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ORIGINAL ARTICLE

Urinary antigen test EIA: A valuable rapid clinical method for the diagnosis of legionella

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ABSTRACT

Background: Community acquired pneumonia (CAP) is a common health concern and the main mortality factor worldwide. Nowadays, legionella pneumophila is one of the most common microorganisms responsible for CAP. We designed this study to investigate the antigen test as a useful, simple and rapid test for early diagnosis of legionella pneumophila.

Materials and methods: We enrolled 118 patients (32 females and 86 males) with CAP, COPD and asthma in Masih Daneshvari medical center during 2004 –5. Clinical and microbiological evaluations were performed. Sputum culture and legionella urinary antigen tests were carried out.

Results: Different types of bacteria were isolated from 118 patients. The most frequently isolated respiratory microorganisms were: streptococcus pneumonia (88%), candida spp. (76.2%), beta-hemolytic streptococcus (61.8%), neiseria spp (44.4%), staphylococcus (40.6%), klebsiella spp (27.1%), fungi (16.1%), E.coli (8.4%), and pseudomonas spp (5.1%). No legionella was detected from sputum specimens. Legionella urinary antigen testing was revealed to be positive in 3 males.

Conclusion: Urinary antigen test is a particularly useful, simple and rapid test since it is often easier to obtain urine in ill patients. The results can be available within hours and it is also reliable enough to commence treatment.

Keywords: *Legionella pneumophila, EIA, Respiratory Infections*.

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INTRODUCTION

Pneumonia, in general, is the sixth leading cause of death in the USA, and is responsible for more than 600,000 hospital admissions per year (1, 2). Streptococcus pneumonia is the most common microbe associated with community–acquired pneumonia (CAP), however, during the last decade, atypical pathogens such as moraxella cataralis, legionella pneumophila and chlamydia pneumonia have been identified more frequently, and several studies have demonstrated the importance of these newly recognized pathogens (1,3,4). Atypical pathogens are responsible for 40% of CAP cases and several studies have ranked L. pneumophila among the three most common microbial causes of CAP in patients admitted to the hospital (5).

In 1976, an outbreak of pneumonia occurred at a hotel at the site of the American legion convention in Philadelphia. A total of 182 persons were diagnosed to have pneumonia, and 34 died.

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Investigators from the Centers for Disease control (CDC) isolated a bacterium from the autopsy of the lung specimens that was ultimately named legionella pneumophila (5).

Legionella pneumophila is an aerobic, gram-negative bacillus. In tissue and clinical specimens, the organisms are coccobacillary. The organism can be visualized by Gram stain with some difficulty in clinical specimens, basic fuchsine serves as a better counterstained than safranin. The organism is fastidious and does not grow on standard bacteriologic media. Buffered Charcoal Yeast Extract (BCYE) (pH 6.9) is the primary medium used for isolation of these organisms and they require 3-5 days for growth (5,6). Five methods are currently employed for the laboratory diagnosis of legionella infection (6): 1) urinary antigen test, 2) isolation of the organism from respiratory secretions, 3) direct fluorescent antibody stain, 4) DNA probe, and 5) indirect fluorescent antibody technique.

Urinary antigen detection of lipopolysaccharide (LPS) by ELISA is a useful diagnostic tool, with a specificity of 100% and sensitivity of 70-100% (7,8). This test is particularly useful because it is often easier to obtain urine than adequate sputum in ill patients and results can be available within hours (5). Since 1979, the detection of specific antigen in urine has been described as a reliable, simple test (3,9), however, this has been limited to the detection of serogroups. The test presented here detects specific legionella antigen and recognizes all Legionella pneumophila serogroups (10).

The purpose of this study is to present legionella urinary antigen assay as a simple, rapid, available, and useful means of identifying patients infected with legionella pneumophila.

PATIENTS and METHODS

This study was a prospective, randomized, comparative, controlled study conducted at Masih Daneshvari hospital in Tehran.

Patients eligible for inclusion in the study met the following criteria: older than 18 years and hospitalized with a primary diagnosis of CAP, chronic obstructive pulmonary disease (COPD), or asthma. The following exclusion criteria were applied at baseline: pregnant women, antimicrobial therapy for 24–72 hours prior to the baseline visit, HIV-positive patients, suspected pneumocystis carinii pneumonia, a known infection with fungi or mycobacterium, receiving immunosuppressive therapy, cystic fibrosis, lung cancer, and any evidences of recent drug or alcohol abuse.

A sputum specimen was obtained from 118 eligible patients. It could be kept at 4ºC for 24 hours. The sputum specimens were cultured in routine bacteriological media. The standard medium for legionella isolation is Buffered Charcoal Yeast Extract (BCYE) agar supplemented with legionella selective supplement gentamycin, polymixin, vancomycin (GPV), gentamycin, polymixin, vancomycin, anisomycin (GPVA) and cycloheximide.

The antimicrobial agents prevent the overgrowth of legionella by competing organisms, while the optimal growth temperature is 35-37ºC and the organism requires 3 to 5 days to produce macroscopically visible colonies. Those that appear sooner than 3 days are not legionella colonies. Meanwhile, for each specimen, direct smear was performed, and stained by gram stain. The Gimnez stain is as rapid as the gram stain and stains the organism more effectively (5).

In addition, urine specimen was obtained from patients. It could be kept in 2-8ºC for 14 days. A lipopolysaccharide antigen of legionella is detectable in urine (5). Urine specimens were prepared and evaluated by legionella urine antigen standard kit.

Principle of urine antigen test: The strips of a microplate are coated with polyclonal antibodies which react with legionella pneumophila antigen of all serogroups as well as further legionella species. Patient urine is added to the wells of the microplate

and legionella antigen, if present, will bind to the specific antibody on the solid phase following the first incubation, then the wells are washed and peroxidase labeled antibody which react with legionella pneumophila antigen is added which binds to free binding sites on the antigen during a second incubation. After a further washing stage, the presence of bound peroxidase is demonstrated in a color reaction with a substrate. Adding sulphuric acid stops the reaction and the optical density (OD) is measured with a spectrophotometer at 450nm and a reference wavelength of 615-690 nm (11).

RESULTS

Totally, 118 patients were enrolled during the study period at Masih Daneshvari hospital with the mean age of 65.8 years. Most of the cases (58.5%) with acute respiratory infection were aged 58-77 years. Baseline demographic data are presented in table 1.

Table 1. *Demographic and clinical characteristics of patients*

Total number of patients	118
Male (%)	86(72.8)
Female (%)	32(27.1)
Signs and symptoms (%)	
Cough	90(76.2)
Shortness of breath	105(89.0)
Increased sputum	87(70.3)
Fever	55(46.6)
Chill	9(7.6)
Heart rate ≥ 95/min	47(39.8)
Respiratory rate ≥20 /min	90(76.3)
Respiratory rate ≥30/ min	28(23.7)

The most frequent underlying diseases were cardiac failure (10.2%), renal failure (1.7%), diabetes mellitus (1.7%), and weight loss (8.5%). Seventy-nine patients (66.9%) were smokers. The major risk factor for illness was being a working smoker man aged 69 years.

Twenty-one suspected colonies were identified in BCYE media through case finding, then we performed bacteriological analysis by differential media to prove the existence of these colonies, however, none revealed to be legionella.

Different pathogens were identified at baseline in our patients. The most common typical bacterial pathogens isolated from sputum specimens were streptococcus pneumonia (88%), candida spp. (76%), streptococcus β-hemolytic (61%), neiseria spp. (47.4%), staphylococcus aureus (40%), klebsiella pneumonia (27%), fungi (16%), E. coli (8.4%), and pseudomonas spp. (5%). Other isolated microorganisms which are not usually considered to be respiratory pathogens were: pseudomonas, E. coli, corynebacterium diphtheria, serratia marceseens, candida albicans, and aspergillus fumigates. None of the patients was subjected to invasive techniques for obtaining sputum samples.

Legionella urinary antigen testing was performed in our study patients and was positive in 3 cases (3 males).

DISCUSSION

CAP is a common health concern and its treatment is challenging since the growing incidence of antibiotic resistance among traditional respiratory pathogens and the increasing recognition of pneumonia caused by atypical respiratory pathogens (2,12).

Rapid diagnosis of the etiologic agents causing respiratory infections is difficult, and the treatment of patients with CAP is often empirical.

The most frequent typical isolated respiratory pathogen was streptococcus pneumonia (2,3,5,13).

We performed legionella urinary antigen test for all our studied subjects while it was positive in 3 (2.5%). This infection rate for legionella pneumophila was in agreement with Garbino (2), but lower than other series (1,14).

Availability and the easy use of the legionella urinary antigen test allow earlier outbreak identification; furthermore, the use of the urinary antigen test means that we are able to have laboratory confirmation on some cases on the day they first present to the physician.

In conclusion, our results demonstrated that, by urine antigen test, most of the cases could be identified and treated appropriately. The urinary antigen test can be a valuable tool in investigations and clinicians should be encouraged to use the legionella urinary antigen test. However, the test should be used in conjunction with culture of the respiratory specimens.

In summary, the urinary antigen test represents a significant improvement in diagnostic testing for legionnaires' disease (LD) over the IFA and DFA methods, but culture should always be performed regardless of other tests available.

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