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The high prevalence of *Legionella pneumophila* contamination in hospital potable water systems in Taiwan: implications for hospital infection control in Asia

Pei-Yi Yu [a], Yusen Eason Lin [a,*], Wei-Ru Lin [b], Hsiu-Yun Shih [a], Yin-Ching Chuang [c], Ren-Jy Ben [d], Wen-Kuei Huang [e], Yao-Shen Chen [e], Yung-Ching Liu [e], Feng-Yee Chang [f], Muh-Yong Yen [g], Ching-Chuan Liu [h], Wen-Chien Ko [h], Hsi-Hsun Lin [i], Zhi-Yuan Shi [j]

[a] *Center for Environmental Laboratory Services, National Kaohsiung Normal University, 62 Shen-chong Rd, Yanchao, Kaohsiung, 824, Taiwan*
[b] *Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*
[c] *Chi Mei Hospital, Tainan, Taiwan*
[d] *Kaohsiung Armed Forces General Hospital, Kaohsiung, Taiwan*
[e] *Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan*
[f] *Tri-Service General Hospital, Taipei, Taiwan*
[g] *Taipei City Hospital, Taipei, Taiwan*
[h] *National Cheng Kung University, Tainan, Taiwan*
[i] *E-Da Hospital/I-Shou University, Kaohsiung, Taiwan*
[j] *Taichung Veterans General Hospital, Taichung, Taiwan*

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KEYWORDS
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Summary
Background: The major sources of Legionnaires' disease (LD) are the potable water systems of large buildings including hospitals, nursing homes, and hotels. Culturing the hospital water system for Legionella allows a preventive approach for hospital-acquired LD. However, hospital-acquired LD is rarely reported in Taiwan, and environmental cultures of Legionella in hospital water systems in Taiwan have never been systematically performed.
Objective: The objective of this study was to determine if Legionella is present in hospital water systems in Taiwan. Water quality analysis was also performed to determine if geographic differences in water quality result in different Legionella positivity rates.

* Corresponding author. Tel.: +886 7 6051036; fax: +886 7 6051379.
E-mail address: easonlin@nknucc.nknu.edu.tw (Y.E. Lin).

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Method: The water systems of 16 hospitals throughout Taiwan were tested for Legionella by culture. Standardized culture procedures were followed.
Results: *Legionella pneumophila* was isolated from 63% (10/16) of the hospital water systems; 19% (3/16) of the hospitals had an *L. pneumophila* positive rate greater than 30%. *L. pneumophila* serogroups 1 and 6 (strains that are most responsible for Legionella infections) were isolated from 80% (8/10) and 60% (6/10), respectively, of the hospitals that yielded *L. pneumophila* in their water distribution systems.
Conclusion: As was shown in epidemiological studies in the USA and Spain, hospital-acquired legionellosis may be prevalent but underdiagnosed in Taiwan.

Introduction

Legionella is an opportunistic pathogen with widespread distribution in the environment. Numerous reports have demonstrated that the major sources for Legionnaires' disease (LD) are the potable water systems of large buildings including hospitals, nursing homes, and hotels. Legionella is a common cause of hospital-acquired pneumonia, especially for immunocompromised patients. Cooling towers were originally thought to be the main reservoir for Legionella, but subsequent reports have identified the water distribution systems as the major source of LD in hospitals.[1–3]

Culturing the hospital water supply for Legionella is the first step in the assessment of the risk for hospital-acquired LD. This approach is widely adopted in the national guidelines[4] for France, Denmark, Germany, Netherlands, Spain, Italy, Norway, Portugal, and Switzerland, and in other regional guidelines and recommendations.[5–7]

Hospital-acquired LD has rarely been reported from Taiwan, and environmental cultures of Legionella in hospital water systems in Taiwan have never been systematically performed. To our knowledge, there was no documented epidemiological investigation in Taiwan to determine the origin of the infecting organism until Chen et al. discovered, by molecular subtyping, that the home water supply of a patient was the source of a case of community-acquired LD.[8] The same group of researchers subsequently discovered that the hospital water supply was responsible for cases of hospital-acquired LD in a medical center.[9]

Environmental surveillance for Legionella in hospital water supplies can provide data that are useful for risk assessment and the prevention of hospital-acquired LD. Thus, we conducted the first environmental surveillance of 16 hospitals throughout Taiwan. The objectives were: (1) to investigate the frequency of Legionella contamination in hospital water systems, (2) to assess the frequency of *Legionella spp* and serogroups involved, and (3) to determine the effect of water quality, if any, on the presence of Legionella in hospital water supplies.

Methods

Study hospitals

Sixteen hospitals voluntarily participated in this survey. They were selected to ensure a geographic representation of the entire country: four in northern Taiwan, two in central Taiwan, eight in southern Taiwan, one in eastern Taiwan, and one on a nearby island (Figure 1). The size of hospital ranged from under 500 beds ($N = 7$), to between 500 and 1000 beds ($N = 3$), to over 1000 beds ($N = 6$). All hospitals were supplied with chlorinated domestic drinking water.

Sample collection and processing

A sterile swab (BBL CultureSwab™, Becton Dickinson, Franklin Lakes, NJ, USA) with transportation medium was inserted into faucet outlets (serving both hot and cold water) and rotated against the interior surface two times clockwise and up-and-down two times to dislodge the sediment. The swab was vortexed vigorously in 2 ml of sterile deionized water to resuspend the sediment from the swab to the aliquot. Two ml of 0.2 mol/l HCl—KCl buffer (pH 2.0) was added to the aliquot for 3 minutes. One tenth of 1 ml acid-treated sample was directly inoculated onto buffered charcoal yeast extract (BCYE) culture medium and selective media containing dyes,

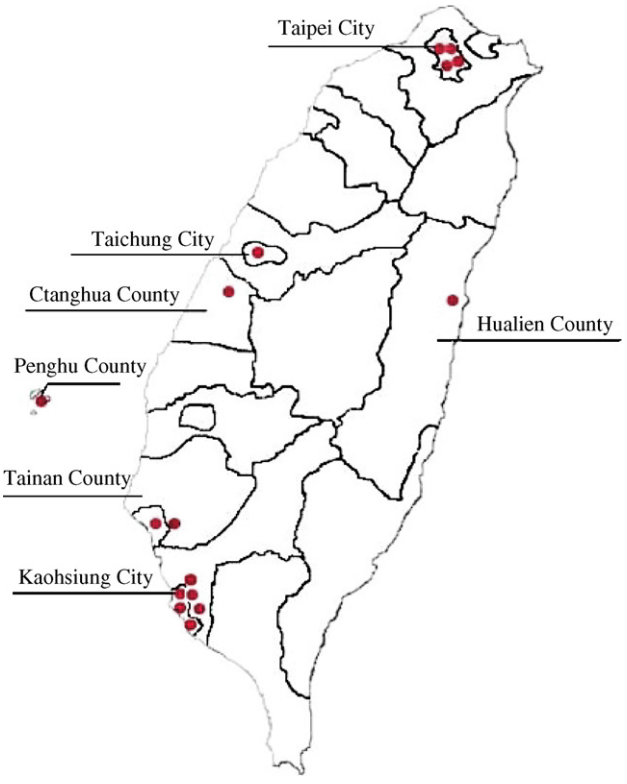

Figure 1 Sampling hospital locations.

glycine, vancomycin, and polymyxin B (DGVP). Culture media were incubated at 37 °C in a humidified atmosphere for 3–7 days.

Sample analysis

Suspect colonies were subcultured in parallel onto BCYE and blood agar plate (BAP) media. Colonies that grew after subculture on BCYE medium but not on BAP were tested with a latex test (Oxoid Ltd, Basingstoke, UK) and confirmed using a DFA monoclonal antibody (Monoclonal Technologies, Inc., Alpharetta, GA, USA). Isolates categorized as *L. pneumophila* serogroup 1 on the latex test were confirmed using a polyvalent *L. pneumophila* serogroup 1 antibody; isolates categorized as *L. pneumophila* serogroups 2–14 were confirmed with a monovalent *L. pneumophila* individual serogroup antibody; Legionella-like organisms were tested with monovalent *Legionella micdadei* antibody.

Water quality analysis

Concentrations of free chlorine, calcium, magnesium, copper, zinc, and iron were measured in the cold water samples. Free chlorine was measured at the site of sample collection using a portable spectrophotometer (Hach DR/2010, Loveland, CO, USA). Concentrations of calcium, magnesium, copper, zinc, and iron were determined by an inductively coupled plasma optical emission spectrometer (Optima 2000DV Perkin Elmer, Boston, MA, USA).

Statistical analysis

Statistical calculations were made using Microsoft Excel for correlation coefficients and *t*-test. Pearson's correlation was used to evaluate the degree of linear relationship between two variables. Correlations between 0 and 0.4, 0.4 and 0.7, and 0.7 and 1 indicate weak, moderate, and strong correlation, respectively. A 'significant' difference among samples was defined by a p value of less than 0.05.

Results

Sixty-three percent (10/16) of the hospital water systems were colonized by *L. pneumophila* (Table 1). For those hospital potable water systems that yielded *L. pneumophila*, serogroups 1 and 6 were isolated from 80% (8/10) and 60% (6/10) of the hospitals, respectively. Among the hospitals with distal site positivity for *Legionella* >30%, the water distribution systems of hospitals A and B yielded *L. pneumophila* serogroups 1 and 6, while hospital C had the most diverse *L. pneumophila* serogroups: 1, 6, 7, and 10.

A correlation was found between the Legionella culture positivity rate and geographical location (Figure 1). Seventy-five percent (6/8) of hospitals in southern Taiwan were colonized by *L. pneumophila*, while only 25% (1/4) of hospitals in northern Taiwan were colonized by Legionella; the distal positivity rate for the only hospital that yielded Legionella in the water distribution system was as low as 6% (2/34). No correlation between Legionella positivity of hospital potable water system and hospital size was found in our study (Table 2).

Table 1 *Legionella pneumophila* in the hospital water distribution system in Taiwan

Hospital	Legionella percent distal site positivity (%)	Serogroups of positive samples
A	100% (12/12)	*Lp* 1, *Lp* 6
B	67% (12/18)	*Lp* 1, *Lp* 3, *Lp* 6
C	40% (10/25)	*Lp* 1, *Lp* 6, *Lp* 7, *Lp* 10
D	27% (3/11)	*Lp* 1, *Lp* 7
E	27% (6/22)	*Lp* 1, *Lp* 6
F	27% (4/15)	*Lp* 1
G	19% (3/16)	*Lp* 1, *Lp* 2, *Lp* 7
H	6% (2/34)	*Lp* 1
I	5% (1/19)	*Lp* 6
J	4% (1/23)	*Lp* 6, *Lp* 7
K	0% (0/15)	NA
L	0% (0/34)	NA
M	0% (0/20)	NA
N	0% (0/13)	NA
O	0% (0/10)	NA
P	0% (0/15)	NA

NA, not applicable; *Lp*, *Legionella pneumophila*.

Table 2 The site positivity for Legionella vs. hospital size

Beds	Number	Percent positive (%)
<500	7	57% (4/7)
500–1000	3	67% (2/3)
>1000	6	67% (4/6)

A moderate correlation was found between calcium (r = 0.620) and magnesium (r = 0.682) concentrations and the presence of Legionella in hospital water systems. Interestingly, a moderate negative correlation was found between iron (−0.621) and the presence of Legionella in hospital water systems, suggesting higher iron concentrations might prohibit the growth of Legionella. No significant correlation was found between concentrations of copper, zinc, and free chlorine and the presence of Legionella in hospital water systems.

Discussion

Hospital potable water systems are the primary reservoirs for hospital-acquired Legionnaires' disease.[10,11] Prevention of hospital-acquired LD has been accomplished by disinfecting hospital water systems.[11–14] The Center for Disease Control in Taiwan has advocated the routine culturing and maintenance of hospital cooling towers even though no epidemiological link between cooling towers and hospital-acquired LD has ever been established in Taiwan. To our knowledge, only one hospital in Taiwan routinely cultures their water distribution system for Legionella; this policy was adopted following the discovery of the first case of hospital-acquired LD that was epidemiologically linked to the hospital water distribution system, not the cooling towers.[9]

Although surveys of Legionella colonization in hospitals have been conducted in the UK,[15–17] Canada,[18,19], USA,[20] and

Spain[21] with positivity rates ranging from 12% (Liu study:[16] two of 17 hospitals were positive) to 85% (Sabria study:[21] 17/20 hospitals were positive), this is the first large-scale hospital survey performed in Asia. In our study, 63% (10/16) of the hospital water systems were colonized by *L. pneumophila*. *L. pneumophila* serogroup 1 was the most frequently isolated serogroup in our surveillance; 80% (8/10) of the hospitals in our surveillance yielded *L. pneumophila* serogroup 1. Nineteen percent (3/16) of the hospitals showed a positivity rate for Legionella greater than 30% and three hospitals had a positivity rate for Legionella of 27%. Hospital A had the highest positive rate with the strains that are most responsible for Legionella infections;[22,23] positivity for *L. pneumophila* serogroups 1 and 6 were 100% and 92%, respectively. Hospital B subsequently detected several cases of hospital-acquired LD after completion of the environmental culture. The full impact of Legionella colonization in hospital B on patient care is still under investigation.

In studies conducted at eight hospitals in the USA and Spain, 100% (8/8) of hospitals colonized with Legionella reported that hospital-acquired LD was uncovered following subsequent clinical surveillances.[2,10,21,24–27] In a USA national surveillance study of 20 hospitals in 13 states, 14 hospitals were colonized with Legionella in the water systems in which 43% (6/14) of hospitals had environmental positive rates for Legionella $\geq$30%. Among these six hospitals with high levels of Legionella contamination, four hospitals discovered hospital-acquired Legionella pneumonia.[28] In a study in Spain of 12 hospitals for which the environmental positive rates for Legionella were $\geq$30%, 92% of hospitals (11/12) found cases of nosocomial legionellosis in the prospective clinical surveillance.[21] Thus, hospital-acquired LD might be underdiagnosed in Taiwan and in Asia given this relatively high colonization rate. For example, not a single case of hospital-acquired legionellosis was detected during the 1998–2002 period in Singapore despite the fact that Legionella bacteria were isolated from the environment at a sample positive rate of 24% (23/97) in tap water.[29] If subsequent clinical surveillance can be conducted followed by environmental cultures for Legionella, cases of hospital-acquired LD would be uncovered in Singapore as in previous studies.[21,28,30] Furthermore, hospital-acquired LD may become more apparent if knowledge of environmental culture results stimulates the awareness of physicians and increases the use of specialized laboratory testing for Legionella in patients with pneumonia, as has been documented elsewhere.[21,31] If Legionella persists in the water system and cases of hospital-acquired LD continue to occur, the infection can be prevented by disinfecting hospital water systems.[12,32]

In summary, *Legionella pneumophila* was found in 63% (10/16) of hospital water systems in Taiwan. Cases of hospital-acquired Legionnaires' disease may have occurred in Taiwan but not been detected. We recommend that the Asian public health agencies consider mandating environmental surveillance of Legionella for all hospitals, as is now implemented in many European countries[4] and elsewhere.[5–7,28] Environmental monitoring followed by clinical surveillance appears to be successful in uncovering previously unrecognized cases of hospital-acquired Legionella pneumonia. Supplemental on-site disinfection of hospital water systems may be necessary if cases of hospital-acquired Legionnaires' diseases continue to occur.

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Conflict of interest: No conflict of interest to declare.

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