to the restricted request of the Environmental Health Directorate, Ministry of Health, with no flexibility to include other facilities and/or increase the number of collected samples.

The hotels are open at a 12 month period with varied rate of occupancy. All of the hospitals considered in the present study are tertiary hospitals. The hotels and hospitals obtain water from intermittent source supplied by the city and is chlorinated at 0.5 mg/L, and from contracted water tankers based on the hotels requests. In both hospitals and hotels, a recirculating pressurized system supplies hot and cold water to the upper floors and rooms.

It is noteworthy to state that in order to comprehensively investigate the seasonal variation effect on *Legionella* occurrence frequency, a number of consecutive years should be studied because climate changes from 1 y to another and these shifts are not easily detectable if a single year is studied.

However, the current study provided a baseline data for risk assessment and management, in addition, control and prevention measures for legionellosis in hospitals and hotels.

4. Conclusions

The statistical comparative assessment results of the present study highlights the significance of conducting a frequent microbiological and environmental surveillance to control the environmental spread of *L. pneumophila* in both of hotels and hospitals, under integrated risk management and water safety plans. The results showed that, out of the total collected sample, high prevalence of 64% positive *L. pneumophila* SG2-14 in the hotels and hospitals water system in Jordan. While, *L. pneumophila* contamination in hotels were significantly higher than hospitals. It is also revealed in the present study that acceptable level of *L. pneumophila* (1,000 cfu/L) was exceeded, which indicates the risk of infection of legionellosis may have occurred in Jordan but not been detected. The seasonal effect of *L. pneumophila* contamination rate was found to be the strongest during spring (76%) and autumn (73%). Finally, the findings of the study may form a cornerstone in risk assessment and management in Jordan by providing the water sector's stakeholders with preliminary data needed for periodic inspection and analysis of *L. pneumophila*, while contributing to increased awareness regarding water quality and safety. However, further in-depth studies are recommended to be conducted to show the contamination patterns, seasonality, risk transition areas, and proper disinfection.

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