CASE REPORT

Diagnosis of Legionnaires’ disease by urinary antigen and DNA detection: A case report

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Abstract

A 38-year-old male patient who was admitted to a private hospital in Kuala Lumpur presented with fever, symptoms of respiratory infection and diarrhoea. On admission, he was febrile, toxic looking, dehydrated with hypotension and tachycardia. No clinical signs of respiratory infection were detected on admission. Initially he was treated as a case of sepsicaemia with fluid therapy and intravenous antibiotic (Perfloxacin). Subsequently, he was noticed to have pneumonia in the right lower zone of the lung. His sputum, stool and blood were sent for culture and the results were negative. Sputum culture for Legionella and serological tests for Mycoplasma and Legionella were also reported negative. Sandwich ELISA performed on his urine sample detected Legionella pneumophila antigen. L. pneumophila mip gene was also detected in his urine by polymerase chain reaction. The patient was commenced on Erythromycin and he responded favourably to the treatment. The present case shows that L.pneumophila should not be overlooked as one of the causative agents of pneumonia and rapid techniques of urinary antigen and DNA detection should be utilized to make an early diagnosis of the infection.

Key words: pneumonia, Legionella pneumonia, urinary antigen, DNA, polymerase chain reaction.

INTRODUCTION

Legionella species may cause a wide range of infections in humans. The clinical manifestations of Legionella infections include pneumonia with or without extrapulmonary involvement known as Legionnaires’ disease, a non-pneumonic flu-like febrile illness also known as Pontiac fever and asymptomatic infections. Legionnaires’ disease varies in severity from a mild pneumonia to an adult respiratory distress syndrome accompanied by major extrapulmonary manifestations. Infections caused by Legionella pneumophila may occur sporadically as well as in well-defined epidemic clusters. The organisms are transmitted to human primarily through aerosolized particles originating from aquatic environment including cooling towers, humidifiers and decorative fountains\(^1,2\). Other modes of transmission include aspiration of contaminated water and direct inoculation by respiratory therapy equipment. However, direct person-to-person spread has not been documented.

The incidence of Legionnaires’ disease would be expected to vary with the degree of environmental exposure of the susceptible population to this pathogen. The incidence of the disease varies geographically\(^3\). Although they are very few reports of legionellosis occurring in Southeast Asia, Legionella has been shown to be present in the environment. In Singapore, environmental surveillance of L. pneumophila showed that 36% of cooling towers, 15-19% of decorative fountains and waterfalls and 2% of spa pools were positive\(^4\). In Malaysia, a survey on cooling towers in Kuala Lumpur showed that L.pneumophila serogroup 1 and 7 were the commonest serogroups isolated\(^5\). Studies have suggested that most of the pneumonia cases were caused by the L. pneumophila serogroup 1\(^6\). Therefore, the local community is exposed to the risk of infection caused by this organism. This paper reports a case of pneumonia caused by L. pneumophila or Legionnaires’ disease admitted to a private hospital in Kuala Lumpur.
CASE REPORT

A 38-year old male patient was admitted to a private hospital in Kuala Lumpur with the presenting symptoms of fever, vomiting and diarrhoea. The symptoms developed one day prior to admission. He also complained of cough and sore throat which developed two days earlier. Fever was associated with chills and cough was with expectoration of purulent sputum.

On clinical examination, he was febrile (39°C), toxic-looking with no anaemia. The lips were dry. The pulse rate was 114/min and blood pressure was 70/50 mm Hg. On admission, examination of the heart and lungs were normal. Abdominal and neurological examinations were also normal.

Initially he was treated in the high dependency ward for septicaemia with fluid therapy to correct the hypotension. The patient was given intravenous antibiotic (Perfloxacin). Since he did not improve, he was transferred to the intensive care unit. He was then noticed to have diminished breath sounds in the base of the right chest posteriorly. Chest x-ray confirmed the presence of pneumonia in the lower zone of the right lung. His sputum, stool and blood were sent for culture and sensitivity. All the culture results were negative. Serological tests for *Mycoplasma* and *Legionella* antibodies were requested and the results were also reported negative. Sputum culture for *Legionella* was also requested and the result was negative. Urine sample of the patient was collected and sent to the Institute for Medical Research for *Legionella* urinary antigen testing. *L. pneumophila* antigen was detected as positive in the patient’s urine by sandwich ELISA using in-house monoclonal antibodies. The results were also reported negative. Sandwich ELISA performed on the serum samples also detected *L. pneumophila* antigen. The levels of antigen were noted to be lower in the serum compared to urine. The results of the sandwich ELISA on the patient’s urine and serum samples are shown in Table 1. The urine and the first serum samples were collected on the fourth day after the onset of the illness, while the second serum sample was sent by the government hospital and was taken about two weeks after the onset of the illness. Bacterial genomic DNA was extracted from the patient’s urine sample using the Geneclean II Spin protocol (BIO 101, USA). The DNA was then used in the polymerase chain reaction (PCR) for amplification of the macrophage infectivity potentiator (*mip*) gene of *L. pneumophila* using a pair of specific primers. *L. pneumophila* (reference strain 11920) obtained from the PHL (Public Health Laboratory), Colindale, United Kingdom was used as a positive control. The PCR amplification gave a positive product of 630 bp size (Fig. 1). For confirmation of the gene, the PCR product was purified with the QIAquick PCR purification kit (Qiagen) and DNA sequencing was subsequently performed on the purified DNA by BigDye chemistry with the ABI Prism 377 Sequencer (PE Applied Biosystem). The DNA sequence analysis using the BLAST software demonstrated 99% identity with the *L. pneumophila* serogroup 1 in the GenBank database (Accession number AJ 496265).

DISCUSSION

The accurate diagnosis of *Legionella* pneumonia has important implications for treatment of the infection. Many first line antibiotics commonly used to treat typical bacterial pneumonia (i.e. beta-lactams) are ineffective against *Legionella* species. This is at least partially due to the fact that *Legionella* bacteria are intracellular pathogens. There is thus a great need for rapid diagnosis of legionella infections.
TABLE 1: Optical density (OD) values of four different monoclonal antibodies using sandwich ELISA

<table>
<thead>
<tr>
<th>Clinical sample</th>
<th>Mab 1F4.10E</th>
<th>Mab 1C7.2B</th>
<th>Mab 2B2.11E</th>
<th>Mab 2B2.10F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>2.007</td>
<td>0.464</td>
<td>0.493</td>
<td>0.490</td>
</tr>
<tr>
<td>First serum</td>
<td>0.541</td>
<td>0.629</td>
<td>0.418</td>
<td>0.466</td>
</tr>
<tr>
<td>Second serum</td>
<td>0.428</td>
<td>0.381</td>
<td>0.338</td>
<td>0.365</td>
</tr>
</tbody>
</table>

Mab- monoclonal antibody

Cut-off OD values for urine:  
- 1F4.10E  0.286
- 1C7.2B  0.282
- 2B2.11E  0.133
- 2B2.10F  0.138

Cut-off OD values for serum:  
- 1F4.10E  0.212
- 1C7.2B  0.380
- 2B2.11E  0.281
- 2B2.10F  0.540

FIG 1: PCR of *mip* gene of *L. pneumophila*. Lane 1- 100 bp ladder marker, lanes 2 &3- positive control, lanes 4&5- duplicate of DNA extracted from patient’s urine sample.
Currently, culture is regarded as the “gold standard” for detection of Legionellae. However, culture of Legionella bacteria has inherent problems. There is a broad range (50-99%) of reported sensitivity of culture in the literature\(^9,10\). This discrepancy is most likely due to differences in the types and qualities of the specimens used in each study. Collection of specimens in saline (bronchoalveolar specimens, bronchial washes) lowers the sensitivity of culture because saline inhibits the growth of Legionellae\(^10\). Sputum samples may be contaminated with oropharyngeal organisms thus masking the presence of Legionella spp. Another contribution to a lowered sensitivity of culture is the use of samples collected from patients being treated with antibiotics.

Due to delay in detecting rising titres of Legionella antibody with respect to the onset of the illness, diagnosis should not rely mainly on antibody detection. Therefore, newer methods including antigen detection in serum and urine have been developed to improve the diagnosis. Detection of soluble antigen can be done using various immunological methods with either monoclonal or polyclonal antibodies. Monoclonal antibodies can detect specific antigen, and therefore serve as an excellent reagent for specific diagnosis of this bacterial infection. Some of the methods have been commercialized. Among the commercially available methods, ELISA and RIA (radioimmunoassay) have demonstrated high sensitivity and specificity\(^11\). A subsequent study demonstrated that immunochromatography gave higher sensitivity and specificity\(^12\). In this study, four in-house monoclonal antibodies used in the antigen detection assay were produced by hybridoma technology following the method of Kohler and Milstein\(^13\). All of the monoclonal antibodies were of IgM class. Characterization by the indirect ELISA, sandwich ELISA and immunoblotting showed that the monoclonal antibodies had specific reactions with antigens of L. pneumophila. Monoclonal antibody 1F4.10E reacted with a specific protein antigenic fraction of a reference strain of L. pneumophila (Knoxville 1) with approximate molecular weight size of 33.0 kDa, while others recognized non-protein epitopes since immunoblot primarily detected protein antigens. The cut-off value for each of the monoclonal antibodies was determined by running the test on control serum and urine samples collected from healthy staff of the Institute for Medical Research and blood donors. The values above the cut-off levels of the monoclonal antibodies were considered positive. The in-house sandwich ELISA using the four monoclonal antibodies detected L. pneumophila antigens in the urine and serum samples of the patient. The ELISA values were noted to be higher in the urine compared to serum, particularly for the monoclonal antibody 1F4.10E. A previous study also reported higher antigen levels in urine than in serum\(^14\). The PCR carried out on the DNA extracted from the patient’s urine gave a positive amplification product of the L. pneumophila mip gene of 630 bp size and was confirmed by DNA sequencing. With the absence of positive culture result, PCR is useful to support the diagnosis of Legionella infection\(^15\). Erythromycin is the antibiotic recommended for treatment of Legionella infection\(^8\). The patient showed clinical improvement after intravenous Erythromycin was instituted.

In view of the reported presence of L. pneumophila in our local environment\(^2\), the present case reminds us that L. pneumophila should be considered as one of the causative agents of pneumonia, particularly if the patient does not respond to the conventional antibiotic treatment for typical pneumonia. Besides culture, rapid techniques including urinary antigen detection and PCR should also be utilized to make an early diagnosis of the infection and thus appropriate treatment can be instituted.

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