Investigation of the presence of *Legionella pneumophila* in water samples from Erzurum and surrounding provinces in Turkey

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### Abstract

**Introduction and objective.** Legionnaires’ disease, which is a waterborne disease leading to pneumonia that can result in death, is one of the major health issues today. The study aims to carry out a three-year retrospective review of routine *Legionella* follow-up analyses in various sample sets taken mostly from hospitals in the province of Erzurum and nearby provinces, and to discover a frequency of isolation of the agent and serogroups from each kind of media.

**Materials and method.** The study included a total of 2,025 water samples taken from hospitals, hotels, Turkish baths and shopping malls in Erzurum, and 13 nearby cities between 2016 and 2018. Samples were filtered by 0.45 μm-diameter membrane filter paper in the Public Health Laboratory of Erzurum and examined for *L. pneumophila* using culture method, according to the criteria set out in ISO 11731-2.

**Results.** The presence of *L. pneumophila* was found in 65 of the 2,025 water samples taken in hospitals, and in none of 40 samples taken in hotels. Turkish baths and shopping malls. *L. pneumophila* serogroup 2–14 was detected in 46 (70.8%) of 65 samples found positive, whereas *L. pneumophila* serogroup 1 was detected in 18 (27.7%). Furthermore, both the *L. pneumophila* serogroup 2–14 and *L. pneumophila* serogroup 1 were detected simultaneously in one example (1.5%). The first three samples indicate that the highest positivity rates were in hot water taps (11.6%), hot water tanks (6.1%) and shower heads (4.8%).

**Conclusions.** The Prevalence rate of *L. pneumophila* was found to be quite low compared to other studies conducted in the western regions of Turkey. *Legionella* positivity was found to be higher in the hot water systems of hospitals and related points, compared to the other sample points.

### Key words

*Legionella pneumophila*, hospital, hotel, water samples

### INTRODUCTION

Legionnaires’ disease was defined for the first time as a result of analyses performed after an outbreak of acute respiratory tract infection in 182 people who attended a meeting of the Pennsylvania American Legion held in Philadelphia in 1976, and caused the death of 29 of the participants. The source of the transmission of the disease were not exactly determined [1]. In the USA, the Center for Disease Control (CDC) revealed that the agent in this outbreak was a gram-negative bacterium and named the agent *Legionella pneumophila* since it causes a pneumonia-like picture [2].

Currently, there are over 60 species of *Legionella* reported; however, the main cause of Legionnaires’ disease worldwide is *L. pneumophila* [3, 4]. *Legionella* is transmitted to humans by aspiration of contaminated water and inhalation of contaminated aerosols and/or air conditioning gases [5–7].

Common factors contributing to *Legionella* persistence in water systems include biofilm formation, growth in amoebae, growth in nutrient-poor environments, and disinfectant resistance or tolerance [8, 9]. Two major hotel-related cases of Legionnaires’ disease have been reported in Turkey, the first of which involved 17 cases in a hotel in Kusadasi in 1994, and the other involved 16 cases in a hotel in Istanbul in 1997 [10].

### OBJECTIVES

Species of *Legionella* survive for a long time in chlorinated city waters, tank waters and on wet surface, and can be transmitted to humans through such media. Therefore, the aim of this study was to carry out a three-year retrospective review of routine *Legionella* follow-up analyses in various samples taken from hospitals, hotels, Turkish baths and shopping malls in the province of Erzurum and surrounding provinces, and to determine the frequency of isolation of the agent, and serogroups from each kind of environments.

### MATERIALS AND METHOD

The study included a total of 2,025 water samples taken from hospitals, hotels, Turkish baths and shopping malls in Erzurum and nearby provinces (Ağrı, Ardahan, Artvin, Bayburt, Bingöl, Erzincan, Giresun, Gümüşhane, Iğdır, Kars, etc.).
Muş, Tunceli and Van) between 2016–2018. Analyses were carried out in the Public Health Laboratory of Erzurum, which is also the reference laboratory in the region, by the method described in the relevant circular issued by the Ministry of Health. All samples were examined for 

*L. pneumophila* using the culture method according to the criteria set out in ISO 11731–2.

The water samples were inoculated both directly and after concentration. Using a sterile 0.45 µm diameter membrane filter paper, 50 ml water samples were filtered. 5 ml of sterile water were added to the filter paper which had sediment on it, and vortexed for 30 seconds. 2 ml of this water were taken and 2 ml of acid buffer added. For culturing, 100 µl water was added after 3 minutes of waiting time to both the BCYE (buffered charcoal yeast extract) and the DGVP (dye-glycine-vancomycin-polymyxin B) agar medium. Inoculated plates were incubated at 36 ± 2°C in an environment of 90% humidity for 10 days. The plates were checked daily for growth after the 3rd day of incubation. Plates with no visible colonies were incubated for the end of the period. Gram staining was performed with suspected *Legionella* cp colonies where the growth was observed within 3–5 days. For the bacillus-shaped bacteria that displayed weak gram-negative staining, 5% sheep blood agar and BCYE agar media was passaged. The passaged samples were incubated at 37 °C for 24–48 hours. Isolates that developed on the BCYE agar, but did not develop on the 5% sheep blood agar, were serologically confirmed by the latex agglutination test (Oxoid), and identified as belonging to the *L. pneumophila* serogroup 1, *L. pneumophila* serogroup 2–14, and *Legionella* species other than pneumophila. ATCC 43111 reference strain was used as a positive control.

**Statistical analysis.** SPSS 22.0 (SPSS Inc, Chicago, IL, USA) statistical package programme was used for evaluation of all data obtained and statistical analysis of the results. Chi-square test was used to evaluate the isolation and positivity of *L. pneumophila*, and the limit of significance was set at p <0.05. The approval of the Ethics Committee was not needed for the study, only an institutional permit was obtained from the Erzurum Health Directorate on 8 April 2019.

**RESULTS**

The number of samples taken in different months is shown in Table 1. Positive test results of *L. pneumophila* were found especially at the highest rate in April, May, and June. In November, no positivity was detected in any of the samples studied (Fig. 1). *L. pneumophila* positivity was detected in 65 (3.2%) of the 2,025 samples of water samples taken from hospitals (94.8%), hotels (1.6%), Turkish baths (0.2%) and shopping malls. The highest positivity rates were found in the hot water taps (11.6%), hot water tanks (6.1%), and shower heads (4.8%). No positivity was detected in any of the samples taken from artesian water tanks or cooling towers (Tab. 3). The difference between the groups was statistically significant p<0.05.

**Table 1. Distribution of sample numbers by month**

| Month       | Number of samples | Percent |
|-------------|-------------------|---------|
| January     | 66                | 3.3     |
| February    | 87                | 4.3     |
| March       | 104               | 5.1     |
| April       | 263               | 13.0    |
| May         | 374               | 18.5    |
| June        | 185               | 9.1     |
| July        | 104               | 5.1     |
| August      | 176               | 8.6     |
| September   | 147               | 7.2     |
| October     | 275               | 13.6    |
| November    | 176               | 8.7     |
| December    | 68                | 3.4     |

**Table 2. Prevalence of *L. pneumophila* in examined facilities**

| Facility               | No. of facilities examined | No. of facilities where positive water samples were found | No. of total samples examined positive/total (percent) |
|------------------------|----------------------------|---------------------------------------------------------|------------------------------------------------------|
| Hospital               | 89                         | 26                                                      | 65/1989 (3.3%)                                       |
| Hotel                  | 3                          | 0                                                       | 0/32 (0)                                              |
| Turkish Bath           | 1                          | 0                                                       | 0/4 (0)                                               |
| Shopping Mall          | 1                          | 0                                                       | 0/4 (0)                                               |
| Total                  | 94                         | 26                                                      | 65/2025 (3.2%)                                       |

**Table 3. Prevalence of *L. pneumophila* at specific sampling points in hospitals**

| Sampling point         | No. of sampling points examined | No. of sampling points where positive water samples were found | No. of water samples examined positive/total (percent) |
|------------------------|---------------------------------|---------------------------------------------------------------|------------------------------------------------------|
| Lavatory faucets       | 848                             | 16                                                           | 16/848 (1.9%)                                         |
| Shower heads           | 546                             | 26                                                           | 26/546 (4.8%)                                         |
| Cold water tank        | 299                             | 4                                                            | 4/299 (1.3%)                                          |
| Hot water tank         | 213                             | 13                                                           | 13/213 (6.1%)                                         |
| Mains water inlet      | 51                              | 1                                                            | 1/51 (2.0%)                                           |
| Hot water tap          | 43                              | 5                                                            | 5/43 (11.6%)                                          |
| Artesian water tank    | 14                              | 0                                                            | 0/14 (0%)                                             |
| Cooling tower          | 11                              | 0                                                            | 0/11 (0%)                                             |
| Total                  | 2025                            | 65                                                           | 65/2025 (3.2%)                                       |

Our study was carried out on water samples taken from 14 provinces in eastern Turkey (Fig. 2). When considering the distribution of positive *L. pneumophila* test results by
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Provinces, the highest positivity rates were detected in the provinces of Tunceli (23.5%), Gümüşhane (11.6%), and Erzurum (4.4%). No positivity was detected in any samples studied found in Ardahan, Bayburt, Giresun, Iğdır, and Van provinces (Fig. 3).

**Figure 3.** Distribution of *L. pneumophila* positive results by provinces

**DISCUSSION**

Legionnaires’ disease is important for preventive medicine because it is a waterborne disease that leads to severe pneumonia which can result in death. Prevention of *Legionella*’s settlement and proliferation in water-related areas and water systems constitutes the basic protection strategy for Legionnaires’ disease [1]. While bacteria cause no health problems in most healthy individuals, they can cause very serious diseases in some individuals [11]. The Center for Disease Control (CDC) has reported that people over 50 years of age, smokers, people with chronic lung disease, and people with a weakened immune system, are at serious risk for Legionnaires’ disease [12]. It is reported that the incidence of Legionnaires’ disease in the USA has increased four times since 2000, and the incidence in Europe has increased approximately three times since 1995 [13]. In 2016, 7,069 cases were reported in 30 European countries, of which 6,560 (92.8%) were verified [14]. The disease is divided into three categories which are reported as follows: 1) approximately 70% of *L. pneumophila* cases are community-acquired, 2) 20% travel-related, and 3) 10% nosocomial [13].

Some studies show that 12% – 70% of the water systems in hospitals involve *Legionella* bacteria colonization [15]. Stagnation of water at the usage points, in other words, at the end points, increases the colonization of *Legionella*. There are studies showing that stagnation in places such as sensor lavatory faucets, lavatory drainage pipes, ice machines, and decorative fountains are associated with nosocomial infection [16].

When evaluated at the national level, it is reported that the number of cases of Legionnaires’ disease is the highest in countries such as Italy, France, Spain and Turkey [17]. In a 15-year study by Lagana et al. [18] conducted in a hospital in Italy,
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This study was conducted using data from 14 provinces in eastern Turkey, and when comparison of the positivity rates of the samples taken from these provinces, the three provinces with the highest positivity rate were found to be Tunceli (23.5%), Gümüşhane (11.6%), and Erzurum (4.4%). No Legionella bacteria were found in water samples taken from the provinces of Ardahan, Bayburt, Giresun, Iğdır and Van. Due to the retrospective nature of the study, no conclusions could be formed for the causes of this difference. The element iron is important for the reproduction and survival of Legionella because of it involvement as a co-factor in bacterial enzymes [10]. However, no information could be obtained about the iron content of the water samples taken from the provinces, the structure of the water systems or the age of the buildings. This was one of the limiting factors of the current study.

A summary of results of this study indicate that they differ from studies conducted by other researchers in different geographical regions of Turkey. The positivity rate of L. pneumophila is quite low compared to the other studies conducted the western part of the country. Since most of the water samples that involved growth of Legionella were taken from a hospital, the study emphasizes the importance of continuous observation, especially in water samples from hospitals to prevent the risk of hospital-induced legionellosis. The incidence rate of L. pneumophila serogroup 2–14 was higher than the incidence rate of serogroup 1. Legionella positivity was found to be higher in hot water systems of hospitals and related points, compared to the other sampling points. Furthermore, it is believed that the data obtained in this study can serve as important epidemiological data for Legionella surveillance control in Turkey. In addition, the results also indicate that further studies, such as molecular identification methods, are needed to identify the types of Legionella isolates and obtain more epidemiological data in Turkey.

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