LEGIONELLA WATER SURVEILLANCE IN SOUTHEASTERN TURKIYE: A FIVE-YEAR EPIDEMIOLOGICAL EVALUATION

Gönenç Çalişkantürk[1], Mehmet Erinmez[2], Esin Dogantekin[3] and Selda Aslan[4]

[1]Department of Medical Microbiology, Nizip State Hospital, Gaziantep Provincial Health Directorate, Gaziantep, Türkiye; [2]Department of Medical Microbiology, Faculty of Medicine, Gaziantep University, Gaziantep, Türkiye; [3]Department of Medical Microbiology, Gaziantep City Hospital, Gaziantep Provincial Health Directorate, Gaziantep, Türkiye; [4]Department of Infectious Diseases, Gaziantep City Hospital, Gaziantep Provincial Health Directorate, Gaziantep, Türkiye

Abstract. *Legionella* is a waterborne Gram-negative bacterium transmitted mainly through inhalation of contaminated aerosol from artificial water systems, such as those found in healthcare facilities and hotels. In this study we aimed to determine the prevalence and temporal trends of *Legionella* in hospitals and hotels in southeastern Türkiye in order to inform prevention strategies at these study sites. Water samples were collected from hot- and cold-water systems from study sites and sent for culture and filtration with acid treatment in order to detect *Legionella* spp from January 2019 to December 2023. Water samples were collected from faucets, shower heads, storage reservoirs, hot and cold-water tanks and were processed within 24 hours of collection. A total of 67 hospitals and 7 hotels were purposively selected for the study. A total of 7,112 water samples were collected and included in the study; 6,934 (97.4%) from hospitals and 178 (2.6%) from hotels. Of these, 624 samples (8.8%) were positive for *Legionella* spp; 596 (95.5%), from hospitals and 28 samples (4.5%) from hotels (p-value<0.001). The most common sites with positive specimens were from faucets (n = 327, 52.4%) and shower heads (n = 182, 29.2%). 430 samples (68.9%) were positive for serogroups 2-14 and 182 samples (29.2%) were positive for serogroup 1 (p-value<0.001). The mean bacterial concentration among positive study samples was relatively low (52 CFU/ml). The numbers of positive samples during the Spring, Summer, Winter and Autumn were 130 (20.8%), 205 (32.9%), 111 (17.8%) and 178 (28.5%) samples, respectively (p-value<0.001). In summary, *Legionella* contamination was widespread in healthcare water systems in Southeast Türkiye with the most common

serogroups being *L. pneumophila* serogroups 2–14. Conclusion, there is a need for improved water management and continuous surveillance at the study sites to reduce the risk of contracting *Legionella* infection at the study institutions. After implementing these prevention measures further studies are needed to determine their efficacy.

Keywords: *Legionella pneumophila,* Legionnaires' disease, Pontiac fever and environmental surveillance

Correspondence: Mehmet Erinmez, Department of Medical Microbiology, Faculty of Medicine, Gaziantep University, Gaziantep 27310, Türkiye
Tel: +90 5978568810 E-mail: mehmeterinmez92@hotmail.com

INTRODUCTION

Legionella is a ubiquitous Gram-negative bacterium naturally present in freshwater environments at low concentrations and infection with *Legionella* occurs primarily through inhalation of contaminated aerosol generated by artificial water systems (Misch, 2016). *Legionella* spp are responsible for Legionnaires' disease, a severe atypical pneumonia, and Pontiac fever, a self-limited flu-like illness; Pontiac fever is underdiagnosed because of its nonspecific, mild presentation (Diederen, 2008). Among more than 60 identified species of *Legionella*, *Legionella pneumophila* is the most common clinically significant species and accounts for the majority of reported cases worldwide, with serogroup 1 responsible for approximately 85% of Legionnaires' disease cases (Cunha *et al*, 2016).

The risk for *Legionella* infection is determined by a combination of environmental, technical and host related factors, such as bacterial proliferation within water systems, aerosol generation and individual susceptibility (Fields *et al*, 2002). Modern urbanization and industrialization have facilitated the colonization of complex man-made water installations in residential buildings, healthcare facilities and hotels (Deiana *et*

al, 2021). Conditions, such as biofilm formation, on pipe surfaces and water temperatures between 20°C and 45°C promote *Legionella* survival and replication within hot- and cold-water distribution systems (Deiana *et al*, 2021).

Healthcare facilities represent particularly high-risk environments for *Legionella* transmission due to the vulnerability of hospitalized patients and the structural complexity of their water systems (Nisar *et al*, 2020). Hospital water systems often include features, such as storage tanks, low flow areas, dead ends and aging infrastructure, all of which favor microbial persistence (Exner *et al*, 2005). Tap water outlets, showers and cooling systems have frequently been implicated as sources of nosocomial legionellosis; drinking water systems are the primary reservoir for healthcare associated *Legionella* infections (Deiana *et al*, 2021; Lin *et al*, 2011). The risk for contracting Legionnaires' Disease is even greater in intensive care, hematology, pulmonology, cardiology and hemodialysis units, where patients are commonly immunocompromised or critically ill (Spagnolo *et al*, 2013; Lombardi *et al*, 2023).

Environmental surveillance of hospital and public building water systems, including routine isolation and quantification of *Legionella* species, is a critical component of infection prevention and control strategies (Decker and Palmore, 2013). Despite this, long term surveillance data remain limited in many regions. Southeastern Türkiye is characterized by a warm climate, expanding healthcare infrastructure, and increasing tourism, all of which may influence *Legionella* colonization of water systems; additionally, the region was affected by two major earthquakes on 6 February 2023 (Moment magnitude scales (Mw) of 7.8 and 7.5), which may have disrupted water supply and sanitation infrastructure impacting *Legionella* ecology (Çakin *et al*, 2024).

In this study we aimed to determine the prevalence and

temporal trends of *Legionella* in hospitals and hotels in southeastern Türkiye in order to inform prevention strategies at these study sites.

MATERIALS AND METHODS

Water sampling

Water samples were collected from hospitals and hotels following Turkish National Guidelines for the Prevention and Control of *Legionella* in Water Systems, issued by the Ministry of Health (TRHM, 2014). All hospitals in Türkiye are routinely check for the presence of *Legionella*, so all the hospitals in the study region were included in our study and the data used in our study were the data obtained from this surveillance. All hotels in the study region with tourism certification are routinely checked for the presence of *Legionella* and the hotels included in our study were purposely selected from these hotels. Sampling sites were selected based on risk assessment, prioritizing water tanks, hospital wards housing high-risk patient populations and water systems in hotels. The hospital units sampled for our study were intensive care units and other high dependency wards. In the study hotels, samples were obtained from central water distribution systems. For each hot water system, samples were collected from the supply line, recirculation line and storage tank bottoms, with at least 3 representative sampling points selected, to include the furthest points in the distribution network and areas with the lowest water temperature. For cold water systems, samples were obtained from tank bottoms at a minimum of two representative points, including the furthest points in the distribution network and areas with the highest temperature. Water samples were also collected from air treatment units when present.

Sample processing and culture

All samples were transported to the laboratory under controlled conditions and processed within 24 hours of collection. Two approaches were used to process water samples: direct plating and filtration followed by acid treatment. The filtration

followed by acid treatment was conducted in order to enhance *Legionella* recovery and reduce interference from background flora.

For direct culture plating, water samples were homogenized by vortexing or manual shaking. Sampled water aliquots of 0.1 ml were directly inoculated onto buffered charcoal yeast extract agar supplemented with L-cysteine and α-ketoglutarate (BCYE) and plated onto selective dye glycine vancomycin polymyxin cycloheximide agar (DGVP). The inoculum was evenly spread over the agar surface using a sterile L-shaped spreader.

For the filtration and acid treatment method, 50 ml of each water sample was filtered through a sterile membrane filter composed of polycarbonate, nylon or nitrocellulose, with a pore size of 0.22 µm, using a filtration apparatus. For samples with a high particulate content, multiple membranes were used sequentially. The membrane was carefully removed with flame sterilized forceps, rolled aseptically, and transferred into a sterile tube (25 × 160 mm) containing 5 ml of sterile water. The tube was vortexed for 30 seconds to detach microorganisms retained on the membrane. For the acid treatment, 2 ml of the vortexed suspension was transferred into a tube containing 2 ml of HCl-KCl acid solution (pH 2.2). The mixture was vortexed and incubated for 3 minutes at room temperature. At the end of the incubation period, the tube was vortexed again, and 0.1 ml aliquots were inoculated onto BCYEE and DGVPP agar plates. All plates were incubated at 36 (±1)°C in a humidified atmosphere with increased CO_2 and examined periodically for up to 10 days for the presence of suspected *Legionella* colonies.

Identification and enumeration

Presumptive *Legionella* colonies were subcultured onto BCYE agar with L-cysteine and blood agar. Isolates demonstrating growth on cysteine supplemented BCYE and no growth on blood agar were considered *Legionella* spp.

Confirmation and serogroup identification were performed using a commercial latex agglutination test (*Legionella* Latex Test; Oxoid Ltd, Cheshire, UK). From each positive sample, at least ten suspected colonies were tested when available and categorized as *L. pneumophila* serogroup 1, *L. pneumophila* serogroups 2-14, or other *Legionella* species associated with human disease.

The concentration of *Legionella* in the water samples was calculated based on the applied culture method and expressed as colony forming units per milliliter (CFU/ml). Colony counts obtained from plates were converted using the following formulas:

Direct plating: CFU/ml = number of colonies × 10

Filtration plus acid treatment: CFU/ml = number of colonies × 2

Final results were reported as CFU/l. Results were interpreted according to national guideline thresholds and classified into three categories: 100-1,000 CFU/l, 1,001-10,000 CFU/l, and >10,000 CFU/l (TRHM, 2014).

RESULTS

A total of 7,112 water samples were collected from 67 hospitals and 7 hotels. Of these, 6,934 (97.4%) were obtained from hospitals and 178 (2.6%) from hotels. Of these, 624 samples (8.8%) were positive for *Legionella* spp; 596 (95.5%) from study hospitals and 28 (4.5%) from study hotels (p-value<0.001). The *Legionella* positivity rate was 8.6% (596/6,934) in study hospitals and 15.7% (28/178) in study hotels, indicating a higher positivity rate in study hotels. Of the hospitals 232 out of 1922 samples (12.1%) from a primary level care hospitals were positive, 171 out of 2764 samples (6.2%) from secondary level care hospitals were positive

and 193 out of 3148 samples (6.3%) from tertiary level hospital were positive. The difference among the different levels of care hospitals was significant (p-value<0.001).

In hospitals, positive samples were most frequently obtained from medical wards (n = 216, 36.2%), followed by surgical wards (n = 176, 29.5%), intensive care units (n = 83, 13.9%) and other hospital areas (n = 120, 20.1%). The most common sites from which positive specimens were obtained were faucets (n = 327, 52.4%) and shower heads (n = 182, 29.2%), followed by hot water tanks (n = 70, 11.2%), cold water tanks (n = 14, 2.2%), storage reservoirs (n = 7, 1.1%) and other sampled areas (n = 8, 1.3%).

The percentages of positive samples were Şanliurfa (n = 300 out of 3140, 9.6%), Gaziantep (n = 190 out of 2465, 7.7%), Kahramanmaraş (n = 93 out of 1046, 8.9%), Kilis (n = 24 out of 270, 8.9%) and Adıyaman (n = 17 out of 191, 8.9%) (p-value<0.001).

430 of 624 positive samples (68.9%) were *L. pneumophila* serogroups 2-14, 182 (29.2%) were serogroup 1 and 12 (1.9%), were other serogroups (p<0.001) (Table 1).

The mean bacterial concentration among positive samples was 52 CFU/ml. Most samples had *Legionella* concentrations below 100 CFU/ml (n = 513, 82.2%), followed by 100-1000 CFU/ml (n = 109, 17.5%) and >1000 CFU/ml (n = 2, 0.3%).

Positive samples were most frequently detected during the Summer (n = 205, 32.9%), followed by Autumn (n = 178, 28.5%), Spring (n = 130, 20.8%), and Winter (n = 111, 17.8%) (p-value<0.001) (Table 2). *L. pneumophila* serogroups 2-14 predominated throughout the year, while *L. pneumophila* serogroup 1 increased in frequency during the final two years of the study (Fig 1).

DISCUSSION

In our study, *Legionella* was detected least frequently during the Winter and the frequency of positivity varied by season. In a

Table 1

Distribution of *Legionella* species (N = 624)

Legionella species	Frequency *n* (%)	*p*-value*
L. pneumophila serogroup 1	182 (29.2)	
L. pneumophila serogroups 2-14	430 (68.9)	<0.001
Other *Legionella* spp	12 (1.9)	

*Chi-square goodness-of-fit test

Table 2

Distribution of Legionella-positive samples by year and season (N = 624)

Year	Frequency *n* (%)	*p*-value*	Season	Frequency *n* (%)	*p*-value*
2019	106 (16.9)		Winter	111 (17.8)	
2020	102 (16.3)		Spring	130 (20.8)	<0.001
2021	68 (10.9)	<0.001	Summer	205 (32.9)	
2022	172 (27.6)		Autumn	178 (28.5)	
2023	176 (28.2)				

*Chi-square goodness-of-fit test

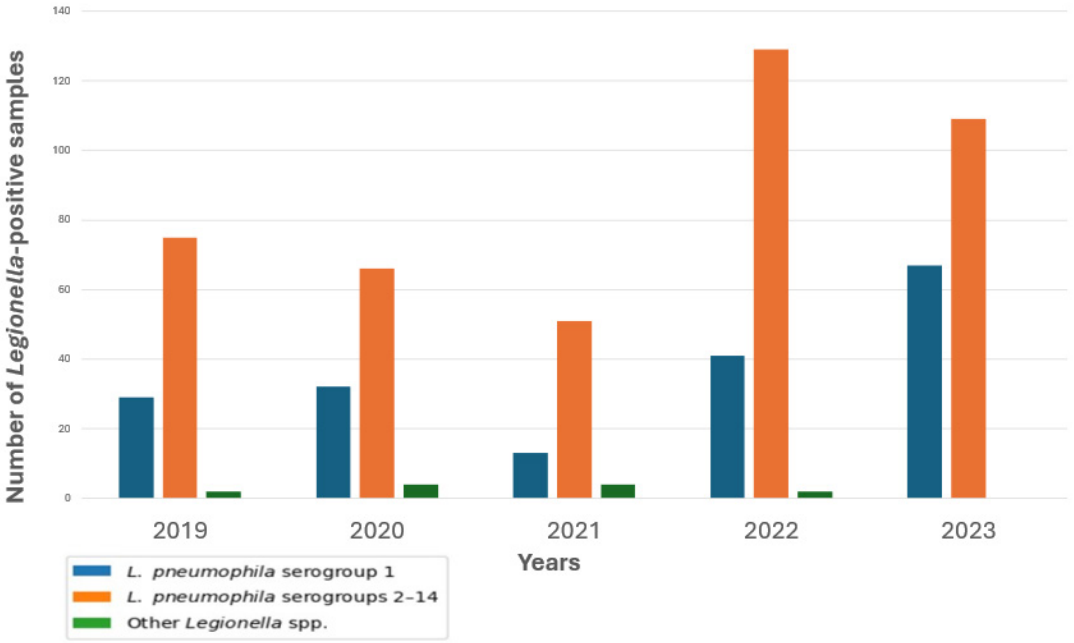

Fig 1 - Annual distribution of *Legionella* species identified in water samples

study from eastern Türkiye, the *Legionella* colonization rate in hospitals was 3.2%, lower than that observed in our study; possibly because the region where that study was conducted has a lower ambient temperature than the region where we conducted our study (Yilmaz and Orhan, 2021). Temperature is an important determinant of *Legionella* colonization since it proliferates most efficiently at a temperature of 20-45°C, and is capable of survival at temperatures of 5.7-63°C (Deiana *et al*, 2021; De Giglio *et al*, 2019). This may explain the seasonal variation in *Legionella* positivity rates seen in our study. Other seasonal factors may also have contributed to differences in positivity by season, such as water use differences, but these could not be controlled for in our study.

In our study, the *Legionella* positivity rate was significantly higher in hotels (15.7%) than

hospitals (8.6%). In a previous hotel study from Greece, the *Legionella* positivity rate 20.8% (Mouchtouri *et al*, 2007). In another hotel study from Türkiye, the *Legionella* positivity rate was 21.2% (Erdogan and Aslan, 2015). In a hotel study from Italy, the *Legionella* positivity rate was 60.5% (Borella *et al*, 2005). However, in another study from Greece, the hotel positivity rate was only 6.9% (Chochlakis *et al*, 2013). These large differences in positivity by study indicate multiple factors involved in positivity. What they all do show is the need for routine surveillance of water systems at hotels and the development of effective control methods for managing these high rates.

In our study, the rate of positivity at primary level care hospitals was 12.1%, while a study from Greece reported a primary level healthcare positivity rate of 85.96% (Papadakis *et al*, 2025). There is limited research data regarding percent positivity by level of the healthcare institution. Further studies are needed to determine if there are differences by level of healthcare in the percentages of positivity as seen in our study.

Our study was conducted after a major earthquake in the study region. The earthquake caused widespread damage to buildings and critical infrastructure, including hospital water distribution systems (Gunasekera *et al*, 2023). Such large-scale disruptions to water supply may create conditions favorable for *Legionella* proliferation through prolonged water stagnation, pressure fluctuations, pipe damage, emergency repairs, and intermittent disinfection practices (Scanlon *et al*, 2020). The largest number of *Legionella*-positive samples was detected in 2023, the year of the earthquake, although this was not significantly different among the other years of the study. This suggests that surveillance should especially take place in years of or just after major disruptions of water systems.

In our study, the most common *L. pneumophila* serogroups were 2-14 (6.89%), similar to the findings

of a previous study from Türkiye (Glažar Ivče *et al*, 2021). In our study, only 29.2% of samples were serogroup 1. However, most cases of hospital-acquired *Legionella* pneumonia are due to *Legionella pneumophila* serogroup 1 (Helbig *et al*, 2002; Yu *et al*, 2002). In Europe, *L. pneumophila* serogroup 1 is thought to be responsible for approximately 70% of legionellosis cases, with serogroups 4 and 6 occurring less frequently thereafter (Glažar Ivče *et al*, 2021). However, the incidence of Legionnaires' disease may be underestimated because some cases have nonspecific clinical manifestations, they have early initiation of empirical antibiotic therapy without etiological confirmation or unavailability of diagnostic tests (Glažar Ivče *et al*, 2021). Environmental monitoring and clinical surveillance may reveal previously unrecognized cases of hospital-acquired *Legionella* pneumonia, suggesting the importance of routine surveillance (Stout *et al*, 2007).

Conventional *Legionella* culture-based diagnostic techniques are labor intensive and slow, yielding results only after several days, and may underestimate *Legionella* in water samples with heavy background microbiota or when organisms enter a viable but nonculturable state (Dunne *et al*, 2017). These diagnostic constraints result in a gap between the incidence of clinical cases and surveillance results.

In our study, 82.2% of positive results had *Legionella* concentrations <100 CFU/ml. Sporadic Legionellosis cases have been reported at water *Legionella* concentration levels of 1,001-10,000 CFU/l, but levels >10,000 CFU/l are more confidently associated with probability of disease (Sikora *et al*, 2015), but infection can occur at any concentration where *Legionella* is present. Contaminated piping has been reported as a source of this exposure (Deiana *et al*, 2021). Again, this shows the need for routine surveillance.

In summary, *Legionella* contamination was common in

both study hotels and hospitals in the study area. The percentage of positive samples was greater in study hotels than hospitals. The most common isolates were *L. pneumophila* serogroups 2-14. The most common units in the hospitals with positive screening were medical and surgical groups. We conclude there is a need for improved water management and continuous surveillance at the study sites to reduce the risk of contracting *Legionella* in the study institutions. After implementing these prevention measures further studies are needed to determine their efficacy.

CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflicts of interest.

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