

Prevalence of *Legionella* and the relationship with heterotrophic(HPC) bacteria in public spas

Kyong Whan Moon, Young Whan Kim

Dept. of Environmental Health, College of Health Sciences, Korea University, Seoul, 136-703 Korea

Abstract

This study was to determine the prevalence of *Legionella* species in public spas and to know that the presence of *Legionella* was correlated with other microbiological parameters. A total of 81 water samples were collected from 30 different public spas and sudatoriums so called zzimzilbang in Korea.

Although *Legionella* was not detected in the influent water and the cold tub water, of the 38 samples of hot and warm water taken from public tubs, 11(28.8%) were found to be positive for *Legionella* spp. All of the isolates were identified as a species *L. pneumophila* serogroups 1 and 2-14 by latex agglutination. And HPC concentrations in all spas water isolated *Legionella* were above the level of 10^4 cfu/ml.

Introduction

Legionella bacteria have been known to cause Legionnaires` diseases(pneumonic legionellosis) and Pontiac fever (severe influenza-like illness). Outbreaks of legionellosis are frequently reported around the world, which are associated with the diffusion of contaminated aerosols. Water systems, such as cooling towers and hot-water systems, have been attributed to be the source of the bacteria.

A particular risk factor has recently been identified in the use of whirlpools and hot tubs in spas and public baths. In July 2002, 295 cases of Legionnaires` diseases occurred in Japan, of whom 7 persons died. It was found that all of the patients had visited the spa in Miyazaki. The event received huge media attention causing considerable alarm in the population.

The legionellae are gram negative bacteria. At least 40 known species and many more serogroups of *Legionella* bacteria have been identified, and many of these have been implicated in human disease. The investigations on *Legionella* in various air conditioning systems and building water facilities had been carried out for

several years in Korea. But heated spas have been investigated less commonly.

Recently, a great number of public spas and sudatoriums so called zzimzilbang are thriving for 24 hours in Korea.

The purpose of the current study was to determine the prevalence of *Legionella* species in public spas and to know that the presence of *Legionella* was correlated with other microbiological parameters.

Materials and Methods

All samples used in this study were obtained during from July to December 2003 from public spas located in Seoul, Korea. A total of 81 water samples were collected from 30 different public spas.

Isolation of *Legionella* spp. was performed in accordance with the selective procedure described by International Standard Method (ISO).

Cysteine dependence positive colonies were confirmed as *Legionella* species by direct immunofluorescence and latex agglutination. The agglutination test was done using a commercially available test kit(Oxoid DR 800M). The reagents supplied with the kit were specific for either *L. pneumophila* SG 1, *L. pneumophila* SGs 2-14, or other *Legionella* species(including *L. longbeacheae* SGs 1-2, *L. bozemanni* SGs 1-2, *L. gormanii* , *L. jordanis*, *L. micdadei* and *L. anisa*). At the same time, total coliforms and heterotrophic plate counts bacteria were determined in water samples by EPA methods.

Results

As shown in Table 1, *Legionella* was not detected in raw water and the cold tub water. Whereas, of the 38 samples of hot and warm water taken from public tubs, 11(28.8%) were found to be positive for *Legionella* spp. The temperatures of the hot tub waters ranged from 38 to 45°C and the cold waters were between 18 and 25 °C. It has been known that the temperatures between 30 to 40 °C provided optimal growth conditions for *Legionella*.

Total coliforms were detected in 45% of the samples and were grew in even raw water. But total coliform was not detected in the majority of *Legionella*-positive samples. The correlation between the presence of *Legionella* and total coliforms was not showed in the tub water. The public health standard for *Legionella* not only in cooling tower systems but also in the spa water was not regulated in Korea. Only

the standard for total coliform has been established primarily to supervise a state of sanitation in the spa water.

Table 1. Prevalence of *Legionella* and total coliform in 81 spas water

Spa category	No.(%) of spa	No.(%) of <i>Legionella</i> -positive	No.(%) of total coliform-positive
raw water	19	0(0)	9(47.4)
Tub water	Cool	24	0(0)
	hot	38	11(28.8)
Total	81	11(13.6)	37(45.7)

All of the isolates were identified as a species *L. pneumophila* serogroups 1 and 2-14 by latex agglutination. HPC bacteria concentrations in all spas isolated *Legionella* were above the level of 10^4 cfu/ml and the total coliforms were detected only on the occasion of four. On the basis of these results, it would appeared that *Legionella* proliferate primarily in inadequate tubs to control the total bacteria numbers. (Table 2)

Table 2. *Legionella* serogroup and the relationship between heterotrophic bacteria and total coliform in the tub water detected *Legionella*.

Tub No.	<i>L.pneumophila</i> Serogroup	HPC bacteria (cfu/ml)	Total coliform (/100ml)
S-1	1	$>10^4$	No detected
S-8	2-14	$>10^4$	No detected
S-13	1	$>10^4$	No detected
S-14	1	$>10^4$	No detected
S-16	1	$>10^4$	No detected
S-18	2-14	$>10^4$	No detected
S-22	2-14	$>10^4$	Detected
S-29	2-14	$>10^4$	No detected
S-35	2-14	$>10^4$	Detected
S-47	2-14	$>10^4$	Detected
S-72	2-14	$>10^4$	Detected

The levels of *L. pneumophila* were fairly distributed over a broad range of

concentrations from <100cfu/100ml to 10,000cfu/ml, with 1 of a spas in the <100cfu/ml, 4 spas in the 100 to 1,000cfu/ml, and 2 spas had levels of culturable *L. pneumophila*. >10,000cfu/ml. (Table 3)

Table 3. Concentrations of *Legionella* detected in the spas

Spa category	No.(%) of <i>Legionella</i> -positive	Concentrations of <i>Legionella</i> (cfu/100ml)			
		<100	<100-1,000	<1,000-10,000	10,000<
Hot water	11(100)	1(9.0)	4(36.4)	4(36.4)	2(18.1)

Conclusion

The study showed that almost one quarter of the tub water in public spas were contaminated with at least 2 *Legionella* species including *L. pneumophila* serogroup 1 and 2-14. It was found that *Legionella* proliferate primarily in inadequate circumstances to control the growth of HPC bacteria. The levels of *L. pneumophila* were fairly distributed over a broad range of concentrations from <100cfu/100ml to 10,000cfu/ml.

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