JCHR (2022) 12(4), 557-562

Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Survey on *Legionella pneumophila* in Water Supply Systems of Qazvin Hospitals

Shaghayegh Mousavi[1], Mohadeseh Choubdar[2], Masoud PanahiFrad[3], Fatemeh Fotoohi[*4]

[1]*Department of Molecular Medicine, Faculty of Medical Sciences, Qazvin University of Medical Sciences, Qazvin, Iran*

[2]*M.Sc. in Occupational Health Engineering, School of Health, Qazvin University of Medical Science, Qazvin, Iran*

[3]*Research Committee and Department of Environmental Health Engineering, School of Health, Qazvin University of Medical Sciences, Qazvin, Iran*

[4]*Department of Microbiology, Faculty of Medical Sciences, Qazvin University of Medical Sciences, Qazvin, Iran*

(Received: 3 February 2020 Accepted: 1 February 2022)

KEYWORDS

Hospital;
Infection;
Legionella pneumophila

ABSTRACT: Hospital infections are one of the important causes of hospital mortality and *Legionella pneumophila* bacteria is considered as one of the common causes of hospital infection. The natural habitat of these bacteria in aquatic environments and can tolerate different environmental conditions in terms of pH, temperature, oxygen content and nutrients. Therefore, this study aimed to identify *Legionella pneumophila* in aquatic systems of hospital environments. This descriptive cross-sectional study was performed in the summer of 2017 to identify *Legionella pneumophila* in hot and cold water systems of important and critical parts of Qods and Kosar Hospital. A total of 120 samples were collected, and after filtration, heat treatment, and acidic treatment, all the samples are cultured on BCYE agar enriched with GPVC supplement, and after incubation, the bacterial growth is investigated. Legionella suspected colonies were approved by complementary tests and their assignment to Legionella was confirmed. The water samples were also investigated for temperature, pH, and residual chlorine (using a DPD kit). The frequency of *Legionella pneumophila* bacteria in water supply systems of Qods Hospital was zero in all hot and cold water showers of NICU, PICU 1, PICU 2, Hematology and Oncology sectors. The frequency of this bacterium was zero in postpartum, childbirth, and women's surgery sectors, but it was positive in the hot and cold water showers of the prenatal sector (8 CFU ml^{-1} and 8.33 CFU ml^{-1}, respectively) and neonatal sector (4 CFU ml^{-1} and 4.16 CFU ml^{-1}, respectively). Although Qods and Kosar Hospitals use the treated water of urban distribution network, 24 cases (40%) of contamination were found in water supply systems of Kosar Hospital, and this contamination can be attributed to reasons such as low residual chlorine content, and appropriate temperature range for growth of this bacteria.

INTRODUCTION

Hospital infections are one of the most important causes of mortality in the world, which cause diseases due to the presence of dangerous bacteria depending on the environment and host. One of the most important causes of hospital infections is Legionella bacteria. Legionella species cause Legionnaires' disease and Pontiac fever and Legionella is a severe form of pneumonia and can be fatal [1-2].

*Corresponding author: fatemeh_fotoohi@yahoo.com (F. Fotoohi)
DOI: 10.22034/jchr.2022.1892531.1101

So far, 49 species of these bacteria have been identified. One of the major species known in this genus is *Legionella pneumophila*, which is detected in more than 15 supergroups and is responsible for 90% of cases of Legionella [3]. Depending on molecular epidemiological studies, it has been found that Legionella is a considerable percentage of pneumonia that has been occurred in hospital environments. Various reports indicate that 1-3% of community-acquired pneumonias and above 30% of hospital-acquired pneumonia are caused by Legionella [4]. The hospital-acquired infections are occurred by inhalation of aerosol particles or aspiration of contaminated water, respiratory equipment, nebulizer, mask, bath shower, water incinerator, water bath, neonatal incubators, use of ice pieces to relieve thirst in patients undergoing cardiac surgery, and NG tube [5]. One of the most important reasons that lead to attention to these bacteria in hospital environments is the presence of vulnerable people in these places. Although any person can be exposed to the pathogenicity of the bacterium, the individuals admitted in hospitals with the sensitive groups such as those with cancer, dialysis patients, those with diabetes and AIDS, and people who had kidney transplants, and in general, those whose immunity level is weakened, are more at risk [6].

The hospital environments are high-potential places for growth and prevalence of this factor in terms of growth ground, aerosol transmission system, and people at risk. The water distribution networks and air conditioners in hospitals are considered an important source for Legionnaires' disease in these centers. The water distribution network, due to the retention time and blind spots, is a suitable environment for the growth of bacteria in most the reservoirs and inner surfaces of water pipes made of PVC, steel, iron, and copper surfaces. The specific ecological characteristics of this bacterium and its coexistence with protozoa, algae, and other bacteria, especially in bacteria of water supply systems, provide the conditions required to tolerate adverse environmental conditions, resistance to disinfections, and growth and replication of bacteria [5].

The center for disease control and prevention (CDC) has reported the prevalence of 25-45% for Legionella disease in the hospital environment and the prevalence of 30% for mortality rate caused by this disease in the hospital cases, but some sources have reported this to be above 40% [7] To reduce the risk of legionella infection, the density of *Legionella pneumophila* bacteria should be less than 1000 CFU/L in water and less than 250 CFU L^{-1} in conditions dealing with vulnerable people. Furthermore, the World Health Organization (WHO) has determined the reference values of 1 CFU L^{-1} for *Legionella bacteria* due to the effect of water quality on the DALY index in drinking water quality guidelines [8]. The primary diagnosis of Legionella and epidemic states in the hospitals is necessary not only for the correct and effective treatment of patients but also for controlling and preventing the occurrence of diseases. Given the high mortality ratio of Legionella disease and the prevalence of resistance to various disinfectants, in order to prevent the release of legionella species in the hospital environment, effective measures should be taken 9. This study was conducted to identify Legionella in a hospital environment.

MATERIALS AND METHODS

This descriptive cross-sectional study was conducted in Qazvin University of Medical Sciences in the hot and cold water systems of critical parts of Qods Hospital (NICU, PICU 1, PICU 2, Hematology and Oncology sectors) and Kosar Hospital (Postpartum, Childbirth, Prenatal, Neonatal, Women's Surgery sectors). A total of 120 samples were collected from five sectors of Qods Hospital and five sectors of Kosar Hospital.

Sampling was carried out over three months of July, August, September 2017, every 15 days. To prepare the sampling sites before sample collection, the water taps of the desired sectors are left open for 1 min, then the head of taps and showers are disinfected, and once again the water taps are left open for 1 min, and then the sampling was conducted. For microbial tests, the water samples were collected in 1.5 L sterile containers and were transferred to the laboratory of the Faculty of Health, Qazvin University of Medical Sciences. Each sample was immediately concentrated using a membrane filtration system of multi

pore nylon membrane filters with the size of 0.2 µm. After concentrating each sample, the filter was isolated from the device and was placed in a clean container containing 20 ml of the same sample. For better isolation of bacteria from the filter, the samples were shaken at medium speed for 30 min. Then until being used for culture, it was stored in the refrigerator at a temperature of 4°C [10]. All components of the filtration device were sterilized after each use by autoclaving and boiling water. To examine and identify the bacteria, two 10 ml components were separated from each sample. First, each component was cultured on Mueller Hinton agar (Merck, Inc) and MacConkey agar (Merck, Inc), and then in order to isolate the Legionella, after thermal treatment (12 min at 56°C) and acidic treatment (with the acidic buffer of HCL/KCL PH=2.2 manufactured by Merck, Inc), all samples are cultured on BCYE agar (Buffered charcoal yeast extra agar) enriched with GPVC supplement (containing antibiotics of polymyxin B, vancomycin, cycloheximideglycine) and are placed in CO_2 incubator at 37°C and an atmosphere containing 5% CO_2. The bacterial growth was examined on days 3, 5, 7, 10, 14. If there is no observation of colony, the result is negative and if there is a growth of suspicious colonies, given the macroscopic characteristics of the colony (color and size, etc.) and biochemical tests (catalase and oxidase), the Legionella is identified, and for approval, the above colonies were re-cultured on BCYE agar and blood agar, and in case of no growth, their assignment to Legionella was confirmed. Meanwhile, the water samples were investigated for temperature, pH, and residual chlorine (using DPD kit). The resulting data were analyzed using SPSS software, tables, and charts.

RESULTS

The frequency of *Legionella pneumophila* bacteria in water supply systems of Qods and Kosar hospitals is presented in Tables 1 and 2.

According to the results of Table 1, a total of 60 samples collected from the water supply systems of Qods Hospital were negative for *Legionella pneumophila* bacteria.

Table 1. Frequency of *Legionella pneumophila* bacteria in water supply systems of Qods hospital (CFU ml^{-1}).

		July		August		September	
		Week1	**Week2**	**Week1**	**Week2**	**Week1**	**Week2**
NICU	hot water valve	0	0	0	0	0	**0**
	cold water valve	0	0	0	0	0	**0**
PICU1	hot water valve	0	0	0	0	0	**0**
	cold water valve	0	0	0	0	0	**0**
PICU2	hot water valve	0	0	0	0	0	**0**
	cold water valve	0	0	0	0	0	**0**
Hematology and oncology	hot water valve	0	0	0	0	0	**0**
	cold water valve	0	0	0	0	0	**0**

Table 2. Frequency of *Legionella pneumophila* bacteria in the water supply systems of Kosar Hospital (CFU ml^{-1})

		July		August		September	
		Week1	**Week2**	**Week1**	**Week2**	**Week1**	**Week2**
Postpartum	hot water valve	0	0	0	0	0	**0**
	cold water valve	0	0	0	0	0	**0**
Childbirth	hot water valve	0	0	0	0	0	**0**
	cold water valve	0	0	0	0	0	**0**
Prenatal	hot water valve	8	7	8	9	8	**8**
	cold water valve	9	8	8	9	8	**8**
Neonatal	hot water valve	4	3	5	4	4	**5**
	cold water valve	3	4	4	4	4	**5**
Women's Surgery	hot water valve	0	0	0	0	0	**0**
	cold water valve	0	0	0	0	0	**0**

According to the results of Table 2, from a total of 60 samples collected from water supply systems of Kosar Hospital, 24 samples (40%) were positive for *Legionella pneumophila* bacteria.

The following table indicates the results of water samples for temperature, pH, and average residual chlorine in the water supply systems in Qods and Kosar hospitals.

According to the results of Table 3, the water samples collected from Qods Hospital had higher temperatures and residual chlorine than the samples obtained from Kosar Hospital.

Table 3. Average values of temperature, pH, and residual chlorine in water supply systems at Qods and Kosar hospitals

		July		August		September	
		Week1	**Week2**	**Week1**	**Week2**	**Week1**	**Week2**
Temperatures	Qods Hospital	55	49	39	45	38	34
	Kosar Hospital	25	29	32	34	34	30
pH	Qods Hospital	7.8	7.6	7.6	7.6	7.8	7.7
	Kosar Hospital	7.8	7.8	7.8	7.7	7.8	7.6
Residual chlorine	Qods Hospital	0.7	0.6	0.5	0.6	0.9	0.9
	Kosar Hospital	0.3	0.2	0.3	0	0	0.4

DISCUSSION

A review of water supply systems in some of the hospitals studied by some researchers represents the positive contamination of water supply systems to *Legionella pneumophila* bacteria. The results obtained from the studies conducted by other researchers have shown that environmental factors such as water temperature, residual chlorine, type of water treatment system, pH, turbidity, and other microbial agents such as cysts can be effective in resistance to *Legionella bacteria* [11].

The results of this research also showed that even though Qods and Kosar hospitals in the city of Qazvin use the urban water treatment system, 24 samples (40%) of all samples were collected from water supply systems of Kosar Hospital were positive for *Legionella pneumophila*. Thus, it can be concluded that the common methods of water treatment and disinfection for purification of the water network from this microorganism might not be enough. The free chlorine of 0.9 mg l^{-1} is sufficient to eliminate Legionella in water reservoirs, but the results of studies have also shown that under some conditions, the *Legionella bacteria* has even resisted against free chlorine of 5 mg l^{-1}. These organisms also survive for a long time under wet conditions and can resist under temperature conditions of 20-50ºC and pH of 2-8.5 [12]. Moreover, the coexistence of Legionella with allergens and other bacteria, participially in the bacteria complex, as well as single cells, can provide the necessary nutritional resources to continue the bacterial life. This relationship provides conditions for bacteria that can easily resist the water treatment operations (chlorine, etc.), in which Legionella can also take shelter before the formation of an amoeba cyst and use it as a biological barrier to tolerate the lethal conditions in the environment. Also, some materials such as black plastic tubes in cold water valve washers and plastic showerheads and the presence of amoeba in showerheads increase the growth of Legionella [13]. Therefore, the growth of this bacterium in some parts of the water supply system of Kosar Hospital can be due to the presence of bacteria in these systems and low residual chlorine than the water supply system of Qods hospital.

According to the results in Table 2, the water samples collected from Qods Hospital have higher temperature and residual chlorine content than the samples obtained from Kosar Hospital, which could be one of the reasons for the growth of this bacterium in the water supply system of this hospital.

Different studies around the world have also shown the positive cases for contamination of water supply systems

and cooling towers of the hospitals. The results of the study that aimed to evaluate the contamination level of Legionella in cooling towers in Southern Australia indicated that 60-75% of these towers were colonized by a variety of Legionella species [14].

Young in Malaysia isolated a total of 28 different strains of Legionella with a study on 20 water samples from 11 cooling towers in different parts of Malaysia, and in general, they reported 35.7% *Legionella pneumophila* in supergroup 1, 39% *Legionella pneumophila* in supergroup 2-14, and 10.7% non-groupable *Legionella pneumophila*. Moreover, in addition to *Legionella pneumophila*, Legionella business, *Legionella gurmaneh*, *Legionella Anisa*, and *Legionella gerisinis* were also isolated [15].

Moghaddam in the study on the frequency of *Legionella pneumophila* in the cold and hot water valve and water reservoir of incubators in the neonatal department of Gilan hospitals in 2012, showed that from a total of 140 hospital samples, about 8.5% of the samples were contaminated, where 11.1% of the water of incubators and 5.8% of cold and hot water were separated [16].

Eslami also conducted a study with an aim to investigate and identify Legionella in water distribution systems in Taleghani Hospital, Tehran. The results of this study showed that from 32 studied samples, 11 samples (34%) were positive for Legionella. The residual chlorine was 0.9 mg l^{-1} and average pH was 7.36. Most of positive samples were related to hot water with chlorine content of 1 mg l^{-1} [17].

According to a literature review that was carried out by Qanizadeh in 2016 on the identification and isolation of legionella species in hospitals in Iran and the world, it was shown that 2.85-41.75% of samples in Iranian hospitals were infected with Legionella. This is reported to be 17-98.7% in the hospital centers of other countries [18].

CONCLUSIONS

Although infection with Legionella in Iran is lower than in other countries, in accordance with WHO standards, the presence of 1 CFU l^{-1} for *Legionella bacteria* for its control and its infections are among the most important health priorities.

Accordingly, the specific properties of this microorganism lead to special attention to this bacterium. The Legionella control methods include thermal disinfection, immediate heating system, hyper chlorination, monochloramine, chlorine dioxide, copper-silver ionization, ultraviolet sterilization, filtration, hospital masks, and disinfection of nebulizers. The coarse filtration in airborne channels and incorporation of lamps in the airborne channels in front of filters and coiling are also suitable methods for controlling these microbes.

Although Qods and Kosar Hospitals use the treated water in the urban distribution network, 24 cases of contamination were found in the water supply systems of Kosar Hospital. This contamination can be attributed to reasons such as low residual chlorine content, presence of bacteria in the water supply system, and appropriate temperature range for growth of this bacterium. According to the results, in order to control *Legionella pneumophila* bacteria, the disinfection methods, residual chlorine with a concentration above 1 mg L^{-1}, flash tanks with water at temperate above 55°C and ultraviolet radiation can be used.

ACKNOWLEDGEMENTS

The authors are grateful to all the experts of the Qazvin University of medical science who helped in conducting this study.

ETHICAL CONSIDERATION

This study was approved by the Ethics Committee, Qazvin University of Medical Sciences (IR.QUMS.REC.1395.145) and project number #28/32/40038.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Thornley C., Harte D., Weir R., Allen L., Knightbridge K., Wood P., 2017. Legionella longbeachae detected in an

industrial cooling tower linked to a legionellosis outbreak, New Zealand, possible waterborne transmission? Epidemiology & Infection. 145 (11), 2382-2389.

2. Kanatani J.I., Isobe J., Norimoto S., Kimata K., Mitsui C., Amemura-Maekawa J., Kura F., Sata T., Watahiki M., 2017. Prevalence of Legionella species isolated from shower water in public bath facilities in Toyama Prefecture, Japan. Journal of Infection and Chemotherapy. 23(5), 265-270.

3. Sánchez-Busó L., Guiral ., Crespi S., Moya V., Camaró M L., Olmos M.P., Adrián F., Morera V., González-Morán, F.,Vanaclocha H., 2016. Genomic investigation of a legionellosis outbreak in a persistently colonized hotel. Frontiers in Microbiology. 6, 1556.

4. Marchesi I., Ferranti G., Mansi A., Marcelloni A M., Proietto A. R., Saini N., Borella P., Bargellini A., 2016. Control of Legionella contamination and risk of corrosion in hospital water networks following various disinfection procedures. Appl Environ Microbiol. 82(10), 2959-2965.

5. Cunha B.A., Burillo A., Bouza E., 2016. Legionnaires' disease. The Lancet. 387(10016), 376-385.

6. Kyritsi M.A., Mouchtouri V.A., Katsioulis A., Kostara E., Nakoulas V., Hatzinikou M., Hadjichristodoulou C., 2018. Legionella Colonization of Hotel Water Systems in Touristic Places of Greece: Association with System Characteristics and Physicochemical Parameters. International Journal of Environmental Research and Public Health. 15(12), 2707.

7. Richardson K., Grigoryeva L., Corsini P., White R., Shaw R., Portlock T., Dorgan B., Fornili A., Cianciotto N., Garnett J., 2019. Structure and functional analysis of the Legionella chitinase ChiA reveals a novel mechanism of metal-dependent mucin degradation. BioRxiv, 687871.

8. Lévesque S., Lalancette C., Bernard K., Pacheco A. L., Dion R., Longtin J., Tremblay C., 2016. Molecular Typing of *Legionella pneumophila* Isolates in the Province of Quebec from 2005 to 2015. PloS One. 11(10), e0163818.

9. Springston J. P., Yocavitch L., 2017. Existence and control of Legionella bacteria in building water systems: A review. Journal of Occupational and Environmental Hygiene. 14(2), 124-134.

10. Khaledi A., Bahrami A., Nabizadeh E., Amini Y., Esmaeili D., 2018. Prevalence of Legionella species in water resources of Iran: a systematic review and meta-analysis. Iranian Journal of Medical Sciences. 43(6), 571.

11. Panahifard M., Mahvi A., Asgari A., Nazemi S., Moradnia M., 2017. A Survey on drinking water quality in qazvin in 2015. Journal of Rafsanjan University of Medical Sciences. 16(1), 3-16.

12. Danila R.N., Koranteng N., Como-Sabetti K.J., Robinson T.J., Laine E.S., 2018. Hospital water management programs for Legionella prevention, Minnesota, 2017. Infection Control & Hospital Epidemiology. 39(3), 336-338.

13. Feazel L.M., Baumgartner L.K., Peterson K.L.; Frank D.N.; Harris J.K.; Pace N.R., 2009. Opportunistic pathogens enriched in showerhead biofilms. Proceedings of the National Academy of Sciences. 106(38), 16393-16399.

14. Bentham R., 1993. Environmental factors affecting the colonization of cooling towers by Legionella spp. in South Australia. International Biodeterioration & Biodegradation. 31(1), 55-63.

15. Yong S.F.Y., Goh F.N., Ngeow Y.F., 2010. Legionella species and serogroups in Malaysian water cooling towers: identification by latex agglutination and PCR-DNA sequencing of isolates. Journal of Water and Health. 8(1), 92-100.

16. Moghadam M.A.J., Honarmand H., Meshginshahr S.A., 2016. Contamination of tap water with Pseudomonas aeruginosa, *Legionella pneumophila*, and Escherichia coli in Guilan, Iran. Journal of Medical Bacteriology. 21-28.

17. Eslami A., Momayyezi M., Esmaili D., Joshani G., 2012.Presence of *Legionella pneumophila* and environmental factors affecting its growth, in the water distribution system in Taleghani hospital, Tehran. Pajoohande. 17(1), 32-37.

18. Ghanizadeh G., Mirmohammadlou A., Esmaeili D., 2016. Survey of legionella water resources contamination in Iran and foreign countries: A Systematic Review. Iranian Journal of Medical Microbiology. 9(4), 1-15.