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Multiple disinfection processes of *Legionella pneumophila* positive in hotels' water distribution systems in Jordan

Motasem N. Saidan[a,b,*], Ahlam I. Abdalla[b], Nivin Al Alami[b], Hanan Al-Naimat[b]

[a]*Chemical Engineering Department, School of Engineering, The University of Jordan, Amman 11942, Jordan, emails: m.saidan@ju.edu.jo/ m.saidan@gmail.com*
[b]*Water, Energy and Environment Center, The University of Jordan, Amman 11942, Jordan, emails: ahlam2ali@gmail.com (A.I. Abdalla), n.alami@ju.edu.jo (N.A. Alami), h.naimat@ju.edu.jo (H. Al-Naimat)*

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ABSTRACT

Legionella pneumophila was detected in 47.4% of the samples taken from three hotels' water distribution systems examined, while in 29.8% counts exceeded 1,000 CFU L^{-1}. The highest positive legionella contamination in point-of-use is observed in cold sink (33%) followed by hot showerhead (22.2%) with higher mean value obtained in: 4.11 and 3.85 CFU L^{-1}, respectively. Multiple disinfection processes (MDP) was applied at *Legionella pneumophila* positive in hotels' water distribution systems while the water system was kept in operation throughout the MDP cycles. The aim of this study is to statistically assess the synergetic effects of MDP at *Legionella pneumophila* positive in hotels' water distribution systems. After the first application of the MDP procedure, the *Legionella pneumophila* prevalence has not achieved complete reduction but only reduced in the three hotels from 69.2% to 23.1%, 16.7% to 6.7%, and 92.9% to 50%, respectively. However, the result from the second application of MDP was satisfactory, and negative *Legionella pneumophila* prevalence was observed in the three hotels. The MDP application seems to be efficient enough to eliminate legionellae when repeatedly applied twice within 21 d.

Keywords: Legionella; Disinfection; Hotels; Water distribution system; Jordan

1. Introduction

Legionella pneumophila is a gram-negative bacterium which can represent a potential fatal pneumonia, mainly through inhalation of contaminated aerosols created by water and air conditioning systems. The presence of legionella and its ability to colonize in water distribution systems is an increasing problem in facilities such as hotels, hospitals, dental clinics, cooling towers, institutional buildings, and spa centers [1–6].

Legionella pneumophila lives in fresh water while warm water provides an ideal habitat for its massive growth [7–9]. *Legionella pneumophila* can reproduce at temperatures between 25°C and 43°C [10], and survive in temperatures of up to 55°C–60°C [11]; while its growth significantly favors temperatures under 55°C [12–14]. Temperatures consistently exceeding 60°C can inhibit the ability of *Legionella pneumophila* to colonize and its detection [15,16].

Legionella pneumophila is dependent on various characteristics of the water plumbing system (WPS) in buildings. For instance, rough and corroded pipe walls promote the biofilms development such as in cast iron pipes [17]. However, Flemming et al. [18] found that *Legionella pneumophila* prefers PVC and polyethylene pipes over stainless steel pipes under the same water conditions. Mathys et al. [13] found that plumbing systems with copper pipes

* Corresponding author.

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were more frequently contaminated than those made of synthetic materials or galvanized steel in hot water system in single-family residences.

When levels of *Legionella* exceeding 1,000 CFU L^{-1} is detected, an action level for control of *Legionella* is recommended by most guidelines [19]. Hence, various disinfection methods have been used to eradicate *Legionella* from water systems [20], including ultraviolet disinfection [21,22]; ozone [23]; hyperchlorination [24,25]; copper–silver ionization [26,27]; hydrogen peroxide [28] and thermal disinfection [29–31]. However, it is reported that not all of these methods have proven to be consistently effective [32]. Nonetheless, thus far data on the efficacy of synergetic disinfection methods is very limited.

Ozone is an oxidant with high reactivity and has been used in water disinfection [33], and ultimately produces the hydroxyl free radical (HO*) which is a stronger oxidizing agent than ozone [34]. It has been suggested that ozone first damages the cell membrane but that it eventually affects the nucleus [35]. Lee et al. [36] indicated that ozone caused rapid damage to nucleic acids and the cell membrane due to the high reactivity, which was similar to the results obtained with the high chlorine concentration tested [36].

Nowadays, disinfection of tap water systems using superheat-and-flush is a quite common method because it requires no special equipment, and it can be initiated expeditiously [3,37]. Superheat-and-flush method can be adopted when the facility's piping components in the water distribution system is well maintained and engineered. However, superheat-and-flush method is not recommended in old facilities due to the risk of distribution system failure. Moreover, it was reported that the application of superheat-and-flush for flush duration of 5 min is ineffective, as well as, it may not be economic for a large facilities (i.e., hotels and hospitals) because it could be costly and labor intensive [37].

Water chlorination is a common action of water disinfection in Jordan [38]. However, chlorine dissipates in the presence of organics and generates toxic by-products (i.e., trihalomethanes) in water distribution systems in Jordan [39–41]. Bodet et al. [25] have investigated the *Legionella pneumophila* transcriptional response to chlorine treatment and indicated that chlorine induces expression of proteins involved in cellular defense mechanisms against oxidative stress that might be involved in adaptation or resistance to chlorine treatment. Others have reported that protection of *Legionella pneumophila* in biofilms and protozoa contributes to bacterial survival in chlorine-disinfected water sources [42,43]. Therefore, sole chlorine disinfection is not effective in real water systems in institutional buildings and large facilities.

The main objective of the present study is to undertake statistical assessment and investigate the synergetic effects of multiple disinfection processes (MDP) at *Legionella pneumophila* positive in hotels' water distribution systems in Jordan. Where water distribution system includes the point where the water enters the facility (i.e., wells [cisterns] and storage tanks) to the cold and hot end points, by which water is served to consumers (i.e., water taps, faucets, showers, sinks, etc.). The multiple disinfection implies forward and backward procedures on consecutive basis: continuous ozone injection in the main onsite storage wells (rectangular cisterns), hydrogen peroxide addition, and regular chlorine doses, in addition to other cleaning and disinfection activities of end points using steam, disinfecting agents, and disinfectants immersing tanks.

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 [44–46], and with the overwhelming pressure on water and sanitation as a result of hosting over 1 million Syrian refugees in Jordan [47–49]. Accordingly, hotel and resorts companies in Jordanian have both a strong commercial and moral imperative for addressing water use and ensuring water quality.

Currently, the World Health Organization (WHO) in Jordan raised the concern about the worldwide increasing incidence of this severe disease in general, and some reported cases in Jordan in particular. Hence, the Ministry of Health (MoH) has increased the attention on the monitoring of *Legionella* contamination in commercial and institutional buildings in Jordan.

2. Materials and methods

2.1. Study site selection and description

Based on three reports (received on November 2016, April 2017, and May 2018) from the European Centre for Disease Prevention and Control (ELDSNet) advising the Ministry of Health in Jordan that there has been a 'cluster' of 'travel-associated instances' of *Legionella* reported by people who were guests at the three hotels selected in the present study during the incubation period. By way of definition, a 'cluster' is defined as at least two instances within a 2-year period; an 'instance' is a report to ELDSNet of symptoms of Legionnaires' disease and the case becomes 'travel-associated' if the patient stayed or visited an accommodation site during the disease incubation period, which is 2–10 d prior to the symptom onset. When a cluster is identified within an EU/EEA country, ELDSNet also notifies the public health authorities in the country about where the accommodation site is located. The public health authority is then expected to investigate and report back to ELDSNet. The report should show that adequate steps have been taken in order to control the risk of further *Legionella* infections at the accommodation site in the future. If ELDSnet is satisfied, then no further action is taken but if not, the name of the accommodation site is published on the ECDC website until adequate measures are in place.

The study was conducted in three hotels (Hotel A and C located in Amman city, and Hotel B in southern part of Jordan). More technical details and characteristics of the drinking WPSs these hotels are shown in Table 1. These three hotels were reported by the Ministry of Health in Jordan to have *Legionella pneumophila* positive in their water distribution systems in different periods: November 2016, April 2017 and May 2018 for Hotels A, B and C, respectively.

The hotels obtain water from intermittent source supplied by the city and is chlorinated at 0.5 mg L^{-1}, and from contracted water tankers based on the hotels requests. In both hotels, a recirculating pressurized system supplies hot and cold water to the upper floors and guests rooms. Some of the water is softened by conventional softening systems,

Table 1
Characteristics of hotels buildings and drinking WPSs

Hotel	Operation year	WPS materials	Number of floors	Number of endpoints	Number of rooms	Number of water wells and sizes	Number of roof tanks and sizes
A	1970s	Plastic, steel, stainless steel, Plastic (PVC-C)	23	924	257	2 (700 m^3, 100 m^3)	–
B	2000s	Copper, steel, plastic, stainless steel	16	789	183	2 (180 m^3, 14 m^3)	–
C	2017	Plastic, steel, stainless steel	11	463	100	2 (80 m^3, 50 m^3)	24 (2 m^3)

stored in a large storage tank and then heated, by steam plate heat exchangers, and distributed as hot water throughout the hospital. The water quality provided in Hotel A by the local water authority is considered hard water (hence the need for softening) and the mean pH is 7.48. However, in Hotel B no softening system is installed and consequently the unsoftened water is delivered from the incoming mains to 24 water tanks (2 m^3 each) on the hotel's roof, from which water is distributed throughout the hotel as unsoftened water.

The hotels' facility comprises the main, multi-storey building, the guest rooms and wings, reception areas, consulting rooms, gymnasium, restaurants, kitchens, swimming pool, etc.

2.2. Samples collection

The sampling scheme within the hotel buildings was following to request of the Environmental Health Directorate, Ministry of Health, and to certain extent it covers the entire plumbing system (sampling at water wells (cisterns) and storage tanks, hot and cold water distribution systems, and outlets including showerheads and taps) of the aforementioned hotels.

Ninety six samples were collected over a period of 20 months, from November 2016 to end of July 2018, according to different MDP stages: 57 potable water samples were collected from water outlets (showers and taps) in of the aforementioned hotels prior MDP activities; 27 samples were collected during MDP activities; and 12 samples were eventually collected after accomplishing MDP activities.

All the samples were collected in 1-L sterile Duran bottles containing 10% sodium thiosulfate that able to neutralize up to 5 mg L^{-1} residual chlorine [50]. The samples' bottles were transported to the Microbiology laboratory in the Water, Energy & Environment Center (WEEC) at the University of Jordan, which is internationally accredited in ISO 17025. Subsequently, all samples were analyzed in duplicate within 6 h of collection, with estimated reproducibility (RSD_{RC}) that has been calculated for the duplicates and found to be (0.0266), and the precision criterion (PC) was (0.256).

2.2.1. Microbiological analysis

Legionella were isolated by using the procedure described in APHA [51] 22nd ed. Culture and identification of *Legionella pneumophila* was carried out by using the APHA [51] 22nd ed. One liter of a water sample was concentrated by membrane filtration (0.2 μm-pore-size polycarbonate filter; Sartorius, Billerica, MA). The filter membrane was resuspended in 10 mL of the sterile distilled water. Aliquots of the original water sample were acid treated and cultured on selective medium (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom). The plates were incubated at 35°C in a humidified environment with 2.5% CO_2 at least for 10 d and examined beginning on day 3. All colonies on plates containing ≤10 colonies and 10–20 random colonies from other plates were subcultured on BCYE (with cysteine) and CYE (cysteine-free) media (Oxoid) for ≥2 d.

2.3. Legionella pneumophila identification and calculation

Legionella pneumophila latex test (DR0800M, Oxoid, UK) has been used according to the manufacturer's instruction to recognize the serogroup type of *Legionella pneumophila* isolates, this agglutination test identifies *Legionella pneumophila* serogroup 1 (SG1), serogroup 2–14 (SG2-14) and other seven *Legionella pneumophila* species. However, Eq. (1) is used as stated in APHA [51] 22nd method, where 1,000 mL are filtered and washed in 10 mL then 0.2 mL inoculated on plate, acid treatment used with dilution factor 10 and limit of detection was 500 CFU L^{-1}.

$$C_s = \frac{a \times V_c}{V \times V_{\text{tot}}} \times D_f \times V_s \tag{1}$$

where C_s is the number of *Legionella* in CFU L^{-1}; a is the number of calculated confirmed *Legionella* colonies; V_c is the (concentrated) sample volume in milliliters, mL; V is the sample volume inoculated per plate in milliliters, mL; V_{tot} is the total tested sample volume in milliliters, mL; D_f is the dilution factor from acid treatment; V_s is the reference volume chosen to express the concentration of the microorganisms in the sample (normally 1,000 mL).

2.4. Reference materials and lab quality control

A Certified Reference Material for *Legionella pneumophila* (IFM, Australia) have been analyzed on Dec 2017, and all the results were within the acceptable ranges. In addition, the microbiology lab of WEEC participated and satisfactory passed the proficiency test (PT) scheme on Jun 2018 (PT-WT-417: 417, LGC, UK) to provide objective evidence on the Lab accuracy and reliability regarding *Legionella pneumophila* detection and enumeration in water.

2.5. Statistical analysis

Statistical analysis was conducted using SPSS, version 20 (IBM SPSS Statistics 20). The bacterial count was transformed to log_{10}, as accordingly, zero converted to positive value by adding 1.0 for (ND) results (log_{10} for 1 = zero). Descriptive statistics were used to summarize continuous variables and presented as mean ± standard deviation (SD), while categorical variables were expressed in count number and percentage. Paired-samples *t*-test and was used to examine the reduction in mean value after treatment. In addition, correlation analysis was used to describe the strength and direction of relationship between the continuous variables. One-way ANOVA were used to explore differences between means in the different locations, whereas Chi-square test was used to compare qualitative data, when appropriate.

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

2.6. Disinfection chemicals and procedures

The MDP hypothesized in the present study is implemented on consecutive basis as follows:

First, main water wells (rectangular cisterns) should be filled with fresh water to its maximum capacity in order to use the water as disinfectants carrier. Since there is normally at least two onsite water storage wells (cisterns), it is preferable to put one of them in operation while to evacuate others from water and undertake cleaning up of the empty one. After a mechanical cleaning of the surfaces from dust and dirt sediments, the walls are brushed with a high-pressure tool.

A chlorinated solution prepared by diluting 10% sodium hypochlorite (Sigma-Aldrich, Milano, Italy) was used. The chlorine concentration of the municipal water is adjusted to maintain between 0.5 and 1 mg L^{-1}. Hence, if water is provided by water tankers, then chlorine must be adjusted to be less than 1 mg L^{-1} when added to the main onsite water wells.

Second, in-situ ozone generator (MT-OZ-3G, China) is installed on the onsite wells with capacity of 3 g h^{-1}. Ozone is dissolved into the water system to achieve a dose of about 1–2 mg L^{-1}, ideally via a generator that produces ozone in proportion to the water flow. However, due to the rapid decomposition of ozone residual in water, its main utility may be limited as a supplemental disinfectant [52]. The water pumps are kept in operation throughout the disinfection procedures and water is allowed to flow to the facility components (rooms, kitchen, gym, outdoor showers, etc.).

Once ozone residual is measured in all facility component mainly in the farthest point from the onsite water wells with residual around 0.7 mg L^{-1} on average basis, then the third disinfection procedure is undertaken by addition of hydrogen peroxide to the main onsite water wells in concentration between 5 and 15 mg L^{-1}. In case of having water tanks (2 m^3 for each) on the hotel roof, then hydrogen peroxide is added directly to these water tanks. Fourth, hot and cold water flushing is recommended for 10 min at least in the farthest water distribution outlets.

Fifth, hyperchlorination or shock chlorination is undertaken by raising chlorine levels to 1.5–2 ppm for at least 24 h. Sixth, after 24 h hyperchlorination is maintained, all water distribution system outlets, fixture components, and endpoints (i.e., faucets, taps, shower heads, aerators, water saver shower heads, etc.), which are temporarily not in use, are disassembled, immersed in 1 m^3 boiling water tank for 30 min at least, and then transferred subsequently into another water tank containing chlorine disinfectant with concentration of 5 mg L^{-1} for another 30 min, and finally after that cleaned the interior parts of these outlets with steam to ensure that no biofilm is still adhered internally. The effect of replacement of outlets (faucets, shower heads, etc.) with new ones appeared to be insignificant in colonization of *Legionella* [37]. However, dead legs of water pipes and rubber gaskets should be eliminated, and fixture components, should be removed and cleaned frequently.

Seventh, prior to assembling of the outlets and endpoints (i.e., faucets, taps, shower heads, etc.) to the water distribution and plumbing system, the interior wall of the connection and joint points shall be disinfected with steam, then assembling can be conducted, and eventually, water flushing through these outlets is recommended for 2–3 min.

Practically, the MDP might take between 14 and 21 d achieve complete elimination of *Legionella pneumophila* contamination. This is influenced by the sample testing method which is normally takes from 8 to 10 d.

3. Results and discussions

Before 2017, there was no regulation in Jordan for environmental *Legionella* monitoring and control. Hence, the water systems in large buildings (i.e., hotels, hospitals, health care centers, etc.) are operated without any *Legionella* monitoring and control intervention or at least awareness of *Legionella* risk.

3.1. Baseline results

The environmental surveillances for *Legionella* were performed on December 2016 for Hotel A, April 2017 for Hotel B, and March 2018 for Hotel C, as requested by Environmental Health Directorate, Ministry of Health. Monitoring of *Legionella pneumophila* was conducted by taking 57 samples in total from the water distribution system of the hotels: hotel A (13 samples); hotel B (30 samples), and hotel C (14 samples). Investigation was directed only to the premise plumbing system which includes the onsite water wells (cisterns) and points of use (water taps in the kitchen and restaurants, room's sinks faucets, shower heads, etc.). Testing of samples reveals that 47.4% of the total examined samples in the three hotels were contaminated with *Legionella pneumophila*. Table 2 shows the positivity of *Legionella pneumophila* (% or CFU L^{-1}) in each hotel and in relative comparison with all samples.

The results in Table 2 show that all hotels A, B and C have positive *Legionella* contamination. However, the highest positive *Legionella* contamination is found in hotel C. For instance, 13 positive samples detected in hotel C which form 22.8% from the overall samples (57 samples in total) and form about 48% from the total positive samples in the three hotels. The lowest positivity of *Legionella* is clearly observed in Hotel B with 8.8% positive samples from the overall samples, and 18.5% from the total positive samples in the three hotels.

Table 3 shows the contamination level of *Legionella pneumophila* (% or CFU L^{-1}) at each hotel before any disinfection intervention took place, and in relative comparison with all samples. Almost half (47.4%) of the water samples taken from the three hotels water networks were positive for *Legionella*, while in 29.8% counts exceeded 1,000 CFU L^{-1}. The data found in the present study are consistent with the literature, according to which positive *Legionella* is found in approximately 50% of large building water systems [53–56].

Fig. 1 presents positive *Legionella pneumophila* results as a function of the type of point-of-use sampled (i.e., onsite water wells and storage tanks, cold and hot end points by which water is served to consumers (i.e., water taps, faucets, showers, sinks, etc.), and water softeners, as shown also in

Table 2
Positivity of *Legionella pneumophila* (%) (prior MDP activities)

		Positive/negative		
		Positive	Negative	Total
Hotel A	Count	9	4	13
	% Within site	69.2%	30.8%	100.0%
	% Within positive/negative	33.3%	13.3%	22.8%
	% of Total	**15.8%**	**7.0%**	22.8%
Hotel B	Count	5	25	30
	% Within site	16.7%	83.3%	100.0%
	% Within positive/negative	18.5%	83.3%	52.6%
	% of Total	**8.8%**	**43.9%**	52.6%
Hotel C	Count	13	1	14
	% Within site	92.9%	7.1%	100.0%
	% Within positive/negative	48.1%	3.3%	24.6%
	% of Total	**22.8%**	**1.8%**	24.6%
Total	Count	27	30	57
	% Within site	47.4%	52.6%	**100.0%**
	% Within positive/negative	100.0%	100.0%	**100.0%**
	% of Total	47.4%	52.6%	**100.0%**

Table 3
Contamination level of *Legionella pneumophila* (% or CFU L^{-1}) of the three hotels

		Contamination level before treatment (CFU L^{-1})				
		ND	<1,000	1,000–10,000	>10,000	Total
Hotel A	Count	4	3	4	2	13
	% Within site	30.8%	23.1%	30.8%	15.4%	100.0%
	% Within level 1	13.3%	30.0%	44.4%	25.0%	22.8%
	% of Total	7.0%	5.3%	7.0%	3.5%	22.8%
Hotel B	Count	25	4	1	0	30
	% Within site	83.3%	13.3%	3.3%	0.0%	100.0%
	% Within level 1	83.3%	40.0%	11.1%	0.0%	52.6%
	% of Total	43.9%	7.0%	1.8%	0.0%	52.6%
Hotel C	Count	1	3	4	6	14
	% Within site	7.1%	21.4%	28.6%	42.9%	100.0%
	% Within level 1	3.3%	30.0%	44.4%	75.0%	24.6%
	% of Total	1.8%	5.3%	7.0%	10.5%	24.6%
Total	Count	30	10	9	8	**57**
	% Within site	52.6%	17.5%	15.8%	14.0%	**100.0%**
	% Within level 1	100.0%	100.0%	100.0%	100.0%	**100.0%**
	% of Total	52.6%	17.5%	15.8%	14.0%	**100.0%**

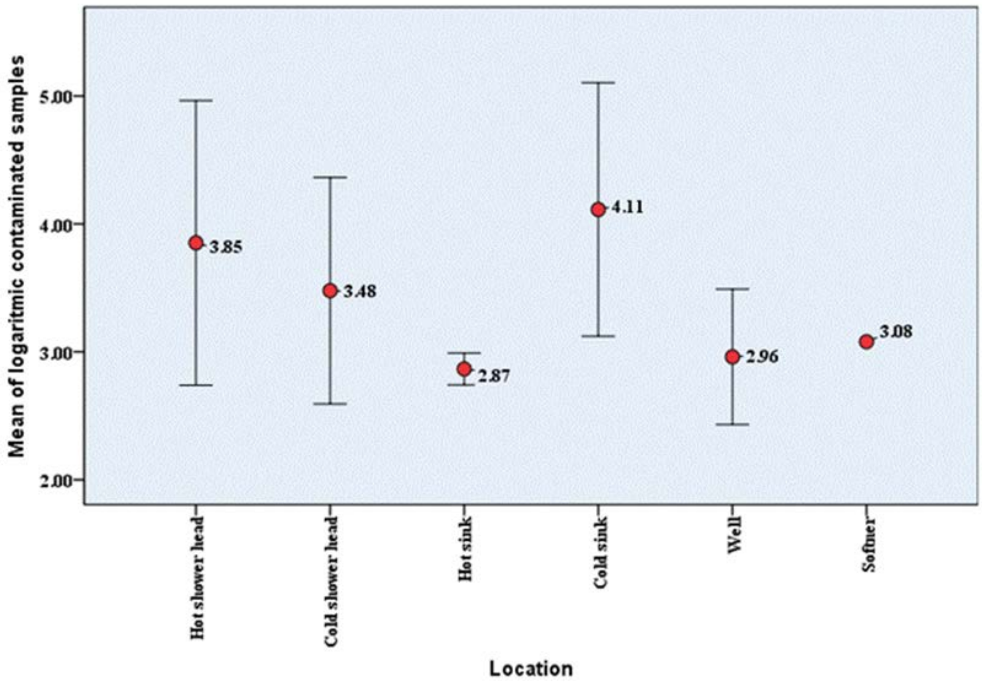

Fig. 1. Positive *Legionella pneumophila* counts (log_{10}) measured by type of point-of-use before corrective measures. Error bars show the minimum and maximum value (excluding outliers).

Table 4 where number of samples for each point-of-use is specified.

As shown in Fig. 1, *Legionella pneumophila* count from contaminated samples only transformed into log_{10}, and distributed according to sampling location. In Fig. 1, red dot represents the mean value, and the error bars indicate the standard deviation. It is crystal clear that the highest positive *Legionella* contamination in point-of-use is observed in cold sink (33%) followed by hot shower head (22.2%) with higher mean value obtained in: 4.11 and 3.85 CFU L^{-1}, respectively. However, there was no statistically significant difference observed at $p < 0.05$ between the different locations by one-way ANOVA: $F(5, 21) = 1.626$, $p = 0.197$.

3.2. Impact of MDP on positive Legionella pneumophila

The relationship between logarithmic contamination count of *Legionella pneumophila* before MDP and after starting of MDP was investigated using Pearson product-moment correlation coefficient. There was a strong positive correlation between the two variables, $r = 0.576$, $n = 57$, $p < 0.0005$.

A paired-samples *t*-test was conducted to evaluate the impact of the treatment on the mean value of log_{10} of all positive results. There was a statistically significant decrease in level of contamination from (mean = 3.598, SD = 0.953) before treatment, to (mean = 1.46, SD = 1.72) after start of treatment, $t(26) = 6.447$, $p < 0.0005$ (two-tailed). The mean decrease in colonization results was 2.137 with 95% confidence interval ranging from 1.456 to 2.819. The eta squared statistic (0.61) indicated a large effect size.

The first round of MDP reduced the *Legionella pneumophila* prevalence in the 27 samples collected from the three hotels as of the following: from 69.2% (9/13) to 23.1% (3/13) for Hotel A, from 16.7% (5/30) to 6.7% (2/30) for Hotel B, and from 92.9% (13/14) to 50% (7/14) for Hotel C, 10 d after the starting of MDP, as shown in Fig. 2.

Table 4
Statistical results of positive *Legionella pneumophila* counts measured by type of point-of-use

Point-of-use	Mean (Log_{10})	Number of samples (*N*)	Standard deviation	% of Total sum	% of Total *N*
Hot shower	3.85167	6	1.112241	23.8%	22.2%
Cold shower	3.47800	3	0.885280	10.7%	11.1%
Hot sink	2.86600	2	0.124451	5.9%	7.4%
Cold sink	4.11300	9	0.991295	38.1%	33.3%
Well	2.96067	6	0.529352	18.3%	22.2%
Softener	3.07900	1	–	3.2%	3.7%
Total	**3.59763**	**27**	**0.953238**	**100.0%**	**100.0%**

However, the first MDP round did not achieve complete reduction of *Legionella pneumophila* in the three hotels. However, the result from the second MDP (10 d) was satisfactory, and negative *Legionella pneumophila* prevalence was observed in all 12 samples collected from the three hotels.

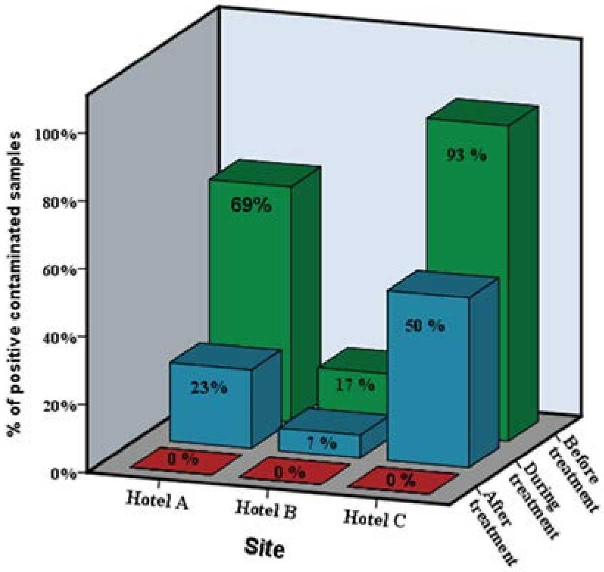

Fig. 2. Percentage of positive Legionella pneumophila before, during (10 d), and after MDP.

This indicates that synergetic effects of MDP needs between 14 and 21 d to achieve complete elimination of *Legionella pneumophila* contamination regardless of the size of the multi-storey building. Taking into account that the water system was kept in operation throughout the MDP cycles (i.e., no isolation of any portion of the water system was done). In this context, it is noteworthy that it was reported that *Legionella pneumophila* can be isolated from water flowing through within days to weeks throughout effective disinfection (i.e., superheat-and-flush), because *Legionella pneumophila* is still present in biofilms throughout the plumbing system [37].

As shown in Fig. 3, a one-way repeated measures ANOVA was conducted to compare means of samples contaminated with *Legionella* with statistics test before treatment, during treatments and after treatment. The means represented with red dots, where the error bar represent SD. There was a significant effect for treatments (Wilks' Lambda = 0.063, $F(2, 28) = 183.323$, $p = 0.0001$, multivariate partial eta squared = 0.937).

Positive contaminated samples before treatment were reorganized into three different groups according to water distribution lines (source (onsite main water well), hot water distribution system, and cold water distributon system) and the reduction in *Legionella pneumophila* log count re-tested, as shown in Fig. 4. Blue, green and gray dots represent

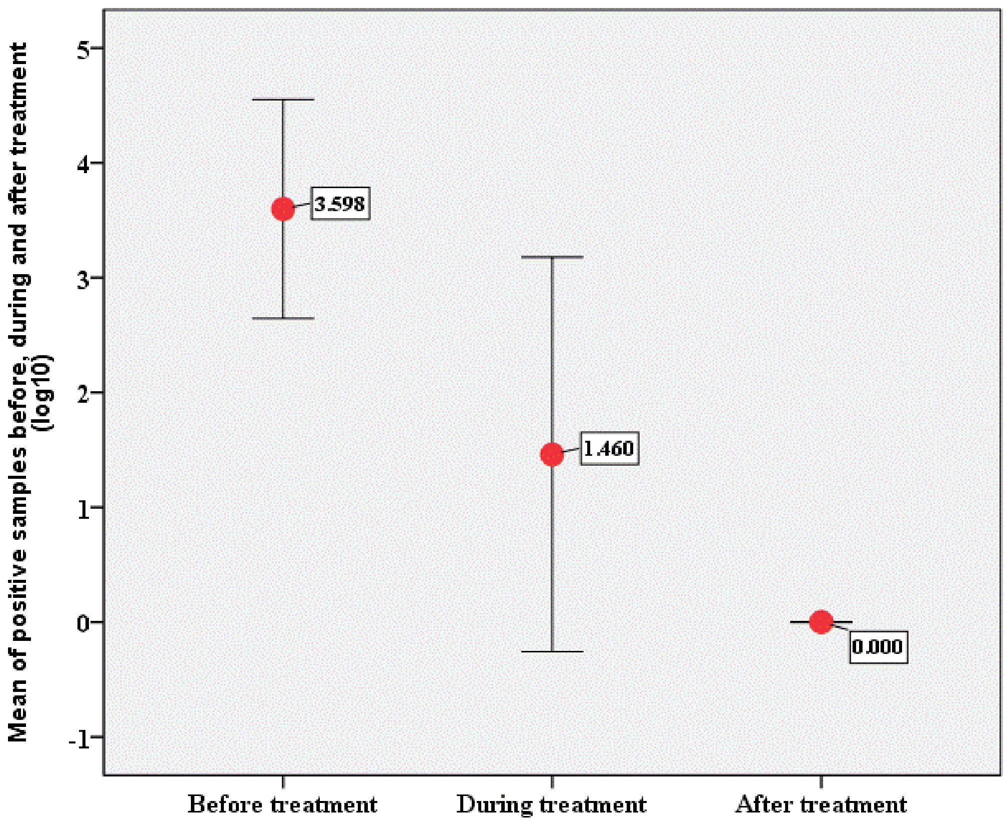

Fig. 3. Total positive *Legionella pneumophila* before, during (10 d), and after MDP. Error bars show the minimum and maximum value (excluding outliers).

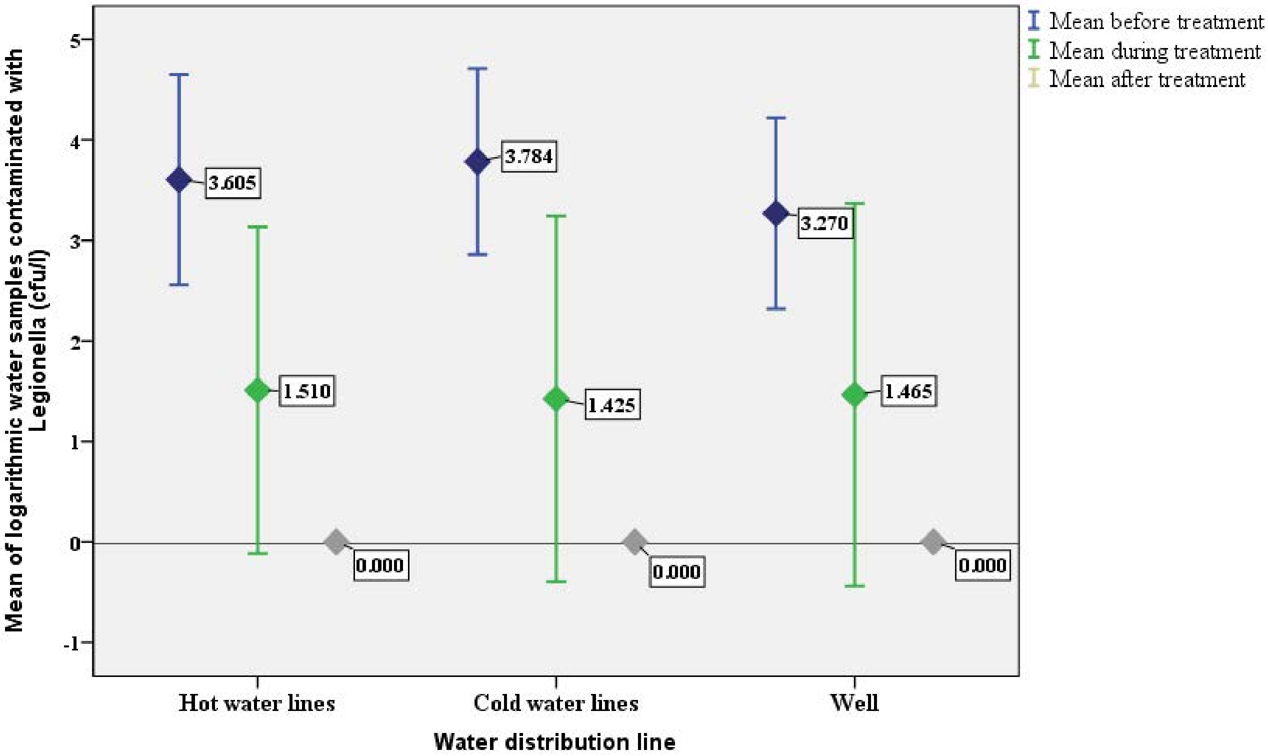

Fig. 4. Signficant effect on *Legionella pneumophila* in source (well), hot, and cold water systems: before, during, and after treatment. Error bars show the minimum and maximum value (excluding outliers).

the mean value (before, during and after treatment, consequently), and the error bars indicate the standard deviation. The higher mean value obtained in the cold water line followed by hot water line: 3.784 and 3.605 CFU L^{-1}, respectively.

4. Conclusions

Water distribution systems in hotels with numerous points of use are frequently subject to the occurrence and concentration of *Legionella*. Environmental surveillances for *Legionella* were performed in three hotels in Jordan. Testing of samples reveals that 47.4% of the total examined samples in the three hotels were contaminated with *Legionella pneumophila*. The synergetic effects of MDP application at *Legionella pneumophila* positive in the three hotels' water distribution systems were investigated. The findings herein indicate that synergetic effects of MDP needs between 14 and 21 d to achieve complete elimination of *Legionella pneumophila* contamination regardless of the size of the multi-storey building. Taking into account that the water system was kept in operation throughout the MDP cycles (i.e., no isolation of any portion of the water system was done).

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