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Factors Affecting Hospital Water System Contamination with *Legionella pneumophila*in Northeast of Iran

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ABSTRACT

Background: Natural and man-made environments are the main sources of *Legionella pneumophila*. Hospitals are considered as at risk environments for legionella contamination. Considering the serious outcome of legionnaire diseasein hospitalized patients, we investigated possible contamination with *Legionella pneumophila* in water system of two main hospital settings in north-east of Iran and affecting factors.
Material and methods: Forty water samples were taken from the different wards of each two main1000 bed university hospitals in Mashhad, Iran. Free chlorine and temperature was measured and recorded at the sampling location. Next, 250 ml of each water sample was filtrated and *Legionella pneumophila* antigen was evaluated by ELISA technique.
Result: Twenty-three out of 40 samples from Imam Reza university hospital and 3 out of 40 samples from Ghaem university hospital were found positive for *Legionella pneumophila* antigens, hence a significantly difference in contamination rate was observed between the two hospitals(P-value<0.001).We did not observe any significant relationship between free chlorine, temperature and contamination with *Legionella pneumophila* (p =0.365and p= 0.753 respectively).
Conclusion: both hospitals had the same environmental hygiene policy, but the older hospital had notably increased water contamination in at risk wards, probably owing to the older water distribution system or its longer water supply piping system. The hospital building age and structure is an effective factor that is generally ignored in decontamination plannings. These findings suggest the needs for more rigorous decontamination protocols in older hospitals.
KEY WORD: water contamination, legionella pneumophila, hospital

INTRODUCTION

Legionella Pneumophila discovered in 1976, causes a wide range of infections including lower repertory tract infection (Legionnaires' disease) as well as a mild illness known as Pontiac fever. *Legionella pneumophila* is a gram negative and non-sporeforming bacterium which is common in aquatic environments(1-3). It can tolerate temperatures up to 5.0°C-63°C, pH range of about5.0–9.2 and low nutrients conditions(3-7). Different natural and man-made environments are known as the sources of *Legionella* including rivers, pools, public drinking water, as well as air-conditioning cooling towers (1, 8-10). *Legionella* may cause human disease when water contaminated with *Legionella* becomes aerosolized and is inhaled or aspirated (11). Hospital water distribution systems have been identified as a source of *Legionella pneumonia* and have been considerate as a serious concern in healthcare settings. Patients particularly those with compromised immune systems are at high risk of *Legionella* infection(11-12).

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Hot tubs have been blamed as one of the most probable sources of Legionella infection in Japanese outbreak investigations(1). In addition, large numbers of *Legionella pneumophila* have been identified in the residual sediment that accumulates at the bottom of hot water tanks (6). Also *Legionella pneumophila* serogroup 1 detected from water storage tanks, shower, and taps water, has been related to the cases of *Legionella pneumophila* infection with the same strain(13).

Data obtained from 1990 to 2005 survey, demonstrate that Legionnaires' cases have significantly increased during recent years, emphasizing the importance of this disease and its environmental sources (14). *Legionella pneumophila* related infections are potentially life threatening in hospitalized patients who are generally susceptible to the infectious pathogens due to their impaired immune status (3-4, 8, 13, 15-16). Also, the outbreak of such infections affecting a large number of people is considered as a great concern in health care authorities(5).

Given the serious outcomes of *Legionella pneumophila* in hospital water systems, we aimed to investigate possible contamination with *Legionella pneumophila* in two main university hospitals, with two distinct building features, located in Mashhad/ northeastern Iran. Here we report the contamination rate along with possible factors affecting hospitals' water contamination.

METHODS

Sampling

Samples of tap water were collected from Ghaem and Imam Reza university hospitals during summer 2015. Samples were taken at the beginning of the working hours (8 am).From each site, hot and cold water were collected separately. Totally 80 samples were taken from high risk hospital wards: Hemodialysis, NICU, Thorax, Nephrology, Pediatric and Emergency unit. Samples were also taken from the only burn ward of the city located in Imam Reza hospital.

For each sampling, a total volume of 250 ml of water was collected in a sterile container. The samples were then concentrated by a 0.22 µm sterile cellulose acetate filter paper. Thereafter, the concentrated samples (≈5ml) were stored at -70 °C until examined by ELISA.

In order to determine any possible effect of residual chlorine on legionella contamination, DPD (diethyl para diphenyle diamin) tablets and relative kits were used, also the water temperature was measured and recorded at the site of sampling.

ELISA assay

Sandwich ELISA technique was used to detect the antigen of Legionella in water samples. ELISA urinary antigen kit (Diagnostic automation/Cortez Diagnostics Inc., California, USA) with specificity and sensitivity as 99.2% and 100% respectively, was used based on manufacturer's instructions. Briefly, samples were vortexes vigorously to release filter bound bacteria into the water. Next,100 µl of samples were added into each antibody coated well and incubated for 30 minutes; plate was then washed away 3 times. Thereafter enzyme-conjugated secondary antibody was added to the wells. Plate was again washed 3 times with kit provided washing solution. Thereafter, substrate was added to the wells for chromogenic reaction. Finally, the plate was read at the wavelength of 450-630 nm by ELISA reader (state fax-2100 awareness plate reader).

Statistical analysis of data

The comparisons between two hospitals and clinical units were performed using mann-whitney test. SPSS version 16 software was used and the significant level was considered as 0.05 in all calculations.

RESULTS

Overally, 23 samples out of 40taken from Imam Reza university hospital were positive while 3 out of 40 samples were positive in Ghaem university hospital (table 1).Therefore water samples obtained from Imam Reza hospital were significantly higher contaminated comparing Ghaem hospital water samples (P-value<0.001).Frequencies of Legionella antigen detection in different wards of each hospital have been shown in table 1. Almost in every wards of Imam Reza hospital the frequency of Legionella was higher than water of Ghaem university hospital. Generally, cold water had 35% positive Legionella and hot water had 26%; however no statistically significant difference was found between hot and cold water samples (P-value >0.1).

Table 1: positive legionella water samples according to wards of each main hospitals

Ward	Emergency	Hemo-dialysis	NICU	Thorax	Nephrology	Pediatrics	Burn unit	Total
Ghaem university hospital	2/19	1/3	-/3	-/5	-/6	-/4	-/-	3/40
Imam Reza university hospital	4/12	2/3	3/3	3/3	3/8	4/7	4/4	23/40

The relationship between frequencies of Legionella antigen detection and residual chlorine was also investigated. As shown in table2, no relationship between these variables was observed (Mann-Whitney U, P=0.365).Similarly, as shown in table 3, no correlation between water temperature and legionella contamination was found (Mann-Whitney U, P= 0.753).

Table2: The relationship between contamination with Legionella and the residual chlorine (Cl^-) mg/L

Legionella infection	Positive (n=26)	Negative (n=54)	Total (n=80)	Mann-Whitney U(P value)
Mean	0.23	0.13	0.16	0.365
Standard deviation	0.36	0.21	0.27	
IQR	0.45	0.2	0.2	

Table3: The relationship between water temperature (℃) water samples for contamination with Legionella

Legionella infection	Positive (n=26)	Negative (n=54)	Total (n=80)	Mann-Whitney U(P value)
Mean	30.23	29.85	29.98	0.753
Standard Deviation	7.71	8.93	7.5	
IQR	15.25	16	15.75	

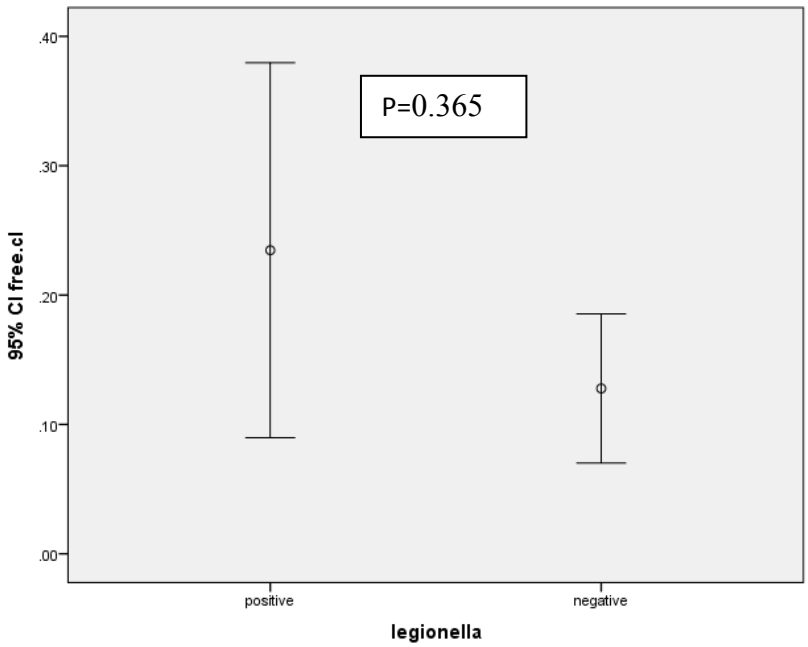

Figure 1: The relationship between contamination with Legionella and the residual chlorine (Cl^-) mg/L

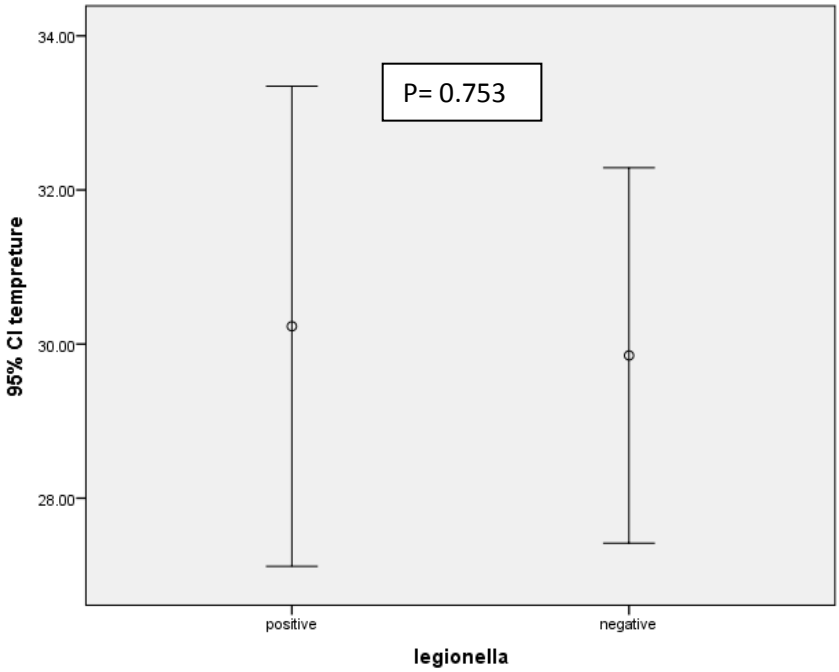

Figure 2: The relationship between temperature (℃) of water samples and contamination with Legionella

DISCUSSION

The study was performed in Mashhad, second largest city of Iran. Two main university hospitals located in the city were included in this study. Imam Reza university hospital is an old large hospital with separated single floor buildings which has been established in 1934. On the other hand, Ghaem hospital is newer (built in 1970) with a modern construction putting all wards in one single large four floor building. We found significantly higher contamination rate in the older Imam Reza hospital (P_{value}<0.001). Both hospitals have almost the same infection control policy and both have similar visiting and patient load. Therefore it seems that the main cause of the difference is the water distribution system of these hospitals. The water piping system is very old and extended in the case of Imam Reza hospital comparing to more compact and newer water distribution system of Ghaem hospital. These structural features make Imam Reza hospital more prone to biofilm formation in different parts of its connected piping system.

Legionella pneumophila is a fastidious bacterium and needs different nutrients including iron salts and several amino acids such as L-cysteine, that are used as carbon, nitrogen and energy sources. These nutritional needs results in hampered *Legionella pneumophila* growth in ordinary laboratory culture media(4).To monitor Legionella we used ELISA as an accurate method, because the traditional culture method is a time-consuming procedure and suffers from several limitations and requires special culture medium with a rather high false negative results (17).

Legionella pneumophila has great ability for survival in natural and artificial aquatic systems. In addition *Legionella pneumophila* is a biofilm forming bacterium and biofilm provides a condition that protects Legionella from different kinds of disinfectant such as chlorine-based biocides which are routinely used as common disinfectants in public buildings(18-21). Our findings highlights several factors that affect the survival of the *Legionella* in water system including type and age of water distribution system, length and form of piping network, horizontal or vertical piping system as well as building structure.

It seems that the decontamination procedure wasn't sufficient in both evaluated hospitals. There are several disinfection methods routinely used in hospitals including thermal and chemical approaches. Heat disinfection has been widely used especially for boiler water systems because *Legionella* was found to be inactivated at >70°C,though some studies have reported that thermal disinfection is not completely effective in removal of Legionella in aquatic environments(22). For the disinfection of drinking water, chemical methods using chlorine have been the most widely used(23). Many studies suggest using disinfectant and hot water simultaneously for more effectiveness(24).

We found no association between free chlorine and Legionella contamination. This was probably because of persistent biofilm formation that makes a suitable bed for the bacteria to resist against chlorine. This finding emphasizes that additional strategies might be needed to eliminate *Legionella*. For example other approaches like monochloramine and electrolysis in water distribution system of critical clinical wards might be beneficial (25-26).
In contrast to other investigations, we did not found any significant difference in *Legionella* contamination between hot and cold water system. That might be due to difference in models of hot and cold water piping systems used around the world.
Water contamination in a very critical ward such as burn unit should be considered as a serious threat. Considering the clinical importance of this ward in terms of nosocomial infections, it is essential to eliminate the contamination sources to prevent life threatening infections. Also water contamination in hemodialysis ward, Emergency department, Pediatric, NICU and renal wards might directly contaminate all devices in the patient room and may directly superimpose a life threatening pneumonia to already immune compromised patients. These issues necessitate the need for more attention and appropriate policies for prevention of infections due to *Legionella pneumophila* in especially old hospital settings.
Some limitations of the study should be noted. First, due to financial reasons only critical wards were included in the study. Second, we are not able to comment on the count of the bacteria because legionella antigen ELISA kit used in the study was a qualitative kit. Third it would be beneficial if sampling could be repeated in all seasons to evaluate any seasonal relationship with bacterial contamination. Regardless of the mentioned limitations, the study highlights a potentially hidden life threatening pathogen that requires more restrict monitoring and elimination policies in hospital settings as critically high risk environments.
To summarize, both hospitals had the same environmental hygiene policy, but the older hospital had notably increased water contamination in at risk wards, probably owing to the older water distribution system or longer water supply piping system; an effective factor that is generally ignored in decontamination action plans. Therefore it is recommendable that the policy makers should include additional underlying factors affecting each individual hospital environment to eliminate *Legionella pneumophila.*

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