NOTES

Detection of *Legionella* DNA in Peripheral Leukocytes, Serum, and Urine from a Patient with Pneumonia Caused by *Legionella dumoffii*

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The polymerase chain reaction (PCR) has been used to detect *Legionella* DNA in respiratory tract, serum, and urine samples from patients with pneumonia. In addition, a preliminary study using a guinea pig model suggested that testing of peripheral leukocytes by PCR may be more sensitive than testing of other samples. We used PCR to detect *Legionella* DNA in serial peripheral leukocyte (buffy coat), serum, and urine samples from a patient with pneumonia caused by *Legionella dumoffii*. *Legionella* DNA was detected in all 3 sample types when first collected. Buffy coat and urine samples remained positive up to 56 days after the onset of symptoms, whereas serum samples were positive from 10 up to 16 days after the onset of symptoms. Sequencing of PCR amplicons indicated the presence of *L. dumoffii* DNA in positive samples. It appears that buffy coat may be a useful sample to test for *Legionella* DNA, but further study is required to determine the precise sensitivity and to make comparisons with other sample types.

*Legionella* pneumonia can be difficult to diagnose. Clinical and radiographic features are indistinguishable from those of other causes of pneumonia, and conventional laboratory tests either lack specificity or provide a retrospective diagnosis only. Recently, PCR has shown promise as a rapid diagnostic test for legionellosis. PCR has been used to detect *Legionella* DNA in bronchoalveolar lavage fluid [1], serum [2, 3], urine [3, 4], and throat swab samples [5] from patients with pneumonia. In addition, we have shown in a guinea pig model that testing of peripheral leukocytes is more sensitive than testing of urine or plasma samples [6]. We describe the detection of *Legionella* DNA in peripheral leukocytes (buffy coat), in addition to serum and urine from a patient with pneumonia due to *Legionella dumoffii*.

Case Report

An 80-year-old man presented with a 1-week history of shortness of breath, fever, right-sided pleuritic chest pain, and hemoptysis. Physical examination and chest radiographs revealed changes consistent with an extensive pneumonia involving the right lung. Cultures of blood were sterile. Sputum collected on admission and cultured on modified Wadowsky-Yee medium yielded *L. dumoffii* after 3 days. The patient’s condition steadily improved with antimicrobial therapy (initially amoxicillin/clavulanate 1.2 g every 8 h; then clarithromycin 500 mg every 12 h after the sputum culture results became available), and when reviewed 7 weeks after admission his symptoms had almost completely resolved and another chest radiograph showed clear lung fields.

Methods

We used a PCR assay to test urine, serum, and leukocyte (buffy coat) samples collected during the illness for *Legionella* DNA. The *Legionella* isolate cultured from sputum was suspended in distilled water at a concentration of ~10⁹ cfu/mL and tested in a similar manner. The PCR system we used is described elsewhere [6]. In brief, DNA was extracted from samples (200 μL urine, 100 μL serum or leukocytes [buffy coat], or 50 μL bacterial suspension) by use of phenol and chloroform, then a 104-bp segment of the *Legionella* SS rRNA gene was amplified. Restriction enzyme analysis confirmed that PCR amplicons were specific for *Legionella* species. Direct sequencing was used to compare the amplified PCR products obtained from each sample [7]. *Legionella pneumophila* serogroup 1 (ATCC 33153) was grown on buffered charcoal yeast extract agar, serially diluted with distilled water, and added to urine, serum, and leukocyte samples from an uninfected human control. The inocula were determined by mea-
measurement of optical density and confirmed by colony counts after subculture to solid agar. These samples were then tested by PCR to determine the analytical sensitivity of the assay. Total genomic DNA from \textit{L. pneumophila} serogroup 1 (ATCC 33153) was added to a urine sample known to be negative and served as a positive control in each DNA amplification run. False positive PCR results were monitored by inclusion of a known negative urine control in each DNA amplification run. \textit{L. pneumophila} serogroup 1 (ATCC 33153) was added to any samples that tested negative by PCR and amplified again to test for the presence of PCR inhibitors.

\textbf{Results}

Table 1 summarizes the PCR, culture, and serology results. The \textit{L. dumoffii} suspension also tested positive. All samples that tested negative by PCR returned positive results after the addition of the stock strain of \textit{L. pneumophila}, which suggested that PCR inhibitors were absent. The PCR assay was capable of detecting $\sim 10$ to $100$ cfu/mL of \textit{L. pneumophila} in each sample type.

Amplicons from urine samples taken on days 16 and 56 failed to produce readable sequences, despite repeated attempts. Another 3 samples showed inconsistent results for 2–4 bases at either end of the 5S rRNA amplicon, but otherwise showed 100% homology with the GenBank 5S rRNA sequence of \textit{L. dumoffii} (accession number X72349), including the central variable region. The remaining sequences, including those from the sputum isolate, showed 100% homology with the GenBank sequence.

\textbf{Discussion}

This report documents the detection of \textit{Legionella} DNA in clinical samples collected from a patient 10 days after the onset of pneumonia to several weeks after resolution and, to our knowledge, is the first to include the testing of human peripheral leukocytes. Although the discriminatory power of the genotyping method used is only moderate [7], the results suggest the presence of the same \textit{Legionella} species in all clinical samples.

These results are consistent with our previous work. In a retrospective study, we detected \textit{Legionella} DNA in urine and serum samples from shortly after the onset of pneumonia and, occasionally, for several weeks thereafter [3]. Although we have since modified our PCR methods to optimize DNA extraction and detection, when we retested all the samples from the original study with the new methodology, we obtained identical results (D. Murdoch, unpublished data); this suggests that the PCR analysis has the same clinical sensitivity and specificity as the older method.

From the present report it appears that testing for \textit{Legionella} DNA in peripheral leukocytes is worthy of further investiga-

\begin{table}[h]
\centering
\caption{Results of \textit{Legionella} PCR and other tests according to sample type and collection time.}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Days after onset of symptoms & \textit{Legionella dumoffii} isolated from sputum & PCR assay & Antibody to \textit{L. dumoffii} \((\text{titre}^a)\) \\
& & Serum & Buffy coat & Urine & \\
\hline
7 & + & ND & ND & ND & ND \\
10 & ND & + & + & ND & 1 : 64 \\
11 & ND & ND & + & + & ND \\
16 & ND & + & + & + & ND \\
56 & ND & ND & ND & ND & > 1 : 2048 \\
86 & ND & ND & ND & ND & > 1 : 2048 \\
\hline
\end{tabular}
\begin{tablenotes}
\item \textbf{NOTE.} +, positive; -, negative; ND, no data or not determined.
\item \textsuperscript{a} Measured by the indirect immunofluorescent antibody test.
\end{tablenotes}
\end{table}

The use of leukocyte samples is also supported by other data [6]. In a guinea pig model, \textit{Legionella} DNA was detected in peripheral leukocytes with greater sensitivity than in plasma or urine; when testing all 3 samples simultaneously, the testing of leukocytes contributed significantly to the overall sensitivity of PCR to diagnose \textit{Legionella} infection.

The precise sensitivity of PCR for testing different sample types (including leukocytes) is yet to be determined. Whether disease severity and isolation of \textit{Legionella} from sputum are associated with PCR positivity is also unknown. Further study testing samples from many patients with \textit{Legionella} pneumonia is required to clarify these issues.

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\textbf{References}