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ORIGINAL ARTICLE

# *Legionella* pollution in cooling tower water of air-conditioning systems in Shanghai, China

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## Abstract

**Aims:** To determine *Legionella* pollution prevalence, describe the amount of *Legionellae* with respect to temperature in Shanghai cooling tower water (CTWs) in various types of public sites.

**Methods and Results:** Six urban districts were selected as the study fields, adopting multiple-phase sampling methods. Routine culture was used to identify *Legionellae.* Of the samples, 58·9% (189/321) were observed to be positive, 19·9% were isolated over 100 CFU $ml^{-1}$. *Legionella pneumophila* serogroup 1 was the most frequently isolated species (155/189, 82·0%), followed by *Leg. micdadei* that was at the second place (44/189, 23·3%). The mean CFU $ml^{-1}$ of *Legionellae* in CTWs reached its peak from July to September. Over all 15·4% of the samples exceeding 100 CFU $ml^{-1}$ were observed in a hospital setting.

**Conclusions:** The prevalence of *Legionella* pollution in CTWs, especially in CTWs of subway stations and hospitals, is worrying, and the positive rate and CFU $ml^{-1}$ of *Legionellae* in CTWs have a close relationship with air temperature.

**Significance and Impact of the Study:** The study demonstrates pollution prevalence rates in different types of sites and various seasons, and provides a proportion of different serogroups of *Legionellae*. It illuminates an urgent need for dealing with the potential risk of legionellosis in Shanghai, through improved control and prevention strategies.

## Introduction

*Legionella* species are aerobic bacteria, and are naturally found in surface water, ground water and aerated biofilms (Edelstein and Meyer 1984; Fields *et al.* 2002; Costa *et al.* 2005). The sources of *Legionellae* may include almost any warm water or devices that disseminate water, particularly aerosols, sprays or mists, that also provide ideal conditions for the growth and amplification of *Legionellae* (Fields *et al.* 2002). *Legionellae* can be inhaled when water is released into the air, through air conditioners, steam or other ways (McDade *et al.* 1977; Breiman *et al.* 1990; Lowry *et al.* 1991; Stout and Yu 1997).

At present, legionellosis, caused by *Legionellae*, is recognized to be an environmentally acquired disease, as almost all outbreaks of legionellosis are because of inhalations of contaminated aerosolized water. Outbreaks of legionellosis have been traced to a wide variety of environmental water sources (Atlas 1999), and occurred in homes, offices, hotels, hospitals, cruise ships and other locations (Leoni *et al.* 2005). Cooling towers have been recognized as the primary source in major outbreaks of legionellosis (Atlas 1999; Garcia-Fulgueiras *et al.* 2003; Kirrage *et al.* 2007). There are increasing evidences that have indicated a direct correlation between the cooling tower *Legionellae* and clinical legionellosis (Jansa *et al.* 2002; van den Hoek *et al.* 2006; Sabria *et al.* 2006). Fortunately, *Legionellae* have been proven to be slow-growing bacteria which are highly affected by environmental conditions, and are incapable of human-to-human transmission. Temperature is an important factor in *Legionella*'s amplification and transmission. Growth of *Legionellae* has been recorded between 20°C and 45°C, while the optimal amplification range is a narrower band of 35–45°C (Berry *et al.* 2006).

Currently, at least 48 species of *Legionellae* have been reported (Fields *et al.* 2002). Although potentially all *Legionellae* may cause human disease, only 19 *Legionella* species are documented as human pathogens (Muder and Yu 2002). Among these, *Leg. pneumophila* (Lp) infection is responsible for about 90% of clinical cases identified (Fields *et al.* 2002; Yu *et al.* 2002). And *Legionella* pneumophila serogroup 1 (Lp-1) is found to be the most important causative species to more than 79–90% of *Legionellosis* cases (Edelstein 1993; Sopena *et al.* 1999; Fields *et al.* 2002).

Shanghai is the largest industrial and commercial city in China, with a high population density and numerous air-conditionng systems. However, data on *Legionella* pollution in this city are very limited. In addition, guidelines for the management of *Legionellae* have not been established at present in China. This study was a cross-sectional study on *Legionellae* in cooling tower water (CTWs) of air-conditioning systems in Shanghai, with a purpose to understand the extent of *Legionella* pollution and the ideal temperature for *Legionellae* growth in the air-conditioning systems.

## Materials and methods

### Study setting

We adopted multiple-phase sampling in Shanghai. Six of the 11 urban districts of Shanghai city – Luwan, Pudong, Jing'an, Huangpu, Changning and Xuhui – were selected as the study fields. Based on distribution of the cooling towers, target sites were selected using probability proportion (around 1/500) to size sampling from the study fields.

### Samples collection and microbiological analysis

In total, 321 CTWs were sampled. The number of CTW varied from 43 in Jing'an district to 77 in Pudong district. Two water samples were collected from each CTW every month from May to November in 2003. No samples were collected from November to April as the temperature was not optimal for bacterial growth (31–68°F). Each sample consisted of 500 ml water. These samples were processed in the laboratory of the Shanghai Municipal Center for Disease Control and Prevention (Shanghai CDC) within 24 h.

The samples were processed as follows: each sample was shaken vigorously and then left for about 15 min, after which 100 ml was concentrated by membrane filtration (0·2-$\mu$m-pore-size polyamide filter; Millipore, Billerica, MA, USA). The filter membrane was resuspended in 5 ml of the original sample and put in vortex for 30 s. One millilitre was treated with an equivalent volume of acid buffer (0·2 mol $l^{-1}$ HCl–KCl, pH 2·2), at 25°C, for 3 and 15 min, then the acid-treated sample was neutralized with KOH (pH = 6·9–7·0). Acid buffer treatment was used as a selective method to reduce the numbers of non-*Legionella* (Bopp *et al.* 1981; Edelstein 1981). After neutralization, 0·1 ml of the sample was spread onto duplicate plates of the GVPC media (composed of buffered charcol yeast extract agar with glycine, vancomycin, polymyxin B, cycloheximide; produced by Shanghai CDC) to be tested, with a sterile glass rod (Wadowsky and Yee 1981).

The inoculated culture media were incubated at 37°C in a humidified atmosphere with 5% $CO_2$ and examined for 3–10 days. Typical colonies of *Legionella* on GVPC agar were slightly convex, circular and entire, with a ground glass appearance. Suspected *Legionella* colonies were examined with a microscope and subcultured onto blood agar and buffered charcol yeast extract (BCYE) for verification (Vickers *et al.* 1981). Colonies grown on GVPC were subsequently identified by an agglutination test (*Legionella* latex test; Oxoid). The test allows a separate identification of *Legionella* pneumophila serogroup 1 and serogroups 2–14 and detection of seven other *Legionella* species which have been implicated in human disease (Brindle *et al.* 1987; Vickers *et al.* 1987; Murray and Baron 2007). Furthermore, Microagglutination test was used to separately identify the *Legionella* pneumophila serogroups 2–6. The reagent (provided by Shanghai CDC) consists of blue latex particles sensitized with specific rabbit antibody reactive with *Legionella* pneumophila serogroups 2–6 antigen. In this study, Lp-1 to Lp-6 and *Leg. micdadei* (L-*micdadei*) had been detected.

### Data analysis

Statistical analysis was done with Intercooled STATA 9·0 (Stata Press 2005) for Windows. Chi-squared tests were used for statistical analysis of qualitative variables, i.e. to examine the significance among positive proportions of different districts, sites and months. Statistical significance was assessed by the two-sided test with a test level ($P$-value) equaling 0·05.

## Results

### The pollution prevalence of *Legionellae* in CTWs

Overall, the data present a high positive rate of *Legionellae* (58·9%, 189/321) in Shanghai CTWs. Of the samples, 19·0% were isolated over 100 CFU $ml^{-1}$ (shown in Table 1), and 0·9% even exceeding 1000 CFU $ml^{-1}$. In addition, as shown in Table 2, totally 58·9% (189/321) of

**Table 1** Distribution of *Legionella* isolates in the sites examined

| Parameter | Changning | Huangpu | Luwan | Pudong | Jing'an | Xuhui | Total |
|---|---|---|---|---|---|---|---|
| No. of negative samples/total number (%) | 26/50 (52) | 15/53 (28·3) | 29/53 (54·7) | 29/77 (37·7) | 16/43 (37·2) | 17/45 (37·8) | 132/321 (41·1) |
| No. of samples with 1–10 CFU ml$^{-1}$/total number (%) | 0 | 5/53 (9·4) | 1/53 (1·9) | 6/77 (7·8) | 4/43 (9·3) | 2/45 (4·4) | 18/321 (5·6) |
| No. of samples with 11–100 CFU ml$^{-1}$/total number (%) | 18/50 (36) | 28/53 (52·8) | 10/53 (18·9) | 23/77 (29·9) | 12/43 (27·9) | 16/45 (35·6) | 107/321 (33·3) |
| No. of samples with 101–1000 CFU ml$^{-1}$/total number (%) | 6/50 (12) | 5/53 (9·4) | 11/53 (20·8) | 18/77 (23·4) | 11/43 (25·6) | 10/45 (22·2) | 61/321 (19·0) |
| No. of samples with >1000 CFU ml$^{-1}$/total number (%) | 0 | 0 | 2/53 (3·8) | 1/77 (1·3) | 0 | 0 | 3/321 (0·9) |

**Table 2** Positive rate and proportion of different serogroups of *Legionellae* in CTWs

| | Proportion of different serogroups in CTWs* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| District | Lp-1 (%) | Lp-2 (%) | Lp-3 (%) | Lp-4 (%) | Lp-5 (%) | Lp-6 (%) | L-*micdadei*† (%) | $\chi^2$, *P* | Positive rate‡ (%) |
| Changning | 18/24 (75·0) | 7/24 (29·2) | 3/24 (12·5) | 1/24 (4·2) | 1/24 (4·2) | 3/24 (12·5) | 6/24 (25·0) | 49·5, <0·01 | 24/50 (48·0) |
| Huangpu | 29/38 (76·3) | 7/38 (18·4) | 4/38 (10·5) | 3/38 (7·9) | 3/38 (7·9) | 3/38 (7·9) | 9/38 (23·7) | 82·3, <0·01 | 38/53 (71·7) |
| Luwan | 19/24 (79·2) | 6/24 (25·0) | 2/24 (8·3) | 2/24 (8·3) | 3/24 (12·5) | 1/24 (4·2) | 6/24 (25·0) | 54·6, <0·01 | 24/53 (45·3) |
| Pudong | 45/48 (93·8) | 8/48 (16·7) | 3/48 (6·3) | 3/48 (6·3) | 5/48 (10·4) | 3/48 (6·3) | 12/48 (25·0) | 161·4, <0·01 | 48/77 (62·3) |
| Jing'an | 22/27 (81·5) | 4/27 (14·8) | 2/27 (7·4) | 4/27 (14·8) | 3/27 (11·1) | 4/27 (14·8) | 5/27 (18·5) | 60·9, <0·01 | 27/43 (62·8) |
| Xuhui | 22/28 (78·6) | 2/28 (7·1) | 5/28 (17·9) | 3/28 (10·7) | 5/28 (17·9) | 4/28 (14·3) | 6/28 (21·4) | 55·5, <0·01 | 28/45 (62·2) |
| Total | 155/189 (82·0) | 34/189 (18·0) | 19/189 (10·0) | 16/189 (8·5) | 20/189 (10·6) | 18/189 (9·5) | 44/189 (23·3) | 448·1, <0·01 | 189/321 (58·9) |

*Proportion of different serogroups in CTWs: the positive number of serogroups of *Legionellae*/the positive sample number of *Legionellae*.
†Lp1-6: *Leg. pneumophila* serogroups 1–6; L-*micdadei*: *Leg. pneumophila* serogroup *micdadei*.
‡Positive rate: the positive sample number of *Legionellae*/the total sample number of each study site; the positive numbers in different serogroups may overlap, therefore not corresponding to the added positives of the rest of the data.

samples were observed to be positive with *Legionella* species. No statistical significance in the positive rate of *Legionella* pollution among the study districts was observed (shown in Table 2, $P > 0·05$, chi-squared test). The positive rate of *Legionellae* in CTWs was highest in Huangpu district (38/53, 71·7%), and lowest in Luwan district (24/53, 45·3%). As indicated in Table 2, Lp-1 was the most frequently isolated species (155/189, 82·0%), followed by L-*micdadei* that was at the second place (44/189, 23·3%).

## Seasonal variations in the level of *Legionellae*

There was a statistical difference in positive rates of *Legionella* pollution in CTWs among various months (Fig. 1, $P < 0·05$, chi-squared test). The mean number of *Legionellae* detected varied with seasons. The mean CFU ml$^{-1}$ of *Legionellae* in CTWs increased with increasing temperatures, reaching its peak in July, August and September (mean CFU ml$^{-1}$ was 180, 170 and 170, respectively), and then dropped gradually in October and November, down to 39·3 and 0·33, respectively. There was a similar trend when it comes to the maximum CFU and the 50th percentile CFU. Figure 1 also shows a positive association between *Legionellae* presence in CTWs and increasing temperatures. In the hottest months, August and September, the positive rates of *Legionellae* reached 81·8% and 79·7%, respectively.

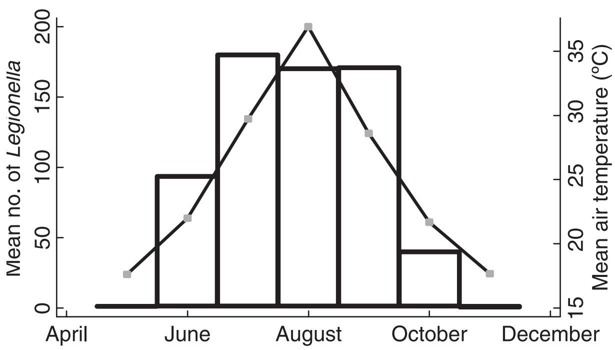


**Figure 1** Mean numbers of *Legionellae* varied with seasons and air temperatures (▭), Mean no. of *Legionella*; (–▪–) Mean air temperature (°C).

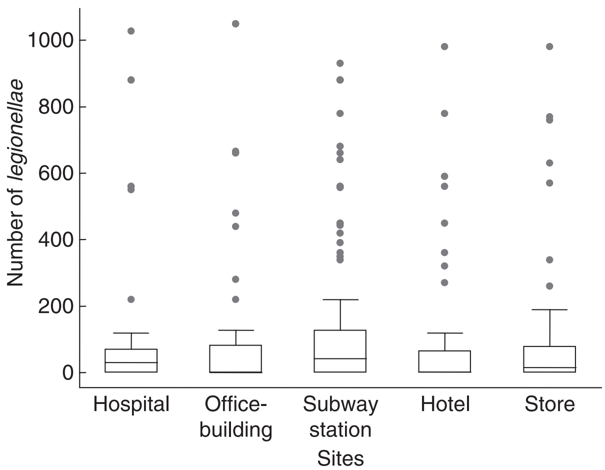


**Figure 2** Numbers of *Legionellae* varied among samples from various types of sites. Each box comprises 50% of data of certain subset. Boxes are notched (narrowed) at the median and return to full width at the lower and upper 95% confidence interval values. The lower and upper limits of the rectangle indicate the quartiles (25th and 75th percentiles, respectively) of the subset. Dots refer to 'outside values' or 'far outside values'.

### Site variations in the level of *Legionellae*

The positive rates were statistically different among various sites included in the study ($P < 0{\cdot}05$, chi-squared test). The median number of *Legionellae* was 31 CFU $ml^{-1}$ for hospitals and 42 CFU $ml^{-1}$ in subway stations, while it was 0 CFU in office buildings (Fig. 2). Subway stations and hospitals had higher *Legionella* pollution positive rates (71·3% and 69·2%, respectively) than other public places, during the study period. All the positive rates, however, whether in hotels, stores or hospitals, exceeded 40%. As shown in Table 3, 15·4% of samples exceeding 100 CFU $ml^{-1}$ were observed in a hospital setting; 18·9%, 27·7%, 12·3% and 22·7% of the samples exceeding 100 CFU $ml^{-1}$ were from office buildings, subway stations, hotels and stores, respectively.

## Discussion

This study illustrates a high pollution prevalence of *Legionellae* in the CTWs of Shanghai's air-conditioning systems, especially in subway stations and hospitals. A recent epidemiological survey conducted by Wang *et al.* (2005) identified that 7·6% of the general population in Shanghai were *Legionella* antibody positive. Because Shanghai has not suffered a *Legionella* outbreak on a large scale before, the measurements for *Legionella* pollution in CTWs are not optimistically prepared. Epidemiological and ecological studies on *Legionellae* and exploring evidence for *Legionella* control in Chinese urban cities such as Shanghai are urgently needed.

### The pollution level of *Legionellae* in CTWs

The result demonstrates that the positive rate of *Legionellae* in CTWs of central air-conditioning systems was 58·9%, in comparison to those of other cities in China (Beijing, 52·4%; Hangzhou, 44·8%; Tianjian, 33·3%; Liu *et al.* 2006; Si *et al.* 2006; Li *et al.* 2007). Although there was no statistical difference among *Legionella* positive rates in different districts, the positive rate itself did show the severity of contamination in Shanghai. If culture methods were considered that might be generally underestimated to indicate the presence of *Legionella* species, the real level of contamination could be greater. In some CTWs, the concentration far exceeded the environmental health standard. This study also observed an increase of rates by comparing the result with another similar study conducted in 1997–1999 in Shanghai that reported a 49·9% detection rate of *Legionella* species (Hu *et al.* 2001). This result fits in with the fact that cooling towers were implicated in outbreaks of legionellosis, particularly at startup or during construction (Atlas 1999).

Although the dose of *Legionellae* required to infect humans remains unknown, the US Association of Water Technologies (AWT) recommended that a decision to disinfect the tower should be made by considering the CFU of *Legionellae*, the location of the tower and the type of population, when results exceed 100 CFU $ml^{-1}$ (indication of a large bacterial presence in the CTWs). In this study, it was found that 19·9% of the specimens were above 100 CFU $ml^{-1}$ and some specimens (0·9%) even greater than 1000 CFU $ml^{-1}$, implying a need for instant disinfection of those cooling towers.

**Table 3** Distribution of *Legionella* isolates in the areas examined

| Parameter | Hospital | Office building | Subway station | Hotel | Store |
|---|---|---|---|---|---|
| No. of negative samples/total number (%) | 16 (30·7) | 32 (55·2) | 27 (28·7) | 36 (49·3) | 21 (47·7) |
| No. of samples with 1–10 CFU $ml^{-1}$/total number (%) | 4 (7·7) | 2 (3·4) | 7 (7·4) | 4 (5·5) | 1 (2·3) |
| No. of samples with 11–100 CFU $ml^{-1}$/total number (%) | 24 (46·2) | 13 (22·4) | 34 (36·2) | 24 (32·9) | 12 (27·3) |
| No. of samples with 101–1000 CFU $ml^{-1}$/total number (%) | 7 (13·5) | 9 (15·5) | 26 (27·7) | 9 (12·3) | 10 (22·7) |
| No. of samples with >1000 CFU $ml^{-1}$/total number (%) | 1 (1·9) | 2 (3·4) | 0 (0·0) | 0 (0·0) | 0 (0·0) |

Moreover, there existed predominant species in CTWs, e.g. Lp-1 and L-*micdadei* were detected from 82·0% and 23·3% of contaminated CTW samples, respectively. It is consistent with previous studies that noted Lp-1 as a dominate specie, and the most common isolates were cultured from environmental samples (Benson and Fields 1998; Drenning *et al.* 2001; Bouvet 2006). In comparison to the proportion range of Lp-1 in other studies in China, the type of *Legionella* species determined in this study was more concentrative in Lp-1. For example, 9 and 14 different types of *Legionella* species were detected from CTWs in Tianjian and Beijing, respectively, and Lp-1 took up 6·3% and 50·0% of all the *Legionella* species (Peng *et al.* 2000; Liu *et al.* 2006).

**Seasonal variations in the level of *Legionellae***

In order to have a better understanding of *Legionella*'s potential to cause disease and methods to control growth in water systems, we have to understand the conditions that favour *Legionella* growth and amplification. The results illustrate that the mean concentration of *Legionella* in CTWs varied with seasons and air temperatures. Furthermore, *Legionella* pollution rates proportionally increased with the growing air temperatures. The results are consistent with the optimal range of temperature (25–35°C) for *Legionella* reproduction, indicating a seasonal effect on growth and implying a need for increased monitoring of CTWs when the air temperature shifts into this range (Fields *et al.* 2002; Bouvet 2006). Such a seasonal variation may be due to the facts that *Legionellae* multiply faster in the warmer waters of summer and that the greater use of cooling towers in summer provides opportunities for dissemination.

**Site distribution variations in the level of *Legionellae***

In this study, *Legionella* pollution appeared very common in subways, hospitals, hotels and stores. Some laboratory results of specimen in these places were observed exceeding 100 CFU ml$^{-1}$. According to US AWT, *Legionellae* in CTWs may have a potential impact on public health when laboratory results exceed 100 CFU ml$^{-1}$. The mean CFU ml$^{-1}$ in CTWs varied among different sites; the rates were relatively high in subway stations and hospitals, especially during summer and autumn, with mean positive rates at 71·3% and 69·2%, respectively. The characteristics of *Legionella* distribution may aggravate the risk of legionellosis outbreaks in subways and hospitals, where are usually crowded with moving population. In addition, hospitalized patients, especially tumour patients and immune-repressed patients are susceptible to legionellosis. In this case, 15·4% of samples with rates exceeding 100 CFU ml$^{-1}$ of *Legionellae* were observed in a hospital setting. Thus, more attention should be paid to the *Legionella* pollution of CTWs in hospitals. Because of the severity of contamination, periodical surveillance of the CTWs in subway stations, hospitals, hotels and big stores should be added into routine measures to guarantee public health.

**Recommendations on the strategy of *Legionella* control in Shanghai**

Because of the potential for air-handling systems to harbour, amplify and disseminate *Legionellae*, control measures need to be considered for all cooling towers and evaporative condenser operations. Cooling tower disinfection is usually recommended as a maintenance action for regularly scheduled tower cleaning. When *Legionella* concentration exceeds 100 CFU ml$^{-1}$; however, an immediate disinfection of the cooling tower should be performed. In addition, additional prevention and control actions are required when a confirmed or suspected legionellosis case is diagnosed. In the months with high risk of *Legionella* pollution, from July to September, more attention should be given. Specifically, a more frequent surveillance with a larger sampling of CTWs may be required. Minimizing transmission from the tower to the host is the second responsible measure in reducing the risk of legionellosis, again recognizing that there are no guarantees to keeping a cooling tower system 100% from *Legionella* species. Health education may also be needed to alert people of the potential pollution status and health threats of *Legionellae* in CTWs of central air-conditioning systems, because there is a general lack of awareness of this problem (Carbonne and Astagneau 2005; Pedro-Botet and Sabria 2005; Bouvet 2006; Udale 2006).

**Limitations**

The findings in this study may be subject to at least two limitations. Firstly, the study provides limited information directly related to human health as a seroepidemiological survey. From the result, it is insufficient to state the relationship between *Legionella* contamination in cooling towers and public health. Further information on *Legionella* infection of the general population may provide the understanding of the risk of disease exposure in public places. Secondly, insufficient samples were collected at each site and, particularly for some sites, only limited samples were obtained. The power of the study would have been compromised by the small sample size. Not withstanding its limitations, this study does provide the meaningful data for *Legionella* control in urban cities of China.

## Conclusions

The results suggest an urgent need for *Legionella* control strategies by the government because of the high positive rate of *Legionellae* in CTWs. It shows that the *Legionella* pollution in CTWs is worrying, and the positive rate and CFU $ml^{-1}$ of *Legionellae* in CTWs had a close relationship with air temperature. To deal with the potential risk of legionellosis, improved control and prevention strategies, i.e. CTW disinfection and control of transmission, are urgently needed.

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## Conflict of interest

None declared.

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