ORIGINAL ARTICLE

# Isolation of *Legionella Pneumophila* from Hospital Cooling Towers in Johor, Malaysia

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**Summary**

Water-based cooling towers and their water supply at two hospitals in Johor were surveyed for the presence of *Legionella pneumophila*. *L. pneumophila* were grown from 19 (76%) out of 25 collected water samples. One hospital cooling tower was contaminated with L. pneumophila serogroup 1.

*Key Words:* *Legionella*, Cooling tower, Hospital, Johor

## Introduction

Legionnaires' disease was the term first used by press and media after an unexplained respiratory outbreak among war veterans attending an American Legion convention in Philadelphia in July 1976. A total number of 221 cases with 34 deaths were reported during the outbreak. The responsible bacterium was identified in January 1977 and later was given a name as *Legionella pneumophila*[1].

*Legionella* bacteria are one of the members of the Legionellaceae *family which thrive in warm* environments. They have been isolated from man-made and natural aqueous environments like drinking water, tap water and shower heads, water from cooling towers and evaporative condensers, river and lake water, and riparian soil. The pathogens are introduced into human lungs via microaspiration or inhalation of contaminated water droplets causing legionellosis manifested as either *Legionella* pneumonia in severe form or Pontiac fever in mild form. *L. pneumophila* accounts for approximately 90% of human infections, mostly associated with serogroup 1[2].

*Legionella* infect human mainly from environmental sources with no person-to-person transmission. Hospital cooling towers had been shown to be associated with a number of Legionnaires' disease outbreaks. In two major outbreaks at a large regional medical center in Burlington, Vermont, USA, epidemiologic and environmental studies supported the association of the so called cooling tower A located 150 metres from the hospital with both outbreaks[3]. Cases were both hospital- and community-acquired whereby cases without previous exposure to the hospital were more likely to occur among those who lived just downwind of the contaminated cooling tower but not throughout the municipal water system. In 1985, *L. pneumophila* serogroup 1 was isolated from the water samples of a cooling tower in the vicinity of the outpatient department in Staffordshire, England causing 60% case-fatality rate (36 of 60 patients died)[4].

A cooling tower removes heat by conduction between water and air as they contact each other. Cooling towers by their designs break water droplets into small particles that multiply the amount of water surface in contact with the air. This increases evaporation and improves the cooling process. Heat from refrigeration cycle produced by the condenser will raise the water temperature to the region that is conducive for the *Legionella* to multiply. Thus, the cooling towers

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provide an ideal environment for the growth of aquatic organisms including *Legionella*, if the cooling towers are not properly maintained.

Water-based cooling towers are extensively being used in the air conditioning system in many buildings including hospitals. As the air and water droplets containing *Legionella* are drawn up through the top of the cooling tower, creating cloud of moist air depending on the humidity, wind may blow this cloud into the inlet of the air conditioning system thus posing a potential hazard to the susceptible person in the building. The cloud may also enter the building through open windows or to those near and directly exposed to the cooling tower.

Although cooling towers were linked to many previous legionellosis outbreaks, recent findings in the field of molecular epidemiology from case-control studies and DNA fingerprinting show that potable water has been the primary environmental source for legionellosis outbreaks[5,6]. The main mode of transmission in nosocomial Legionnaires' disease is microaspiration[7].

This survey was conducted to determine the presence of *Legionella* in cooling towers at two selected hospitals in Johor, Malaysia. Their presence, if any, would be compared between cooling towers with and without cleaning and/or treatment maintenance programme, and correlated with the water variables namely temperature, pH, turbidity, and free chlorine content.

## Materials and Methods

Five cooling towers in Hospital A and 2 cooling towers in Hospital B were surveyed for *Legionella* between 1st July 2002 and 26th July 2002. All the water samples were collected on different days with no less than 3 days from the date of the cooling tower treatment. The water sampling was done between 8.30 am and 9.30 am and all the water samples were immediately sent to the Microbiology Laboratory in Hospital Sultanah Aminah Johor Bahru (HSAJB) at ambient temperature and kept away from sunlight. The water samples were given codes and not revealed to the laboratory personnel.

In each cooling tower, water samples were collected using 500ml sterilized bottles from: i – the cooling tower water cascades that were dropped down; ii - the cooling tower water at the mid-depth of the sump; iii - the deliberately stirred cooling tower water near the bottom of the sump and; iv - the make-up water i.e. the cooling tower water supply. Water variables were measured *in situ*. Water pH and temperature were measured using "Sension 1" pH meter from Hach and, water turbidity and free chlorine content were measured using "DR 890" colorimeter/spectrophotometer.

Each water sample was filtered by negative pressure through 47mm cellulose nitrate membrane filters of 0.22 µm pore size before undergoing three different treatment methods namely direct plating, heat and acid buffer. Treated samples were plated onto the Buffered Charcoal Yeast Extract (BCYE) agar plates with added selective and growth supplements, and subsequently incubated at 35°C in a moist chamber to grow the colonies. *Legionella*-like colonies which grew on the BCYE agar but not on Horse Blood agar would undergo Gram stain microscopy before being confirmed by the Direct Fluorescent Antibody (DFA) test under the immunofluorescence (IF) microscopy. Further identification was done by serogrouping technique using specific antisera at the Monash University Malaysia.

Trial run was conducted in the month of June 2002 to find out whether the BCYE culture media prepared by the Microbiology Laboratory HSAJB were able to grow the *Legionella* bacteria. Three plates of *L. pneumophila* isolates were gained from the School of Science and Engineering, Monash University Malaysia, Bandar Sunway, Petaling Jaya as control isolates coded as *L. pneumophila* MO 1. Few of the control isolates were subcultured onto the tested BCYE culture media which successfully grew *L. pneumophila* colonies.

Data gained from the environmental sampling of the cooling tower water and the laboratory results were computed and analysed using SPSS Version 10.0 from SPSS Inc.

## Results

*L. pneumophila* were grown from 19 (76%) out of 25 samples of water collected. All cooling towers except cooling tower 7 in Hospital B (86%) had *L. pneumophila* bacteria isolated at sampling sites i, ii and iii, and by all treatment methods namely direct plating, heat and acid buffer (Table I). L. pneumophila were also found in the water supply for cooling tower 1, cooling tower 2 and cooling tower 5 at Hospital A.

Confirmation was done by the Direct Fluorescence Antibody (DFA) test using specific reagents for *L. pneumophila* serogroup 1 to 7 with the immunofluorescence (IF) grades seen were between 2+ to 4+. All the *Legionella* isolates were sent to the School of Science and Engineering, Monash University Malaysia, for quality control and further identification. By serogrouping technique using specific antisera, isolate from the cooling tower 5 was identified as *L. pneumophila* serogroup 1 which is the most virulent strain of the L. pneumophila.

The cooling tower 7 which is the only cooling tower spared from the *Legionella* bacteria had its water samples collected 3 days after undergoing cooling tower treatment as compared to other cooling towers with more than 6 days in interval between the cooling tower treatment and the water sampling (Table II).

**Table I: Isolation of *L. pneumophila* from Hospital A and Hospital B cooling towers by different sampling site and treatment method**

| Sampling premise | | Sampling site[f] | Treatment method (1 = Detected; 0 = Not detected) | | |
|---|---|---|---|---|---|
| | | | Direct Plating | Heat | Acid Buffer |
| Hospital A | CT 1 | i. | 1 | 1 | 1 |
| | | ii. | 1 | 1 | 1 |
| | | iii. | 1 | 1 | 1 |
| | | iv. | 1 | 1 | 0 |
| | CT 2 | i. | No sample | No sample | No sample |
| | | ii. | 1 | 1 | 1 |
| | | iii. | 1 | 1 | 1 |
| | | iv. | 1 | 1 | 1 |
| | CT 3 | i. | 1 | 1 | 1 |
| | | ii. | 1 | 1 | 1 |
| | | iii. | 1 | 1 | 1 |
| | | iv. | 0 | 0 | 0 |
| | CT4 | i. | 1 | 1 | 1 |
| | | ii. | 1 | 1 | 1 |
| | | iii. | 1 | 1 | 1 |
| | | iv. | 0 | 0 | 0 |
| | CT 5 | i. | 1 | 1 | 1 |
| | | ii. | 1 | 1 | 1 |
| | | iii. | 1 | 1 | 1 |
| | | iv. | 1 | 1 | 1 |
| Hospital B | CT 6 | i. | No sample | No sample | No sample |
| | | ii. | 1 | 1 | 1 |
| | | iii. | 1 | 1 | 1 |
| | | iv. | No sample | No sample | No sample |
| | CT 7 | i. | 0 | 0 | 0 |
| | | ii. | 0 | 0 | 0 |
| | | iii. | 0 | 0 | 0 |
| | | iv. | 0 | 0 | 0 |

CT = Cooling tower

fSampling site :-

i. the CT water cascades that were falling through the fill or directly from the nozzle (depending on the type of CT)

ii. the stagnant CT water at the mid-depth of water level in the sump

iii. the deliberately stirred up CT water near the bottom of the sump where sediments and biofilms could be found on the sump surface

iv. the make-up water i.e. the water supply to the CT

Multiple regression analysis shows that free chlorine content is correlated with number of days between cooling tower treatment and water sampling, p value < 0.001 (Table III). Although the analysis could not suggest any single water variable responsible for the presence of L. pneumophila in the cooling tower water, the bacteria were not detected in water samples at pH 8.42 and 8.68 ($p < 0.05$), and free chlorine content of 0.15 mg/L ($p < 0.01$) - Table IV.

**Table II: Isolation of *L. pneumophila* by number of day intervals between cooling tower treatment and water sampling**

| Day intervals[d] | Isolation of *L. pneumophila* from CT water samples[c] (n = 25) | |
|---|---|---|
| | Not detected | Detected |
| 3 (CT 7) | 3 | 0 |
| ≥ 7 (other CTs) | 3 (3) | 19 (3) |

CT = Cooling tower ( ) = sampling site no. iv. i.e. the make-up water

c versus d: p value = 0.001

Note: All water samples from the water supply (make-up water) were not treated by the cooling tower treatment method

**Table III: Correlation between water variables and number of day intervals between cooling tower treatment and water sampling**

| Water Variable | Unstandardized Coefficients | | Standardized Coefficients | t | Significance | 95% Confidence Interval for B | |
|---|---|---|---|---|---|---|---|
| | B | Standard Error | Beta | | | Lower Bound | Upper Bound |
| pH | -0.169 | 0.148 | -0.051 | 0.269 | -0.479 | -1.140 | 0.141 |
| Temperature | -1.078E-02 | 0.014 | -0.016 | 0.437 | -0.039 | -0.794 | 0.018 |
| Chlorine | -25.703 | 0.977 | -0.957 | 0.000 | -27.748 | -26.304 | -23.657 |
| Turbidity | -0.114 | 0.067 | -0.065 | 0.105 | -0.253 | -1.704 | 0.026 |

Dependent Variable: Number of day intervals between CT treatment and water sampling

**Table IV: Univariate analysis of *L. pneumophila* isolations by water variables**

| Water variable | | Isolation of *L. pneumophila* from CT water samples[c] (n = 25) | |
|---|---|---|---|
| | | Not detected | Detected |
| Temperature[e], °C | 26.0 – 29.8 | 6 | 10 |
| | 30.4 – 32.2 | 0 | 9 |
| pH[f] | 7.25 – 8.26 | 2 | 19 |
| | 8.42 & 8.68 | 4 | 0 |
| Free chlorine[g], mg/L | 0.00 – 0.01 | 2 | 19 |
| (Missing value = 1) | 0.15 | 3 | 0 |
| Turbidity[h], NTU | 1.23 – 2.40 | 5 | 15 |
| (Missing value = 1) | 3.00 – 3.80 | 0 | 4 |

CT = Cooling tower

c versus e: p value = 0.035; c versus f: p value = 0.022

c versus g: p value = 0.001; c versus h: p value = 0.008

(Multiple Regression Analysis is not significant)

## Discussion

The presence of the *Legionella* bacteria found in this survey was unexpectedly high. All the 5 cooling towers in the Hospital A and 1 out of 2 cooling towers in the Hospital B (86% of all the cooling towers surveyed) were colonized by *L. pneumophila* serogroup 1 to 7. Further identification conducted by the School of Science and Engineering, Monash University Malaysia, showed that the cooling tower 5 at the Hospital A was colonized by *L. pneumophila* serogroup 1. The serogroup 1 subgroup Pontiac 1a had been known to be the most frequently associated with Legionnaires' disease outbreaks[8].

*L. pneumophila* had also been isolated from the water supply for the cooling towers 1, 2 and 5, all located at the Hospital A. The water supply for both cooling towers 1 and 5 came from the respective cooling tower water tanks whereas the water supply for the cooling tower 2 came directly from the public water supply pipeline. Further investigation showed that the water sample for cooling tower 2 water supply was actually mixed with the cooling tower 2 sump/basin water sample since the outlet of cooling tower 2 water supply came directly in contact with the sump water.

It has become a routine for building owners/managers to treat their cooling towers with at least one chemical compound i.e. the corrosion and scale inhibitor, and two microbiocides at regular intervals – one microbiocide (represented as Microbiocide I in Table V) is intended to control the growth of common bacteria, fungi and algae, and the purpose of the other microbiocide (represented as Microbiocide II in Table V) is to control the growth of *Legionella* bacteria in addition to the control of common bacteria, fungi and algae in the aquatic environment. At least two different microbiocides are used to avoid the microorganisms from becoming resistant.

Cooling tower maintenance records had been sought after from both selected hospitals in this study and the summary of treatment and cleaning schedules are presented as follows:-

**Table V: Isolation of *L. pneumophila* and summary of cooling tower maintenance programmes at Hospital A and Hospital B.**

| Sampling site | | *Legionella* isolation* | Frequency of treatment & cleaning programme | | | |
|---|---|---|---|---|---|---|
| | | | Chemical | Microbiocide I | Microbiocide II | Manual cleaning |
| Hospital A | CT 1 | 1 | Two weekly | Two weekly | Monthly | None |
| | CT 2 | 1 | Two weekly | Two weekly | Monthly | None |
| | CT 3 | 1 | Two weekly | Two weekly | Monthly | None |
| | CT4 | 1 | Two weekly | Two weekly | Monthly | None |
| | CT 5 | 1 | Two weekly | Two weekly | Monthly | None |
| Hospital B | CT 6** | 1 | None | None | None | None |
| | CT 7 | 0 | Weekly | Weekly | Monthly | Weekly |

CT = Cooling tower

* 1 = Detected; 0 = Not detected

** CT 6 was used as a backup cooling tower therefore it was not operated at the time of sampling. It would undergo treatment before putting back on operation.

All the cooling towers at the Hospital A underwent regular chemical and microbiocidal treatments only in every two weeks and it was apparent that all the cooling towers had never underwent manual cleaning. Both factors may contribute to the high isolation (100%) of L. pneumophila at the Hospital A. At the Hospital B, most of the time the cooling tower 7 was used to cool down the heated water from the air conditioning system whereas the cooling tower 6 was used as a backup cooling tower when the cooling tower 7 was shut down for any reason including cleaning purposes. The cooling tower 6 was not operated at the time of sampling (the cooling tower 6 was not operated most of the time), therefore the Hospital B management did not treat the cooling tower 6 which obviously contributed to the detection of the *Legionella* bacteria in the tower. Nonetheless, the contaminated cooling tower 6 was thought to pose low hazard to the public health since aerosols were not produced when the cooling tower fan was switched off.

It was apparent that the cooling tower 7 was spared from the *Legionella* bacteria since it had underwent weekly treatment and manual cleaning. However, water samples from the cooling tower 7 were collected only 3 days after undergoing cooling tower treatment as compared to other water samples that were collected no less than 6 days after treatment. Table II showed significant association between *Legionella* detection and number of day intervals between cooling tower treatment and water sample collection with p value = 0.001.

Tables III and IV subsequently pointed out that higher free chlorine content, which was correlated with less number of day intervals between the cooling tower treatment and the water sampling (i.e. 3 days), was significantly associated with the failure to detect the *Legionella* bacteria in the cooling tower 7 (p value = 0.001). So, it was unsure whether the treatment efficacy or the manual cleaning intervention or high free chlorine content in the cooling tower system by virtue of the interval duration between the cooling tower treatment and the water collection had contributed to zero detection of *Legionella* strains in the cooling tower 7. The *Legionella* bacteria might be detected at the cooling tower 7 should the water samples were collected at day 6. Certainly, the cooling tower 7 would repeat another cycle of treatment and manual cleaning at day 7, thus eliminating microbial growth in the previous cycle. In Singapore, the isolation rate of the *Legionella* bacteria in cooling towers with cleaning/treatment programme (52.6%) was lower than the cooling towers without the maintenance programme (78.3%), but the difference was not statistically significant[9].

*L. pneumophila* were known to survive in a wide range of environmental conditions with temperature ranges from 0°C to 63°C and pH from 5.0 to 8.5[10]. Although the Legionella bacteria were chlorine tolerant, some survived the water treatment process and passed into the water distribution system. Table IV showed that the Legionella bacteria could not survive in the water pH at 8.42 and 8.68 (p value = 0.022), and the free chlorine content of 0.15 mg/L (p value = 0.001). The water pH at 8.42 was the make-up water for the cooling tower 3 at the Hospital A whereas the water pH at 8.68 were the cooling tower 7 water samples for sampling sites i, ii and iii at the Hospital B. However, all the cooling tower water samples with free chlorine content of 0.15 mg/L actually came from the same cooling tower 7 which gave the highest pH reading of 8.68 that were also significantly associated with zero Legionella detection. Therefore, it was very likely that the relatively high chlorine and pH readings from the cooling tower 7 was due to the sampling time conducted rather at early days (3-day apart) after the cooling tower treatment. The Legionella bacteria were all detected at the surveyed water temperature ranged from 26.0oC to 32.2°C and water turbidity ranged from 1.23 NTU to 3.80 NTU.

Although these findings suggest that the pH for the cooling tower water is best to be maintained at higher pH level, too high pH will allow alkalinities in the cooling tower system that in turn contribute to scale and fouling formations. Certainly, low water pH will promote microorganism growth including *L. pneumophila* and too high acid concentration in the tower will eventually strip away the protective film (formed by anodic and cathodic inhibitors) thus increase the potential for corrosion in the cooling tower system. Therefore, the overall pH level in the cooling tower water should consider the optimal function of the cooling tower while not compromising the presence of *L. pneumophila*.

This survey also found an unexpected isolation of *L. pneumophila* in the cooling tower water supply since other studies conducted in this region[9,11] apparently did not include the cooling tower water supply in their environmental sampling. In light of other published reports that hospital water supply has been attributed

to nosocomial Legionnaires' disease,[6,7] the findings in this survey raised the need to screen our hospital water supply and its distribution system for *Legionella contamination.*

The disease burden of legionellosis in Malaysia is unknown since legionellosis is not a notifiable disease. Although no outbreaks of hospital-acquired legionellosis in Malaysia has been documented, however, potable water has been implicated in an outbreak of Legionnaires' disease at Taman Sinar Harapan, Tampoi, Johor Bahru in May 1997. A retrospective record search conducted by the Epidemiology Unit, Johor State Health Department on the reported outbreak at Taman Sinar Harapan, which is a rehabilitation center for people with mental retardation, showed that the outbreak started on the 17th May 1997 when a 20-year-old male inmate with Down's Syndrome was referred to the Hospital Sultanah Aminah Johor Bahru (HSAJB) with the complaints of high grade fever and cough for one week and chest x-ray showed pneumonic changes. The patient died 19 hours after admission.

On the next day (18th May 1997), a 21-year old male inmate with Down's Syndrome from the same rehabilitation center was admitted to the HSAJB with a history of fever for 5 days. On presentation, the patient was drowsy and gasping with signs of central cyanosis, acidotic breathing and low blood pressure. The patient died shortly after admission. During the same day, another 21 inmates from the Taman Sinar Harapan were admitted to the hospital with the main complaints of fever, cough and vomiting for the duration of between one to two days.

In total, 29 inmates were screened for the immunoglobulin M (IgM) for Legionella. Their paired sera were sent to the Serology Unit, Institute for Medical Research (IMR), Kuala Lumpur and tested for the *Legionella* IgM using the immunofluorescent antibody test. Sixteen of them (55.2%) were found to have IgM positive for *Legionella* which indicates recent *Legionella* infection. Environmental tests conducted by the Johor Bahru Health Office during the outbreak showed that one out of six (16.7%) water samples collected from 6 respective water tanks at the rehabilitation center was positive for *Legionella* bacteria. The positive water sample was collected from Asrama Bunga Raya (Asrama 6).

There were neither cooling towers nor hot water system at the center. The common bathroom at Asrama 6 did not have any head shower but only water taps with in-built concrete water containers. Interviews conducted on the carers revealed that the inmates like to splash water repeatedly onto their faces while taking baths. Therefore, microaspiration has been postulated to be the major mode of transmission in the Taman Sinar Harapan legionellosis outbreak. This finding supports the fact that showering is not a mode of transmission[7].

## Conclusion

In conclusion, *L. pneumophila* were prevalent in the water-based cooling towers at both selected hospitals in Johor especially the Hospital A with *L. pneumophila* serogroup 1 had been isolated which may lead to nosocomial legionellosis outbreak. Although high pH level and free chlorine content would eliminate *Legionella* in the cooling tower, however, high pH level may lead to scale and fouling formations in the cooling tower whereas free chlorine content would quickly dissociate with time.

Isolation of *L. pneumophila* in the cooling tower water supply was equally alarming which could be regarded as a tip of an iceberg on our hospital water supply and its distribution network. The cooling tower and its water tank should be treated and physically cleaned at least by a weekly basis. In addition, the hospital water supply shall be routinely screened for *Legionella* bacteria by environmental culture that has been proven as an important strategy in prevention whereas an application of copper-silver ionisation systems has emerged as the most successful long-term disinfection method for the hospital water systems[7].

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