Detection of *Legionella pneumophila* from domestic water and their antibiotic resistance profiles

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**ABSTRACT**

**Objective:** To investigate the presence of *Legionella pneumophila* (*L. pneumophila*) in domestic water in Bitlis province and to determine the *in vitro* susceptibility of the isolates against several antibiotics.

**Methods:** A total of 320 tap water samples were collected from the urban areas and villages of Bitlis province during the period from May to December 2010. All samples were cultured on plates of buffered charcoal yeast extract agar. *L. pneumophila* strains were tested for antimicrobial susceptibility by the disk diffusion method.

**Results:** *L. pneumophila* strains were isolated from six (1.9%) domestic water samples. All isolates were typed as *L. pneumophila* serogroup 1 by latex agglutination test. Four of strains were isolated in July and two of them were detected in August. Antibiotic susceptibility testing was carried out on six *L. pneumophila* serogroup 1 isolates. Of the six strains, two was resistant to erythromycin and streptomycin, four were resistant to ampicillin and gentamicin, but all were sensitive to chloramphenicol and doxycycline.

**Conclusions:** Our results indicate that *L. pneumophila* serogroup 1 is the most common type in the domestic water samples and threats public health. This is the first report of *L. pneumophila* in domestic water samples from Bitlis province.

**KEY WORDS**

*Legionella pneumophila*, Domestic water, Antibiotic susceptibility testing

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1. Introduction

*Legionella pneumophila* (*L. pneumophila*) is a Gram-negative, small, aerobic, and intracellular microorganism. It causes pneumonia, pontiac fever or mild upper respiratory tract infections¹. This bacterium has been firstly isolated and identified from an outbreak of severe pneumonia in 1947. *L. pneumophila* species have over 15 serogroups, of which serogroup 1 is responsible for the infections in humans². This bacterium is ubiquitous in water environments worldwide and can survive for a long period in low nutrient environments in appropriate conditions³. *L. pneumophila* is found in bathrooms, swimming pools, fountains, tap water, hot water systems and cooling towers⁴⁻⁵.

*L. pneumophila* is transmitted from a contaminated water or environmental source to a host via inhalation of aerosols. The diameter of aerosol particles is important for transmission, because aerosols with diameter less than 5 μm can enter deeply the respiratory system⁶. It has been demonstrated that infection may be spread at even longer distances. However, it cannot be transmitted from human to human⁷.

To indicate the presence of *L. pneumophila*, numerous studies have been conducted on different sources worldwide. *L.
Legionella pneumophila strains were isolated from 8%, 22.6%, 30%, 26%, and 6% of domestic water samples in Catalonia, Italy, Finland, Germany, and Greece, respectively [8-12]. In Turkey, Akkaya and Özbal isolated L. pneumophila strains from 8 out of 120 domestic water samples [13]. Erandaç and Elsalih examined 93 tap water samples and Legionella spp. was not detected in the samples [14]. There are limited studies on investigation of L. pneumophila in domestic water or different sources in Turkey.

The aims of this study were to investigate the presence of L. pneumophila in domestic water in Bitlis province and to determine the in vitro susceptibility of isolates against several antibiotics.

2. Materials and methods

2.1. Sample collection

A total of 320 tap water samples were collected from the urban areas and villages of Bitlis province during the period from May to December 2010. Collection of samples was done according to the “Standard Methods for Examination of Water and Wastewater” [15]. The water samples (500 mL) were collected in sterile dark glass bottles, in which had been added 1 mL sodium thiosulfate solution at 1% to inactivate the residuals chlorine. The samples were transferred to the laboratory in isothermal coolers. The concentrations of chlorine of water samples were immediately measured by a chlorine meter.

2.2. Bacterial isolations

The water samples were filtered by membrane filtration (0.45 μm pore-sized filter, Sartorius) and with vacuum. Membrane filter paper was transferred in 10 mL Ringer’s solution and was mixed for 2 min. A few drops of 0.1 mL of suspensions were inoculated on plates of buffered charcoal yeast extract agar with supplements (Oxoid). The cultures were incubated at 37 °C in a humidified environment with at least 2.5% CO₂ for 10 days. Suspected colonies were subcultured on selective buffered charcoal yeast extract agar (containing polymyxin B, vancomycin, anisomycin, and cefamandole). L. pneumophila were identified using standard classification tests including oxidase, catalase, nitrate reduction, motility, gelatin liquefaction, urease and hippurate test [16]. The slide-agglutination test (Oxoid, UK) was used for confirmatory identification of L. pneumophila to serogroup 1 and serogroups 2-15 [2].

2.3. Antimicrobial susceptibility testing

L. pneumophila strains were tested for antimicrobial susceptibility by the disk diffusion method [17]. The following disks (Oxoid) were used: doxycycline (30 μg), erythromycin (15 μg), streptomycin (10 μg), gentamicin (10 μg), chloramphenicol (30 μg) and ampicillin (10 μg). The Mueller–Hinton agar (Oxoid) plates were incubated at 37 °C overnight. The diameter of zone of inhibition of each antimicrobial agent was measured and recorded as resistant, sensitive or intermediate according to the manufacturer’s table.

3. Results

A total of 320 samples from various points of Bitlis province were cultured and L. pneumophila were isolated from six (1.9%) of the domestic water samples. All isolates were typed as L. pneumophila serogroup 1 by latex agglutination test. Four strains were isolated in July and two of them were detected in August. The chloride levels of L. pneumophila positive samples were measured as 0-0.04 mg/L. The pH of domestic water samples was detected to be between 7.1-7.6. The temperature of water samples was measured between 19.2-23.8 °C.

Antibiotic susceptibility testing was carried out on six L. pneumophila serogroup 1 isolates. Of the six strains, two were resistant to erythromycin and streptomycin, four were resistant to ampicillin and gentamicin, but all the six isolates were sensitive to chloramphenicol and doxycycline.

4. Discussion

The genus of Legionella has 42 species and L. pneumophila is the most common pathogenic species of this genus. It is recognized as an important cause of atypical pneumonia [2]. In the present study, the presence of L. pneumophila in domestic water was investigated.

In our study, a total of 320 domestic water samples were cultured and six (1.9%) L. pneumophila strains were isolated. In another study from Turkey, 93 tap and shower water samples were examined for L. pneumophila and it was not demonstrated in the samples [14]. L. pneumophila Strains were isolated from 8 (12%) out of 100 domestic water in Greece [12]. In another work in Catalonia, bacteria was detected in 8% of the water samples which were collected from the houses of patients with Legionella [10]. Ghotasdou et al. [18] announced that four L. pneumophila were isolated from 140 water samples collected from hospitals. The rate of the isolation of L. pneumophila from water samples have been found to be 0-12% in different regions of the world as reported by some researchers. The reason of the different percentage may be due to differences in the biofilms formed in the distribution pipe networks. Biofilms are very important for growth and proliferation of L. pneumophila [19].

L. pneumophila species have over 15 serogroups, of which serogroup 1 is predominant in water and environmental samples [12]. In the present study, all isolates were typed as L. pneumophila serogroup 1 by latex agglutination test. Similar results have been obtained by other researchers. Al-Sulami et al. [20] reported that 258 L. pneumophila strains were isolated from tap water and tankers in Iraq and 77.1% of the isolates were serotyped as serogroup 1. In another study, six out of eight L. pneumophila strains from tap water samples were detected as serogroup 1 in Turkey [13]. Erdoğan and Arslan [5] announced that 11 L. pneumophila strains isolated from patients in Turkey and all of the isolates were typed as serogroup 1. Codony et al. [10] reported that L. pneumophila Serogroup 1 was detected in six out of nine L. pneumophila strains that were isolated from water samples of patient houses in Catalonia. These results indicated that L. pneumophila serogroup 1 which is responsible for infections in humans has high frequencies in water of buildings and threatens public health.

Antimicrobial resistance in bacteria has been raised in the last decades due to increasing use of drugs for medical and agricultural purposes. It threatens the effective treatment and prevention of infections. In the present study, antibiotic susceptibility test was carried out on all isolates and two out of the six strains were resistant to erythromycin and streptomycin. Erythromycin is usually used in the treatment of legionnaires disease and is a less toxic antibiotic [21]. Four of six isolates were resistant to ampicillin and gentamicin in this study. Legionella spp. isolates showed ampicillin resistance, because of beta lactamase production [22]. Also, all strains in our study were sensitive to chloramphenicol and doxycycline. The efficacy of doxycycline on Legionella spp. strains has previously been demonstrated [23].

In conclusion, our results indicate that L. pneumophila serogroup 1
is the most common type in the domestic water samples and threats public health. This is the first report of \textit{L. pneumophila} in domestic water samples from Bitlis province.

**Conflict of interest statement**

We declare that we have no conflict of interest.

**Related reports**

In the present study, the authors investigated the presence of \textit{L. pneumophila} in domestic water and assessed antibiotic susceptibility pattern of the isolates found. Studies from the literature indicates a wide variability in the rate of recovery of \textit{L. pneumophila} species in domestic water samples. In Turkey, two different studies revealed the presence of 6.7% and 0%, respectively, \textit{L. pneumophila} strains from water samples.

**Applications**

This scientific study provides interesting data and the recovery of \textit{L. pneumophila} type 1 from domestic water has important clinical implications on the public health.

**Peer review**

The paper is interesting in this field. The manuscript is technically sound and the conclusions inferred are well supported by the data presented.

**References**

[1] Cloud JL, Carroll KC, Pixton P, Enali M, Hillyard DR. Detection of \textit{Legionella} species in respiratory specimens using PCR with sequencing confirmation. \textit{J Clin Microbiol} 2000; 38(5): 1709-1712.

[2] Forbes BA, Sahm DF, Weissfeld A.S. Bailey and Scott’s diagnostic microbiology. 12th ed. St Louis, Missouri: Mosby Elsevier; 2007.

[3] Chang CW, Hwang YH, Cheng WY, Chang CP. Effects of chlorination and heat disinfection on long-term starved \textit{Legionella pneumophila} in warm water. \textit{J Appl Microbiol} 2007; 102:1636-1644.

[4] Brooks T, Oswicki R, Springthorpe V, Sattar S, Filon L, Abril D, et al. Detection and identification of \textit{Legionella} species from groundwaters. \textit{J Toxicol Environ Health A} 2004; 67: 1845–1849.

[5] Erdoğan H, Aşlan H. [Evaluation of a Legionella outbreak emerged in a recently opening hotel]. \textit{Mikrobiyol Bal} 2013; 47(2): 240-249. Turkish.

[6] FitzGeorge RB, Baskerville A, Broster M, Hambleton P, Dennis P. J. Aerosol infection of animals with strains of \textit{Legionella pneumophila} of different virulence: comparison with intraperitoneal and intranasal routes of infection. \textit{J Hyg (Lond)} 1983; 90(1): 81-89.

[7] Nguyen TM, Ileif D, Jarraud S, Roull L, Campese C, Che D, et al. A community-wide outbreak of legionnaires disease linked to industrial cooling towers–how far can contaminated aerosols spread? \textit{J Infect Dis} 2006; 193(1): 102-111.

[8] Zacheus OM, M artikainen Pj. Occurrence of \textit{Legionella} in hot water distribution systems of Finnish apartment buildings. \textit{Can J Microbiol} 1994; 40(12): 993-999.

[9] Zietz B, Wiese J, Brengelmann F, Dunkelberg H. Presence of \textit{Legionellaceae} in warm water supplies and typing of strains by polymerase chain reaction. \textit{Epidemiol Infect} 2001; 126(1):147-152.

[10] Codony F, Alvarez J, Oliva JM, Ciurana M, Company M, Camps N, et al. Factors promoting colonization by \textit{Legionella} in residential water distribution systems: an environmental case-control survey. \textit{Eur J Clin Microbiol Infec Dis} 2002; 21(10): 717-721.

[11] Borella P, Montagna MT, Romano-Spica V, Stampi S, Stancanelli G, Triassi M, et al. \textit{Legionella} infection risk from domestic hot water. \textit{Eur J Infect Diseases} 2004; 10(3): 457-464.

[12] Dimitriadis D, Velonakis E. Detection of \textit{Legionella} spp. from domestic water in the prefecture of Arta, Greece. \textit{J Pathog} 2014; doi: 10.1155/2014/207385.

[13] Akkaya Z, Özbal Y. [\textit{Legionella} resencing in water depots’ of different buildings in Kayseri]. \textit{J Health Sci} 2011; 2011: 9-17. Turkish.

[14] Erandaç M, Elałąı N. [Investigation of \textit{Legionella} spp. in tap and shower water of the hospital]. \textit{Camihiyet Med J} 2001; 23(2): 81-83. Turkish.

[15] American Public Health Association/ American Water Works Association/ Water Environment Federation. \textit{Standard methods for the examination of water and wastewater}. 20th ed. Washington DC: American Public Health Association/ American Water Works Association/ Water Environment Federation; 1995.

[16] Bonadonna L, B riancesco R, Della Libera S, Lacchetti I, Paradiso R, Semproni M. Microbial characterization of water and biofilms in drinking water distribution systems at sport facilities. \textit{Cent Eur J Public Health} 2009; 17(2): 99-102.

[17] Stokes EJ. Ridgway G, eds. \textit{Clinical bacteriology}, 5th ed. London: Arnold; 1980, p. 215.

[18] Ghotaslou R, Yeganeh Sefidan F, Akhi MT, Hejazi MS. Detection of \textit{Legionella} Contamination in fabriz hospitals by PCR assay. \textit{Adv Pharm Bull} 2013; 3(1): 131-134.

[19] Lin YS, Stout J, Yu VL, Vidic RD. Disinfection of water distribution systems for \textit{Legionella}. \textit{Semin Respir Infection} 1998; 13: 147-159.

[20] Al-Sulami AA, Al-Taee AM, Yehyazarian AA. Isolation and identification of \textit{Legionella pneumophila} from drinking water in Basra governorate, Iraq. \textit{East Mediterr Health J} 2013; 19(11): 936-941.

[21] Baltch AL, Bopp LH, Smith RP, Michelsen PB, Ritz WJ. Antibacterial activity of levofloxacin against contemporary clinical isolates of \textit{Legionella pneumophila}, \textit{Mycoplasma pneumoniae} and \textit{Chlamydia pneumoniae} from North America and Europe. \textit{Clin Microbiol Infect} 2002; 8: 214–221.