New Microbes and New Infections 65 (2025) 101584

Contents lists available at ScienceDirect

New Microbes and New Infections

journal homepage: www.journals.elsevier.com/new-microbes-and-new-infections

Original Article

Quantitative microbial risk assessment of *Legionella pneumophila* in a drinking water distribution system: A case study

Seyed Mohammad Ranjdoost [a], Mina Owrang [b,*]

[a] Department of medical Laboratory sciences, Faculty of medicine, Sari branch, Islamic Azad University, Sari, Iran
[b] Biological lab Expert, Water and Wastewater Quality Monitoring and Supervision Center East Mazandaran Water and Wastewater Company, Sari, Iran

ARTICLE INFO

Handling Editor: Patricia Schlagenhauf

Keywords:
Quantitative microbial risk assessment
Legionella
Season

ABSTRACT

Background: *Legionella pneumophila* poses a significant health risk in hospital water systems. This study assessed the risk associated with *Legionella* contamination in a hospital drinking water system in Sari, Iran, over one year.
Methods: Water samples were collected seasonally from various hospital taps, including patient room showers and toilet faucets. Both cold and warm water sources were analyzed. Water quality parameters, including pH, chlorine levels, and temperature, were measured. *Legionella* spp. were isolated and enumerated using standard microbiological techniques, and species identification was confirmed via 16S rRNA gene sequencing. A Quantitative Microbial Risk Assessment (QMRA) model was employed to estimate the infection risk from shower and faucet use.
Results: *Legionella* counts were significantly higher in warm water samples and during the summer season. A positive correlation was observed between *Legionella* counts and water pH, whereas negative correlations were found with chlorine levels and water temperature. QMRA results indicated that the estimated annual infection risk exceeded the acceptable limits set by the World Health Organization (WHO) and the United States Environmental Protection Agency (USEPA), particularly during summer.
Conclusions: The findings suggest that existing water management practices may be inadequate for controlling *Legionella* growth and transmission. Seasonal variations significantly impact infection risk, emphasizing the need for improved monitoring and control strategies. However, limitations related to sampling methodology, geographic specificity, and dose-response modeling should be considered when interpreting the results.

1. Introduction

One of the most common causes of drinking water-associated disease outbreaks is *Legionella pneumophila* [1]. This bacterium causes legionellosis, which can result in a pneumonia-like illness known as Legionnaires' disease (LD) or a milder, influenza-like illness known as Pontiac Fever [2]. This aquatic bacterial species inhabits both natural and man-made freshwater environments worldwide [3,4]. Inhalation of contaminated water aerosols is the primary source of LD in humans [3]. The LD occur sporadically and epidemically worldwide, with an average case-fatality rate of 10 % [5,6]. Time-series analysis of LD incidence rates shows an increasing global burden, making it a significant cause of preventable morbidity and mortality [6,7].

Over 65 species of *Legionella* have been identified, but only about 25 are associated with disease [6]. *Legionella pneumophila* (Lp), particularly serogroup 1 (Lp1), is the most common cause, responsible for 80–90 % of cases in Europe and the US, followed by serogroups 3 and 6 [5].

Despite the global prevalence of LD, its true incidence is often undetermined due to inadequate surveillance. A major concern with LD is its potential to cause outbreaks affecting many people, including fatalities. Such outbreaks have been linked to the presence of *Legionella* in domestic hot water supplies, showerheads, cooling towers, and other water systems [8–10]. These man-made aquatic environments share a common characteristic: the generation and spread of inhalable aerosols [11].

Man-made water systems that support *Legionella* growth share not only aerosol-generating properties but also specific physicochemical conditions that facilitate bacterial proliferation. *Legionella pneumophila* responds to key water quality parameters, including pH, temperature, conductivity, chlorine concentration, and carbonate levels. These factors interact to create microenvironments that either promote or inhibit *Legionella* colonization and biofilm formation [12]. Understanding these

* Corresponding author.
E-mail address: mi_owrang@yahoo.com (M. Owrang).

https://doi.org/10.1016/j.nmni.2025.101584
Received 8 August 2024; Received in revised form 1 April 2025; Accepted 2 April 2025
Available online 3 April 2025

interactions is crucial for developing effective control strategies in drinking water distribution systems.

Health Impact Assessment (HIA) has become integral to public health practice within the framework of national and international policies and guidelines for building design and disinfection practices [13,14]. A crucial component of HIA is assessing disease risk through Quantitative Microbial Risk Assessment (QMRA). QMRA is a valuable tool for estimating the risk associated with exposure to microorganisms, providing insights into the probability of infection from pathogens in drinking and bathing water—information that epidemiological studies alone may not offer [15]. QMRA was effectively utilized in a pilot study to predict infection risk from waterborne pathogens in drinking and bathing water under various climate change scenarios [15]. Consequently, QMRA can aid in designing effective microbial monitoring systems and implementing robust water safety plans [16,17].

QMRA is a structured methodology comprising four main phases: hazard identification, exposure assessment, dose-response modeling, and risk characterization [18]. This approach evaluates the likelihood of infection and subsequent illness following exposure to pathogens within drinking-water distribution systems (DWDSs), offering insights beyond those accessible through epidemiological studies alone [19]. In the case of LD, QMRA has utilized dose-response data from guinea pigs as a substitute for unavailable human data, a method documented in several studies [19,20].

To evaluate whether the risk calculated by QMRA exceeds acceptable levels, it should be compared to a standard based on the global burden of the disease, measured by illness, morbidity, and mortality. The Disability-Adjusted Life Years (DALY) index is a key parameter for this purpose. DALY quantifies disease burden by summing the years of life lost (YLL) due to death and the years lived with disability (YLD) due to illness, with one DALY equating to one year of 'healthy' life lost [21,22]. The DALY index is normalized for comparisons across health conditions, making it a useful tool for public health management. The WHO reference level for waterborne diseases is set at 10^{-6}per person per year, while the USEPA's acceptable infection risk level from pathogens in drinking water is 10-4 per person per year [23]. The DALY index assigns a severity weight to each health effect, which is multiplied by the effect's duration and the number of affected individuals to calculate maximal risk [21]. However, a specific DALY value for *Legionella* has not yet been established.

Although QMRA has been applied to assess the risk of *Legionella* infection from various man-made water systems, studies reporting QMRA for *Legionella* are scarce [24–26]. The aim of this study was to utilize one-year survey data on *Legionella* prevalence in a drinking water system to conduct a QMRA in Sari city of Iran. This analysis models public health risks associated with common water usage practices, enabling the assessment of risks posed by *Legionella* in potable water distribution systems.

2. Materials and methods

2.1. Water sampling

Samples were collected from three taps at different points within a hospital in Sari city, the capital of Mazandaran, a northern province of Iran. These sampling points were selected to cover the water route within the hospital's DWDS. Point A (bathing in patient rooms) is the closest to the water entry point and was sampled for one year (from summer 2017 to spring 2018). Points B-E followed the subsequent course of the water. Toilet faucets (points D, F, G) and the second bathing in patient room (point E) were sampled seasonally for one years, concluding in spring 2018. Seasons were defined based on the weather patterns in Northern Iran from 2017 to 2018: Summer (mid-May to mid-September), Autumn (mid-September to mid-December), Winter (mid-December to mid-March), and Spring (mid-March to mid-May). Fifteen water samples were collected per season on the same day. Hot water was included when possible because water heaters use a different pipeline than cold water. The taps were opened for 10–15 min before sampling to clear any accumulated dust and dirt. All samples were collected in sterile polystyrene bottles containing sodium thiosulfate (100 mg/L per liter of water) to neutralize residual chlorine. Further details are available in Rodríguez-Martínez et al. [27].

2.2. Water quality measurements

Abiotic parameters were promptly monitored post water collection for accuracy. Conductivity, temperatures and pH were measured using a MultiMeter MM40+ (Crison, Spain). Free chlorine levels were assessed via the Aquaquant Colorimetric DPD test (Merck, Germany), with a detection limit of 0.01 ppm. In the subsequent sampling period, nitrite, nitrate, manganese, iron, total water hardness, and carbonic hardness were analyzed using Quantofix semi-quantitative test strips (Macherey–Nagel, Germany). Detection limits were 1 mg/L for nitrite, 10 mg/L for nitrate, 1 mg/L for manganese, and 5 mg/L for iron. Total water hardness and carbonic hardness both had detection limits of 5°d.

2.3. Enumeration and isolation of Legionella spp. from water samples

A 1-L water sample was filtered through a 0.2 mm cellulose nitrate filter (Sartorius Stedim Biotech, Germany), following the procedures outlined in ISO 11731:1998 [28]. The filter was transferred to a 15 ml Falcon tube containing 10 ml of the same water sample and was vortexed at maximum speed for 10 min. For *Legionella* detection, aliquots of 0.5 ml and 0.1 ml from the untreated water were plated on *Legionella* -selective GVPC media (Glycine-Vancomycin-Polymyxin-Cycloheximide medium, Becton Dickinson GmbH, Germany). Additionally, a 1 ml sample, after being heated at 50 °C for 30 min, was plated in 0.5 ml volumes onto two GVPC plates. The detection limit for *Legionella* was established at 10 colony-forming units per liter (cfu/l). All plates were incubated at 37 °C, with colonies counted on days seven and fifteen. From each positively identified sample, five colonies were isolated repeatedly, five times, and subsequently preserved in LB medium supplemented with 30 % glycerol at −80 °C.

2.4. Legionella identification and typing

Isolates were identified by amplifying the *Legionella* genus-specific 16S rRNA genes, using the approach described by previous studies [29,30]. The primers employed were Lgsp28R (5′-CACCGGAAATTCCACTACCCTCTC-3′) and Lgsp17F (5′-GGCCTACC AAGGCGACGATCG-3′). Serogroup typing of the *Legionella* isolates utilized the *Legionella* latex test (Oxoid, UK).

2.5. Risk assessment

The potential health risks of *Legionella* exposure from showers and toilet faucets in a water distribution system were assessed using the Quantitative Microbial Risk Assessment (QMRA) method. This evaluation utilized average *Legionella* concentrations in water and data from existing literature. Exposure durations for showers and toilet faucets were derived from previous studies. The average daily shower duration was approximately 7 min [31], resulting in a weekly exposure of 49 min. For toilet faucets, the average use was 7 s per hand-wash, with four hand-washes per day [32], leading to a weekly exposure duration of 2 min and 48 s in the QMRA model.

2.6. Concentration of airborne Legionella and inhalation exposure-dose

The concentration of airborne *Legionella* (C_{air}) was estimated using established emission factor (EF) specific to each exposure point and the average *Legionella* spp. concentrations in water (C_{water}) observed at each sampled location. The EF, defined as the ratio of bacterial concentration

in air to that in water, facilitates the estimation of airborne concentrations from known water concentrations. These factors were instrumental in calculating the weekly exposure oint, as detailed in Equation (1).

$$C_{air} = C_{water} \times EF_{faucets/showers} \quad \text{Equation (1)}$$

C_{air} is the baclable aerosols.

C_{water} is the bacterial concentrations in water.

EF is the emission factor ($EF_{faucets}$: 5.6×10^{-4} and $EF_{showers}$: 3.4×10^{-4} (L/m^3) [33,34].

The EF for faucets was derived from experimental data on aerosols generated by fourteen sink faucets [33]). For showers, the EF applied in the exposure assessment was based on previous studies [19,35].

Inhalation exposure dose was estimated using *Legionella* concentrations in the air, exposure duration, inhalation rate, and the respirable aerosol fractional retention rate [19] Equation (2)

$$IED = C_{air} \times ED \times RR \times IR \quad \text{Equation (2)}$$

IED is the weekly inhaled exposure-dose.

C_{air} is the bacterial concentrations in inhalable aerosols.

ED is weekly exposure duration (2.8 min for Faucets and 49 min for shower) [31,32].

RR is the retention rate of aerosols in the lungs (0.5) [26,36].

IR is the inhalation rate (1.05 m^3/h)[23].

2.7. Risk for L. pneumophila infection

The risk of *L. pneumophila* infection was estimated for each sampling point, with average concentrations calculated for each season. This estimation used the dose–response relationship for Legionnaire's disease and the calculated inhalation exposure. For disease transmission modeling, infection is considered a more protective and useful endpoint than illness or death [26]. The infection risk was calculated using the exponential model in Equation (3) [19].

$$R_w(d) = 1 - e^{(-\gamma dR)} \quad \text{Equation (3)}$$

Here, $R_w(d)$ is the predicted risk given the weekly dose, d (CFU), and γ is the model parameter for *Legionella* infection risk, set at 0.06 (1/CFU) [19]. Additionally, the 95 % confidence intervals for the γ parameter (0.039 and 0.131) were used to calculate the minimal and maximal risks of infection. These confidence intervals were obtained from QMRAwiki, established by the Center for Advanced Microbial Risk Assessment at Michigan State University [37]. These values are based on 10,000 bootstrap iterations of experimental data from Muller et al. (1983), who exposed outbred, specific pathogen-free Hartley strain Guinea pigs to aerosols of the *Legionella* Philadelphia 1 strain in a modified aerosol infection chamber (Tri-R Instruments, Rockville Centre, NY) [38].To evaluate the risk from organisms in water or air, each day is considered an exposure event, so the dose entered in the dose-response model is the total number of microorganisms per day. In this study, each week was considered an exposure event. The cumulative annual risk was calculated using Equation (4).

$$R_A(d) = 1 - \prod_{w=1}^{n} [1 - R_w(d)] \quad \text{Equation (4)}$$

where R_A is the seasonal or annual cumulative risk given $R_w(d)$, and n is the total number of exposure events (13 weeks per season and 52 weeks per year).

2.8. Statistical analysis

Statistical analyses were performed using SPSS (version 24) and Excel 2019. Graphs were prepared with R software (version 4.1.0). Data normality was confirmed with the Kolmogorov-Smirnov test. Significant differences between sampling sites were assessed using one-way ANOVA followed by Tukey's post-hoc test. Pearson's correlation analysis was employed to evaluate the relationships between *Legionella* counts and water quality parameters. A significance level of $p < 0.05$ was set for all tests.

3. Result

3.1. Water quality measurements

Water quality factors are presented in Table 1. The highest conductivity values were observed in the spring. The pH of cold water was highest in summer and lowest in winter. Cold water temperatures significantly differed between the seasons ($P = 0.01$), with the maximum temperature in summer and the minimum in winter, while there was no significant difference between autumn and spring temperatures ($P = 0.230$). Chlorine concentration varied between sampling points but did not show any significant differences among them ($P = 0.121$). Manganese, iron, nitrate, and nitrite were not detected in either cold or warm water. Total hardness and carbonate hardness showed no differences between sampling points, water types, or the two sampling seasons. Total hardness was consistently high, ranging from 3.58 to 4.25 mmol/l. Carbonate hardness was also high, consistently measured at 3.6 mmol/l.

3.2. Enumeration, isolation and identification Legionella spp

The highest count of *Legionella* was recorded in the summer season, with a maximum mean of 2.40E+03 and a minimum mean of 1.00E+02 (Table 2). Conversely, the lowest *Legionella* distribution occurred in the winter season. *Legionella* distribution in points A and E (warm water) was significantly higher across all seasons compared to points B, C, and D (cold water) ($P = 0.02$) (Table 2).

16S rRNA gene sequencing of isolates from various seasons and sampling points confirmed that most isolates were *L. pneumophila*, showing over 99.9 % gene similarity (Table 3).

3.3. The effect of water quality parameters on Legionella counts

Multiple correlations between *Legionella* counts and water quality parameters were analyzed (Fig. 1). A significant positive correlation was found between *Legionella* counts and water pH ($p = 0.01$). Conversely, significant negative correlations were observed between *Legionella* counts and both chlorine levels and water temperature ($p = 0.01$ and $p = 0.03$, respectively). The correlations between *Legionella* counts and both carbonate hardness and conductivity were not significant.

3.4. Risk assessment

Seasonal average concentrations of *Legionella* species in water (C_{water}) and air (C_{air}) are provided in Table 4. The highest bacterial concentrations in both water and air were found in warm water (showers), and the lowest were in cold water (faucets) during winter. Weekly inhalation exposure doses (cfu/week) for faucet and shower use were calculated based on air concentrations of *Legionella*, exposure durations, inhalation rates, and respirable aerosol retention rates, as described in the methods section. The calculated inhaled exposure doses (IED) for both warm water (showers) and cold water (faucets) are also presented in Table 4. Weekly inhaled exposure doses were applied to the exponential risk characterization model. The annual risk levels associated with both exposure events (warm water (shower) and cold water (faucets)) exceeded the WHO's acceptable annual DALY for waterborne diseases (1×10^{-6} per person per year). Even the minimal annual risk values (1×10^{-4}) surpassed the acceptable infection risk level (Table 4). The annual risk values also exceeded the threshold set by the United States Environmental Protection Agency (USEPA) ($1 \times 10-4$ per person per year). Significant seasonal differences were observed, with the highest risks occurring in summer and the lowest in winter (Table 4). Annual risk levels for warm water (shower) were higher than those for

Table 1
Water quality data of the drinking water.

Sampling point	Conductivity (µS/cm)	pH	Temp (°C)	Chlorine (ppm)	Carbonate hardness (mmol/l)	Total hardness (mmol/l)
Summer						
A warm	980	7.99	51.3	0.01	3.69	3.61
B	820	7.70	28.3	>0.3	4.11	3.60
C	1021	7.71	27.8	0.05	4.13	3.62
						3.6
						3.6
						0.3
D warm	990	7.87	52.3	0.08	3.58	3.60
E	980	7.69	27.3	0.05	4.00	3.61
Autumn						
A warm	1000	7.80	51.4	0.01	3.80	3.62
B	1003	7.46	25.1	0.06	4.15	3.61
C	970	7.47	24.3	>0.2	4.11	3.62
						3.
D warm	820	7.82	51.3	<0.01	3.80	3.60
E	1001	7.51	24.4	0.05	4.15	3.60
Winter						
A warm	960	7.71	50.4	<0.01	3.70	3.62
						3.61
B	978	7.30	17.3	0.025	4.21	3.60
C	1001	7.29	17.8	0.08	4.20	3.60
					3.7	
D warm	950	7.73	50.2	0.01	3.80	3.60
E	990	7.31	17.1	>0.2	4.25	3.60
Spring						
A warm	1170	7.82	51.3	0.01	3.98	3.61
B	1230	7.51	25.1	0.03	4.18	3.60
C	1029	7.49	24.6	0.08	4.12	3.60
D warm	1047	7.80	51.5	<0.01	3.79	3.60
E	1089	7.45	25.5	>0.2	4.17	3.60

Table 2
Legionella counts (cfu/L) in water by sampling point and season. The detection limit for *Legionella* isolation was 10 cfu/L.

	Type of sample	Summer	Autumn	Winter	Spring
A	Warm water (Shower)	1.30E+03	1.00E+03	1.00E+03	1.10E+02
B	Cold water (Faucets)	1.20E+03	[a]ND	ND	1.00E+03
C	Cold water (Faucets)	1.10E+03	1.10E+03	3.00E+02	ND
E	Warm water (Shower)	2.40E+03	2.10E+03	ND	1.01E+03
D	Cold water(Faucets)	1.00E+03	1.20E+03	1.00E+02	1.05E+02

[a] ND: not detected.

Table 3
Accession numbers of *Legionella* isolates sequenced for the 16S rRNA gene.

Season	Sampling point	16S rRNA Genbank accession number
Summer	B	KF537550
	B	KF537557
	C	KF537552
	D	KF537545
	E	KF537540
	A	KF537546
	A	KF537549
	D	KF537543
	E	KF537544
	D	KF537555
Autumn	A	KF537549
	A	KF537553
	C	KF537550
	D	KF537559
	D	KF537558
Winter	C	KF537544
	D	KF537565
	E	KF537543
Spring	B	KF537550
	B	KF537543
	A	KF537546
	D	KF537544

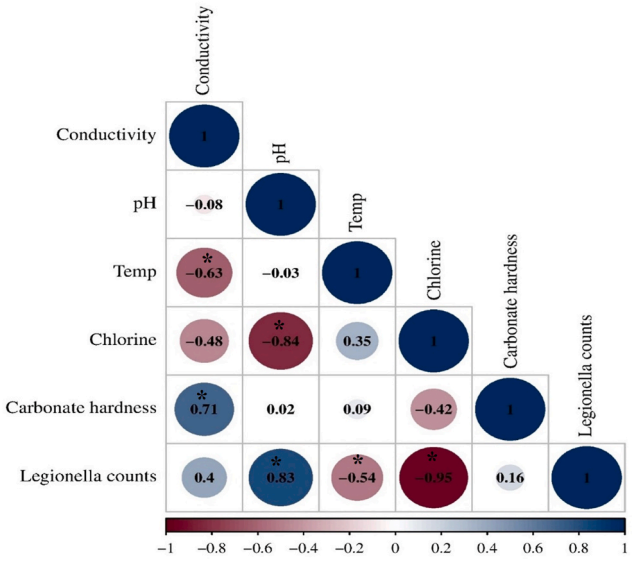

Fig. 1. Pearson correlations of *Legionella* counts and water quality parameters in the water samples. *: Indicates significant difference among parameters.

cold water (faucets) use (Table 4).

3.5. Limitations of the study

The study's sampling design aimed to represent the hospital's water distribution system but had inherent limitations. The selected sampling points (A–G) may not fully capture microbial dynamics, particularly in peripheral areas not directly connected to these locations. Additionally, reliance on three primary taps may not adequately reflect the system's heterogeneity. Temporal variations beyond seasonal trends could also have been overlooked due to the sampling frequency. Future studies

Table 4
Average seasonal *Legionella* spp. concentrations in water (C_{water}) and air (C_{air}), weekly inhaled *Legionella* exposure dose (IED), and seasonal and annual risk levels expressed in DALYs (Disability-Adjusted Life Years) for warm water (shower) and cold water (faucet) use.

Parameters	Type of sample	Summer	Autumn	Winter	Spring
water (C_{water}) (CFU/L)	Warm water (Shower)	2.35E+03	2.20E+03	1.00E+03	6.15E+02
	Cold water (Faucets)	2.63E+03	1.50E+03	3.00E+02	1.04E+03
air ($_{Cair}$)(CFU/L)	Warm water (Shower)	8.46E-01	7.92E-01	3.60E-01	2.21E-01
	Cold water (Faucets)	1.47E+00	8.40E-01	1.68E-01	5.80E-01
IED (CFU/week)	Warm water (Shower)	2.18E+01	2.04E+01	9.26E+00	5.69E+00
	Cold water (Faucets)	2.16E+00	1.23E+00	2.47E-01	8.53E-01
Risk (DALY)	Warm water (Shower)	5.50E-03	2.60E-03	9.80E-04	2.20E-03
	Cold water (Faucets)	3.50E-03	1.20E-03	6.90E-04	1.30E-03

should expand the number of sampling points and increase sampling frequency to provide a more comprehensive assessment of *Legionella* prevalence. The study followed ISO 11731:1998 protocols for *Legionella* detection, a widely accepted standard. However, culture-based methods may underestimate *Legionella* populations, as they fail to detect viable but non-culturable (VBNC) bacteria. Molecular techniques, such as qPCR, offer more accurate quantification by identifying both culturable and VBNC forms. Future research should integrate culture-based and molecular approaches to achieve a more comprehensive assessment of *Legionella* abundance and viability. The findings of this study are specific to the hospital in Sari, Iran, and may not be directly generalizable to other healthcare facilities. *Legionella* colonization and proliferation can be influenced by factors such as plumbing infrastructure age, water treatment protocols, hospital size, and patient demographics. Variations in these factors across institutions may result in differing *Legionella* dynamics. Therefore, caution is warranted when extrapolating these results to other settings. This study was conducted in Mazandaran Province, northern Iran, where climate conditions may influence seasonal variations in *Legionella* counts. However, the generalizability of these findings to hospitals in other climatic zones remains uncertain. Future research should examine *Legionella* prevalence across diverse geographic regions to better understand the impact of climate on its dynamics in hospital water systems.

The QMRA model utilized a dose-response relationship based on guinea pig studies due to the absence of human data, a common but uncertain approach. Physiological differences between guinea pigs and humans may influence *Legionella* susceptibility, potentially affecting risk estimates. Developing human-specific dose-response models remain a critical research need. The exposure assessment relied on assumptions about shower and faucet usage durations, derived from previous studies. However, their accuracy in reflecting actual water usage patterns among hospital patients and staff is uncertain. Additionally, variations in aerosol generation, influenced by water pressure, fixture type, and user behavior, were not explicitly considered. Future research should incorporate direct measurements of water usage patterns and aerosol concentrations for more precise exposure assessments.

4. Discussion

This study was conducted in northern Iran, along the southern coast of the Caspian Sea, which has a mild and humid climate. Water temperatures between 27 °C and 42 °C favor *Legionella* growth, making this region ideal for *Legionella* survival throughout most of the year [39]. *Legionella* spp. was detected in the monitored water system along the whole year in three cold water (Faucets) and two warm waters (Shower) out of the five sampling points.

The influence of physical and chemical water characteristics on the presence of *Legionella* in water systems has been extensively studied in recent years. This study found a significant positive correlation between *Legionella* counts and water pH. The positive correlation between water pH and *Legionella* counts can be attributed to several factors. *L. pneumophila* thrives in slightly alkaline conditions (pH 6.8–8.0), with growth significantly declining below pH 5.5 and above pH 9.2 [12]. At the molecular level, pH influences the proton motive force across bacterial membranes, which is essential for energy generation and nutrient transport [40]. It also modulates the activity of key metabolic enzymes, most of which function optimally in slightly alkaline environments [41]. Additionally, pH affects *Legionella* 's interactions with protozoan hosts, particularly amoebae. Studies indicate that phagocytosis and intracellular replication are more efficient at pH 6.5–8.0, enhancing bacterial virulence and resistance to disinfectants [42]. Water with a pH of 7.45 or higher is associated with a 4.05 times higher risk of *Legionella* colonization in cold water systems [12], suggesting that slightly alkaline conditions may favor *Legionella* growth. Conversely, significant negative correlations were observed between *Legionella* counts and both chlorine levels and water temperature. In cold water systems, a free chlorine concentration below 0.375 mg/L increases the risk of *Legionella* presence by 9.76 times, highlighting the importance of proper water disinfection and maintaining appropriate chlorine levels to inhibit bacterial growth [12,43]. Additionally, high water temperatures above 50 °C were found to reduce the growth of *Legionella* [43].

The negative correlation between chlorine levels and *Legionella* counts observed in this study reflects chlorine's potent antimicrobial properties. Free chlorine (HOCl and OCl^-) penetrates bacterial membranes and induces oxidative damage to essential cellular components, including proteins, enzymes, and nucleic acids, ultimately causing cell death [44]. However, *Legionella* can develop resistance through various mechanisms. In biofilms, extracellular polymeric substances (EPS) form a protective barrier that limits chlorine penetration [45]. Moreover, *Legionella* residing within amoebae gain substantial protection, requiring 10–100 times more chlorine for inactivation than free-living bacteria [46]. These factors underscore the challenge of maintaining effective chlorine levels for *Legionella* control in distribution systems.

The observed increase in *Legionella* counts in warm water is consistent with its known temperature preferences. Several molecular mechanisms underlie this relationship. Within the optimal range of 25°C-42 °C (peak at 35°C-37 °C), *Legionella* activates virulence factors through temperature-responsive regulatory systems [47]. The heat shock response, including the expression of heat shock proteins (HSPs), facilitates adaptation to temperature fluctuations [48]. Temperature also influences *Legionella* replication within protozoan hosts. Between 30 °C and 40 °C, increased metabolic activity in both *Legionella* and amoebae accelerates bacterial replication [48]. However, at temperatures above 50 °C, replication ceases, and prolonged exposure induces bacterial death through protein denaturation and membrane damage [49].

The influence of water quality parameters on *Legionella* growth and

survival directly affects risk estimates in the QMRA model. Seasonal variations in infection risk, for example, can be partially attributed to temperature-dependent proliferation, particularly during summer when water temperatures align with *Legionella* 's optimal growth range. Likewise, the effectiveness of chlorination as a control measure depends on understanding chlorine resistance mechanisms, especially within biofilms and amoebae hosts. Quantitative microbial risk assessment models were used to evaluate the health risks posed by *Legionella* spp. in the DWDS. Seasonal and annual risk levels were characterized and expressed using the DALY index for two common exposure scenarios: warm water (shower) and cold water (faucet) usage. The highest seasonal infection risk values were observed for both warm and cold water use in summer. In both cases, these values exceeded the WHO's accepted risk level for waterborne pathogens of 1×10^{-6} per year by three orders of magnitude [50].

Additionally, the annual risk values for *Legionella* infection calculated in this study in both warm water (shower) and cold water (faucet) exceeded the accepted infection risk limit for pathogenic microorganisms in drinking water, set at 1×10^{-4} per year by the US Environmental Protection Agency [23]. The acceptable risk levels set by the WHO and EPA are primarily defined for members of the Enterobacteriaceae family, which have different infection transmission routes in humans. Unlike Enterobacteriaceae, which enter the body through the skin or digestive system, *Legionella* infects via the lungs. Thus, traditional infection risk calculations may not be well suited for *Legionella*. Recently, Azuma et al. (2013) suggested an accepted risk level specifically for *Legionella* infections. They performed a QMRA based on residential bathrooms in the Adachi outbreak area in Japan and estimated risk levels. Their study concluded that the QMRA model developed by Armstrong and Haas (2007) was adequate for predicting infection risks at $1 \times 10\hat{}-4$ per year [20]. The EPA has also adopted this value as the accepted risk level for waterborne diseases [23]. The annual infection risk values calculated in this study align with these findings. This study demonstrates that *Legionella* prevalence in water systems and the associated infection risk are season-dependent and can vary significantly between closely located points. Therefore, a single sampling point in an annually sampled area does not accurately represent the overall prevalence of *Legionella* or the infection risk in the DWDS. Routine sampling and control measures should be intensified during the summer, when the risk of infection and illness is significantly higher.

A range of *Legionella* counts has been observed in water distribution systems, and the predicted QMRA risk may be inaccurate if a significant portion of the *Legionella* species present differs in virulence traits [35]. However, the main causative agent of Legionnaires' disease, accounting for over 90 % of reported cases [3,51]. Legionnaires' disease is a serious and potentially life-threatening illness. The population potentially exposed in the studied water system includes about 900000 people, some of whom are elderly adults, smokers, and individuals with weakened immune systems, making them particularly susceptible [51]. Therefore, these findings underscore the need for preventive measures, such as disinfection of the water distribution system.

5. Conclusion

The study demonstrates that *Legionella pneumophila* poses a significant health risk in the studied hospital's water system, with seasonal variations in prevalence and infection risk. The QMRA model effectively predicted these risks, which were found to exceed recommended safety levels, especially in summer. This work underscores the importance of seasonally adjusted water management and disinfection strategies to protect susceptible individuals from *Legionella* -associated diseases.

CRediT authorship contribution statement

Seyed Mohammad Ranjdoost: Writing – review & editing, Writing – original draft, Software, Formal analysis, Data curation. **Mina Owrang:** Supervision, Software, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

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

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