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Serotyping of *Legionella* Bacteria Isolated from Various Water Systems in the Central Anatolia Region

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ABSTRACT

Objective: *Legionella* bacteria are waterborne environmental pathogens that are considered a public health problem because they cause Legionnaires' disease, which is a nationally notifiable disease.

Materials and Methods: *Legionella* analysis was performed in a total of 651 water samples collected during the years 2015 (450) – 2016 (201). Water samples were collected from hospitals (64.66%), hotels (15.05%), the automotive industry (14.43%) and from the buildings (5.83%) in the Central Anatolia Region. After the isolation of *Legionella* by the filtration and culturing method, serogroup and subtypes were determined via latex agglutination tests and the direct fluorescent antibody method.

Results: In 2015, the *Legionella* positivity rate was 8.6%, where 28.2% of detections were from *L. pneumophila* serogroup-1. Six isolates were found to be Philadelphia, four were Olda, and one was Bellingham subgroup. Overall, 64.1% were *L. pneumophila* serogroup 2-14. Moreover, 14 isolates were SG-5, 10 were SG-6, and one was SG-10. 7.7% were unidentified *Legionella* species. The *Legionella* species identified were *L. micdadei* and *L. longbeachae*. In 2016, the *Legionella* positivity rate was 10.4%, with 28.6% of them being from *L. pneumophila* serogroup-1. Four were found to be Olda and two were Philadelphia subgroups. Overall, 66.6% of them were *L. pneumophila* serogroup 2-14. Moreover, six of them were SG-5, four were SG-6, and four were SG-2. 4.7% were unidentified *Legionella* species. There was only one species detected as *L. micdadei*.

Conclusion: It has been observed that the distribution of *Legionella* has exhibited diversity in different water systems throughout the Central Anatolia Region.

Keywords: *Legionella*, water systems, DFA method, virulence, serotyping

INTRODUCTION

Legionella causes either influenza-like Pontiac fever or Legionnaires' disease, which is more dangerous due to the potential for pneumonia developing in the lungs. In 1976, during the Legion Congress, epidemic diseases and deaths were revealed in a hotel in the city of Philadelphia, USA. It was thought the outbreak was caused by the water used in the hotel. In 1977, a bacterium isolated from the hotel's water was revealed to be pathogenic and called *Legionella* by McDade et al. (1). Additionally, the disease was called *Legionnaires' disease* by Fraser et al. (1). *Legionella* is recognized as a waterborne environmental pathogen (2, 3). *Legionella* lives and proliferates by forming colonies in the biofilms of water systems (2-4). Thus, they represent a great danger to humans (5, 6). Additionally, they can resist environmental conditions by forming an exopolysaccharide matrix or by entering a "viable but non-culturable" state. These are important strategies that enable *L. pneumophila* to adapt to different environmental characteristics such as water temperature, flora, nutrients, chemicals, and chlorination, while also gaining resistance to disinfectants (7). For this reason,

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the European Legionnaires' Disease Surveillance Net and the World Health Organization emphasized that it is necessary for potentially dangerous water systems to be checked for *Legionella* at regular intervals.

The number of reported *Legionnaires' disease* cases has increased in the United States and in Europe (1). The industrialization of cities and the use of pools, spas, jacuzzis, air conditioning, shower systems, sports centers, and elderly care centers have caused the increased pollution of water resources, which has increased *Legionnaires' disease* prevalence (4). It has been reported that *Legionella* especially affects elderly patients (8). Also, it more effectively invades suppressed immune systems and chronic disease patients. Additionally, health workers, smokers, agricultural workers, car washers, frequent travelers, and those staying in hotels are at risk (9, 10).

Legionella has 62 different species (11) and more than 80 serogroups (12), at least 21 of which cause infections in humans (3, 4). *L. pneumophila* is the most well-characterized strain, which causes 70-90% of all cases of legionellosis (13). The SG-1 serogroup of *L. pneumophila* is the most prevalent disease-causing variant (2), while serogroups 1, 4 and 6 are the causative agents of 85% of human infections (14). Other *Legionella* types that cause disease are *L. micdadei, L. bozemanii, L. dumoffii, L. gormanii,* and *L. longbeachae* (9). Additionally, *L. pneumophila* SG-1, SG-6, SG-7, and the species L. *micdadei, L. feeleii,* and *L. anisa* cause Pontiac fever (15).

In 2014, the largest *Legionella* outbreak occurred in Portugal. During this outbreak, 14 out of 400 cases resulted in death. It was reported that the disease was transmitted from person to person and involved the *L. pneumophila* SG-1 1905 strain (16). Moreover, in Japan, there were four major outbreaks caused by public baths (17). Notably, hot springs and baths are more common sources of *L. pneumophila* than cooling towers, according to the Japan National Center for Epidemiological Surveillance and Infectious Diseases (18). In Poland, although it has been a nationally notifiable disease since 2002, low-cost techniques are preferred for the diagnosis of *Legionella*. Thus, cases of disease are only usually noticed in an advanced stage, which has resulted in a paucity of relevant data (8). In Turkey, *Legionnaires' disease* was accepted as a nationally notifiable disease with the circular published by the Ministry of Health in 1996, and Turkey was included in the European *Legionella* Infections Working Group in 2001 (19). Detection of Legionella species in domestic, hotel and hospital hot water systems is very important (20).

Legionella bacteria are pleomorphic structures and thus have different morphological and physiological characteristics in the same species, and this results in varied virulence characteristics (21). Additionally, they have advanced mechanisms to reproduce and survive in different hosts and environmental conditions (22). For this reason, it is important to understand the virulence characteristics so that the disease can be detected more quickly and easily to apply necessary treatments.

The aim of this study is the determination of the species, serogroups, and subtypes of *Legionella* bacteria found in the water systems of hospitals, hotels, auto industry buildings, and buildings in different cities in the Central Anatolian Region. For this purpose, the serological typing of *Legionella* found in water systems was performed to detect *Legionella* contamination.

MATERIALS AND METHODS

Sample Collection

Legionella analysis in water systems has been routinely conducted in Turkey by the National Respiratory Pathogens *Legionella* Reference Laboratories of the General Directorate of Public Health in Ankara. In the present study, water samples were taken in 2015 and 2016 from hospitals, hotels, automotive industry buildings, and other buildings. The samples were then analyzed for *Legionella*. Water samples were collected from cooling towers, water tanks, and faucets/showerheads by qualified personnel in sterile containers. All samples were labeled, stored in a cool box at a temperature of up to 5 (±3)°C, and delivered directly to the laboratory within 24 h. Detailed information about water sample collection is available in the *Legionnaires' Disease* Laboratory Diagnosis Guide (23).

Filtration and Culture

Since water samples were taken from different water systems (cooling towers, water tanks, and faucets/showerheads), the filtration methods performed were also different. The filtration processes for water were carried out in accordance with standardized methods by the General Directorate of Public Health (previously known as the Refik Saydam Hygiene Center Presidency) National Respiratory Pathogens *Legionella* Reference Laboratories, 1999 (19-23). According to this method, the filtration of the 50 ml water samples was performed in three different ways. Each sample was inoculated on two different media. One medium is inhibitor-free Buffered Charcoal Yeast Extract (BCYE), while the other is BCYE-based agar with dyes, vancomycin, polymyxin B, and glycine (a chemical used to prevent the growth of environmental flora in water systems). We prepared all media for cultivating *Legionella* in our laboratory as described in the *Legionnaires' Disease* Control Program guide. After cultivation, plates were incubated at 37°C in a humidity incubator. For determination, suspected colonies were randomly chosen for subculture on both BCYE and 5% sheep blood medium (non-cysteine) at the same time. If colonies only grew on BCYE but did not grow on the blood medium, they were considered to be *Legionella*.

Isolation of *Legionella*

Firstly, gray-green and bright colonies that are similar to *Legionella* were examined under a colony microscope (Olympus SZ-40) for morphological assessment. After confirming the characteristic *Legionella* bacteria under the colony microscope, a second identification was made according to the protocol of the commercial agglutination kit (Oxoid, Latex; Oxoid Limited, UK). The latex agglutination test was performed for the identification of predominant *Legionella* species grown on plate

media with suspected *Legionella* bacteria. The Oxoid *Legionella* Latex Test allows the separate identification of *Legionella pneumophila* serogroup 1 and serogroups 2–14, as well as the detection of seven other *Legionella* species (*L. longbeachae*-1 and 2, *L. bozemanii*-1 and 2, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. micdadei*, and *L. anisa*). The kit contains blue latex particles that are sensitive to *Legionella* species and serotypes. It allows specific *Legionella* cell walls to bind together and form visible clusters. This ensures that the test is completed rapidly and easily for pathogenic *Legionella* species and serotypes identification. The result is positive if the agglutination of blue latex particles occurs within one minute and with no agglutination in the control circle. A positive reaction indicates the presence of antigens against the suspected *Legionella* in the sample and which serogroup or species it belongs to.

Direct Fluorescence Antibody Testing

Following identification, direct fluorescence antibody (DFA) testing was applied to identify serogroups 2–14, subtypes, and *Legionella* spp. using an m-TECH assay kit (m-TECH, Monoclonal Technologies Inc. Milton, GA). The DFA test is particularly useful for a rapid microscopic diagnosis to detect the presence of specific antigens of bacteria based on fluorescent-labeled antibodies binding to the target antigen of *Legionella*. According to the manufacturer's protocol, *Legionella* bacteria are tagged with a monoclonal antibody treated with a fluorescein dye to form labeled antibody reagents against *Legionella* antigens. After the antibody binds to the antigen on the bacterial cell wall, it glows green under a fluorescence microscope. Firstly, *Legionella* sp. specimens were plated onto BCYE agar. The plates were then incubated at 37°C in 2.5% CO_2 and in a humidified environment for 24–48 h. A clear suspension (McFarland No.1) in phosphate buffer from pure *Legionella* cultures were made to assist in the attachment of the cells to the diagnostic microscope slides, which have a black epoxy coating that includes a few circles. The bacterial isolates to be tested were fixed to a microscope glass slide, air dried, gently fixed with heat, and overlaid with the Fluorescein isothiocyanate (FITC)-labeled antibody reagents directed against *Legionella* antigens. The diagnostic microscope slides were incubated for 20–30 min at 37°C in 2.5% CO_2 and in a humidified environment. They were then gently and individually rinsed with phosphate buffered saline (PBS) to remove the conjugates, resulting in the unbound antibodies being washed away. The slides were then immersed in individual jars containing PBS for five min. All slides were then rinsed with distilled water and air-dried. A mounting medium was then dropped onto the slides and coverslips were applied. The slides were then examined using a fluorescence microscope without delay (24). Both a polyvalent positive control antigen and a negative control conjugate were run with each test.

Fluorescence Microscopy

The microscope slides were examined under a fluorescence microscope (Leica DM1000). The FITC-labeled antibody binds specifically to any *Legionella* antigen in the sample isolate. If no *Legionella* antigen is present, the antibody reagent does not bind and is removed during the washing steps. The FITC-labeled antibody-antigen complex is detected by glowing a bright green color, and *Legionella* cells appear to glow as green bacilli under a fluorescence microscope.

RESULTS

Microscopy and Isolation of *Legionella*

Overall, 651 water samples were examined for *Legionella* between 2015 and 2016. In 2015, 278 hospitals, 98 hotels, 53 automotive industry buildings and 21 other buildings were analyzed. In 2016, 143 hospitals, 41 auto industry buildings and 17 other buildings were analyzed (Figure 1). After 3–5 days from filtration to cultivation, the plates were checked and the outer morphologies (edges) of colonies were examined under a colony microscope. Colonies suspected of being *Legionella* have a smooth surface and are slightly convex with a gray-white center as well as green, blue, purple, and pink edges with a cut glass appearance.

Identification of *Legionella*

Identification tests were performed for the typing of *Legionella* (Figure 2). According to the typing with the latex agglutination test for 450 samples in 2015, 39 (8.6%) samples tested positive for *Legionella*. The serogroups found were as follows: *L. pneumophila* SG-1, 11 (28.2%); *L. pneumophila* SG-2-14, 25 (64.1%); *Legionella* spp. 3 (7.7%) (Figures 2a, 3a). According to the typing of 201 samples in 2016, 21 (10.4%) samples tested

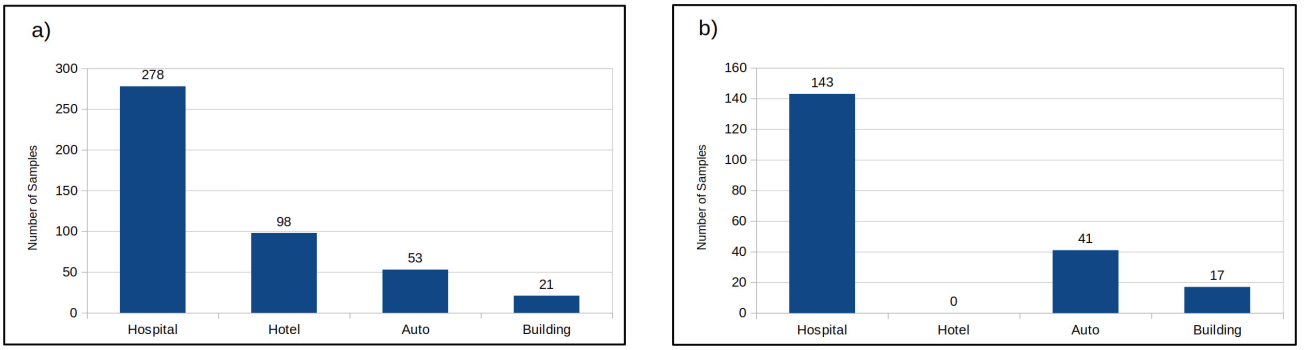


Figure 1. Distribution of water sample sources for the years 2015 (a) and 2016 (b).

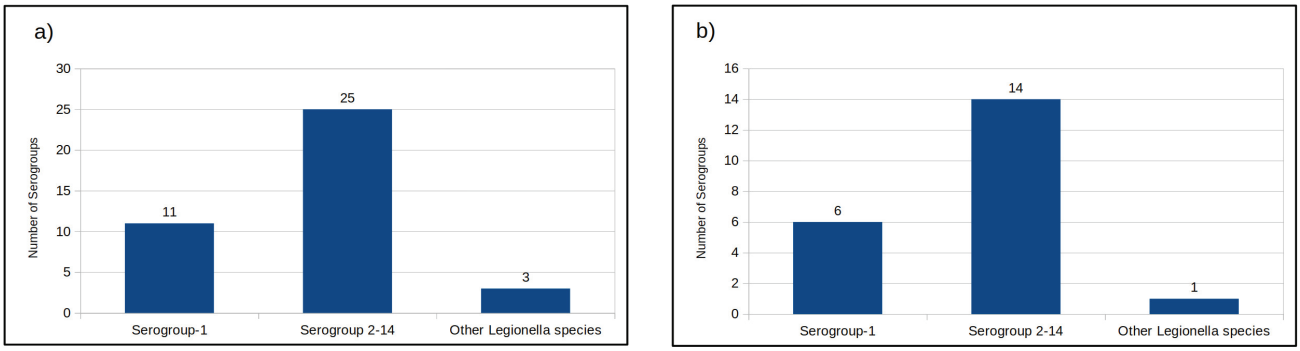


Figure 2. Distribution of serogroups for the years 2015 (a) and 2016 (b).

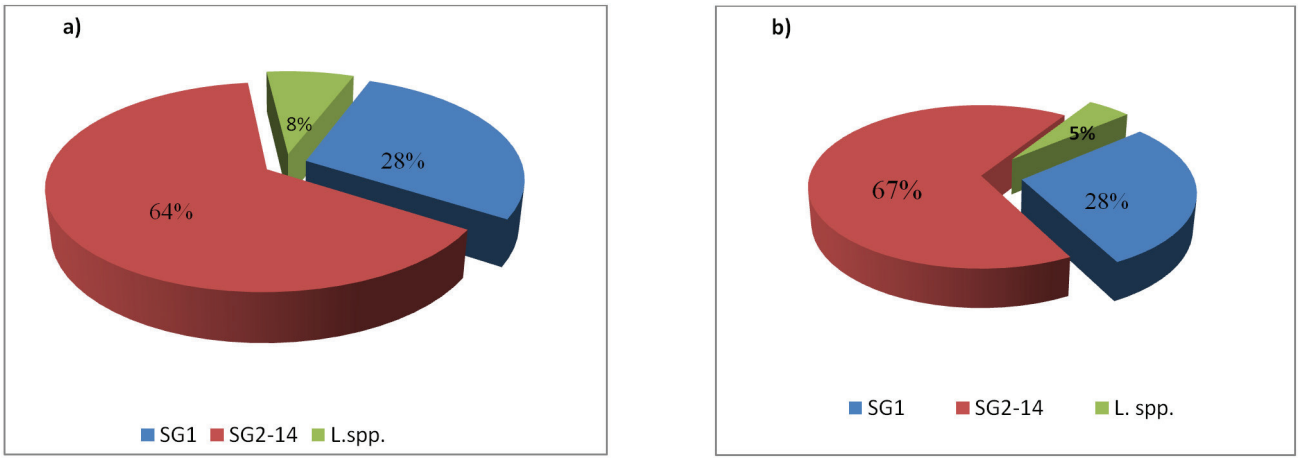
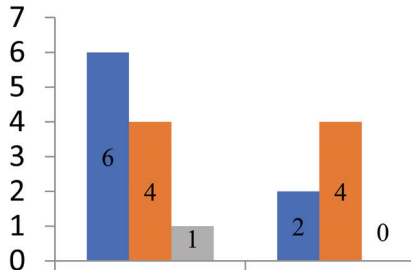


Figure 3. Percent distribution of serogroups for the years 2015 (a) and 2016 (b).

positive for *Legionella*. The serogroups found were as follows: *L. pneumophila* SG-1, six (28.6%); *L. pneumophila* SG-2-14, 14 (66.6%); *Legionella* spp. 1 (4.7%) (Figures 2b, 3b).

Serotyping with DFA and Fluorescence Microscopy

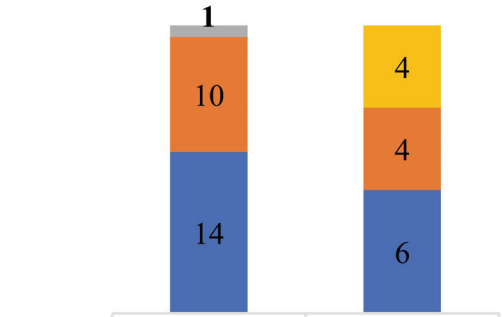
According to the subtyping of samples collected in 2015, the following results were found. Overall, 11 cases of *L. pneumophila* serogroup-1 were found: six as Philadelphia (hotel washstand, automotive industry building shower, hospital room water); four as Olda (hospital collector, heated shower in an automotive industry building); one as Bellingham (intensive care washstand of a hospital) (Figure 4). Overall, 25 cases of *L. pneumophila* serogroup 2-14 were found: 14 were SG-5 (in the rooms, toilets, and showerheads of a hospital, water boiler); 10 were SG-6 (hospital rooms, water boiler); one was SG-10 (hospital room) (Figure 5). Two other *Legionella* species were found (*L. micdadei* and *L. longbeachae*) in automotive industry cooling towers (Figure 6). According to the subtyping of samples collected in 2016, six of them were *L. pneumophila* SG-1—two were Philadelphia and four were Olda subgroups—and they were in the showerheads of automotive industry buildings (Figure 4). Overall, 14 samples with *L. pneumophila* serogroup 2-14 were found (6 were SG-5, in the hot water tank of a hospital, purification tower, and pool



	2015	2016
Philadelphia	6	2
Olda	4	4
Bellingham	1	0

Figure 4. SG-1 Serogroups.

in a chemical industry building). Four of them were SG-6 (from the bathroom tap of a hospital, showerhead of a hospital room, and a hot water boiler). Four of them were SG-2 (from a tap, tap head, and showerhead from a hospital room) (Figure 5). Only one other *Legionella* species was found (*L. micdadei*) in an automotive industry cooling tower (Figure 6).



	2015	2016
sg2	0	4
sg10	1	0
sg6	10	4
sg5	14	6

Figure 5. SG-2-14 Distribution.

	2015	2016
L. micdadei	1	1
L. longbeachae	2	0

Figure 6. *Legionella* Species Distribution.

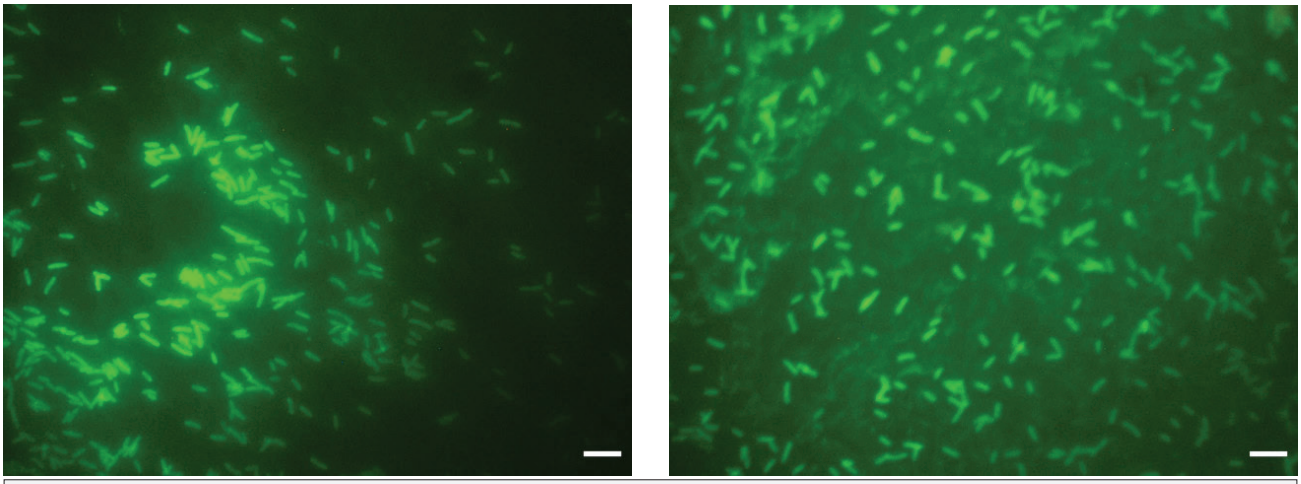


Figure 7. *Legionella* bacteria glowing green under a fluorescence microscope. Scale bar, 20 µm.

According to the DFA method, *Legionella* bacteria glow green in color under a fluorescence microscope (Figure 7).

Locations and numbers of all serogroups and subtypes are shown in Table 1.

DISCUSSION

Legionella bacteria are waterborne pathogens that cause community-acquired Legionnaires' disease, therefore many studies have been conducted to investigate the level of *Legionella* colonization of water systems both in the world and in our country.

Leoni et al. studied 137 hot water samples from apartments, hotels, and hospitals. In apartments, there were 13 samples of *L. pneumophila* (four of them were SG-3 and SG-9, three of them were SG-6, two of them were SG-8), while four of them were other *Legionella* species (one *L. micdadei*, one *L. bozemanii*, and two unidentified *Legionella*). In hotels, there were four positive cases of *Legionella* (three cases of SG-1 and SG-6, one case with both SG-3 and SG-6). In hospitals, seven positive *Legionella* cases were found (five cases of SG-3 and two cases of other *Legionella* species (*L. anisa* and *L. bozemanii* were found at low rates) (20). The *Legionella* serogroups identified in the current study are compatible with the results of Leoni et al.

Afacan et al. assessed water samples collected from touristic hotels in İzmir, Aydın, and Muğla provinces as well as Bodrum. *Legionella* analysis was performed on samples taken from the water systems of hotels (e.g., faucets, cooling towers, Jacuzzis). *Legionella* presence was determined by latex agglutination, while serogroups SG-2–14 were determined by DFA. It was noted that all serogroups (SG-3,-6,-8,-10,-11,-13) were detected in

Table 1. Locations and numbers of all serogroups and subtypes.

2015			2016			
Serogroups Subgroups	**Number**	**Locations**	**Serogroups Subgroups**	**Number**	**Locations**	***L. pneumophila* Serogroups**
Philadelphia	**4**	Hotel washstand	*Philadelphia*	**2**	Automotive industry showerhead	*L. pneumophila* SG-1
	1	Automotive industry shower				
	1	Hospital room tap				
Olda	**2**	Hospital hot water collector	*Olda*	**4**	Automotive industry showerhead	
	1	Automotive industry hot shower				
	1	Residential hot water collector				
Bellingham	**1**	Hospital intensive care washstand				
SG-5	**6**	Hospital hot water tank	**SG- 2**	**2**	Hospital room tap	***L. pneumophila* SG 2-14**
	5	Hospital room showerhead				
	2	Hospital room tap		**1**	Hospital room tap head	
	1	Hospital toilet		**1**	Hospital room showerhead	
SG-6	**4**	Hospital hot water tank	**SG-5**	**2**	Chemical industry purification pool	
	4	Hospital room tap		**2**	Chemical industry purification tower-1	
	1	Hospital room showerhead		**1**	Chemical industry purification tower -2	
	1	Hospital toilet		**1**	Hospital hot water tank	
SG-10	**1**	Hospital room tap	**SG-6**	**2**	Hospital room showerhead	
				1	Hospital hot water tank	
				1	Hospital bathroom tap	
L. micdadei	**1**	Automotive industry cooling tower	***L. micdadei***	**1**	Automotive industry cooling tower	***Legionella* spp.**
L. longbeachae	**2**	Automotive industry cooling tower				

İzmir, while SG-11 was detected in Aydın, SG-6 and SG-8 were detected in Muğla, and only SG-6 was detected in Bodrum. It was emphasized that although SG-1 is the most important cause of *Legionnaires' disease*, other serogroups are also common in tourism regions of Turkey (25).

Tesauro et al. surveyed 271 samples from the hot water systems of two hospitals between 2004 and 2009. *L. pneumophila* prevalence was 37%, SG-2–14 prevalence was 68.3%, and SG-1 prevalence was 18.8%. Moreover, 12.9% of the water samples were positive for both SG-1 and SG-2–14 serogroups. After disinfection with chlorine dioxide five times, *L. pneumophila* concentration reached acceptable limits. This suggests that chlorine dioxide application is effective at keeping *L. pneumophila* concentration within acceptable limits (26). In the current study, due to the high prevalence of SG2-14 in water systems, SG 2-14 incidence was found to be 64% in 2015 and 67% in 2016; these findings are similar to the results of Tesauro et al.

Akkaya et al. analyzed samples taken from various water systems in hospitals, schools, hotels, and residences in Kayseri, Turkey. Serogroups were determined by latex agglutination tests. *Legionella* was detected in eight (6.7%) of 120 water samples, with six of them being *L. pneumophila* SG-1 and two being other *Legionella* species. It was determined that the samples containing other *Legionella* species were taken from the showerheads of hotels, while those containing *L. pneumophila* SG-1 were from a warehouse and the shower systems of hospitals (27). The method used in this study was similar to our study but the distribution of *Legionella* species was different.

In a study conducted by Burak et al., a total of 122 hot water and swab samples were taken from the showerheads of 61 houses that were investigated for the presence of *L. pneumophila* and free-living amoebas in 2009, in Istanbul. According to the results of this study, *L. pneumophila* was isolated from 13 houses (21.3%) and free-living amoebas were isolated from 19 (31%). *L. pneumophila* was isolated from 12 (19.6%) of the water samples and four (6.5%) of the swab samples. Among the isolated *L. pneumophila*, it was reported that 87.5% were *L. pneumophila* serogroup 2-14, while 12.5% were *L. pneumophila* serogroup 1. Although there is no correlation between the presence of *L. pneumophila* and free-living amoebas, it was stated that there was a significant correlation between the presence of *L. pneumophila* and the presence of a central heating system (28). Due to the high SG 2-14 ratio, our results can be considered to be compatible with Burak et al.

İğnak et al. detected *Legionella* colonization in 7% of 100 water samples taken from showerheads, taps, and tank water in different locations within Istanbul University Medical Faculty Hospital. A total of seven *Legionella* strains were isolated. Notably, three SG-1 and three *Legionella* spp. were found in the showerheads and faucets, while only one other *Legionella* species was found in tank water. *Legionella* SG-1 and other *Legionella* species were especially found in pediatric departments. Additionally, *Legionella* spp. were found in the tap water of anesthesiology and reanimation units. *Legionella* growth was not observed in the water systems of clinical units more distant from the water tank where growth was observed (29).

Ulleryd et al. investigated 61 environmental samples from 15 cooling towers and 138 clinical samples. Overall, 84 patients linked to the study were hospitalized in an epidemic that lasted for weeks in the city of Lidköping, Sweden. Overall, two of 32 patients died. Moreover, in an isolated sample, *L. pneumophila* SG-1 Benidorm and Bellingham were found. In three cooling towers, *L. pneumophila* SG-1 Benidorm, Bellingham, Portland, and Olda subgroups were found. Notably, cooling towers effectively spread *Legionella* via aerosols during outbreaks (30). The identification of Bellingham and Olda subgroups in the current study is similar to the findings of these researchers.

In a study by Erdoğan et al., *Legionella* was isolated from 11 out of 13 water samples in a newly opened hotel during a small *Legionella* outbreak in Alanya in 2009. The hotel's water systems and clinical samples were studied together. Water samples taken from 10 different parts of the hotel were examined. Moreover, six patients and 26 suspected cases staying in the same hotel were also examined. *L. pneumophila* SG-1 was found in 11 out of 13 water samples. All six patients were positive for *L. pneumophila* SG-1 in their urine. Moreover, SG-1 antibody positivity was observed in the serum of only one patient. No SG-1 positivity was observed in the urine of patients who applied with complaints of 26 other diseases. Although data could not be adequately collected and detailed tests could not be performed, it was thought that this outbreak was caused by the water systems in hotels, with water systems in newly opened hotels being at higher risk for *Legionnaires' disease* (31).

Sepin Özen et al. studied a total of 1403 water samples from 56 different hotels during January-December 2010 in Antalya, Turkey. *L. pneumophila* was isolated from 37.5% of the hotels and 10.1% of water samples. It was reported that 85% of the samples were positive for *L. pneumophila* serogroup 2–14, while 15% of the samples were positive for *L. pneumophila* serogroup 1 (32).

Quero et al. described and compared 528 isolates collected between 1989 and 2016. Typing studies were carried out using monoclonal antibodies (MAb) and sequence-based typing (SBT) methods. A total of 266 samples (109 clinical samples and 157 environmental samples) were compared to each other. Clinical samples were divided into seven Dresden subgroups. It was indicated that Philadelphia (26.61%), Knoxville (19.27%), Olda (14.68%) and Benidorm (14.68%) were the most frequent subgroups. In typing environmental samples using the Dresden Panel, Olda (33.1%) and non-SG-1 *L. pneumophila* (17.2%) were frequently detected. Although there is a high incidence of *Legionnaires' disease* in Spain, this study was comprised of the Catalan and Valenciana regions. An *L. pneumophila* population was found in clinical and environmental samples in the Valenciana region, while *L. pneumophila* was found only in clinical samples from Catalan. It has been reported that this study is an important term of comparison for *L. pneumophila* typed with MAb and SBT for both clinical and environmental samples in Catalan (33). In our results, Olda subgroup in 2015 was 36%, non-SG-1 *L. pneumophila* was 8%; while in 2016 Olda was 67% and non-SG-1 *L. pneumophila* was 1%.

Zeybek et al. investigated *Legionella* and free-living amoebae in swimming pool samples from Istanbul using different methods. In this study, free-living amoebae were identified/found in four of the water samples and two of the biofilm samples via the culture method. Free-living amoebae were found in three water and three biofilm samples that could not be detected via the culture method. *Legionella* was only found in one biofilm sample via the culture method. By using the fluorescence in situ hybridization (FISH) method, *Legionella* was detected in six water and seven biofilm samples. According to the results of this study, it was stated that the FISH method could be a more effective method for *Legionella* detection. However, it has been expressed that the culture method should be used for the correct isolation of *Legionella* bacteria (34).

Papadakis et al. inspected and tested 3311 water samples from the hot and cold water systems of 132 hotels between 2000 and 2019. The serogroups of *L. pneumophila* were determined via latex polyclonal antisera as SG-1 (27.92%) and SG-3 (17.08%). Moreover, it was found that 25.96% occurred in hot water distribution systems, 16.98% in cold waters, and 13.51% in swab samples. It has been reported that more than 80% of *Legionnaires' disease* cases are caused by *L. pneumophila* serotype 1 (35). The incidence of SG-1 in the current study, 28% in 2015 and 2016, is consistent with the findings of Papadakis et al.

In another study by Yılmaz et al. in Turkey, the presence of *L. pneumophila* was determined in water samples taken from hospitals, hotels, Turkish baths, and shopping centers in Erzurum and nearby provinces. *L. pneumophila* was found in 65 of the 2025 water samples. *L. pneumophila* serogroup 2–14 was detected in 46 (70.8%) of 65 positive samples, while *L. pneumophila* serogroup 1 was detected in 18 samples (27.7%). Additionally, *L. pneumophila* serogroup 2–14 and *L. pneumophila* serogroup 1 were detected (1.5%) in one water sample. It was indicated that the highest positivity rates were in hot water taps (11.6%), hot water tanks (6.1%), and showerheads (4.8%) (36).

Since clinicians need to recognize hospital-acquired *Legionnaires' disease*, the identification of this disease is important. To achieve this, both the phenotypic and genotypic characteristics of the bacterium should be known in greater detail and should be detected more quickly. To understand the pathogenic effect of *Legionella* in humans, mechanisms such as attachment, entry to cells, and avoidance of the host's immune system should be very well known. For these reasons, it is important to define the serogroups, subgroups, and strains of *Legionella* (8). Due to most nosocomial infections originating from water systems, hospitals are risky environments. Thus, *L. pneumophila* infections must be given greater attention in health institutions and organizations (20). All public buildings (including hotels, businesses, schools, apartments, and government buildings) and cooling towers should have water management plans. These plans should establish a program team, identify control measures and where they should be applied to stay within limits, monitor certain parameters to determine whether control measures are working, verify and validate the program, and document everything. It is also important that water entering domestic and public buildings should have a minimum disinfectant residual. In Turkey, the Ministry of Health issues guidelines about legionnaires' disease control programs.

In our study, there are limitations and drawbacks. First of all, the clinical conditions of the people and the patients exposed to *Legionella* positive environments have not been evaluated. Secondly, in other studies, serotyping and subtyping were performed using molecular methods for sequence analysis. Thirdly, the *Legionella* positivity of water samples after disinfection has not been analyzed to demonstrate the success of the disinfection process.

On the other hand, the identification of *Legionella* in our samples indicates that water management plans should be implemented in these locations.

CONCLUSION

Various studies have been carried out in Turkey and abroad for the serological typing of *Legionella* bacteria. It was observed that there was much variation in the distribution of serogroups and subgroups. Since *Legionella* is a waterborne, travel-related, and community-acquired infection, it will be possible to observe different serogroups between countries. Identifying serogroups and subgroups is important for the diagnosis of infections. For this reason, it is important to conduct comprehensive and detailed studies. This study is important because it shows the distribution of serogroups and subtypes of *Legionella* bacteria for at least one region of Turkey. Thus, it should help inform further studies on this subject.

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