LEGIONELLA PNEUMOPHILA ANTIGEN DETECTION IN URINE BY RAPID IMMUNOCHROMATOGRAPHIC TEST - IMPACT ON PATIENT OUTCOME

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ABSTRACT

BACKGROUND

Legionella species are non-sporing, gram-negative bacilli varying in length from short coccobacillary forms to longer filamentous forms. The clinical spectrum of Legionellosis comprises of two kinds of clinical manifestations - Legionnaires disease and Pontiac fever. The diagnosis can be made by various staining methods, culture, Direct Fluorescent Antibody (DFA), antigen detection in urine and body fluids and molecular detection.

The aim of this study was to determine the presence of legionella antigen in urine of patients with clinical suspicion of legionellosis.

MATERIALS AND METHODS

This is a retrospective study done in Department of Microbiology, Christian Medical College and Hospital, Ludhiana. A total of 126 urine samples from patients with suspected legionellosis were sent to the Microbiology Laboratory for detection of Legionella pneumophila antigen. The samples were processed by rapid immunochromatographic antigen detection test using BinaxNow urinary Legionella antigen detection kit and results interpreted as per kit instructions.

RESULTS

A total of 126 urine samples were tested, out of which 54 were from ICU and 72 from ward patients. Legionella antigen was detected in urine of 3 (2.4%) patients. All the patients tested positive were inpatients and 2 of these were from ICU.

CONCLUSION

The immunochromatographic tests like BinaxNow are rapid, easy and especially useful for immunocompromised patients and patients who do not produce adequate sputum for culture; therefore, can be applied for establishing rapid diagnosis in patients not responding to adequate antibiotic therapy and prevent complications.

KEYWORDS

Urinary Legionella Antigen Detection, BinaxNow.


BACKGROUND

Pneumonia is the most serious infection of the respiratory tract and one of the leading infectious causes of mortality and morbidity worldwide. Streptococcus pneumoniae still remains the most common cause of community acquired pneumonia, atypical respiratory pathogens account for 30 - 40% of these infections.[1] Atypical pneumonia is the infection of lower respiratory tract, but the classical symptoms of pneumonia are not present. The important pathogens causing atypical pneumonia are Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella pneumophila. As infections due to atypical pathogens do not present with classical signs and symptoms of pneumonia, they are often underdiagnosed and the patients are prone to develop associated complications like acute respiratory distress syndrome and respiratory failure.[2]

MATERIALS AND METHODS

This is a retrospective study done in Department of Microbiology, Christian Medical College and Hospital, Ludhiana from 1st December 2014 to 28th February 2016. A total of 126 urine samples from patients with suspected legionellosis were sent to the Microbiology Laboratory for detection of Legionella pneumophila antigen.

The samples were processed by rapid Immunochromatographic Test (ICT) for antigen detection using Binax Now urinary Legionella antigen detection kit and results interpreted as per manufacturer's instructions. A swab was dipped in the urine sample, removed and then inserted into the test card. Two drops of Reagent A were added and the card was closed, bringing the specimen into contact with the test strip. The positive and negative results were read visually in 15 minutes and were interpreted by the presence or absence of visually detectable pink to purple coloured lines. Detection of both control and patient lines was interpreted as positive and only control line was interpreted as negative for presence of legionellosis antigen in urine.[3]
Legionella species are slender gram negative bacilli measuring 0.3 μm to 0.9 μm in breadth and their length varies from short coccobacillary to longer filamentous forms. They are motile by means of one or more polar or sub-polar flagella, non-sporing organisms, slow growing and requires aerobic environment and nutritional enrichment.

The conventional culture medium used for isolation of Legionella species is Buffered Charcoal Yeast Extract (BCYEa) Agar medium. It contains yeast extract for nutrition, activated charcoal for removal of toxic oxygen radicals, ACES buffer and α-ketoglutarate. Biochemical characteristics helpful in identification include weakly catalase and peroxidase positive and assacharolytic. A positive hippurate hydrolysis test for L. pneumophila helps to differentiate it from most other species isolated in clinical specimen, which are hippurate negative.[4] Other methods of diagnosis includes Direct Fluorescent Antibody (DFA) staining (Sensitivity - 25 - 70%, Specificity - > 95%), antigen detection in urine (Sensitivity - 70 - 90%, Specificity - > 99%), serological testing (Sensitivity - 60 - 80%, Specificity - > 95%) and PCR (Sensitivity - 30 - 100%, Specificity - > 90%).[5]

Detection of Legionella antigen in urine samples is a rapid diagnostic method that provides an early detection of Legionella infection. Commercial kits based on principles of Radioimmunoassay (RIA) and Enzyme Immunoassay (EIA) methodologies and immunochromatographic assay are available with similar sensitivity and specificity. The major disadvantage with the immunochromatographic assays is their inability to reliably detect organisms other than L. pneumophila serogroup 1. Legionella antigenuria can be detected as early as 1 day after onset of symptoms and persists for days to weeks. Other samples that can also be used for detection of Legionella antigens include sputum, lung tissue, serum and pleural fluid, although the use of such samples has not been fully evaluated.[5]

The incidence of atypical pathogens in community acquired pneumonias was reported to be 22% globally and 20% in Asia in a meta-analysis by Bartlett J. G.[6,2] In the same study, the global and Asia incidence of Legionella pneumophila was reported to be 5% and 6% respectively. A study conducted in our institute based on serological examination of paired serum samples in acute and convalescent periods for antibodies against Mycoplasma and Chlamydia by ELISA has reported the total incidence of atypical pneumonia to be 34% with the presence of antibodies against Mycoplasma in 16.5% and Chlamydia in 17.6% samples, respectively.[7]

In another Asian study by Ngeow Y et al, Legionella pneumophila cases were found to be 62%.[8] A study conducted in a tertiary centre in South India that compared the results of cultivation of Mycoplasma pneumoniae and Legionella pneumophila, detection of Legionella antigen in urine and serological detection of Mycoplasma pneumoniae IgM and Chlamydia pneumoniae IgM antibodies reported the presence of IgM antibodies against Mycoplasma pneumoniae in 6.5% and Chlamydia pneumoniae IgM antibodies in 5.6% samples. All the samples were culture negative for Mycoplasma pneumoniae and Legionella pneumophila and also negative for the presence of Legionella antigen in urine.[2] In a systematic review by L. Peto et al, the weighted average of data reported for Legionella species in various Indian studies between 1987 - 88 and 2010 was reported to be 6%.[9]
A study in a tertiary care hospital in North India reported 17.69% urinary Legionella antigen positive cases by ELISA, but all were negative by BinaxNow antigen detection ICT kit.[10]

In this study, legionella antigen was detected in the urine of 2.4% patients with suspected legionellosis, which is less than other studies using ELISA for its detection. The limitation of this study is use of a single method for diagnosis as most of the studies have reported higher positivity rates with ELISA as compared to ICTs.

CONCLUSION
The diagnosis of legionella pneumonia is difficult due to non-specific clinical features and non-availability of reliable diagnostic methods. The immunochromatographic tests like BinaxNow are non-invasive, rapid and easy to perform and hence can be applied for establishing rapid diagnosis in patients not responding to adequate antibiotic therapy and immune-compromised patients, which can help in decreasing complications, improved outcome due to reduced morbidity and mortality and also in early detection of outbreak in hospital settings.

Since the prevalence of legionella infection is lesser than the other causes of atypical pneumonia like Mycoplasma, Chlamydophila infections, a negative test should prompt investigations to look for these organisms and also viral infections. Also such pathogens causing atypical pneumonia do not respond to the beta-lactam antibiotic agents, which are the mainstay empirical antibiotics; hence, diagnostic methods for detection of these organisms are required in tertiary care settings.

REFERENCES
3. Binax Now Legionella Kit Insert