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A Study on The Presence of Legionella pneumophila in Hospital Water Samples from Eastern Turkey

Elif Aydin[1], Yalçin Dicle[2], Duygu Kübra Tuna[3]

1 Kütahya Health Sciences University, Tavsanlı Health Services Vocational School, Kütahya, Turkey

2 Mardin Artuklu University, Faculty of Medicine, Department of Medical Microbiology, Mardin, Turkey

3 Edremit State Hospital, Microbiology Laboratory, Balıkesir, Turkey

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Abstract

Objective: Legionnaires' disease is a fatal form of pneumonia brought on by an infection mostly caused by the 60 different species of Legionella pneumophila. Legionnaires' illness is caused by Legionella spp.-contaminated water systems. In this work, we sought to analyze Legionella species, serogroups (SG), and contamination in the water sources of hospitals in five regions in eastern Turkey.

Methods: Between January 2017 and December 2018, a total of 1008 samples were examined, including 2 cooling towers, 62 hot water tanks, 104 cold water tanks, and 840 faucet shower heads. Samples were collected by the standard culture method L. pneumophila SG 1, it was analyzed for L. pneumophila SG 2-16 and Legionella spp. The samples were inoculated into BCYE and GVPC medium, and the colonies were assessed using a latex agglutination test, followed by species- and serotype-level identifications.

Results: In our study, a total of 1008 water samples were examined, of which 35.31% (356) belonged to 2017, while 64.68% (652) belonged to 2018. 83.33% of the water samples were taken from faucets and shower heads, 10.32% from the cold water tank, 6.15% from the hot water tank, and 0.2% from the cooling tower, and the highest positivity rate was observed in the hot water tank with 12.60%. 7.04% (71) of the samples were positive, and 16.9% (12) of the positive samples were L. pneumophila SG 1, 77.46% (55) L. pneumophila SG 2-14 was detected, while 5.63% (4) were nonpneumophila (Legionella spp) it has been determined as.

Conclusions: Legionella disease remains a significant public health threat. The water tanks of hospitals and hotels should be investigated more thoroughly, the necessary disinfection procedures should be carried out frequently. All hospitals should have water management policies, and towns and large buildings should establish comprehensive water system management programs that decrease Legionella growth and transmission. To enhance prevention measures and clinical diagnosis, we also need quicker ways of detecting Legionella in water systems and clinical samples.

Keywords: Legionella pneumophila, water samples, hospital, BCYE, GVPC

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Correspondence / Yazışma Adresi: *Yalcin Dicle, Department of Medical Microbiology, Faculty of Medicine, Mardin Artuklu University, 47100, Mardin, Turkey, e-mail: yalcindicle@hotmail.com*

Türkiye'nin Doğusundaki Hastanelerden Alınan Su Örneklerinde Legionella pneumophila Varlığı

Öz

Amaç: Lejyoner hastalığı, 60 türü tanımlanan, ağırlıklı olarak Legionella pneumophila' ya bağlı enfeksiyonun neden olduğu ölümcül bir pnömonidir. Legionella spp. ile kirlenmiş su sistemleri lejyoner hastalığının sorumlu kaynaklarıdır. Bu çalışmada Türkiye'nin doğusunda bulunan beş ilin hastanelerinin su kaynaklarında, Legionella kontaminasyonunu, Legionella türlerini ve sero gruplarını (SG) retrospektif olarak değerlendirmeyi amaçladık.

Yöntemler: Ocak 2017 ile Aralık 2018 arasında, 2 soğutma kulesi, 62 sıcak su tankı, 104 soğuk su deposu, 840 musluk duş başlığı örneği olmak üzere toplam 1008 örnek incelenmiştir. Örnekler, standart kültür yöntemi ile L. pneumophila SG 1, L. pneumophila SG 2-16 ve Legionella spp açısından analiz edilmiştir. Örnekler BCYE ve GVPC besiyerlerine inoküle edilmiş ve koloniler lateks aglütinasyon testi kullanılarak değerlendirilmiş, ardından tür ve serotip düzeyinde tanımlamalar yapılmıştır.

Bulgular: Çalışmamızda, toplam 1008 su örneği incelenmiş olup, bunların %35.31'i (356) 2017 yılına ait iken, %64.68'i (652) 2018 yılına aittir. Su örneklerinin %83.33 musluk ve duş başlıklarından, %10.32'si soğuk su deposundan, %6.15'i sıcak su tankından ve %0.2' si ise soğutma kulesinden alınmış olup en fazla pozitiflik oranı %12.60 ile sıcak sun tankında görülmüştür. Örneklerin %7.04'ü (71) pozitif olup, pozitif örneklerin %16.9'u (12) L. pneumophila SG 1, %77.46 'sı (55) L. pneumophila SG 2-14 olarak tespit edilirken, % 5.63'ü (4) nonpneumophila (Legionella spp.) olarak tespit edilmiştir.

Sonuçlar: Legionella hastalığı, önemli bir halk sağlığı tehdidi olmaya devam etmektedir. Hastanelerin ve otellerin su depoları daha kapsamlı araştırılmalı, gerekli dezenfeksiyon işlemleri sık sık yapılmalıdır. Bu enfeksiyonları önlemek için, belediyelerin ve büyük binaların personeli, Legionella büyümesini ve bulaşmasını azaltan etkili su sistemi yönetimi programları uygulamalı ve tüm hastanelerin su yönetimi politikaları olmalıdır. Ayrıca, önleme stratejilerini ve klinik teşhisi geliştirmek için su sistemlerinde ve klinik örneklerde Legionella'yı tespit etmek için daha hızlı yöntemlere ihtiyacımız var.

Anahtar kelimeler: Legionella pneumophila, su örnekleri, hastane, BCYE, GVPC.

INTRODUCTION

Legionnaires' disease (LD), first appeared in the United States in 1976 and was described in 1977, it is a disease with an L. pneumophila effect that can range from a mild lower respiratory tract infection to a coma, more often with pneumoniae[1]. Legionella bacteria, LD, Pontiac fever, and extrapulmonary syndrome are the causative agents. Pontiac fever is a community-acquired, travel-related, and nosocomial-related opportunistic pathogen that leads to a febrile picture with flu-like symptoms, LD lung, and extrapulmonary syndrome leading to serious clinical pictures involving extrapulmonary organs[2]. It is noted that ~70% of LD cases are of community origin, ~20% are travel-related, and ~10% are nasocomial[3]. Studies conducted show that 74-91% of patients are 50 years of age or older, and men are 1.4 to 4.3 times more common than women[4].

The prevalence of Legionnaires' disease is rising on a global scale. From 4921 in 2011 to 11.343 in 2018, the number of reported cases in Europe grew by 300%, and from 2301 in 2005 to 7104 in 2018 in the USA[5,6]. Globally, the death rate for legionnaires' disease ranges from 2.2 to 10.3%, with Singapore reporting the lowest incidence and European nations reporting the highest rate[4]. Hospital epidemics have a mortality rate that can reach 48%[7].

There are 60 species and 80 distinct serotypes of Legionella[8]. WHO estimates that only 5-10% of illnesses are brought on by other Legionella species (L. bozemanii, L. dumoffii, L. micdadei, and L. longbeachae), whereas 20–30% are brought on by other L. pneumophila serogroups. The majority of clinical cases of LD are caused by L. pneumophila (L. pneumophila 1), a member of serogroup 1 (SG)[9]. L. pneumophila accounts for around ~90% of LD cases. Legionella species may persist for a long period in aquatic environments and grow in the presence of free-living protozoa and biocides, such as chlorine[10]. More and more reports of Legionella contamination in cooling towers, spas, foot spas, and drinking water

systems of lodgings, nursing homes, and healthcare institutions are being made[11]. By inhaling or aspirating polluted aerosols or water, transmission can happen. Aerosols are solid or liquid particles suspended in air and can contain particles of any size[12]. The biofilm layers in the water and the amoeba and the cilia protozoa serve as both a food source and a shelter in adverse conditions and are survival surfaces for Legionella[13]. Studies show that 20 amoeba and two ciliated protozoa are hosts for Legionella. Although the number of studies related to water samples is limited in Turkey, colonization was detected in hotels by 10-76.2%, hospitals by 7-27.2%, houses by 21.3%, cooling towers by 26%, and hot spring thermal waters by 11%[14].

Legionnaires' disease surveillance is carried out to prevent cases and outbreaks at specified intervals in hospital-acquired Legionnaires' disease and to investigate the source in case of reported cases. Within the scope of the "Control Program" of the Ministry of Health, General Directorate of Public Health, Department of Infectious Diseases, Legionella research, prevention of the disease in all accommodation units, especially hotels, and hospitals, and the measures to be taken when necessary are carried out within the legal framework. The procedures and principles regarding the measures to be taken to be prepared against Legionnaires' disease, to prevent and combat the disease, and the procedures and principles regarding the notification of the disease are implemented and in this context, the regulation published on "Legionnaires' Disease Control Procedures and Principles" is complied with. "Legionnaires' Disease Control Program Guide" has been developed to ensure standardization of the work to be carried out within the scope of the regulation. In the light of the regulations made with this guide; it is aimed to ensure that Legionnaires' Disease is diagnosed within the framework of certain standards, to obtain accurate notifications, to organize the work to be carried out in accommodation units within the scope of environmental surveillance, and to reveal the extent of the disease in Turkey. In addition, Legionella samples are usually taken every 3 months by the Environmental Health Unit of Public Health or Community Health Centers and analyzed by the relevant Public Health Laboratory[1].

In this study, we aimed to retrospectively evaluate the Legionella contamination in the water systems of hospitals belonging to 5 eastern cities of our country, related Legionella species, and serogroups.

METHODS

Between January 2017 and December 2018, a total of 1008 water samples taken from various water systems of hospitals located in the provinces of Van, Bitlis, Muş, Igdır, and Hakkâri were examined for Legionella (Table I). In our study, samples were taken from a total of 35 hospitals, 13 from Van, 6 from Hakkâri, 7 from Bitlis, 7 from Muş, and 2 from Iğdır.

Table I: Culture and serogrouping results of samples collected from water systems

Sample type	Samples n (%)	*Legionella* reproduce n (%)	Lateks aglütinasyon serogrup results	n
Faucet Shower Head	840 (83.33)	62 (7.38)	*L. pneumophila* SG 1	9
			L. pneumophila SG 2-16	49
			Legionella spp.	4
Cold Water Tank	104 (10.32)	1 (0.98)	*L. pneumophila* SG 1	1
Hot Water Tank	62 (6.15)	8 (12.60)	*L. pneumophila* SG 1	2
			L. pneumophila SG 2-16	6
Cooling Tower	2 (0.2)	0		0
Total	**1008**	**71**		**71(7.04%)**

Water samples (100 ml) were taken in sterile, sealed, and twist-capped bottles. Buffered Charcoal Extract Agar (BCYE) and Glycine Vancomycin Polymyxin Cycloheximide (GVPC) media were used for Legionella isolation[15].

Taking into account the characteristics of the sampling point, water was sown "directly" and/or "after acid treatment" and/or "after filtering and acid treatment" on the culture plates. Water samples taken from the tank, fountain, faucet, and shower heads were divided into two growth media, using a drigalsik spatula to spread one with 0.1 ml BCYE and the other one with 0,1 ml GVPC. 50 ml of water was taken from the same sample and passed through a 0.2 µm membrane filter. The resulting filter was placed in 5 ml of sterile water and vortexed for 30 sec. After adding 2 ml of the 5 ml solution in which the filter was located to the 2 ml HCl-KCl (pH 2.2) acid solution prepared in advance, 3 minutes have waited and 0.1 ml BCYE and GVPC media were taken and cultivation was performed by spreading with a drigalski spatula. Two to six plaque media were used for each water sample (Figure 1).

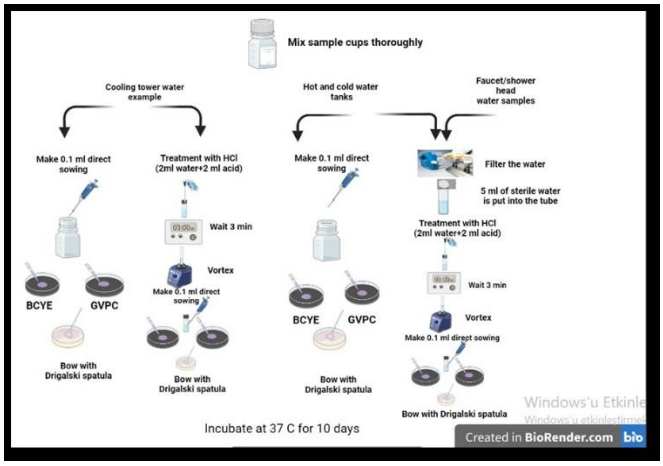


Figure 1: Flow chart for the preparation of samples taken from different sampling points for culture in the laboratory and their cultivation in primary culture media.

The media that were spread were placed in an incubator with a temperature of 36-37 °C and humidity of at least 85% and left for incubation. First of all, starting on day 3, assessments were started and followed by days 5, 7, and 10. Colonies that are considered suspicious were assessed after the 3rd day, the colony was evaluated using a microscope and UV light; when examined with the naked eye, its surfaces are smooth, slightly dished, gray-white, 1-3 mm in diameter; under a microscope, colonies with pink, purple, green or frosted glass edges are evaluated as the target colony (HSGM 2014). A single colony passage was made from suspected Legionella colonies to the BCYE and blood agar medium (Figure 2).

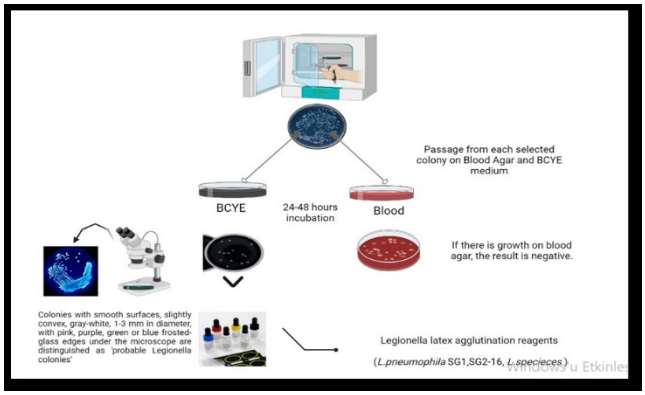


Figure 2: Parallel passage method and subsequent identification steps from suspicious colonies determined by colony microscope in primary culture of water sample to blood agar and BCYE agar plates.

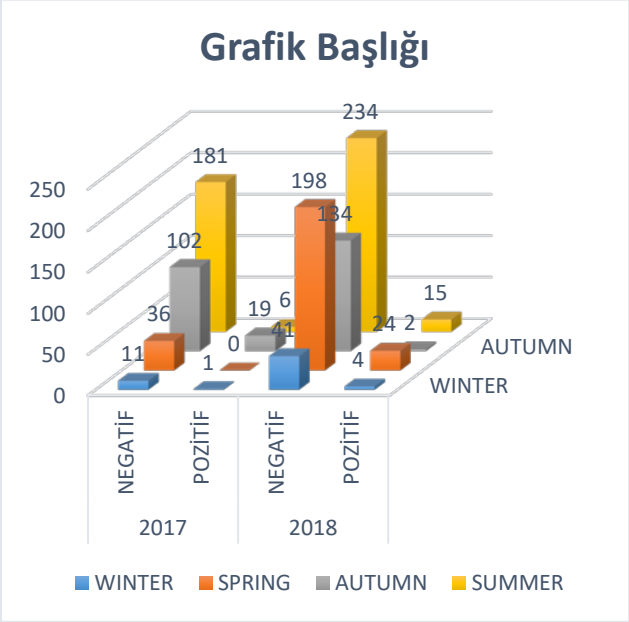
The passages left for incubation were evaluated a day later, but if there was no reproduction, they were incubated for another day. Colonies that did reproduce in BCYE media and did not reproduce in blood agar were evaluated as suspected Legionella. Colonies that were considered to be Legionella were typed by performing a latex agglutination test (Migrogen M45, UK) for serological examination.

This study was approved by the local institutional ethical board (University Kafkas-Interventional Clinical Research Ethics Committee; File Number: 2018/11).

RESULTS

Between January 2017- December 2018, a total of 1008 water samples belonging to various water systems of hospitals located in the eastern provinces of Turkey were bacteriologically and serologically investigated for Legionella bacteria in our study. Of these, 35.31% (356) belong to the year 2017, while 64.68% (652) belong to the year 2018. When

the seasonal distribution of the samples was examined, 25.49% (257) of them were taken in autumn, 5.65% (57) in winter, 25.59% (258) in spring, and 46.23% (466) in summer. The positivity rate in the autumn, winter, spring, and summer seasons are: 29.57 % (21), 7.04 % (5), 33.8% (24), 29.57% (21), and the greatest positivity was observed in the spring months (Graphic 1).



Graphic 1. The distribution rate of Legionella samples according to seasons.

As a result of the study, the results of the latex agglutination test and serogroup (SG) from Legionella colonies are shown in Table II. 7.04% (71) of the samples were positive and 16.9% (12) of the positive samples were L. pneumophila SG 1, 77.46% (55) of them were L. pneumophila SG 2-14, while 5.63% (4) were nonpneumophila.

Table II: Serogrouping results of samples collected from water systems according to cities and years

City	Year	Negative n (%)	Positive n (%)	Latex agglutination serogrup results	n
Van	2017	232	8	*L. pneumophila* SG 1	4
				L. pneumophila SG 2-16	2
				Legionella spp.	2
	2018	292	15	*L. pneumophila* SG 1	8
				L. pneumophila SG 2-16	7
				Legionella spp.	0
Bitlis	2017	31	16	*L. pneumophila* SG 1	0
				L. pneumophila SG 2-16	15
				Legionella spp.	1
	2018	167	23	*L. pneumophila* SG 1	0
				L. pneumophila SG 2-16	22
				Legionella spp.	1
Muş	2017	67	2	*L. pneumophila* SG 1	0
				L. pneumophila SG 2-16	2
				Legionella spp.	0
	2018	74	4	*L. pneumophila* SG 1	2
				L. pneumophila SG 2-16	4
				Legionella spp.	0
Hakkâri	2017	0	0	*L. pneumophila* SG 1	0
				L. pneumophila SG 2-16	0
				Legionella spp.	0
	2018	33	0	*L. pneumophila* SG 1	0
				L. pneumophila SG 2-16	0
				Legionella spp.	0
Iğdır	2017	0	0	*L. pneumophila* SG 1	0
				L. pneumophila SG 2-16	0
				Legionella spp.	0
	2018	41	3	*L. pneumophila* SG 1	0
				L. pneumophila SG 2-16	3
				Legionella spp.	0
Total		**937**	**71**		**71 (%)**

DISCUSSION

Bacteria of the genus Legionella, which maintain their viability by colonizing water systems at different temperatures, cause Legionnaires' disease outbreaks, but also reduce the quality of life and cause economic losses. In addition, these bacteria are not affected much by adverse conditions, as they can live as intracellular parasites in unicellular creatures that can be found in waters with biofilm layers formed in water sources. The presence of bacteria in public living spaces such as hospitals poses a risk of infection, especially in patients with immune system problems. In addition, it can maintain its vitality for two hours in cooling systems such as air conditioning, it can easily multiply in water pipes and spread to the environment.

Although water-borne diseases are significantly reduced by water management and sanitation, outbreaks continue to occur. The US Centers for Disease Control and Prevention (CDC) blamed Legionella for 66% of the outbreaks and 6% of the diseases that occurred between 2011 and 2012. It has been determined that 66% of Legionella outbreak was caused by building plumbing and 13% was caused by untreated groundwater[15].

When the studies conducted in our country were examined; In their study on 43 samples belonging to Aktaş's environmental and hospital water systems, they isolated 69.76% of Legionella. Of these, 26.66% were isolated from shower heads, 30% from faucets, and 43.33% from samples they took from warehouses. In addition, there are 8 Legionella spp from the warehouses they have isolated. They isolated bacteria from samples taken from the largest number of warehouses[16]. In Ayhan's thesis study conducted on 215 samples, they detected Legionella in 13.74% of them, reported that 26.43% were from warehouses and 16.85% were from faucet shower heads[17]. In a different investigation with 2.025 samples, 3.2% of the water samples from hospitals (94.8%), hotels (1.6%), Turkish baths (0.2%), and shopping malls (0.2%) had Legionella. Of the 65 samples that tested positive, L. pneumophila serogroup 2-14 was discovered in 46 (70.8%) of them[18]. Mouchtori and co isolated Legionella in 20.8% (Mouchtouri et al. 2007) of 385 hot and cold water samples belonging to hotels in Greece, Borella and co isolated Legionella in 22.6% of shower heads and tap water in Italy, Zietz and co isolated Legionella in 26% of hot water samples in Germany[19,20]. In our study, 1008 water samples were examined and Legionella was isolated from 7.04% of them. 83.33% of the samples were taken from faucets and shower heads, 10.32% from the cold water tank, 6.15% from the hot water tank, and 0.2% from the cooling tower, and the highest positivity rate was observed in the hot water tank with 12.60%. This situation has shown similarities with the literature and has been interpreted as an advantage for the colonization of these organisms by warm and stagnant waters.

Of the 8 Legionella isolates obtained from a water sample of 150 units made in our country, 6 were found to have L. pneumophila SG 2-15, and 2 of them have Legionella spp.[21]. Again, in another study conducted in schools, hospitals, and hotels, they detected Legionella in 29.71% of 313 samples, 79.56% of them were L. pneumophila SG 2-14, and 20.43% were L. pneumophila SG 1[22]. In another study, Legionella has detected in 13.74% of the samples taken, and 61.11% were L. pneumophila SG 2-15, Legionella spp in 27.77%, and L. pneumophila SG 1 at 11.11%[23]. When the studies conducted are examined worldwide; Laganà and co, detected Legionella in 64% of 92 water samples, 36% of which were L. pneumophila SG 1, 51%, L. pneumophila SG 2-14, and 13% detected both serogroups[24]. According to Goutziana and co a total of 96 Legionella isolates were detected in the study of 90.6% and 44.8% of L. pneumophila SG 1 account for 44.8% of it was noted L. pneumophila SG 2-14 and the remaining 9.4% were also detected as Legionella spp.[25]. In our study, 7.04% of the samples were positive and 16.9% of the positive samples were L. pneumophila SG 1,77.46% of L. pneumophila SG 2-14, 5.63% Legionella spp. it has been determined that the most we have detected L.

pneumophila SG 2-14 and our results are consistent with the literature.

When a literature review was conducted, there was no similar study in which a study was conducted by month. In our study, the highest positivity rate was observed in the spring months (33.8%) and then in the summer and autumn at the same rate. We attribute this to the fact that the spring seasons are a transitional period and it is the period when the heaters do not burn, so they start to heat up with air conditioners. In the summer season, we think that it is due to the fact that it is preferred to cool down with the air conditioning in the extreme temperatures.

CONCLUSIONS

Legionnaires' disease is considered a preventable infection, especially hospital-acquired LD, due to its potential to cause an epidemic, and is a disease of public health importance and is monitored under the control program. Legionella infections are not well known in our country and are not considered by clinicians in the first place. To be examined for Legionella, especially in cases of nosocomial pneumonia, it should be passed to the system as a routine test in the microbiology laboratories of all hospitals. In Legionella analyses performed by Health Directorates at specific intervals with water samples taken from hospitals, routine use of urinary antigen test should be mandatory in nosocomial pneumonias encountered in the hospital in case of pneumophila SG 1 reproduction.

To prevent the disease, it is also recommended that hospitals conduct risk assessments, implement routine preventive measures, conduct a program with active case surveillance, and periodically clean and decontaminate these areas at frequent intervals.

Legionella culture analysis results from a minimum of 5 and maximum of 12 days, which would cause it to fail in managing an outbreak of the epidemic that results in more comprehensive epidemiological research in laboratories for PCR, DFA, PCR, and FISH and especially that we believe should be done routinely supported by molecular techniques. In the studies, seasonal differentiation of samples, evaluation of the sampled waters in terms of temperature, pH, and chlorine value; it is proposed to evaluate the sampled structures and the water system in terms of parameters such as the year of use, material, and the way the water is heated.

Ethics Committee Approval: This study was approved by the local institutional ethics committee (Van Training and Research Hospital Clinical Research Ethics Committee; File no: 2018/11).

Conflict of Interest: The authors declared no conflicts of interest.

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