Veteriner Farmakoloji ve Toksikoloji Derneği Bülteni

Bulletin of Veterinary Pharmacology and Toxicology Association

e-ISSN: 2667-8381

PRESENCE OF LEGIONELLA PNEUMOPHILA IN TAP WATER AND ITS IMPORTANCE FOR PUBLIC HEALTH

Omer CAKMAK[1a*]
Tuba ALDEMIR[2b]
Erdi ERGENE[1c]
Ulas ACAROZ[3,4d]
Damla ARSLAN-ACAROZ[5,6e]
Nuri TAS[3f]

[1]Istanbul Esenyurt University, Faculty of Arts and Social Sciences, Gastronomy and Culinary Arts Department, Istanbul, Türkiye
[2]Istanbul Esenyurt University, Vocational School, Cooking Program, Istanbul, Türkiye
[3]Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Afyonkarahisar, Türkiye
[4]Kyrgyz-Turkish Manas University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Bishkek, Kyrgyzstan
[5]Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Biochemistry, Afyonkarahisar, Türkiye
[6]Kyrgyz-Turkish Manas University, Faculty of Veterinary Medicine, Department of Biochemistry, Bishkek, Kyrgyzstan

ORCID[a]: 0000-0001-7658-1284
ORCID[b]: 0000-0001-7419-3640
ORCID[c]: 0000-0001-7555-5148
ORCID[d]: 0000-0002-1533-4519
ORCID[e]: 0000-0001-9230-6725
ORCID[f]: 0009-0004-7035-1444

***Sorumlu Yazar:** Omer CAKMAK
E-Posta: omercakmak@esenyurt.edu.tr

Geliş Tarihi: 06.02.2024
Kabul Tarihi: 24.06.2024

15 (1): 64-76, 2024
DOI: 10.38137/vftd.1432171

Makale atıf

Çakmak, Ö et.al. (2024). Presence of Legionella pneumophila in tap water and its importance for public health, Veteriner Farmakoloji ve Toksikoloji Derneği Bülteni, 15 (2), 64-76. DOI: 10.38137/vftd.1432171.

ABSTRACT. *Legionella pneumophila* is one of the *Legionella* species that has been implicated in outbreaks of Legionnaires' disease in many countries, particularly in the United States of America (USA), which is caused by tap water, resulting in severe pneumonia and a mortality rate of 7 to 10%. The majority of outbreaks were linked to buildings with plumbing systems (healthcare facilities, hospitals, large buildings, etc.) and specially designed water systems (cooling towers, fountains, showers, etc.), which are optimal sources for the growth of *Legionella* spp. Infections caused by *L. pneumophila* are more likely to be caused by hot water in showers, while *L. anisa* appears to be more likely to cause infections from cold water in taps. Aging municipal water infrastructure, main line outages and leaks in the system are cited as reasons for the transmission of microorganisms, which are difficult to remove from plumbing systems. In addition, climatic changes such as temperature and precipitation are other factors that contribute to the spread of *Legionella* spp. in water systems and expose more people to the pathogen. In 2023, the outbreak of *L. pneumophila* in Poland, which resulted in the death of 14 people, brought the importance of Legionnaires' disease back to the agenda. In this review; the epidemiology, transmission routes and detection methods of *L. pneumophila*, the most important agent of the disease, were revealed in order to prevent and control Legionnaires' disease outbreaks by searching the current literature. Also included is the treatment of Legionnaires' disease.

Keywords: *Legionella pneumophila*, Legionnaires' disease, tap water, water pollution.

ŞEBEKE SULARINDA LEGIONELLA PNEUMOPHILA VARLIĞI VE HALK SAĞLIĞI AÇISINDAN ÖNEMİ

ÖZET. *Legionella* pneumophila; başta Amerika Birleşik Devletleri (ABD) olmak üzere bir çok ülkede şebeke suyu kaynaklı olarak ortaya çıkan, ciddi zatürre ile seyreden ve %7 ila 10 ölüm oranına sahip Lejyoner hastalığı salgınlarına neden olan *Legionella* türlerinden biridir. Görülen salgınlarının çoğunluğu, *Legionella* spp.'nin gelişimi için en uygun kaynaklar olan sıhhi tesisat sistemleri (sağlık tesisleri, hastaneler, büyük binalar vb.) ve özel tasarlanmış su sistemlerine (soğutma kuleleri, çeşmeler, duşlar vb.) sahip binalarla ilişkilendirilmiştir. *L. pneumophila*'nın neden olduğu enfeksiyonlar daha çok duşlardaki sıcak su kaynaklı iken, *L. anisa*'nın musluklardaki soğuk sudan kaynaklanan enfeksiyon yapabilme riskinin daha yüksek olduğu görülmektedir. Eskiyen şebeke suyu altyapısı, ana hat kesintileri ve sistemdeki sızıntılar mikroorganizmaların bulaşmasına neden olarak gösterilmekte ve bulaşının tesisat sistemlerinden uzaklaştırılması oldukça zor olmaktadır. Bununla birlikte sıcaklık ve yağış gibi iklimsel değişiklikler de *Legionella* spp.'nin su sistemlerinde yayılmasına ve daha fazla kişinin etkene maruz kalmasına zemin hazırlayan diğer etkenlerdir. 2023 yılında Polonya'da yaşanan ve 14 kişinin ölümüyle sonuçlanan *L. pneumophila* kaynaklı salgının ortaya çıkması Lejyoner hastalığının önemini yeniden gündeme getirmiştir. Bu derlemede; güncel literatür taraması yapılarak Lejyoner hastalığı salgınlarının önlenmesi ve kontrol altına alınması amacıyla hastalığın en önemli etkeni *L. pneumophila*'nın epidemiyolojisi, bulaşma yolları ve tespit yöntemleri ortaya konulmuştur. Ayrıca Lejyoner hastalığının tedavisine de yer verilmiştir.

Anahtar Kelimeler: *Legionella pneumophila*, *Lejyoner hastalığı*, şebeke suyu, su kirliliği.

INTRODUCTION

Legionella pneumophila is a gram-negative, aerobic, non-spore-forming, non-encapsulated, water-borne pathogenic bacterium, 0.5 µm wide and 2 µm long. Currently, 61 Legionella species have been identified, more than half of which are associated with human diseases (Dybwad et al., 2016). *Legionella pneumophila*, which is the most important factor in the formation of Legionnaires' disease that threatens human life, has the ability to multiply inside and outside the host cells (Fraser et al., 1977). *L. pneumophila* is a bacterial species consisting of 16 different serogroups. It has been determined that the infection in humans is mostly caused by *L. pneumophila* serogroup 1, serogroup 4 and serogroup 6 (Fields et al., 2002). The agent, which survives planktonically, colonises in biofilms and can infect permissive protozoa, is widely found in natural and artificial water systems (Fields et al., 2002; Hilbi et al., 2011). Outbreaks caused by *L. pneumophila* have been reported in many countries around the world, including North America, Europe, Asia and Oceania (Phin et al., 2014; Cunha et al., 2016). Outbreaks of Legionnaires' disease have put public health at risk in these countries and created a social and economic financial burden. Legionnaires' disease outbreaks in the USA were reported to be caused by drinking water (Zhang et al., 2021); in 1 in 10 cases, the disease progressed to fatal pneumonia (Shah et al., 2018). In 2018, the Centers for Disease Control and Prevention (CDC) reported 10000 cases of Legionnaires' disease, although the actual number of cases is estimated to be ~1.8-2.7 times higher (Benedict et al., 2017). Among confirmed cases of Legionnaires' disease, the per capita cost of hospitalization is estimated to be between $26,000-38,000 and costing a total of $340 million annually (Colier et al., 2012; Naumova et al., 2016). 7800 cases of Legionnaires' disease were reported in European Union countries in 2020 (ECDC, 2022), The economic cost of the 143 cases of Legionnaires' disease reported in New Zealand between 2016 and 2020 was reported to be $2.1 million (Graham and Baker, 2023).

Legionella spp., and especially *L. pneumophila* cause epidemic and sporadic cases of pneumonia, mostly affect immunocompromised individuals and are a common cause of nosocomial pneumonia (Kanarek et al., 2022). In a study conducted in Italy, 36.8% of water samples collected from nursing homes were positive for *Legionella* spp. (De Filippis et al., 2018). Although this finding indicates that the elderly are in the high risk group, it has been reported that *Legionella* spp. associated pneumonia cases have also been observed in newborns (Perez-Ortiz et al., 2021). Legionnaires' disease mortality rate ranges from 7.0-24.0% (Fliermanns, 1996), transmission occurs through inhalation of contaminated aerosolized water particles (Fields, 1996). The areas where *Legionella* spp. are most commonly detected and which contain favorable living conditions for them are central air conditioning and ventilation systems, hot water tanks, cooling towers, water hardness tanks, shower heads and hot water taps, thermal baths, muds and spas, respirators in hospitals, ornamental pools and garden fountains, springs, evaporators and nebulizers used in fire extinguishing. Aerosols containing *Legionella* spp. released from cooling towers and central air conditioning systems pose a danger especially for people with weak immune systems and risk groups (ECDC, 2022).

Especially in crowded living spaces such as hotels, hospitals, schools and dormitories, old plumbing systems and high water temperatures can increase the growth and reproduction of bacteria and cause epidemics (Garrison et al., 2016). Water samples should be taken from the water tank, water tanks, water circulating in the air conditioning system, shower heads of at least one room on each floor of the building, faucets and thermal pools in the building where the person or persons diagnosed with Legionnaires' disease are located. In hospital buildings, samples should also be taken from the water reservoirs of the respiratory equipment of patients hospitalized in intensive care and operating rooms and clinics to represent the entire building (Huang et al., 2011).

LEGIONNAIRES' DISEASE

History

The first outbreak of Legionnaires' disease occurred in 1976 at the American Legion Conference held at the Bellevue-Stratford Hotel in Philadelphia. As a result of the outbreak, 182 people developed pneumonia, 142 were hospitalized and 29 died (Fraser et al., 1977). After nearly a year of epidemiological investigations, Dr. Joseph McCade of the CDC identified *Legionella pneumophila* as the cause of the outbreak. The source of the outbreak was the hotel's water tower, which spread *Legionella*-contaminated water to the air conditioners in the building (McDade et al., 1979). After this outbreak in Philadelphia, Legionnaires'

disease became more recognized worldwide (Anon, 2020). A retrospectively investigated outbreak led to the identification of Pontiac Fever, a less severe *Legionella*-related illness. In 1978, a group of patients with flu-like symptoms in Pontiac, Michigan, were found to have been infected by exposure to an air conditioning system contaminated with *Legionella* spp. (Thacker et al., 1978). Following the identification of outbreaks of Legionnaires' disease and Pontiac Fever, knowledge about this infection (Legionnaires' disease and Pontiac Fever), referred to as Legionellosis, has gained importance with increasing data on detection techniques, discovery of multiple species, common exposure sites and clinical diagnostic methods (Burstein et al., 2016; Bai et al., 2023).

Symptoms

In humans, infection occurs mainly through inhalation of aerosols contaminated with *Legionella* spp. Infection occurs when the causative agent replicates within macrophages and monocytes in alveolar sacs. Following replication within pulmonary immune cells, a hyperactive inflammatory response occurs that can damage lung tissue and cause pulmonary damage (Fraser et al., 1977). Legionnaires' disease is characterized by pneumonia, fever, cough, shortness of breath and muscle pain (Barskey et al., 2022). Pontiac fever typically presents with fever, chills and headache without pneumonia and is usually limited (Fraser et al., 1977). Elderly individuals (≥50 years of age) with predisposing risk factors such as smoking habit, chronic cardiovascular or respiratory disease, diabetes, alcoholism, and immunosuppression have a higher risk of developing Legionnaires' disease. (Phin et al., 2014; Mondino et al., 2020). The US has seen a steady and consistent increase in Legionnaires' disease cases over the last two decades. In 2018, in particular, there was a reported more than five-fold increase in Legionnaires' disease cases per 100,000 people compared to the early 2000s (Garrison et al., 2016; Cassell et al., 2021). However, it is noteworthy that the incidence of Legionnaires' disease cases is not confined to a geographically limited region. Indeed, in the early 2000s, the Mid-Atlantic region saw a large increase and geographically heterogeneous spread of Legionnaires' disease (Neil & Berkelman, 2008). The virulence of *Legionella* species, which are the source of infection, is also related to the severity of infections (Fraser et al., 1977).

Sources of Contamination

Legionella species have been found in rivers, lakes and thermal waters, which are known as natural water sources (McDade et al., 1979). In the U.S., about 1.2 million miles of pipes supply drinking water to more than 300 million people through a municipal water system (Anon, 2020). According to the US Safe Drinking Water Act, local governments are required to control microorganisms in municipal water through disinfection at treatment plants (Tiemann, 2014). In addition, under the US EPA (Environmental Protection Agency) treatment law, free chlorine levels (0.2-4.0 mg/L) in drinking water are monitored upstream and throughout the distribution system to prevent the risk of waterborne infection (EPA, 2002). In the last decade, however, waterborne diseases caused by infrastructure deficiencies such as main line breaks, leaks and corrosion have increased (Craun and Calderon, 2001; Benedict et al., 2017). Aging infrastructure leads to microbial contamination, especially the re-emergence of opportunistic plumbing pathogens (Selvakumar and Tafuri, 2012). These pathogens, including *Legionella* species, can enter water distribution systems and form colonies (Beer et al., 2015). In addition, equipment (such as shower heads, hot water taps, toilets) or systems (such as cooling towers, fountains, hot tubs) can aerosolize *Legionella* and cause illness through inhalation of contaminated water droplets (Anon, 2020). *L. pneumophila*, a waterborne pathogen, has the ability to persist in drinking water and to multiply when combined with biofilms (Falkinham, 2020). The agent has the ability to survive in water for months or even years without losing its ability to infect host cells (Ohno et al., 2003; Söderberg et al., 2008). The increasing burden of waterborne Legionellosis indicates the need for a better understanding of how the *Legionella* agent survives in water and protozoa to identify vulnerable points and design remediation strategies (Abbott et al., 2015). Transcriptomic and proteomic screens have identified an increase in *L. pneumophila* genes as a result of prolonged exposure to water stress (Aurass et al., 2016). *Legionella* species have been isolated from natural water sources and municipal water distribution systems, as well as from man-made environments such as air conditioning systems, fountains, wastewater, ice and ice machines, room humidifiers, fog machines, etc. (Fields et al., 2002; Lapierre et al., 2017).

L. pneumophila can be found in biofilms or native

protozoa in natural reservoirs and aquatic environments. Consequently, biofilms and their intracellular location lead to higher resistance to sterilizing chemicals and environmental suppressants (Abdel-Nour et al., 2013; Sciuto et al., 2021). For example, *L. pneumophila* which persists in amoebae, has a higher nutritional intake, a longer water survival time, and a greater ability to infect mammalian cells (Fields et al., 2002). *Legionella* spp. were isolated in various water supply systems, including water towers (Fitzhenry et al., 2017), in these environments, *Legionella* spp. are known to proliferate in unicellular hosts and survive in biofilms (Abu Khweek and Amer, 2018; Mondino et al., 2020). The potential for *Legionella* spp. to proliferate in municipal water may pose a serious public health risk. Infection is most commonly caused by inhalation of aerosols containing *Legionella* in contaminated water supplies. Once inhaled, the agent is taken up by macrophages in the lung alveoli, where bacteria multiply within the macrophages, causing a severe pneumonia known as Legionnaires' disease (Mondino et al., 2020).

Since *Legionella* spp. are found in freshwater reservoirs, waterways, moist soils, fertilized material and various natural aquatic environments, it is easy to enter the network systems (Fliermanns,1996; van Heijnsbergen et al., 2015). The causative agent can survive at temperatures between 0-68°C and can continue its development at temperatures between 20-42°C. *Legionella* species can survive and reproduce in various protozoa groups such as amoebozoa, percolozoa and ciliophora (Boamah et al., 2017). Although *L. pneumophila* can be found in planktonic form in freshwater systems, it is mostly present as an active component in existing biofilms (Koide et al., 2014).

Legionella species show optimum growth in the temperature range of 25-45°C. The optimum temperature for infection and transmission is between 25-30°C (Wullings and Van Der Kooij, 2006; Buse et al., 2017). Specific nutrient sources containing amino acids and ferric ions are also required for the growth of the causative agents of *Legionella* species. (George et al., 1980; Ristroph et al., 1981). Water stagnation (reduced water use and shrinking plumbing spaces) is another factor affecting and promoting the spread of *Legionella* species. Plumbing systems experiencing water stagnation can lead to lower water temperatures, reduced dissolved oxygen and increased organic matter concentrations, which can lead to increased growth of *Legionella* (Fisher-Hoch et al., 1982; Wang et al., 2012). *Legionella* spp. can multiply in free-living amoebae, increasing their pathogenicity and ability to survive in unfavorable environments (Fields et al., 2002).

Epidemiology

Approximately 90% of Legionnaires' disease cases in the United States are caused by L. pneumophila (Dooling et al., 2015). Other pathogenic species, including *L. bozemanii, L. dumoffi, L. longbeachae* and *L. micdadei*, have been widely isolated from immunocompromised populations (Cunha et al., 2016; Rucinski et al., 2018). *Legionella* spp. is the most frequently reported cause of waterborne diseases, responsible for 43% of outbreaks, 94% of hospitalizations and all outbreak-related deaths (Collier et al., 2012). In particular, *L. pneumophila* is an opportunistic bacterium that causes Legionnaires' disease, which is caused by inhalation of contaminated water and water systems (cooling towers, fountains and humidifiers, etc.) (Rucinski et al., 2018). The annual treatment cost of the disease, which has a high mortality rate, is reported to be over 340 million dollars (EPA, 2002; Tiemann, 2014). In 2019, the National Academy of Sciences (NAS) reported that between 52000-70000 cases of Legionnaires' disease occur in the US each year (Dooling et al., 2015).

Legionnaires' disease surveillance data in the European Union/European Economic Area (EU/EEA) are held at the European Centre for Disease Prevention and Control (ECDC), with annual reporting by EU/EEA countries through the European Legionnaires' disease Surveillance Network (ELDSNet). The annual Legionnaires' disease notification rate in the EU/EEA was reported to have increased slowly between 2012 and 2016, from 1.2 to 1.4 cases per 100,000 population (ECDC, 2018; ECDC, 2021). In 2001, Murcia, Spain, was reported to be the largest outbreak of Legionnaires' disease in the world to date, with more than 800 people affected and 449 confirmed cases of *L. pneumophila* (Garcia-Fulgueiras et al., 2003).

In 2017, the notification rate increased to 1.8 cases per 100,000 population (9260 cases), followed by an even higher rate of 2.2 cases per 100,000 population in 2018 (11403 cases) and 2019 (11298 cases) (ECDC, 2021). Since 2011, cases of Legionnaires' disease have

been consistently reported annually in the EU/EEA by Italy, France, Germany and Spain (ECDC, 2022).

Plumbing fixtures such as shower heads and hot water taps are convenient sources for bacteria to colonize and spread (Bollin et al., "Bacterial counting by the traditional smear plate culture method is the gold standard approach to quantify *Legionella* species isolated from drinking water (APHA, 2007). In addition, the International Organization for Standardization (ISO) 11731:2017 culture method is widely used in outbreak investigations and environmental monitoring worldwide (ISO, 2017). For outbreak investigations in the US, the CDC uses a buffered charcoal yeast extract (BCYE) agar smear plate method enhanced with selective agents and pretreatment steps designed to specifically isolate *Legionella* and inhibit the growth of non-target organisms (Benedict et al., 2017). Isolation of *Legionella* by the smear plate method is challenging due to the need for multiple complex steps as well as reducing potential competition from non-specific microbial flora present in environmental samples (Ditommaso et al., 2015; Fisher et al., 2020). Due to the slow growth rate of *Legionella* species, culturing *Legionella* requires a long incubation period (Whiley and Taylor, 2016; Monteiro et al., 2021). *Legionella* colonies become visible on culture plates after at least 5-7 days of incubation. An additional 3 days of incubation is needed to confirm the colonies as positive (Leoni and Legnani, 2001). In the analyses carried out in relation to the outbreak cases, the average number of *Legionella* species isolated from water samples was 160 cfu/mL (<1 to 11500 cfu/mL) (Shelton et al., 1994). To ensure that sufficient *Legionella* are present prior to the culture method, 0.1-1 L water samples are filtered to concentrate typically viable *Legionella*. Another way to increase the growth of *Legionella* bacteria in the culture method is by acid treatment, which suppresses the surrounding biota (Fiume et al., 2005).

Molecular methods based on selective and highly sensitive Polymerase Chain Reaction (PCR) are used for the detection of *Legionella* agents from environmental samples (Joly et al., 2006). Amplification of specific target DNA genes can achieve higher sensitivity and specificity for *Legionella* and provide rapid results within 24 hours. However, since these molecular techniques cannot determine whether the *Legionella* present is dead or alive, the number of live *Legionella* in the analyzed samples is overestimated (Scaturro et al., 2016). PCR analysis results need to be confirmed by smear plaque culture method. However, very few reports have been published on this subject (Collins et al., 2017). In addition, due to inhibitors such as metals and acids, Legionella PCR in municipal water samples can also give false negative results (Tronel and Hartemann, 2009).

The Most Probable Number (MPN) technique is an alternative to the culture method, based on liquid-based culture and enumeration of bacterial growth. In the traditional culture method, colony forming units (cfu) are determined by direct counting of visible colonies. The MPN method can produce slightly higher numbers of microorganisms compared to the plate count technique (Oblinger and Koburger, 1975). In the MPN technique, samples are divided into aliquots of the same volume in different serial dilutions and inoculated with growth medium. After incubation, positive samples are detected by the typical turbidity or colorimetric change seen as a result of growth in each aliquot (Woomer, 1994). In positive sample dilutions, growth varies and a statistical contingency table is then used to calculate the concentration of viable bacteria present in the sample. The greater the number of replicates, the greater the presence of bacteria in the samples (Oblinger and Koburger, 1975).

Legiolert (IDEXX Laboratories, ME, USA) is a liquid-based culture method capable of quantifying *L. pneumophila* based on the MPN approach. With this method, results can be obtained after 7 days of incubation with simple and rapid sample preparation (Rech et al., 2018). First, the water samples are divided into independent split wells of two different volumes. After incubation, each well is individually scored as positive or negative for *L. pneumophila*. The total concentration of *L. pneumophila* is calculated using an MPN statistical table developed by IDEXX based on the statistical probability of the proportion of positive and negative fraction observed (Hurley and Roscoe, 1983).

Effective *L. pneumophila* detection methods are important for controlling the bacteria and preventing outbreaks of Legionnaires' disease. However, all existing methods have disadvantages. Culture for up to 10 days is the gold standard method for confirming and monitoring the presence of *L. pneumophila* (Tronel and Hartemann, 2009). Consequently, this long waiting period during which contaminated droplets can continue to spread poses

a risk to public health. Therefore, PCR, a rapid method for *L. pneumophila* detection, is preferred to reduce the waiting time. Despite its high sensitivity, the high cost due to the need for professional expertise and expensive equipment limits the use of PCR (Collins et al., 2015). Therefore, ELISA (Enzyme Linked Immunosorbant Assay) tests against *L. pneumophila* antigens have been developed as an alternative (Albalat et al., 2014). The use of lateral flow (LF) technologies for *L. pneumophila* detection has also been investigated. Duopath, a portable and cost-effective kit, was created with LF. However, this method can only detect 10^7 cfu of *L. pneumophila*, which is 100 times higher than the contamination threshold of water samples (Koide et al., 2007). It can also provide only qualitative results as a lateral flow assay (Anon, 2014).

DNAzymes are single-stranded DNA sequences with catalytic functionality. RNA-cleaving DNAzymes are a class of DNAzymes that cleave at an RNA site embedded in a DNA substrate in the presence of target molecules. RNA-cleaving DNAzymes are widely used in molecular-based biosensors for environmental and human health monitoring (Mc Connell et al., 2021). DNAzymes that cleave RNA can be artificially selected for bacterial targets from a pool of randomly sequenced DNA using in vitro selection (Ellington and Szostak, 1990). An RNA-degrading fluorogenic DNAzyme LP1 has been produced for *L. pneumophila*. Extensive gel analyses show that this DNAzyme is highly specific and broadly sensitive to *L. pneumophila* strains. The incorporation of LP1 into a simple and rapidly usable biosensor for the detection of *L. pneumophila* is shown to be promising (Rothenbroker et al., 2021; Qian et al., 2023).

Treatment

Legionella pneumonia progresses faster than other atypical pathogen pneumonias. With timely diagnosis of the disease and appropriate antibiotic treatment, *Legionella* patients can recover significantly (Wang et al., 2024). Macrolides and respiratory quinolones are the most preferred therapeutic agents in treatment (Viasus et al., 2013). However, studies have shown that it is important to consider drug resistance in the treatment of *Legionella*, especially when quinolones are ineffective. Although antibiotic resistance is not a major concern in *Legionella*, resistance to ciprofloxacin has been observed in treated patients (Shadoud et al., 2015). In a study involving clinical isolates, a relationship was found between decreased sensitivity to erythromycin and azithromycin and the presence of *lpeAB* genes, which encode an efflux pump involved in macrolide resistance (Vandewalle-Capo et al., 2017; Natas et al., 2019).

Omadacycline, a new tetracycline, has shown anti-*Legionella* activity in both *in vivo* and *in vitro* studies (Dubois et al., 2020). The concentration in alveolar macrophages (AM) and alveolar epithelial cell lining fluid (ELF) exceeds the concentration of other tetracyclines, making it a suitable option in the treatment of pneumonia (Burgos and Rodvold, 2019). Omadacycline does not have the adverse effects on the central nervous system caused by quinolones (Stahlmann and Lode, 1999). It is also used as a new reliable alternative in the treatment of severe *Legionella pneumonia* in individuals with quinolone intolerance or liver and kidney failure (Wang et al., 2024).

The optimal duration of antibiotic use in the treatment of Legionnaires' disease has not yet been precisely determined. In this situation; Factors such as the specific antimicrobial agent used, the severity of the disease, and the patient's response to treatment are effective (Viasus et al., 2022). The total recommended duration of antibiotic treatment in mild cases of *Legionella pneumonia* is 3-7 days. or until the patient is clinically stable for at least 48 hours and fever-free (Lanternier et al., 2017; Viasus et al., 2022). In the treatment of moderately severe cases of disease, administration of levofloxacin or azithromycin for 7-10 days is recommended. On the other hand, for individuals with suppressed immune systems, it is generally recommended to administer 21 days of levofloxacin or 10 days of azithromycin antibiotic treatment (Chahin and Opal, 2017; Viasus et al., 2022).

Prevention and Control

Controlling *Legionella* agents in plumbing systems is challenging and costly due to their ability to survive in nature for extended periods (Anon, 2020). Regulation of the hot water system, nutrient limitation and prevention of aerosol formation are standard controls carried out by building managers to prevent *Legionella* growth in water systems (CDC, 2017; Anon, 2020). Water treatment is often used in systems where *Legionella* is highly colonized and requires immediate corrective action (EPA, 2016). *Legionella* species are resistant to disinfection because they can survive throughout the water system by settling

on biofilm and free-living protozoan hosts (Flemming, 1993; EPA, 2016; Anon, 2020). In order to decontaminate *Legionella* in water systems; various methods such as thermal shock method, keeping hot water between 55-60°C, chlorination, bromide, monochloramine, chlorine dioxide, copper (Cu) - silver (Ag) ions, ozonization, ultraviolet (UV) light application, terminal filtration are used (Anon, 2020).

Despite an increase in environmental monitoring of *Legionella* over the last decade, there is still a need for improvement as detection methods remain limited. While various methods can be used to isolate *Legionella* species from the environment, the culture method is considered the gold standard for detecting the agent after a possible outbreak. Currently, there are no regulations for routine monitoring of *Legionella* in water systems. If monitoring becomes a standard practice, a quantitative method will be necessary to accurately detect these pathogens and prevent the development of Legionnaires' disease (Anon, 2020).

A threshold of 5 x104 cfu/L has been proposed by the National Academy of Sciences (NAS) for the potential risk of Legionnaires' disease from municipal water systems by compiling data from a large number of studies with reportable *Legionella* concentrations (ASHRAE, 2015). This concentration value of *Legionella* indicates that the water system requires remediation. Additional monitoring data on environmental exposures is also recommended by the NAS to accurately determine the risk of *Legionella* infection in plumbing systems (Anon, 2020).

Although the US has a robust municipal water treatment system, in the last decade nearly 63 million people, one-fifth of the population, have been exposed to the agent in violation of the EPA's safe drinking water law (Benedict et al., 2017). In 2015, around 82 waterborne outbreaks and 1276 cases of disease were reported to be associated with contaminated drinking water. Most of the outbreaks were caused by water distribution system deficiencies (such as main line outages, pipe corrosion and leaks, etc.) that resulted in microbial proliferation in tap water (Collier et al., 2012; Anon, 2020). Addressing aging municipal water infrastructure as a major public health threat is critical to improving water quality and human health (Bai et al., 2023).

Disinfectant residues should be monitored at the entry points of municipal water systems and throughout the distribution system and an average disinfectant residue level of 0.2-4.0 mg/L should be maintained (Cross et al., 2016). However, opportunistic plumbing pathogens can emerge as a result of infrastructure deficiencies. Their disinfectant resistance, their presence in biofilms and their proliferation in amoebic hosts in municipal water distribution systems and installations (pipes and sanitary installations in buildings and residences) are of concern in terms of affecting microbial water quality (Dooling et al., 2015; Cunha et al., 2016). This is a potential indicator of water quality from a microbial perspective, as factors that support *Legionella* survival and proliferation also contribute to the overall microbial increase in plumbing. Studying plumbing water quality is important to determine the impact of aging municipal water infrastructure on the spread of waterborne diseases, including *Legionella* outbreaks (Beer et al., 2015).

CONCLUSION

Data regarding the status and worldwide prevalence of infection caused by *Legionella* spp. are limited. The World Health Organization (WHO) reports that this pathogen is rarely reported in developing and underdeveloped countries due to neglect, lack of information, inadequate diagnosis of the causative agent and the lack of an active Legionnaires' disease surveillance system. Therefore, the symptoms of the disease should be better known. It is important to periodically examine water systems for the presence of *Legionella* spp. especially in places where immunocompromised individuals such as hospitals, kindergartens, old people's homes, nursing homes and schools are located. In addition, air conditioning devices connected to water systems where *Legionella* species are detected as a result of the analysis should be maintained and disinfected in order to prevent the spread of the agent. *Legionella* is found in natural water sources such as rivers, lakes and thermal waters. Water systems such as the water system of buildings, cooling towers, fountains for decorative purposes are contaminated with *Legionella* and cause disease in susceptible people. Water stagnation, temperature, commensal microflora, sediment accumulation and biofilm layer play an important role in the contamination of water systems.

Controlling *Legionella* in building plumbing is critical in preventing infection. For this purpose, it is very important to apply disinfection treatments (thermal, chemical and physical) to tap water to control or eliminate

the agent in building plumbing systems. Disinfection of water systems such as hospitals, spas and cooling towers with chlorine dioxide, a highly effective disinfectant with high temperature and wide pH range, is widely used to control *Legionella*. While the application of existing water treatment methods has been successful in reducing the concentration of *Legionella* in building sanitary ware, it is not sufficient for its complete eradication from municipal water systems. This situation is characterized by the persistence of *Legionella* in biofilms that protect against disinfection. As a result, old water pipes create more biofilms, allowing microbes that support the survival of *Legionella* to take up residence in the environment. For this reason, the process of renewing the pipes in building sanitary installations is very important in the fight against the causative agent.

REFERENCES

Abbott, Z. D., Yakhnin, H., Babitzke, P. & Swanson, M. S. (2015). csrR, a paralog and direct target of CsrA, promotes *Legionella pneumophila* resilience in water. *MBio*, 6(3), e00595.

Abdel-Nour, M., Duncan, C., Low, D. E. & Guyard, C. (2013). Biofilms: the stronghold of *Legionella pneumophila*. *International Journal of Molecular Sciences*, 14(11), 21660-21675.

Abu Khweek, A. & Amer, A. O. (2018). Factors mediating environmental biofilm formation by *Legionella pneumophila*. *Frontiers in Cellular and Infection Microbiology*, 8, 38.

Albalat, G. R., Broch, B. B. & Bono, M. J. (2014). Method modification of the Legipid® *Legionella* fast detection test kit. *Journal of AOAC International*, 97(5), 1403-1409.

American Public Health Association (APHA) (2007). 9260J: detection of pathogenic bacteria: *Legionella*. Stand. Methods Exam. Water Wastewater, 22nd edn. American Public Health Association. 28–32.

American Society of Heating Refrigeration and Air Conditioning Engineers (ASHRAE) (2015). Legionellosis: risk management for building water systems. ANSI/ASHRAE Standard 188–2015. Atlanta. ANSI/ASHRAE Addendum h to ANSI/ASHRAE Standard188-2015.

Anonymous (2014). "Safety Code," can be found under. Erişim Adresi: http://www.legisquebec.gouv.qc.ca/en/document/cr/B-1.1,%20r.%203/20140712#se:4172014.

Anonymous (2020). National Academies of Sciences Engineering Medicine. *Management of Legionella in Water Systems.* Washington, DC: The National Academies Press, 290 p.

Aurass, P., Gerlach, T., Becher, D., Voigt, B., Karste, S., Bernhardt, J., Riedel, K., Hecker, M. & Flieger, A. (2016). Life stage-specific proteomes of *Legionella pneumophila* reveal a highly differential abundance of virulence-associated Dot/Icm effectors. *Molecular & Cellular Proteomics*, 15(1), 177-200.

Bai, L., Yang, W. & Li, Y. (2023). Clinical and Laboratory Diagnosis of *Legionella Pneumonia. Diagnostics*, 13(2), 280.

Barskey, A. E., Derado, G. & Edens, C. (2022). Rising incidence of Legionnaires' disease and associated epidemiologic patterns, United States, 1992–2018. *Emerging Infectious Diseases*, 28(3), 527-538.

Beer, K. D., Gargano, J. W., Roberts, V. A., Hill, V. R., Garrison, L. E., Kutty, P. K., Hilborn, E. D., Wade, T. J., Fullerton, K. E. & Yoder, J. S. (2015). Surveillance for waterborne disease outbreaks associated with drinking water-United States, 2011-2012. *Morbidity and Mortality Weekly Report*, 64(31), 842.

Benedict, K. M., Reses, H., Vigar, M., Roth, D. M., Roberts, V. A., Mattioli, M., Cooley, L. A., Hilborn, E. D., Wade, T. J., Fullerton, K. E., Yoder, J. S. & Hill, V. R. (2017). Surveillance for waterborne disease outbreaks associated with drinking water-United States, 2013–2014. *Morbidity and Mortality Weekly Report*, 66(44), 1216.

Boamah, D. K., Zhou, G., Ensminger, A. W. & O'Connor, T. J. (2017). From many hosts, one accidental pathogen: the diverse protozoan hosts of *Legionella*. *Frontiers in Cellular and Infection Microbiology*, 7, 477.

Bollin, G. E., Plouffe, J. F., Para, M. F. & Hackman, B. (1985). Aerosols containing *Legionella pneumophila* generated by shower heads and hot-water faucets. *Applied and Environmental*

Microbiology, 50(5), 1128-1131.

Burgos, R. M. & Rodvold, K. A. (2019). Omadacycline: a novel aminomethylcycline. *Infection and Drug Resistance*, 12, 1895-1915.

Burstein, D., Amaro, F., Zusman, T., Lifshitz, Z., Cohen, O., Gilbert, J. A., Pupko, T., Shuman, H. A. & Segal, G. (2016). Genomic analysis of 38 *Legionella* species identifies large and diverse effector repertoires. *Nature Genetics*, 48(2), 167-175.

Buse, H. Y., Ji, P., Gomez-Alvarez, V., Pruden, A., Edwards, M. A. & Ashbolt, N. J. (2017). Effect of temperature and colonization of *Legionella pneumophila* and *Vermamoeba vermiformis* on bacterial community composition of copper drinking water biofilms. *Microbial Biotechnology*, 10(4), 773-788.

Cassell, K., Davis, J. L. & Berkelman, R. (2021). Legionnaires' disease in the time of COVID-19. *Pneumonia*, 13, 1-3.

Centers for Disease Control and Prevention (2017). Developing a water management program to reduce *Legionella* growth and spread in buildings: A practical guide to implementing industry standard. Legionella Toolkit-Updated June 5, 2017 Version 1.1 (proasysinc.com).

Chahin, A. & Opal, S. M. (2017). Severe pneumonia caused by *Legionella pneumophila*: differential diagnosis and therapeutic considerations. *Infectious Disease Clinics*, 31(1), 111-121.

Collier, S. A., Stockman, L. J., Hicks, L. A., Garrison, L. E., Zhou, F. J. & Beach, M. J. (2012). Direct healthcare costs of selected diseases primarily or partially transmitted by water. *Epidemiology & Infection*, 140(11), 2003-2013.

Collins, S., Jorgensen, F., Willis, C. & Walker, J. (2015). Real-time PCR to supplement gold-standard culture-based detection of *Legionella* in environmental samples. *Journal of Applied Microbiology*, 119 (4), 1158-1169.

Collins, S., Stevenson, D., Walker, J. & Bennett, A. (2017). Evaluation of *Legionella* real-time PCR against traditional culture for routine and public health testing of water samples. *Journal of Applied Microbiology*, 122(6), 1692-1703.

Craun, G. F. & Calderon, R. L. (2001). Waterborne disease outbreaks caused by distribution system deficiencies. *Journal-American Water Works Association*, 93(9), 64-75.

Cross, K. E., Mercante, J. W., Benitez, A. J., Brown, E. W., Diaz, M. H. & Winchell, J. M. (2016). Simultaneous detection of *Legionella* species and *L. anisa, L. bozemanii, L. longbeachae* and *L. micdadei* using conserved primers and multiple probes in a multiplex real-time PCR assay. *Diagnostic Microbiology and Infectious Disease*, 85(3), 295-301.

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

De Filippis, P., Mozzetti, C., Messina, A. & D'Alò, G. L. (2018). Prevalence of *Legionella* in retirement homes and group homes water distribution systems. *Science of the Total Environment*, 643, 715-724.

Ditommaso, S., Giacomuzzi, M., Ricciardi, E. & Zotti, C. M. (2015). Viability-qPCR for detecting *Legionella*: Comparison of two assays based on different amplicon lengths. *Molecular and Cellular Probes*, 29(4), 237-243.

Dooling, K. L., Toews, K. A., Hicks, L. A., Garrison, L. E., Bachaus, B., Zansky, S., Carpenter, L. R., Schaffner, B., Parker, E., Petit, S., Thomas, A., Thomas, S., Robert Mansmann, R., Morin, C., White, B. & Langley, G. E. (2015). Active bacterial core surveillance for legionellosis-United States, 2011-2013. *Morbidity and Mortality Weekly Report*, 64(42), 1190-1193.

Dubois, J., Dubois, M. & Martel, J. F. (2020). *In vitro* and intracellular activities of omadacycline against *Legionella pneumophila*. *Antimicrobial Agents and Chemotherapy*, 64(5), e01972-19.

Dybwad, M., Aarskaug, T., Fykse, E. M., Henie Madslien, E. & Blatny, J. M. (2016). Complete genome sequences of six *Legionella pneumophila* isolates from two collocated outbreaks of legionnaires' disease in 2005 and 2008 in Sarpsborg/Fredrikstad, Norway. *Genome Announcements*, 4(6), e01367-16.

Ellington, A. D. & Szostak, J. W. (1990). In vitro selection of RNA molecules that bind specific ligands. *Nature*, 346(6287), 818-822.

Environmental Protection Agency (EPA) (2002). National

primary drinking water regulations: long term 1 enhanced surface water treatment rule. Final rule. *Federal Register*, 67(9), 1811-1844.

Environmental Protection Agency (EPA) (2016). Technologies for Legionella Control in Premise Plumbing Systems: Scientific Literature Review. EPA 810-R-16-001. Technologies for Legionella Control in Premise Plumbing Systems: Scientific Literature Review (epa.gov)

Erdogan, H. & Arslan, H. (2007). Colonization of *Legionella* species in hotel water systems in Turkey. *Journal of Tavel Medicine,* 14(6), 369-373.

Erdoğan, H. & Arslan, H. (2013). Yeni Açılan Bir Otelde Ortaya Çıkan *Legionella* Salgınının İrdelenmesi. *Mikrobiyoloji Bülteni,* 47(2), 240-249.

Erdogan, H., Turan, H., Hasimoğlu, R., OK Azap, O. K. & Arslan, H. (2009). Colonization of *Legionella* species in hospital water systems in Turkey. 7th International Conference *Legionella*, Paris, 2009, P184.

Euronews (2023). Eleven people die from Legionnaires' disease outbreak in Polish town of Rzeszow. Eleven people die from Legionnaires' disease outbreak in Polish town of Rzeszow | Euronews.

European Centre for Disease Prevention and Control (ECDC) (2018). Legionnaires' disease: Annual Epidemiological Report for 2016. Stockholm: ECDC.

European Centre for Disease Prevention and Control (ECDC) (2021). Legionnaires' disease: Annual Epidemiological Report for 2019. Stockholm: ECDC.

European Centre for Disease Prevention and Control (ECDC) (2022). Legionnaires' disease: Annual Epidemiological Report for 2020. Stockholm: ECDC.

Falkinham III, J. O. (2020). Living with *Legionella* and other waterborne pathogens. *Microorganisms,* 8(12), 2026.

Fields, B. S. (1996). The molecular ecology of legionellae. *Trends in microbiology*, 4(7), 286-290.

Fields, B. S., Benson, R. F. & Besser, R. E. (2002). *Legionella* and Legionnaires' disease: 25 years of investigation. *Clinical Microbiology Reviews*, 15(3), 506-526.

Fisher, K. E., Wickenberg, L. P., Leonidas, L. F., Ranz, A. A., Habib, M. A., Buford, R. M. & McCoy, W. F. (2020). Next Day *Legionella* PCR: a highly reliable negative screen for *Legionella* in the built environment. *Journal of Water and Health*, 18(3), 345-357.

Fisher-Hoch, S. P., Smith, M. G. & Colbourne, J. S. (1982). *Legionella pneumophila* in hospital hot water cylinders. *The Lancet*, 319(8280), 1073.

Fitzhenry, R., Weiss, D., Cimini, D., Balter, S., Boyd, C., Alleyne, L., Stewart, R., McIntosh, N., Econome, A., Lin, Y., Rubinstein, I., Passaretti, T., Kidney, A., Lapierre, P., Kass, D. & Varma, J. K. (2017). Legionnaires' disease outbreaks and cooling towers, New York city, New York, USA. *Emerging Infectious Diseases*, 23(11), 1769-1776.

Fiume, L., Bucca Sabattini, M. A. & Poda, G. (2005). Detection of *Legionella pneumophila* in water samples by species-specific real-time and nested PCR assays. *Letters in Applied Microbiology*, 41(6), 470-475.

Flemming, H. C. (1993). Biofilms and environmental protection. *Water Science and Technology*, 27(7-8), 1-10.

Fliermans, C. B. (1996). Ecology of *Legionella*: from data to knowledge with a little wisdom. *Microbial Ecology*, 32, 203-228.

Fraser, D. W., Tsai, T. R., Orenstein, W., Parkin, W. E., Beecham, H. J., Sharrar, R. G., Harris J., Mallison, G. F., Martin, S. M. & McDade, J. E. (1977). Legionnaires' disease: description of an epidemic of pneumonia. *New England Journal of Medicine*, 297(22), 1189-1197.

Garcıa-Fulgueıras, A., Navarro, C., Fenoll, D., Garcıa, J., Gonzalez-Dıego, P., Jımenez-Bunuales, T., Rodrıguez, M., Lopez, R., Pacheco, F., Ruız, J., Segovıa, M., Baladron, B. & Pelaz, C. (2003). Legionnaires' disease outbreak in Murcia, Spain *Emerging Infectious Diseases,* **9(8),** 915-921.

Garrison, L. E., Kunz, J. M., Cooley, L. A., Moore, M. R., Lucas, C., Schrag, S., Sarisky, J. & Whitney, C. G. (2016). Vital signs: deficiencies in environmental control identified in outbreaks

of Legionnaires' disease-North America, 2000–2014. *Morbidity and Mortality Weekly Report (MMWR),* 65(22), 576-84.

George, J. R., Pine, L., Reeves, M. W. & Harrell, W. K. (1980). Amino acid requirements of *Legionella pneumophila*. *Journal of Clinical Microbiology*, 11(3), 286-291.

Graham, F. F. & Baker, M. G. (2023). Epidemiology and direct health care costs of hospitalised legionellosis in New Zealand, 2000–2020. *Infection, Disease & Health*, 28(1), 27-38.

Hilbi, H., Hoffmann, C. & Harrison, C. F. (2011). *Legionella* spp. outdoors: colonization, communication and persistence. *Environmental Microbiology Reports*, 3(3), 286-296.

Huang, S. W., Hsu, B. M., Chen, N. H., Huang, C. C., Huang, K. H., Chen, J. S. & Kao, P. M. (2011). Isolation and Identification of *Legionella* and Their Host Amoebae from Weak Alkaline Carbonate Spring Water Using a Culture Method Combined with PCR. *Parasitology Research,* 109(5),1233-1241.

Hurley, M. A. & Roscoe, M. E. (1983). Automated statistical analysis of microbial enumeration by dilution series. *Journal of Applied Microbiology*, 55(1), 159-164.

International Organization for Standardization (ISO) (2017). ISO 11731: 2017 Water Quality-Enumeration of Legionella. *International Organization for Standardization: Geneva, Switzerland*.

Jinna, S. & Gaikwad, U. N. (2018). Environmental surveillance of *Legionella pneumophila* in distal water supplies of a hospital for early identification & prevention of hospital-acquired legionellosis. *The Indian Journal of Medical Research*, 147(6), 611-614.

Joly, P., Falconnet, P. A., André, J., Weill, N., Reyrolle, M., Vandenesch, F., Maurin, M., Etienne, J. & Jarraud, S. (2006). Quantitative real-time *Legionella* PCR for environmental water samples: data interpretation. *Applied and Environmental Microbiology*, 72(4), 2801-2808.

Kanarek, P., Bogiel, T. & Breza-Boruta, B. (2022). Legionellosis risk-an overview of *Legionella* spp. habitats in Europe. *Environmental Science and Pollution Research*, 29(51), 76532-76542.

Koide, M., Haranaga, S., Higa, F., Tateyama, M., Yamane, N. & Fujita, J. (2007). Comparative evaluation of Duopath Legionella lateral flow assay against the conventional culture method using *Legionella pneumophila* and *Legionella anisa* strains. *Japanese Journal of Infectious Diseases*, 60(4), 214-216.

Koide, M., Higa, F., Tateyama, M., Cash, H. L., Hokama, A. & Fujita, J. (2014). Role of Brevundimonas vesicularis in supporting the growth of *Legionella* in nutrient-poor environments. *The New Microbiologica*, 37(1), 33-39.

Lanternier, F., Ader, F., Pilmis, B., Catherinot, E., Jarraud, S. & Lortholary, O. (2017). Legionnaire's disease in compromised hosts. *Infectious Disease Clinics*, 31(1), 123-135.

Lapierre, P., Nazarian, E., Zhu, Y., Wroblewski, D., Saylors, A., Passaretti, T., Hughes, S., Tran, A., Lin, Y., Kornblum, J., Morrison, S. S., Mercante, J. W., Fitzhenry, R., Weiss, D., Raphael, B. H., Varma, J. K., Zucker, H. A., Rakeman, J. L. & Musser, K. A. (2017). Legionnaires' disease outbreak caused by endemic strain of *Legionella pneumophila*, New York, New York, USA, 2015. *Emerging Infectious Diseases*, 23(11), 1784-1791.

Leoni, E. & Legnani, P. P. (2001). Comparison of selective procedures for isolation and enumeration of *Legionella* species from hot water systems. *Journal of Applied Microbiology*, 90(1), 27-33.

McConnell, E. M., Cozma, I., Mou, Q., Brennan, J. D., Lu, Y. & Li, Y. (2021). Biosensing with dnazymes. *Chemical Society Reviews*, 50(16), 8954-8994.

McDade, J. E., Brenner, D. J. & Bozeman, F. M. (1979). Legionnaires' disease bacterium isolated in 1947. *Annals of Internal Medicine*, 90(4), 659-661.

Mondino, S., Schmidt, S., Rolando, M., Escoll, P., Gomez-Valero, L. & Buchrieser, C. (2020). Legionnaires' disease: state of the art knowledge of pathogenesis mechanisms of *Legionella*. *Annual Review of Pathology: Mechanisms of Disease*, 15, 439-466.

Monteiro, S. N., Robalo, A. M. & Santos, R. J. (2021).

Evaluation of Legiolert™ for the Detection of *Legionella pneumophila* and Comparison with Spread-Plate Culture and qPCR Methods. *Current Microbiology*, 78(5), 1792-1797.

Natas, O. B., Brekken, A. L., Bernhoff, E., Hetland, M. A. K., Löhr, I. H. & Lindemann, P. C. (2019). Susceptibility of *Legionella pneumophila* to antimicrobial agents and the presence of the efflux pump LpeAB. *Journal of Antimicrobial Chemotherapy*, 74(6), 1545-1550.

Naumova, E. N., Liss, A., Jagai, J. S., Behlau, I. & Griffiths, J. K. (2016). Hospitalizations due to selected infections caused by opportunistic premise plumbing pathogens (OPPP) and reported drug resistance in the United States older adult population in 1991–2006. *Journal of Public Health Policy*, 37, 500-513.

Neil, K. & Berkelman, R. (2008). Increasing incidence of legionellosis in the United States, 1990–2005: changing epidemiologic trends. *Clinical Infectious Diseases*, 47(5), 591-599.

Oblinger, J. L. & Koburger, J. A. (1975). Understanding and teaching the most probable number technique. *Journal of Milk and Food Technology*, 38(9), 540-545.

Ohno, A., Kato, N., Yamada, K. & Yamaguchi, K. (2003). Factors influencing survival of *Legionella pneumophila* serotype 1 in hot spring water and tap water. *Applied and Environmental Microbiology*, 69(5), 2540-2547.

Perez Ortiz, A., Hahn, C., Schaible, T., Rafat, N. & Lange, B. (2021). Severe pneumonia in neonates associated with *Legionella pneumophila*: case report and review of the literature. *Pathogens*, 10(8), 1031.

Phin, N., Parry-Ford, F., Harrison, T., Stagg, H. R., Zhang, N., Kumar, K., Lortholary, O., Zumla, A. & Abubakar, I. (2014). Epidemiology and clinical management of Legionnaires' disease. *The Lancet Infectious Diseases*, 14(10), 1011-1021.

Qian, S., McConnell, E. M., Rothenbroker, M., Gu, J., Alungulesa, S., Godbout, L. & Li, Y. (2023). Detecting Legionella pneumophila in Cooling Tower Water Samples with a DNAzyme/ Bead-Based Fluorescence Assay. *Analysis & Sensing*, 3(6), e202300020.

Rech, M. M., Swalla, B. M. & Dobranic, J. K. (2018). Evaluation of Legiolert for quantification of *Legionella pneumophila* from non-potable water. *Current Microbiology*, 75, 1282-1289.

Ristroph, J. D., Hedlund, K. W. & Gowda, S. (1981). Chemically defined medium for *Legionella pneumophila* growth. *Journal of Clinical Microbiology*, 13(1), 115-119.

Rothenbroker, M., McConnell, E. M., Gu, J., Urbanus, M. L., Samani, S. E., Ensminger, A. W., Filipe, C. D. M. & Li, Y. (2021). Selection and characterization of an RNA-cleaving DNAzyme activated by *Legionella pneumophila*. *Angewandte Chemie International Edition*, 60(9), 4782-4788.

Rucinski, S. L., Murphy, M. P., Kies, K. D., Cunningham, S. A., Schuetz, A. N. & Patel, R. (2018). Eight years of clinical *Legionella* PCR testing illustrates a seasonal pattern. *The Journal of Infectious Diseases*, 218(4), 669-670.

Scaturro, M., Fontana, S., Dell'eva, I., Helfer, F., Marchio, M., Stefanetti, M. V., Cavallaro, M., Miglietta, M., Montagna, M. T., De Giglio, O. & Ricci, M. L. (2016). A multicenter study of viable PCR using propidium monoazide to detect *Legionella* in water samples. *Diagnostic Microbiology and Infectious Disease*, *85*(3), 283-288.

Sciuto, E. L., Laganà, P., Filice, S., Scalese, S., Libertino, S., Corso, D., Faro, G. & Coniglio, M. A. (2021). Environmental management of *Legionella* in domestic water systems: consolidated and innovative approaches for disinfection methods and risk assessment. *Microorganisms*, 9(3), 577.

Selvakumar, A. & Tafuri, A. N. (2012). Rehabilitation of aging water infrastructure systems: key challenges and issues. *Journal of Infrastructure Systems*, 18(3), 202-209.

Shadoud, L., Almahmoud, I., Jarraud, S., Etienne, J., Larrat, S., Schwebel, C., Timsit, J. F., Schneider, D. & Maurin, M. (2015). Hidden selection of bacterial resistance to fluoroquinolones in vivo: the case of *Legionella pneumophila* and humans. *EBioMedicine*, 2(9),1179-1185.

Shah, P. P., Barskey, A. E., Binder, A. M., Edens, C., Lee, S., Smith, J. C., Schrag, S., Whitney, C. G. & Cooley, L. A. (2018). Legionnaires' disease surveillance summary report, United States: 2014-1015.

Shelton, B. G., Flanders, W. D. & Morris, G. K.

(1994). Legionnaires' disease outbreaks and cooling towers with amplified *Legionella* concentrations. *Current Microbiology*, 28, 359-363.

Söderberg, M. A., Dao, J., Starkenburg, S. R. & Cianciotto, N. P. (2008). Importance of type II secretion for survival of *Legionella pneumophila* in tap water and in amoebae at low temperatures. *Applied and environmental microbiology*, 74(17), 5583-5588.

Sreenath, K., Dey, A. B., Kabra, S. K., Thakur, B., Guleria, R. & Chaudhry, R. (2021). *Legionella pneumophila* in Patients with Pneumonia at a Referral Hospital, New Delhi, India, 2015–2020. *The American Journal of Tropical Medicine and Hygiene*, 104(3), 854-860.

Stahlmann, R. & Lode, H. (1999). Toxicity of quinolones. *Drugs*, 58, 37-42.

T.C. Ministry of Health (2015). Lejyoner Hastalığı Kontrol Usul ve Esasları Hakkında Yönetmelik. *Resmi Gazete:* 13.05.2015 tarih ve 29354 sayılı. Erişim Adresi: https://www.mevzuat.gov.tr/mevzuat?MevzuatNo=20750&MevzuatTur=7&MevzuatTertip=5.

Thacker, S. B., Bennett, J. V., Tsai, T. F., Fraser, D. W., McDade, J. E., Shepard, C. C., Williams, K. H., Jr., Stuart, W. H., Dull H. B. & Eickhoff, T. C. (1978). An outbreak in 1965 of severe respiratory illness caused by the Legionnaires' disease bacterium. *Journal of Infectious Diseases*, 138(4), 512-519.

Tiemann, M. (2014). *Safe drinking water act (SDWA): a summary of the act and its major requirements (pp. 7-5700). Washington, DC: Congressional Research Service.*

Tronel, H. & Hartemann, P. (2009). Overview of diagnostic and detection methods for legionellosis and *Legionella* spp. *Letters in Applied Microbiology*, 48(6), 653-656.

Van Heijnsbergen, E., Schalk, J. A., Euser, S. M., Brandsema, P. S., den Boer, J. W. & de Roda Husman, A. M. (2015). Confirmed and potential sources of *Legionella* reviewed. *Environmental Science & Technology*, 49(8), 4797-4815.

Vandewalle-Capo, M., Massip, C., Descours, G., Charavit, J., Chastang, J., Billy, P. A., Boisset, S., Lina, G., Gilbert, C., Maurin, M., Jarraud, S. & Ginevra, C. (2017). Minimum inhibitory concentration (MIC) distribution among wild-type strains of *Legionella pneumophila* identifies a subpopulation with reduced susceptibility to macrolides owing to efflux pump genes. *International Journal of Antimicrobial Agents*, 50(5), 684-689.

Viasus, D., Di Yacovo, S., Garcia-Vidal, C., Verdaguer, R., Manresa, F., Dorca, J., Gudiol, F. & Carratala, J. (2013). Community-acquired *Legionella pneumophila* pneumonia: a single-center experience with 214 hospitalized sporadic cases over 15 years. *Medicine*, 92(1), 51-60.

Viasus, D., Gaia, V., Manzur-Barbur, C. & Carratalà, J. (2022). Legionnaires' disease: Update on diagnosis and treatment. *Infectious Diseases and Therapy*, 11, 973-986.

Wang, H., Masters, S., Hong, Y., Stallings, J., Falkinham III, J. O., Edwards, M. A. & Pruden, A. (2012). Effect of disinfectant, water age, and pipe material on occurrence and persistence of *Legionella, mycobacteria, Pseudomonas aeruginosa,* and two amoebas. *Environmental Science & Technology*, 46(21), 11566-11574.

Wang, Y., Yi, S. M., Huang, S. M., Xu, W. X., Wei, Y. W., Qu, Q. & Qu, J. (2024). Efficacy of omadacycline in the treatment of *Legionella pneumonia*: a case report. *Frontiers in Cellular and Infection Microbiology*, 14, 1380312.

Whiley, H. & Taylor, M. (2016). *Legionella* detection by culture and qPCR: comparing apples and oranges. *Critical Reviews in Microbiology*, 42(1), 65-74.

Woomer, P. L. (1994). Most probable number counts. *Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties*, 5, 59-79.

Wullings, B. A. & Van Der Kooij, D. (2006). Occurrence and genetic diversity of uncultured *Legionella* spp. in drinking water treated at temperatures below 15 C. *Applied and Environmental Microbiology*, 72(1), 157-166.

Zhang, C., Struewing, I., Mistry, J. H., Wahman, D. G., Pressman, J. & Lu, J. (2021). *Legionella* and other opportunistic pathogens in full-scale chloraminated municipal drinking water distribution systems. *Water Research*, 205, 117571.