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Original Article

# Laboratory Diagnosis of *Pneumonia* by Detection of Urinary Antigen Test of *Legionella Pnuemophilia*

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**A B S T R A C T**

**Background and Aims:** Legionnaires' disease is seen in sporadic and epidemic form. The most prevalent cause is *Legionella pnuemophilia (L.pneumophila),* which produces a severe disease in vulnerable individuals with a high fatality rate. This study was conducted due to the wide and universal distribution of the Legionnaires' disease, lack of conventional and accessible diagnostic method, unresponsiveness to conventional antibiotic therapy in some pneumonia patients and the lack of any report about it in Kashan city, Iran.

**Materials and Methods:** This descriptive study was performed on 117 pneumonia patients hospitalized in Kashan Shahid Beheshti hospital. After obtaining the consent form, urine specimens of the patients for isolating and detecting of *L.pneumophila* were examined using the enzyme-linked immunosorbent assay method. A questionnaire containing information about demographic, clinical and para-clinical findings was filled out by the researcher.

**Results:** From a total of 117 cases, 11 (9.4%) urinary antigens of *L.pnuemophilia* were detected. 48 cases (41%) were males and 69 (59%) were females. The most cases were in ≥60 age group. There was no significant association between sex, age, job with number of *L.pneumophila* cases, but there was a significant correlation between smoking, disturbance of consciousness, increase of aspartate aminotransferase and the prevalence of Legionella.

**Conclusion:** The prevalence of *L.pnuemophilia* among the pneumonia patients was 9.4%. Considering that there is a significant association between smoking and disturbance of consciousness, education about avoiding of smoking and considering *L.pnuemophilia* as a cause of pneumonia in patients with disturbance of consciousness is recommended.

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## Introduction

Legionnaires' disease (LD) is an acute pneumonic illness caused by gram-negative bacilli of the genus *Legionella*, the most common of which is *Legionella pneumophila* (*L.pneumophila*) [1]. *L.pneumophila* is a motile, gram-negative, rod-shaped facultative intracellular bacteria belonging to the genus *Legionella*. The genus *Legionella* consists of 48 species and 70 serogroups. There are currently at least 16 serogroups of *L.pneumophila*. Serogroup 1. *L.pneumophila* is the most common cause of Legionnaires' disease; it causes 80% of all reported cases of legionellosis [2]. It distributes in the natural aquatic environment and spreads by inhalation of aerosolized biofilm droplets containing the bacteria and by invading the lungs, cause pneumonia. Pneumonia due to *L.pneumophila* is termed Legionnaires' disease. The Legionnaires' disease is a severe, life threatening pneumonia [3, 4]. Legionella infections are responsible for 2-15% of community-acquired pneumonia. Morbidity and mortality depends on the underlying diseases of the patient, early treatment and whether the disease is sporadic, nosocomial or part of an outbreak. Outbreaks occur in community acquired and nosocomial settings. Due to the increased tolerance to chlorine, it can proliferate in thermal habitats, including air-conditioning towers, hot water systems, showerheads, taps, spas and respiratory ventilators [2]. There are some specific diagnostic tests for legionnaires' disease caused by *L.pneumophila* including:

1- Culture, which done in sputum or other lower respiratory tract secretions, blood and extra pulmonary tissues or fluids. Sensitivity and specificity of the culture of Sputum is 20-95% and 100%, respectively. May be positive up to several days after treatment; it requires special media and expertise [1].

2- Urine antigen testing: This specific test can be easily performed by those without special skills and is often positive when other tests are negative. The test is not perfect because it is most sensitive for the detection of the Pontiac subtype of *L.pneumophila* serogroup 1 (up to 90%). Sensitivity and specificity of urinary antigen is 60-95% and >99%. The highest sensitivity is for *L.pneumophila* serogroup 1, pontiac type; may remain positive for days to months

3- Immunofluorescent microscopy: Sensitivity and specificity is 20-50% and 99%. The highest specificity is associated with monoclonal antibody; but it requires high level of technical expertise

4- Serology: Sensitivity and specificity of antibody-paired serum is 20-70% and 95-99%. The highest specificity is for *L. pneumophila* serogroup 1. Serology is the method most commonly used for the diagnosis of *Legionella* infections. The sensitivity of serology is generally limited by the time required to develop a detectable antibody response during the course of the infection and by the proportion of infected patients who respond immunologically. Approximately 20-30% of

patients do not develop significantly elevated antibody titres, even after prolonged observation. This limits the overall sensitivity of serology to 70-80%. Some patients (10%) even seroconvert as late as 6-9 weeks after onset of the disease. The specificity of seroconversion (Fourfold titre rise) using *L.pneumophila* SG 1 antigen in the immunofluorescence assay test has been reported to be approximately 99%.

5- Molecular amplification: Sensitivity and specificity of molecular amplification sputum, other lower respiratory tract secretions; urine is 70-95% and 90-95%, it is not well standardized [1].

The frequency of *L.pneumophila* is different in different societies, which can be derived from environmental, health, social or samples and diagnostic methods used to determine the frequency of this microorganism [5]. Garbino et al., in a Swiss study in 2002, reviewed 318 patients with community acquired pneumonia in a prospective study. The prevalence of *Legionella* antigen in this study was 1.8% [6]. In the study of Den Boer, *L.pneumophila* was responsible for 2-5% of acquired pneumonia cases in different regions [7]. Limited studies conducted in Iran (Tehran and Isfahan) have obtained this rate from 2.5 to 8.8% [8, 9].

In spite of dramatic improvement in infectious medicine, but the prominence of pneumonia as a clinical entity remains. The clinical challenge of community-acquired pneumonia (CAP) includes the increasing number of microbial agents that can cause infectious diseases, the difficulty in making etiologic diagnosis due to lack of all new laboratory diagnostic method, and the fact that no single antimicrobial regimen can cover all the possible causes. Because a specific etiologic diagnosis is often not possible at the beginning of the treatment, the empirical therapy is most appropriate. The increasing prevalence of antibiotic resistance among many of the most common pathogens has made this challenge more difficult. Understanding of microbial etiology aids to reasonable therapy [1].

The information about the prevalence of this bacterium in the patients with pneumonia in Kashan is not available, and that the study of urine antigens is a simple method, while also having high sensitivity and high resolution. This study was designed to determine the frequency of legionell pneuomonia and identification of *L.pneumophila* among the patients with CAP referred to Kashan Shahid Beheshti Hospital by using urinary antigen test.

## Materials and Methods

### Study population and setting

This descriptive cross sectional study was conducted in Kashan Beheshti Hospital, in 2015. Our sampling strategy was simple. Inclusion criteria were recorded pneumonia as the primary diagnosis in medical record according to the clinical symptoms and signs (fever, cough, chest pain, sputum, dyspnea) and infiltration in chest X ray in a hospitalized patient aged above 13 years and the presence of signed consent form. Exclusion criteria were as follows: age under 13, and normal chest X ray. Participants were given information about the objectives of the study and informed consent was obtained. 10 ml of

urine sample was collected and sent to laboratory where the urine was used to be tested by enzyme-linked immunosorbent assay (ELISA) method. The Legionella urinary antigen EIA (Binax, Inverness Medical: Scarborough, Maine) with sensitivity of 70-90% and specificity approaching 100% for *L.pneumophila* serogroup 1 was used. A questionnaire consisting the demographic information, risk factors, clinical symptoms and physical findings was filled out through direct interview with the patients and physical examination.

**Interpretation**

Interpretation of results was done by referring to the enclosed visual read card for color comparisons.

**Interpretation of results - visual (Manual)**

Any sample well that had obvious and significant yellow color interpreted reactive and any sample well that does not have obvious and significant yellow color was non-reactive: A sample well must be obviously darker than the negative control well to be called a positive result.

**Interpretation of results**

ELISA Reader: Read all wells bichromaticallyat 450 nm and 620-650 nm.

Reactive: Absorbance reading of 0.15 OD units and above indicates the sample contains *Legionella* antigen.

Non-reactive: Absorbance reading less than 0.15 OD units indicates the sample does not contain detectable levels of *Legionella* antigen.

Limitation of procedure: Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves. A negative result can occur from an antigen level lower than the detection limits of this assay. Multiple samples over time may be indicated for those patients that are suspected of being positive for *Legionella*.

Excretion of *Legionella*: Antigen in urine may vary depending on the individual patient and the stage of the disease. Some individuals have been shown to excrete antigen for an extended period of time, so a positive ELISA reaction may reflect a recent but not active infection. Early treatment with appropriate antibiotics may also decrease antigen excretion in some individuals. Antigen excretion may begin as early as 3 days after onset of symptoms and persist for up to a year afterwards.

**Statistical analysis**

Following the recording of all the data, they were analyzed using statistical SPSS version 16. Descriptive statistics, including mean, standard deviation in addition to frequency rate was calculated; Chi-square test was used to test the associations. The significance level was set to 0.05.

## Results

In this study, 69(59%) from 117 pneumonia patients were males and 48(41%) were females. The most patients were above 60 years old (Mean age 61.66±1.7 min: 18 max: 92). The most common job was housekeeper 53.8%. Most of the patients were Iranian (99.9%) and residing in urban (71.8%) and literate (64.1%). The most cases (85.5%) were living and working (73.6%) in humid environments. There were 13(11.1%) smokers and 5 (4.3%) addicts (Table1).

Cardiovascular diseases were the most common underlying diseases. The most common chief complaint was productive cough (74.4%), dyspnea (64.1%), myalgia (46.9%), loss of consciousness (11.1%), and diarrhea (6%). Physical examaniation showed rale (52.1%), fever (40.2%), tachypnea (40.2%) and tachycardia (6.4%). There was infiltration in chest X ray of all the patients. There was increased aspartate aminotransferase (38.5%) and alanine aminotransferase (61.5%), leukocytosis 31.6%, increased erythrocyte sedimentation rate 49.9% and positive C-reactive protein 54.7%. There was no significant association between age, sex, job, level of education and humidity in residential site with detection of urinary antigen *L.pneumophila*, only there was found significant association between smoking and detection of urinary antigen *L.pneumophila* (Table 2). The study was approved by the Ethics Committee of the Research Deputy in Medical Sciences University of Kashan.

**Table 1.** Demographic characteristics of studied patients

| Variables | | Ag positive No. (%) | Ag negative No. (%) | p-value |
|---|---|---|---|---|
| **Age** | <60 | 6 (54.5) | 47 (44.3) | 0.538 |
| | ≥60 | 5 (45.5) | 59 (55.6) | |
| **Sex** | Male | 6 (54.5) | 42 (39.6) | 0.521 |
| | Female | 5 (45.5) | 64 (60.4) | |
| **Job** | Jobless | 0 (0) | 9 (8.5) | 0.310 |
| | Worker | 3 (27.3) | 11 (10.4) | |
| | Farmer | 1 (9.1) | 8 (7.5) | |
| | Housewife | 4 (36.4) | 59 (55.7) | |
| | Other | 3 (27.3) | 19 (17.9) | |
| **Level of education** | Illiterate | 2 (18.2) | 40 (37.7) | 0.384 |
| | Under diploma | 6 (54.5) | 38 (35.8) | |
| | Diploma and Higher | 3 (27.3) | 28 (26.4) | |
| **Smoking** | Yes | 7 (63.6) | 16 (14.1) | 0.001 |
| | No | 4 (36.4) | 90 (84.9) | |
| **Humidity in residential site** | Wet | 11 (100) | 89 (84) | 0.217 |
| | Dry | 0 (0) | 17 (16) | |
| **Humidity in working site** | Wet | 6 (66.7) | 58 (74.4) | 0.694 |
| | Dry | 3 (33.3) | 20 (25.6) | |

**Table 2.** Relationship between clinical symptoms and laboratory finding with detection of urinary antigen *L.pneumophilla*

| Clinical symptoms and laboratory findings | | Ag positive No. (%) | Ag negative No. (%) | Total No. (%) | p- value |
|---|---|---|---|---|---|
| **Loss of consciousness** | Yes | 5 (45.5) | 8 (7.5) | 13 (11.1) | 0.002 |
| | No | 6 (54.5) | 98 (92.5) | 104 (88.9) | |
| **Dyspnea** | Yes | 6 (54.5) | 69 (65.1) | 75 (64.1) | 0.561 |
| | No | 5 (45.5) | 37 (34.9) | 42 (35.9) | |
| **Diarrhea** | Yes | 2 (18.2) | 5 (4.7) | 7 (6) | 0.214 |
| | No | 9 (81.9) | 101 (95.3) | 110 (94) | |
| **Mialgia** | Yes | 7 (63.6) | 51 (48.1) | 58 (49.6) | 0.362 |
| | No | 4 (36.4) | 55 (51.9) | 59 (50.4) | |
| **Anemia** | Yes | 5 (45.5) | 26 (24.5) | 31 (26.5) | 0.157 |
| | No | 6 (54.5) | 80 (75.5) | 86 (73.5) | |
| **Sodium** | Normal | 6 (54.6) | 91 (85.9) | 97 (64.1) | 0.17 |
| | Hyponatremia | 5 (45.5) | 15 (14.2) | 20 (35.9) | |
| **Alanine aminotransferase** | Normal | 3 (27.3) | 69 (65.1) | 72 (61.5) | 0.21 |
| | Increased | 8 (72.7) | 37 (34.9) | 45 (38.5) | |
| **Aspartate aminotransferase** | Normal | 2 (18.2) | 70 (66) | 72 (49.6) | 0.03 |
| | Increased | 9 (81.8) | 36 (34) | 45 (38.5) | |

## Discussion

The prevalence of *L.pneumophila* in our study was 9.4%. In the study carried out by Goudarzi et al., which done on 210 hospitalized children in Tehran (2011), the sputum of 12(5.7%) children with acute respiratory infections was positive for *L.pneumophila* [5]. In a study of Yazdani et al among the 96 bronchoscopic specimens, 4 strains of gram negative bacilli were isolated. Further specific direct flourcent antibody revealed that they were *L.pneumophila* [8]. Among 80 serum samples from CAP patients in Ahvaz, 12 cases (15%) were positive for *L.pneumophila* [10]. In a study done by Benito et al, seroconversion was observed in 54.8% of 97 studied patients [11]. In a study conducted by Garbino et al in Switzerland on 318 CAP patients, *L.pneumophila* was isolated in 4.4% of cases [6]. In a study of Viasus et al. among 3934 non-immunosuppressed hospitalized patients with CAP, 214 patients (5.4%) had *L.pneumophila* pneumonia [12]. In the research of Kanavaki et al. in Greek on 88 respiratory infection patients by examination on sputum, serum and urine, *L.pneumophila* was isolated in 2 (4.3%) in sputum and 6 (6.8%) in urine [13]. From 204 CAP patients in Thailand, only 3 (1.5%) had urinary antigen of *L.pneumophila* [14]. In a study performed by Dionne et al., 1154 tests were performed on 1007 patients. Seven patients had nine positive *Legionella* urinary antigen tests. Three of these patients had confirmed *L.pneumophila* pneumonia. Three others had probable or possible *L.pneumophila* pneumonia [15]. The prevalence in our study was more than other studies, which is probably due to

the non-standardization of our heating and cooling systems.

In addition, it seems that the difference in the prevalence may be due to several factors, the most important of which are: a) The diversity of studied societies in terms of social conditions, climate and season of study time. b) Seroprevalence of *legionella* infection in general population, reflecting the rate of exposure to this organism, for example, in European countries, Spain has the highest prevalence and Austria has the lowest. c) Demography of the patients and the presence of people with underlying illnesses and risk factors such as smoking [16-18]. In our study, among *legionella* positive cases, 45.5% of cases were over 60 years old and the frequency of *legionella* prevalence was not significantly correlated with age. In the study carried out by Alavi and Mirkalantary et al., also, there was no significant difference between the positive and negative serology, in this respect, we are in agreement with this study [9, 10]. In our study, 54.5% of positive cases were male. The prevalence of legionella was not significantly correlated with the gender. In Alavi et al, positive antibody in males was more than females, but the statistical analysis did not show a significant difference and in this respect, our results were consistent with this study [10]. In our study, the prevalence of legionella was not significantly correlated with habitat and workplace humidity and underlying diseases; these results were consistent with study of Alavi et al. [10]. 54.5% of the positive cases were smokers and 9.1% were hookah users, and there was a significant relationship between *legionella* prevalence and tobacco use. In Alavi et al study, all the patients with positive serology had a positive history of smoking [10]. Therefore, cigarettes are risk factors for the development of *legionella* pneumonai. Criteria to identify the patients at high risk for *L.pneumophila* pneumonia are as follows: male gender, cigarette smoking, chronic heart or lung disease, diabetes, end-stage renal failure, organ transplantation, immunosuppression, some forms of cancer, and age older than 50 years [19]. Despite there are some risk factors and clinical features and laboratory findings, which are helpful to suggest a diagnosis of *L.pneumophila* pneumonia, but exact clinical differentiation from other causes of pneumonia is not possible and the rate of correct diagnosis is about 3% and many cases are often not considered [15]. While *L.pneumophila* is increasingly recognized as a significant cause of CAP in many countries, it becomes an important public health problem worldwide. Since clinical signs and symptoms are not reliable to diagnose Legionnaires' disease, the use of diagnostic laboratory tests for *Legionella* is necessary [20]. According to the importance of disease caused by *L.pneumophila*, laboratory diagnosis of this organism has increased. The value and sensitivity of culture for *L.pneumophila* has decreased because of the need to the specific environments and specialists for working with it, inability to obtain sputum from half of the patients, inability of microorganism to survive in respiratory secretion for long time, inability of microorganism to grow in culture after starting the antimicrobial treatments [13]. Serology,

polymerase chain reaction tests, urinary antigen test are other laboratory diagnostic tests for CAP. Urinary antigen testing has grown in popularity for several significant respiratory infections, particularly *Legionella pneumophila*, *Streptococcus pneumoniae*, and *Histoplasma capsulatum* [21]. Rapid urine antigen tests are very useful to determine CAP etiology in adults. A positive urinary antigen test for *Legionella spp.* allows an early switch from empiric to targeted treatment in hospitalized, community-acquired pneumonia patients [22]. In a research conducted by Kanavaki et al., sensitivity and specificity of urinary antigen g test was calculated 68-90% and 100%, respectively [13]. Guerrero et al. compared the Bartels enzyme immunoassay, Biotest enzyme immunoassay, and Binax NOW immunochromatographic test urinary antigen kits for the detection of *L.pneumophila* serogroup 1 using 178 frozen urine samples. When non-concentrated urine samples were used, the sensitivity levels of both enzyme immunoassays were significantly higher than the sensitivity level of the immunochromatographic test. After concentration of the urine samples, no significant differences in sensitivity were found among the three tests [23]. The advantages of *L.pneumophila* urinary antigen tests are prompt diagnosis due to rapid performing, high specificity, usually detectable at the time of presentation. Urinary antigen tests are quick and simple tests helping to provide an etiological diagnosis in community-acquired pneumonia. *Legionella* urinary antigen test is the most commonly method used for the diagnosis of legionellosis, but must be prescribed in a specific clinical context [24].

## Conclusion

The rate of *L.pneumophila pneumonia* was 9.4%. Using urinary antigen test could help us to detect *L.pneumophila* simple and rapid. Urinary antigen test should be the first diagnostic method in our hospital because it is often easier to obtain urine in ill patients and the results could be available within hours and reliable to commence treatment.

## Conflict of Interest

The authors have no conflict of interest.

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