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

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PEER REVIEW

Peer reviewer

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Comments

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

Details on Page 275

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.

KEYWORDS

Legionella pneumophila, Domestic water, Antibiotic susceptibility testing

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 infections[1]. 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 humans[2]. This bacterium is ubiquitous in water environments worldwide and can survive for a long period in low nutrient environments in appropriate conditions[3]. *L. pneumophila*

is found in bathrooms, swimming pools, fountains, tap water, hot water systems and cooling towers[4,5].

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 system[6]. It has been demonstrated that infection may be spread at even longer distances. However, it cannot be transmitted from human to human[7].

To indicate the presence of *L. pneumophila*, numerous studies have been conducted on different sources worldwide. *L.*

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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 Elaldi 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 bottle, 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. Aliquots 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, bacterium was detected in 8% of the water samples which were collected from the houses of patients with *Legionella*[10]. Ghotaslou *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 predominate 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 threats 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* ssp. 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 *L. pneumophila* in domestic water samples from Bitlis province.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

The authors aimed to investigate the presence of *L. pneumophila* in domestic water from a province in Turkey and to assess antibiotic susceptibility of strains isolated. A total of 6 (1.9%) domestic water samples were found to be positive for *L. pneumophila*, and all were of serogroup 1, which is the most common among those recovered in clinical settings. Overall, 2 out of 6 strains showed resistance to erythromycin and streptomycin, and 4 were resistant to ampicillin and gentamicin; all 6 isolates were susceptible to chloramphenicol and doxycycline.

Research frontiers

The present research reports the presence of *L. pneumophila*, all strains being of serotype 1, in domestic water in a percentage of approximately 2% from a province in Turkey. While all 6 isolates were susceptible to chloramphenicol and doxycycline, 2 showed resistance to erythromycin and streptomycin, and 4 were resistance to ampicillin and gentamicin.

Related reports

In the present study, the authors investigated the presence of *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 *L. pneumophila* species in domestic water samples. In Turkey, two different studies revealed the presence of 6.7% and 0%, respectively, *L. pneumophila* strains from water samples.

Applications

This scientific study provides interesting data and the recovery of *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.

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