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Source: Air, Soil and Water Research, 7(1)

Published By: SAGE Publishing

URL: https://doi.org/10.1177/ASWR.S12972

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Air, Soil and Water Research

Predictive Factors of *Legionella pneumophila* Contamination in Cooling Tower Water

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ABSTRACT: A cross-sectional study of 160 water samples collected from 72 cooling towers in 4 hospitals, 7 department stores, and 3 hotels in Bangkok was carried out to investigate *Legionella pneumophila* contamination and its predictive factors. All water samples were cultured for *Legionella* spp. and tested for *L. pneumophila* by real-time polymerase chain reaction (PCR). Some cooling tower parameters were measured and recorded. Data were analyzed using χ^2-test, odds ratio and stepwise logistic regression analysis at the significant level of $\alpha = 0.05$. Results revealed that the *Legionella* spp. contamination was 20.0% (32/160) and for *L. pneumophila* was 61.3% (98/160). The sensitivity of real-time PCR was higher than that of the culture. Factors significantly associated with *L. pneumophila* contamination by χ^2-test were: the cooling tower model, size, use duration, pH, water temperature, use of ozone, and residual free chlorine (95% CI of OR > 1.0, $P < 0.05$). After stepwise logistic regression analysis, four predictive factors remained. These included the cooling tower model being a cross-flow type (adjusted OR = 3.1, 95% CI = 1.2–7.8, $P = 0.017$), use duration >5 years (adjusted OR = 3.6, 95% CI = 1.3–10.1, $P = 0.016$), water temperature <29.4°C (adjusted OR = 7.9, 95% CI = 2.1–29.6, $P = 0.002$), and residual free chlorine <0.2 ppm (adjusted OR = 8.5, 95% CI = 2.1–34.9, $P = 0.003$). Additionally, the risk probability for *L. pneumophila* contamination was estimated to be 13.9–97.1%, depending on the combination of predictive factors.

KEYWORDS: cooling tower, *Legionella pneumophila*, risk of contamination, real-time PCR

CITATION: Luksamijarulkul et al. Predictive Factors of *Legionella pneumophila* Contamination in Cooling Tower Water. *Air, Soil and Water Research* 2014:7 11–17 doi:10.4137/ASWR.S12972.

RECEIVED: October 8, 2013. **RESUBMITTED:** December 8, 2013. **ACCEPTED FOR PUBLICATION:** December 13, 2013.

ACADEMIC EDITOR: Carlos Alberto Martinez-Huitle, Editor in Chief

TYPE: Original Research

FUNDING: This research project was supported by the Thailand Research Fund (TRF) and Square Cooling Tower Co., Ltd. for the TRF-Master Research Grants (Window I), 2011, and received a publication grant from the China Medical Board, Faculty of Public Health, Mahidol University.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

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Introduction

A Gram-negative bacterium, *Legionella* spp. is a non-spore forming, aerobic bacillus with a single polar flagellum, unable to capsulate.[1,2] The shape will increase in length if experiencing starvation and it thrives in areas where there are high concentrations of rust, sludge, algae, and organic particles.[3] The bacterium survives in water environments with a primary host and free-living protozoa, and can be found in waters ranging from cold to very hot.[1,4] It has been found to possess the ability to survive in tap water at room temperature for more than a year, but temperatures approaching 55°C start to kill the organism.[5] There are 42 species and 64 serogroups among *Legionella* spp.[6,7] The species that most frequently causes human disease is *Legionella pneumophila*. It can cause legionellosis, which includes two distinct syndromes, Legionnaires' disease (a form of pneumonia) and Pontiac fever. In most developing countries, including Thailand, the total case numbers of the disease are generally underestimated. *L. pneumophila* serogroup 1 is the most common etiological agent, and causes about 80–90% of legionellosis cases.[7,8] The innate ability of *L. pneumophila* to replicate inside eukaryotic cells and its capacity to avoid regular pathogen control mechanisms in the host have led to its emergence as an important cause of community-acquired pneumonia and hospital-acquired pneumonia in adults.[9] General risk factors for the illness include being male and older than 50 years, cigarette

smoking, excess consumption of alcohol, chronic lung disease, chronic degenerative diseases, and immunodeficiency states. Usually people who are exposed will develop the disease at a rate of less than 5%, but the fatality rate may reach 15%.[10,11]

Common sources of *L. pneumophila* include cooling towers, which are moist, evaporative condensers that form part of air conditioning systems, domestic hot water systems, fountains, and similar disseminators that tap into a public water supply. Cooling towers have been reported as the primary source in major outbreaks of legionellosis.[9,12,13] Cooling towers can be a particular hazard because fine water droplets are readily generated and transported via air current. Microorganisms can survive in aerosols and have been found as far as 200 meters away from the aerosol source. *Legionella* infections have been associated with cooling towers at distances of up to 7 kilometers.[4] Particles with a diameter of less than 5 µm can be deeply inhaled into the respiratory system and finally cause legionellosis. Many buildings in Bangkok, including hospitals, department stores, hotels and others, have central air conditioning units with cooling tower systems that may be contaminated with *L. pneumophila*. These likely create and transmit aerosols, which can in turn increase the risk of legionellosis. This study investigates *L. pneumophila* contamination and its predictive factors in cooling tower water collected from selected buildings in Bangkok.

Materials and Methods

Study sites and study samples. This cross-sectional study was carried out to investigate *L. pneumophila* contamination in 160 water samples collected from 72 cooling towers located at 4 hospitals (21 cooling towers), 7 department stores (35 cooling towers), and 3 hotels (16 cooling towers) in Bangkok. The study was reviewed and approved by the ethics committee of the Faculty of Public Health, Mahidol University, with a Research with Exemption category.

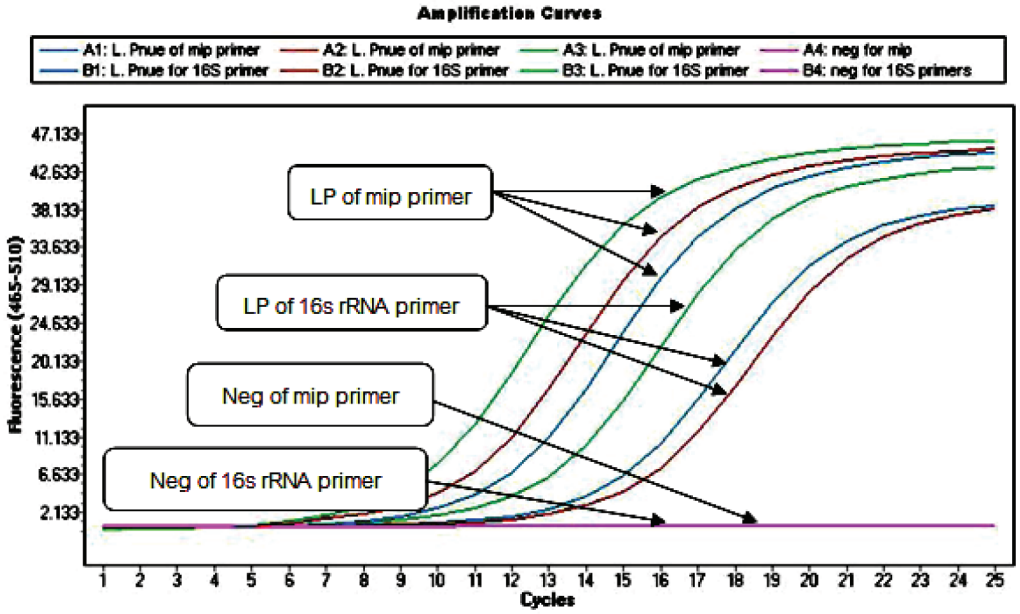
Approximately 2 L of water in sterile screw-cap bottles was collected aseptically from two or three points on each cooling tower (water-in, water-out, and reservoir). The sample size was calculated from a formula: $n = Z^2_{\alpha/2}\, PQ/d^2$ (Z is the standard score of normal distribution at $\alpha = 0.05$ and two tails = 1.96, d = allowable error at 0.075, P is the probability of *L. pneumophila* positivity in cooling tower water by real-time PCR from a previous study = 63%[14] or P = 0.63, Q = 1-P, 0.37). Each water sample was divided into two equal parts, one to test for the *Legionella* culture and one to perform real-time PCR for *L. pneumophila,* following a study by Yaradou et al (2007).[14] Cooling tower characteristics, including design or model and size, maintenance system, and duration of use were recorded. Some water parameters including temperature, pH, and residual free chlorine were measured. All water samples were transferred using ice-boxes to the Department of Microbiology at the Faculty of Public Health, Mahidol University, within four hours and kept at 4–10°C until total bacterial count (TBC), *Legionella* culture, and real-time PCR were performed.

Detection of *Legionella* spp. by culture. To each water sample, 0.5 mL of 0.1 mol/L sodium thiosulfate ($Na_2S_2O_3$ 0.5 mL/sample water 1000 mL) was added, to neutralize disinfectants, following Majid et al (2007).[15] The water samples were concentrated by filtration through 0.45 µm pore-size cellulose nitrate filters, following the study of Yaradou et al (2007).[14] Each filter was cut and transferred into a micro-centrifuge tube. Then, 1.5 mL of sterile distilled water was added to the tube and vortexed for two minutes to recover bacterial cells. Portions of the concentrated sample were treated with 0.2 mol/L HCl-KCl, pH 2.2 at room temperature for 20 minutes to eliminate non-*Legionella* organisms. Next, 100 µL of the concentrate (undiluted and 10-fold diluted samples) were plated onto modified buffered charcoal yeast extract (MBCYE) agar. The inoculated plates were kept in a humidified incubator at 35–37°C for 7 days, and the grayish-white, shiny colonies were counted as suspected *Legionella* spp. Then, suspected colonies were subcultured onto normal BCYE agar and BCYE agar without L-cysteine for verification. If the isolate could grow only on BCYE and the Gram stain was negative, it was determined to be a *Legionella* spp.

Detection of *L. pneumophila* by real-time PCR.

Sample preparation for PCR. Following the Roche method, 1000 mL water samples were concentrated by filtration through 0.45 µm pore-size cellulose nitrate filters. The filter was placed into a centrifuge tube. Then, 1.5 mL of sterile distilled water was added to each centrifuge tube and vortexed for bacterial release for 2 minutes. Next, 1 mL of the concentrate was transferred to a 1.5 mL micro-centrifuge tube and centrifuged at 10,000 g for 5 minutes. The supernatant was discarded. The sediment was used for DNA extraction with a High pure PCR template preparation kit (Roche, Germany). The Roche kit protocol was followed step by step for enzymatic lysis and DNA extraction. Then, the supernatant containing the DNA was stored at –20°C until use.

Real-time PCR. Real-time PCR used in this study followed the study of Yaradou et al (2007).[14] The method is highly specific and sensitive for environmental water samples.[14,16] LightCycler version 1.1 instruments from Roche Applied Science (Roche, Mannheim, Germany) were used with a final volume of 20 µL of total reaction mixture, which consisted of 15 µL of master mix and 5 µL of template. The master mix was prepared with the following final concentrations per capillary tube: 0.5 µL (both) primers, 10 µL LightCycler 480 SYBR Green I Master, 2x conc. (2x-concentrated master mix that contains FastStart Taq DNA polymerase, reaction buffer, nucleotides—dATP, dCTP, dGTP, dUTP—and SYBR Green I), 4 µL PCR-grade. Primers for PCR amplification of *Legionella* spp. (16S rRNA gene) and *L. pneumophila* (mip gene) were used in the reaction. The experimental LightCycler protocol consisted of 10 minutes at 95°C for *Taq* polymerase activation, 45 cycles of PCR amplification (95°C for 15 seconds, 60°C for 15 seconds, and 72°C



The optimal condition for *L. pneumophila* detection by the real-time PCR

	Primers used in the reaction	Optimal Tm
16S-rRNA		
Forward primer	451 AGG GTT GAT AGG TTA AGA GC 470	
Reverse primer	836 CCA ACA GCT AGT TGA CAT CG 817	81°C
mip gene		
Forward primer	110 GCA TTG GTG CCG ATT TGG 126	
Reverse primer	276 G[CT]T TTG CCA TCA AAT CTT TCT GAA 254	86°C
PCR program		
Pre-denaturation	95°C 10 min	1 cycle
Denaturation	95°C 15 s	45 cycles
Annealing	60°C 15 s	
Polymerization	72°C 20 s	

Figure 1. The optimal condition for *L. pneumophila* detection by real-time PCR.

for 20 seconds), melting (65 to 97°C at 0.1°C/second), and a cooling step (40°C for 30 seconds). This process is shown in Figure 1. A positive control (*L. pneumophila* Sg1 strain ATCC 33152) and a negative control (purified PCR grade water) were included in all PCR assays. All the runs were completed with a melt curve analysis to confirm the specificity of amplification by slowly ramping the instrument from a low temperature to a high temperature. The range of between 80°C and 81°C was mip gene. The range of between 84°C and 85°C was 16s rRNA gene. Crossing point (Cp) values were determined by LightCycler 480 software version 1.2 and put into an MS Excel data sheet (Microsoft) for analysis after background subtraction. Cp cycles were plotted with log of input DNA quantities to calculate the slope (see Fig. 1).

Detection of physicochemical parameters and total bacterial counts of water samples. Water temperature, pH (direct reading pH meter, probe and meter) and residual free chlorine using the DPD method (N,N-diethyl-p-phenylenediamine, and colorimeter Protronics Intertrade Co., Ltd) were determined at the time of water sample collection. Additionally, the total bacterial count was detected using 0.1 mL of water samples with a 10-fold dilution series of the concentrated water samples (10^{-1}, 10^{-2} and 10^{-3}) inoculated on plate count agar in duplicate tests. All plates were incubated at 35°C for 24–48 hours. The number of colonies were counted, and reported in colony forming units/mL (CFU/mL).

Statistical analysis. Data about cooling tower characteristics and parameters of water samples with and without *L. pneumophila* were compared and analyzed to search for factors associated with the contamination and predictive factors using univariate analysis (χ^2-test), odds ratio and 95% confidence interval, and stepwise logistic regression analysis. The statistically significant level was set at $\alpha = 0.05$.

Table 1. Summary of physicochemical and biological parameters of studied cooling towers and water samples.

PHYSICOCHEMICAL AND BIOLOGICAL PARAMETERS	MEAN OR RATIO	RANGE (MIN–MAX)
Model: counter-flow/cross-flow ratio (16:56 cooling towers)	1:3.5	NC
Size (tons)	450	300–600
Duration of use (years)	7	3–12
Maintenance (time per year)	6	1–12
Biocide or ozone: use/no use	1:2.6	NC
pH	7.0	6.0–8.5
Water temperature (°C)	32.2	16.7–38.2
Residual free chlorine (ppm)	0.1	0.0–0.5
Total bacterial count (CFU/ml)	6.6×10^5	2.0×10^3–5.0×10^6

NC = Not calculated.

Results and Discussion

Cooling tower characteristics and parameters of studied water samples. The present study found two models of cooling towers used in the studied buildings, a counter-flow model (16 cooling towers) and a cross-flow model (56 cooling towers). Approximately 50% of studied cooling towers had a size less than 500 tons, ranging from 300 to 600 tons (average = 450 tons). Half of them had been in use for at least 10 years, and ages of towers ranged from 3 to 12 years (average = 7 years). The mean maintenance frequency was 6 times a year, ranging from 1 to 12 times a year. However, most of the studied cooling towers were not treated with biocide or ozone to inhibit the growth of *Legionella* spp. The ratio of using biocides, ozone, or none was 1:2.6. It was found that the mean value of residual free chlorine was 0.1 ppm, ranging from 0.0 to 0.5 ppm. The mean pH was 7.0, ranging from 6.0 to 8.5. Average water temperature was 32.2°C, and average total bacterial count was 6.6×10^5 CFU/mL, ranging from 2.0×10^3 to 5.0×10^6 CFU/mL, as shown in Table 1.

***Legionella* spp. and *L. pneumophila* contamination in cooling tower water.** In total 160 water samples, 32 (20.0%) were positive for *Legionella* spp. by culture, and 98 (61.3%) were positive for *L. pneumophila* by real-time PCR. The sensitivity of real-time PCR was higher than that of the culture. Water samples collected from the reservoirs of the cooling towers had the highest percentage of *L. pneumophila* contamination by real-time PCR compared with those collected from the water-in and the water-out flow areas of the cooling towers (reservoirs: 76.0%, water-in: 44.9%, and water-out: 52.8%). It was statistically significant with $P < 0.05$ as shown in more details in Table 2.

Predictive factors and risk probability of *L. pneumophila* contamination. From univariate analysis, the cooling tower characteristics and parameters found to be significantly associated factors for *L. pneumophila* contamination were: (1) cross-flow model, $P = 0.005$ (crude OR = 2.82, 95% CI = 1.26–6.37); (2) size <500 tons, $P = 0.010$ (crude OR = 2.37, 95% CI = 1.16–4.88); (3) use duration >5 years, $P = 0.005$ (crude OR = 3.79, 95% CI = 1.32–11.23); (4) pH > 6, $P = 0.019$ (crude OR = 2.88, 95% CI = 1.07–7.87); (5) no use of biocide or ozone, $P = 0.018$ (crude OR = 2.32, 95% CI = 1.08–4.97); (6) residual free chlorine <0.2 ppm, $P = 0.009$ (crude OR = 4.52, 95% CI = 1.22–20.53); and (7) water temperature <29.4°C, $P = 0.005$ (crude OR = 4.45, 95% CI = 1.40–18.52). After stepwise logistic regression analysis, 4 significantly predictive factors remained including: the cooling tower model as a cross-flow type, $P = 0.017$ (OR = 3.09, 95% CI = 1.22–7.84); use duration >5 years, $P = 0.016$ (OR = 3.59, 95% CI = 1.27–10.14); residual free chlorine <0.2 ppm, $P = 0.003$ (OR = 8.49, 95% CI = 2.06–34.93); and water temperature <29.4°C, $P = 0.002$ (OR = 7.87, 95% CI = 2.09–29.59). (See Table 3). Additionally, the risk probability for *L. pneumophila* contamination was estimated using four predictive factors. It was found that the risk probability ranged from 13.9 to 97.1%, depending on the combination of predictive factors, as shown in Table 4.

A cooling tower is a heat rejecting device, which includes both direct (open) and indirect (closed) circuits. It transmits waste heat to the atmosphere through the cooling of a water stream to a lower temperature.[17] In general, there are two models of cooling towers used in buildings, a cross-flow type and a counter-flow type. In a cross-flow cooling tower, air flows horizontally through the fill as the water moves downward, and in a counter-flow model, air flows upwardly through the fill or tube bundles, opposite to the downward movement of the water.[17,18] The present study found that most of the studied cooling towers were cross-flow types and the ratio of cross-flow models to counter-flow models was 3.5:1. The findings from analysis of risk factors for the contamination of *L. pneumophila* in cooling tower water indicated that the cross-flow model significantly increased the risk of *L. pneumophila* contamination when compared to the counter-flow model (adjusted OR = 3.09, 95% CI = 1.22–7.84, $P = 0.017$). The water movement in the cross-flow model probably increased the settlement of organic particles and sludge in the reservoir of the cooling

Table 2. Prevalence of *Legionella* spp. and *L. pneumophila* contamination in water samples by culture and real-time PCR distributed by places and sites of water collection.

SELECTED PLACES	NO. OF STUDIED WATER SAMPLES	NO. (%) OF WATER SAMPLES WITH POSITIVE CULTURE FOR *LEGIONELLA* SPP.	NO. (%) OF POSITIVE *L. PNEUMOPHILA* BY REAL-TIME PCR
Hospitals	**55**	**17 (30.9)**	**30 (54.5)**
Water-in	16	5	7
Water-out	15	4	7
Reservoir	24	8	16
Department stores	**73**	**13 (17.8)**	**49 (67.1)**
Water-in	24	5	13
Water-out	14	2	7
Reservoir	35	6	29
Hotels	**32**	**2 (6.3)**	**19 (59.4)**
Water-in	9	0	2
Water-out	7	1	5
Reservoir	16	1	12
Total	**160**	**32 (20.0)**	**98 (61.3)**
Water-in	49	10 (20.4)	22 (44.9)[a]
Water-out	36	5 (13.9)	19 (52.8)[b]
Reservoir	75	17 (22.7)	57 (76.0)[c]

Notes: [a,c]and [b,c]were statistically significant, $p < 0.05$.
[a,b]was not significant, $p > 0.05$.

towers. Sediment, sludge, and some organic matter could be nutrient sources for *Legionella* spp.[19,20]

Cooling towers frequently generate droplets by distributing water over a packing material through which there is a counter-current flow of air.[17,18] A previous study reported that major *Legionella* outbreaks have been associated with relatively small systems (i.e. <100 tons).[21] This present study supported that finding, with the smaller cooling towers (<500 tons) having higher risk of *L. pneumophila* contamination in the cooling tower water (crude OR = 2.37, 95% CI = 1.16–4.88, $P = 0.010$), but it was not statistically significant after multivariate analysis ($P > 0.05$). Additionally, the recommended schedule for cooling tower maintenance is at least twice a year and determination of total bacterial count should be frequently performed to evaluate proper water treatment. In this study, the average maintenance frequency was six times per year, and surprisingly, the total bacterial count in cooling tower water was

Table 3. Risk factors for *Legionella pneumophila* contamination in cooling tower water detected by real-time PCR, univariate and multivariate analyses.

PARAMETERS	CRUDE OR (95% CI OF OR)	p-VALUE FROM χ^2-TEST	ADJUSTED OR (95% CI OF OR)	p-VALUE FROM STEPWISE LOGISTIC REGRESSION
Model: cross-flow type	2.82 (1.26–6.37)	0.005*	3.09 (1.22–7.84)	0.017*
Size: <500 tons	2.37 (1.16–4.88)	0.010*		
Maintenance: <2 times per year	2.67 (0.51–26.49)	0.209		
Duration of use: >5 years	3.79 (1.32–11.23)	0.005*	3.59 (1.27–10.14)	0.016*
pH: >6	2.88 (1.07–7.87)	0.019*		
Biocide or Ozone: No	2.32 (1.08–4.97)	0.018*		
Residual free chlorine: <0.2 ppm	4.52 (1.22–20.53)	0.009*	8.49 (2.06–34.93)	0.003*
Water temperature: <29.4°C (85.0°F)	4.45 (1.40–18.52)	0.005*	7.87 (2.09–29.59)	0.002*
TBC: $\geq 10^5$ CFU/ml	0.59 (0.18–1.75)	0.304		

*Statistical significance at $\alpha = 0.05$.

Table 4. The risk probability of *L. pneumophila* contamination in cooling tower water.

COOLING TOWER MODEL	DURATION OF USE (YEARS)	RESIDUAL FREE CHLORINE (ppm)	WATER TEMPERATURE (°C)	RISK PROBABILITY (%)
Cross-flow	>5	<0.2	<29.4	97.1
		≥0.2	≥29.4	57.8
	≤5	<0.2	<29.4	91.5
		≥0.2	≥29.4	26.1
Counter-flow	>5	<0.2	<29.4	87.6
		≥0.2	≥29.4	26.1
	≤5	<0.2	<29.4	75.0
		≥0.2	≥29.4	13.9

rather high with a mean of 6.6×10^5 CFU/ml. However, both parameters showed no significant association with the *L. pneumophila* contamination ($P > 0.05$) after univariate and multivariate analyses. These findings supported previous reports from World Health Organization (2007)[17] and Bentham and Broadbent (1993).[21]

Two other factors, including duration of use and pH, had an effect, since findings indicated that water pH was a significant risk factor for contamination after univariate analysis ($P = 0.019$) and was not significant after multivariate analysis ($P > 0.05$), whereas, the use duration of cooling towers was a significant risk factor for the contamination after univariate and multivariate analyses (crude OR = 3.79, 95% CI = 1.32–11.23, $P = 0.005$ and adjusted OR = 3.59, 95% CI = 1.27–10.14, $P = 0.016$). This might be due to the longer usage, the more accumulation of sediments, sludge, nutrients, and biofilm formation.[19,20] Cooling tower users frequently apply biocides to the circulating cooling tower water for controlling the growth of micro-organisms and other macro-organisms. Oxidizing biocides such as chlorine and bromine are more widely used in the electrical power and refining industries because of their effectiveness, moderate cost and easy treatability. Ozone is also highly effective, but there is more difficulty concerning maintenance than using chlorine.[22–25] Results from this study showed significance in univariate analysis ($P = 0.018$), however there was no significance in multivariate analysis ($P > 0.05$). In Thailand, the Metropolitan Waterworks Authority recommended the standard level of free chlorine to be at 0.2–0.5 ppm for inhibition of microbial growth in the water.[26] This recommendation was supported by data from the present study since residual free chlorine less than 0.2 ppm was a risk factor for *L. pneumophila* contamination (crude OR = 4.52, 95% CI = 1.22–20.53, $P = 0.009$ and adjusted OR = 8.49, 95% CI = 2.06–34.93, $P = 0.003$). Nevertheless residual free chlorine 0.5–1.0 ppm was recommended by Zhang, et al (2007) for controlling the *L. pneumophila* growth in hospital water systems.[23]

Operating temperatures in the range of 25.0°C to 42.2°C are likely to be favorable in amplifying the growth of *Legionella* spp.[27,28] This study found that water temperature at 16.7–38.2°C, with the average of 32.2°C, probably supported the growth of *Legionella* spp. When a cut-off temperature of 29.4°C (85°F) was used for determining the risk factors in *L. pneumophila* contamination of cooling tower water, it was found that water temperature less than 29.4°C was a significant risk factor by univariate and multivariate analyses (crude OR = 4.45, 95% CI = 1.40–18.52, $P = 0.005$ and adjusted OR = 7.87, 95% CI = 2.09–29.59, $P = 0.002$). This evidence remains unexplained.

Additionally, the risk probability for *L. pneumophila* contamination was estimated using four significant factors from multivariate analysis (cooling tower model, use duration, residual free chlorine, and water temperature). It was found that the risk probability ranged from 13.9 to 97.1% depending on the combination of predictive factors. The lowest risk for contamination (13.9%) was for counter-flow type cooling towers, use duration <5 years, residual free chlorine ≥0.2 ppm, and water temperature ≥29.4°C. Alternatively, the highest risk for contamination (97.1%) was the condition of the cooling tower model being a cross-flow type, use duration ≥5 years, residual free chlorine <0.2 ppm, and water temperature <29.4°C. Therefore, cooling tower users should provide the optimal conditions and maintenance methods to minimize the risk of *L. pneumophila* contamination in cooling tower water.

Finally, the findings in this study showed higher sensitivity using real-time PCR than using the culture (61.3% and 20.0%). The culture remains the standard method for detecting *Legionella* spp. from environmental sources, but this technique yields low sensitivity and requires up to 10 days to complete. The PCR method provides a very sensitive and powerful screening test for the detection of *L. pneumophila* in environmental samples and requires a few hours to complete.[14,16,29] Even though the PCR method does not distinguish between living and dead cells, it is usually indicative of an existing or potential future problem and helps to prevent future exposure when it is positive.

The cleanliness of cooling towers is important for public health and should be ensured.[14,29] Results of the present

study revealed that water samples collected from the reservoir of the cooling towers had significantly higher percentages of *L. pneumophila* contamination compared to those collected from the water-in and water-out areas of the cooling towers (reservoir: 76.0%, water-in: 44.9%, and water-out: 52.8%, $P < 0.05$). For routine maintenance of cooling towers, testing for *L. pneumophila* contamination should be done frequently and the reservoirs of cooling towers should receive focus as the predominant sampling point.

Conclusion

This study found 20.0% prevalence of *Legionella* spp. contamination and 61.3% of *L. pneumophila* contamination in cooling tower water. Real-time PCR showed higher sensitivity than culture. Additionally, 4 predictive factors for *L. pneumophila* contamination included cross-flow cooling tower model ($P = 0.017$), use duration >5 years ($P = 0.016$), water temperature <29.4°C ($P = 0.002$), and residual free chlorine <0.2 ppm. ($P = 0.003$). The risk probability for *L. pneumophila* contamination was estimated to be 13.9–97.1%, depending on the combination of each predictive factor. Optimal conditions and maintenance methods should be created and followed to minimize the risk of *L. pneumophila* contamination in cooling tower water.

Acknowledgements

The authors gratefully acknowledge the officers from the studied buildings and wish to extend our deep appreciation to the Thailand Research Fund (TRF). Special thanks to Mr. Isaac Olsan for English proofreading and valuable improvement.

Author Contributions

PL conceived and designed the study, completed funding proposals, and did manuscript preparation. SK conducted the data collection and laboratory experiments. CS obtained approval of the experiments. DS did the data analysis. All authors reviewed and approved the final manuscript.

DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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