

Contamination of Water Sources of Karaj Hospitals with *Legionella pneumophila* and *Campylobacter jejuni*



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Abstract

Background: One of the most common routes of infection development in humans is contaminated water. *Legionella pneumophila* and *Campylobacter jejuni* are the important causes of community- and hospital-acquired pneumonia and gastroenteritis that are transmitted to humans via the inhalation of contaminated water droplets and consumption of contaminated water, respectively. Thus, continuous monitoring of the water supply systems for these pathogens has great importance in public health.

Objective: This study aimed to evaluate the water contamination of Karaj hospitals with these two bacterial species.

Materials and Methods: In this study, 62 water samples were obtained from different parts of the hospitals of Karaj from April to September 2019, including air conditioning systems, dialysis equipment, ventilation tanks, and different wards of a hospital such as infectious diseases, pediatrics, gastroenterology, dialysis, and intensive and neonatal intensive care units. The samples were collected in sterile containers and immediately transferred to the laboratory for further analysis. The culture on specific media, staining, and biochemical tests were performed to identify the *L. pneumophila* and *C. jejuni*.

Results: Out of 62 water samples, 25.8% (16 samples) were positive for *L. pneumophila*; 68.75% were observed in hot water samples, and 31.25% were attributed to cold water samples. Among 62 samples, 4.84% (3 samples) were positive for *C. jejuni*, which were all detected in hot water samples.

Conclusion: Considering that the methods of water refinery of municipal water have no high efficiency, the quality improvement of the water sources of hospitals seems to be necessary.

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Background

Legionella pneumophila is known as heterotrophic gram-negative bacilli.¹ *L. pneumophila* was first reported in Philadelphia in 1976, and afterward, the bacterium has been isolated from many sources in the environment. Water sources, especially hot water systems are the optimum condition for the growth of this bacterium.² Legionnaires' disease is mainly caused by the inhalation and ingestion of aerosol contaminated with *Legionella*. Furthermore, wound infection is another route of infection with this bacterium.³ Hospitalized patients, especially immune-deficient people are at a high risk of infection with *L. pneumophila* because they are exposed to these bacteria through bath showers, air conditioning, and drinking water systems.⁴ According to the reports

of the Centers for Disease Control, the prevalence of legionellosis in hospital settings is 25%-45% and the mortality rate of these diseases is about 30% among hospital-acquired cases.⁵

Given the high mortality rate of Legionnaires' disease in hospitals and the considerable prevalence and resistance of *Legionella* to various disinfectants, the identification of this bacterium in different wards of the hospital is crucial for preventing the spreading of infection among sensitive people.⁶ There are two widely used methods for the isolation of *L. pneumophila*, including the cultivation of samples on buffered charcoal yeast extract (BCYE) and molecular detection by the polymerase chain reaction. However, culture is still the gold standard method for the detection of *L. pneumophila*.⁷

Campylobacter jejuni is an important cause of campylobacteriosis in humans.⁸ According to global statistics, it accounts for 2%-25% of diarrhea cases compared to *Salmonella* infections (3.5%).⁹ In some cases, the infection with this bacterium can also lead to Guillain-Barre syndrome, which is a neurological disorder and muscle paralysis.¹⁰ In developed countries, *C. jejuni* is the leading cause of over 80% of food-borne diarrhea while in developing countries, most cases often remain unknown due to the lack of diagnostic methods.¹¹ Campylobacteriosis is usually detected in immunodeficient people because the bacteria are more pathogenic in these groups.¹²

Unsanitary water is one of the main sources of the transmission of infection to humans. Some outbreaks have been reported because of drinking water with insufficient chlorination.¹³ *C. jejuni* can occasionally survive from a few weeks to several months at 4°C.¹² Therefore, water systems have the potential for the transmission of *C. jejuni* to susceptible patients. Due to the importance of *L. pneumophila* and *C. jejuni* in bacterial water contamination, this study aimed to investigate the presence of these pathogens in the water samples of the selected hospitals of Karaj.

Materials and Methods

Sampling and Preparation

In this descriptive study, water samples were obtained from the hospitals of Alborz University of Medical Sciences. More precisely, samples were mainly collected from the hot and cold water resources of different parts of hospitals between April and September 2019. A total of 62 water samples were collected in 1.5 L sterile containers from 4 different hospitals in Karaj. The water samples of different wards were evaluated, including intensive care unit (ICU), infectious, burnt, surgery, operation room, and gynecology, as well as water sources such as hot bath shower, air conditioning systems, and dialysis equipment. The samples were then referred to the microbiology laboratory for microbial examinations. The membrane filtration system was used for water condensation. The samples were immediately concentrated using 0.45- μ m membrane filters, and then the filters were broken into 50 mL glass containers and were shaken for 30 minutes to release bacteria from the filter. The obtained samples were centrifuged at 12000 rpm for 15 minutes. Finally, the supernatants were discarded and sediments were suspended in 4 mL sterile buffer saline.

Cultivation of *Legionella pneumophila* and *Campylobacter jejuni*

In addition, 2 mL of samples were used for the detection of each *Legionella* and *Campylobacter*. The samples for *Legionella* were heated at 56°C for 12 minutes. *Legionella* is resistant to this temperature, thus all bacteria were eliminated at this stage except for *Legionella*.¹⁴

BCYE agar (Difco Company) supplemented with L-cysteine (40 μ g/mL) and ferric pyrophosphate (250 μ g/mL), as well as antibiotics including vancomycin (5 mg/mL) and polymyxin (2 mg/mL) were applied for culturing *L. pneumophila*. One hundred microliters of specimens were cultured on BCYE agar and incubated at 36°C in the presence of 5% CO₂ in the flow chamber. Wet cotton pads were also placed in the jar for providing humidity.¹⁵ Skirrow agar (Merck, Germany) supplemented with vancomycin, polymyxin, and trimethoprim were used for culturing *C. jejuni* specimens. Next, 100 μ L of specimens were transferred to the culture medium, and the plates were placed in a jar containing Gaspak systems (BD Diagnostics, the USA) and incubated at a temperature of 42°C for 48 hours.

BCYE plates were monitored for 3 days after inclusion, and if no colonies were observed, plate incubation continued for 14 days. Then gram-staining was conducted for microscopic examination.

Colonies on Skirrow plates were examined with direct microscopic after gram-staining for the initial detection of *Campylobacter*.

Identification of Bacterial Isolates

Biochemical tests including catalase and oxidase, nitrate reduction, and hippurate hydrolysis tests were employed to confirm *C. jejuni*.¹⁶ The suspected colonies of *Legionella* were also cultured on the blood agar. The lack of bacterial growth on blood agar was due to the presence of *Legionella*. Moreover, colonies were confirmed with biochemical tests such as oxidase and catalase tests.¹⁷

Statistical Analysis

The data of this study were analyzed using the GraphPad Prism software, version 8.0 (GraphPad, Canada). The chi-square or Fisher exact test was used for categorical variables, and $P < 0.05$ was considered statistically significant.

Results

Distribution of *Legionella pneumophila* in the Water Sources of Hospitals

Out of 62 collected samples from the cold and hot water systems in 4 selected hospitals in Karaj, 16 (25.8%) cases were contaminated with *L. pneumophila* (Figure 1), and the contamination rate varied in different hospitals. Hospitals A, B, C, and D were 20% (3 samples), 50% (5 samples), 23.8% (5 samples), and 18.8% (3 samples) positive for *Legionella*, respectively. The results showed no significant differences between the water quality of selected hospitals in Alborz province in terms of *Legionella* infection ($P < 0.05$, Table 1).

Of 3 positive cases of *Legionella*, 66.7% and 33.3% were from hot and cold water samples in hospital A, respectively. Moreover, 60% and 40% of hot and cold water samples were positive in hospital B. Out of 5 *Legionella* positive

cases, 60% and 40% were from hot and cold water samples in hospital C, respectively. Finally, all 3 cases of *Legionella* (100%) were isolated from hot water samples in hospital D. Of the 16 *Legionella* positive cases, 68.75% and 31.25% were in the hot and cold water samples. The distribution of *Legionella* in different sources of evaluated hospitals is shown in Table 2. Figure 2 displays the distribution of *Legionella pneumophila* in different parts of hospitals.

Among 16 *Legionella* positive samples, 25% (4 samples), .75% (7 samples), 12.5% (2 samples), and 18.75% (3 samples) belonged to the cooler, the ICU ward, and gynecology ward, respectively, and 18.75% (3 samples) were observed in the operation room, dialysis ward, and chiller instrument.

Distribution of Campylobacter jejuni in the Water Sources of Hospitals

Out of all studied samples, 3 cases were positive for *C. jejuni* (Figure 1) and were all collected from one hospital. Only 3 (18.75%) positive samples for *Campylobacter* were detected in hospital A. All 3 samples were collected from the hot water systems, 33.3% (1 sample) and 66.7% (2 samples) were observed in dialysis and ICU wards, respectively.

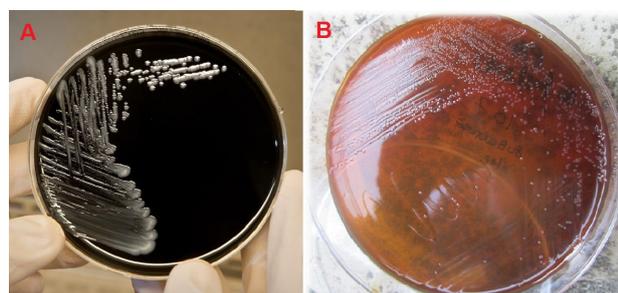


Figure 1. A Sub-culture of a Grown *Legionella pneumophila* Isolate in the BCYE Medium (A) and the Sub-culture of an Isolate of *Campylobacter jejuni* in Skirrow Agar (B). Note. BCYE: Buffered charcoal yeast extract.

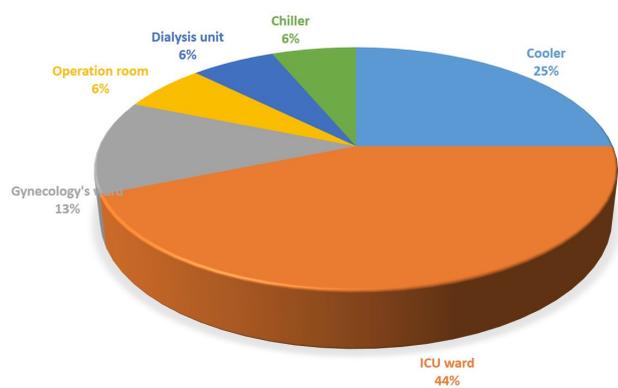


Figure 2. Total Distribution of *Legionella pneumophila* in Different Parts of Hospitals. Note. ICU: Intensive care unit.

Table 1. Information on *Legionella* Samples in Different Hospitals in Alborz Province

Hospital	Samples	Legionella Positive Samples	%	% Total
A	15	3	20	4.8
B	10	5	50	8.1
C	21	5	23.8	8.1
D	16	3	18.8	4.8
Total	62	16	25.8	25.8

Note. P value: 0.32; F-value: 3.43.

Table 2. Positive *Legionella* Samples in Different Hospital Wards

Source	Legionella Positive Samples (n=16)	Ward/Unit
Hospital A	n=3	Cooler water
		Internal ICU hot shower
		Internal ICU cold shower
Hospital B	n=5	Cooler warm water
		NICU warm water
		NICU cold water
		Women hot shower
Hospital C	n=5	Women cold shower
		Cooler water
		Neurosurgery ICU hot shower
		Trauma ICU cold shower
		Surgery room warm water
Hospital D	n=3	Chiller cold water
		Dialysis water
		ICU warm water
		Cooler storage water

Note. ICU: Intensive care unit.

Discussion

Legionnaires' disease is recognized as a fatal cause of bacterial pneumonia and its mortality rate depends on the severity of the disease, rate of diagnosis, the velocity of treatment, and the patient's immune system. The most common cause of death is respiratory failure.¹⁸

The results of the present study showed that 25.8% (16 samples) of the collected water samples from Karaj hospitals were contaminated with *L. pneumophila*, and only 3 water samples were positive for *Campylobacter* contamination. Although all hospitals in Karaj use the refinery tap water, the water samples of these hospitals are still contaminated with these bacteria. The current methods of water refinement seem to be insufficient for eradicating the bacterial contamination of water supply systems. Mirmohammadlo et al reported that 56 water samples (37.33%) of three military hospitals in Tehran were contaminated with *L. pneumophila*.¹⁹

Moradzadeh also reported *Legionella* in 15% of water samples in Ahwaz hospitals among a total of 100 samples.²⁰ Similarly, Yaslianifard et al examined the quality of water in terms of *Legionella* contamination

in Tehran hospitals and found that ICU water samples had the most *Legionella* contamination, and hot showers were a potential source of *Legionella* infections.¹⁷ *Legionella* can lead to serious respiratory complications in immunocompromised patients who were admitted to the ICU. Borella et al surveyed 119 hot water samples from Italian hotels and concluded that *Legionella* was observed in 85% of the samples. Furthermore, they reported that the survival of *Legionella* highly relies on water temperature. They further demonstrated that *Legionella* was the most prevalent bacterium in hot water sources, which could be the reason for the higher frequency of these bacteria in hot water sources in this study (21). In another study by Eslami et al, 34% of samples were *Legionella* positive in the water supply systems of Taleghani hospital in Tehran, implying that 72% and 28% of samples were related to hospital hot and cold water systems, respectively.²² Darelid et al indicated that adjusting the hospital's hot water tank temperature over 55 °C significantly reduced the occurrence of pneumonia and it was recognized as an effective control method.²³ Given that the temperature is one of the major influencing factors on *Legionella* growth, cold water samples were also considered as a source of bacterial growth in Fragou et al study.²⁴ The results suggest that bacteria can exist in both hot and cold water systems. Although most studies have reported more contamination of hot-water systems, the results of this study indicated that *Legionella* bacteria are found and isolated from both hot and cold water systems. According to the obtained results from the present study, *Legionella* was found in air conditioning and cooling systems of all 4 hospitals. The contamination of air conditioning systems directly affects the people in the hospital, especially immunocompromised patients. In France, Campese et al studied the importance of cooling tower contamination from 1998 to 2008. They identified 11 147 cases of legionellosis over 10 years and reported the cooling towers as the most likely source of infection.⁵ A systematic review performed by Wewalka et al confirmed the association of 104 legionellosis deaths with cooling tower contamination.²⁵

Campylobacteriosis was mostly reported as a zoonotic disease between 2007 and 2013, followed by *Salmonellosis* and *Yersiniosis*.²⁶ *Campylobacter* is estimated to cause more than 2 million diseases (or 1% of the population), 13 000 hospitalizations, and about 100 deaths annually in the USA.²⁶ Due to the sensitivity of *Campylobacter* to environmental harshnesses such as the effect of oxygen and the absence of growth requirements, as well as the difficulties of specimen transfer, culture, isolation, and identification, this bacterium is ignored by clinical laboratories. Diergaardt et al identified *C. jejuni* in the South African water resources and reported the prevalence of this bacterium in 13.6% of water samples.²⁷ Likewise, Karikari et al²⁸ investigated the presence of *Campylobacter* in water sources and found that 42 (22.3%) out of 188

water samples were contaminated with *Campylobacter*. More precisely, 35.7%, 21.4%, 5.9%, and 1.7% of isolates were obtained from rivers, wells, basins, and boreholes, respectively. Moreover, 64% of isolates were *C. jejuni*.

Many scientists reported poor water quality as one cause of disasters at dialysis centers. In this condition, the dialysis inoculates contamination in the blood directly during hemodialysis.²⁹ A study in Central America demonstrated that 35% of the water samples and 19% of the dialysis fluid samples had no passable standards such as obsolescence and low quality of the pipes, or system fittings leading to water contamination. Based on the obtained results from the present study, 4.84% of water samples were contaminated with *C. jejuni*.³⁰ Some studies showed that this bacterium is resistant to disinfectants, leading to the survival of the bacterium in inappropriate environmental conditions.³¹ It appears that the disinfection methods of the water distribution system of Karaj are not efficient for eliminating this microorganism. Water sanitation routes such as heat exchangers, monochloramination, copper-silver ionization, and hyper chlorination can help in improving the refinement of water sources and eliminating these microorganisms. Furthermore, adjusting the pH of the water at the range of 5.5-8 may be helpful in this regard.

Conclusion

Regarding the water contamination of municipal water sources of Karaj with *Legionella* and *Campylobacter*, it is suggested that the water systems of hospitals be regularly monitored and more efficient methods (e.g., hyperpolarization in the range of 3-5 mg/L, ozonization, or copper-silver ionization method) be used by hospitals to remove more resistant bacteria from the water distribution systems.

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Authors' Contributions

NG: Sample collection and project administration; SY: Project director, conceptualization, and original drafting; MM: Original drafting, reviewing, and editing; MD: Review and editing; MN: Project director, original drafting, reviewing, and editing.

Conflict of Interest Disclosures

The authors report no conflict of interests.

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