Rapid Detection of *Streptococcus pyogenes* in Pleural Fluid Samples from Pediatric Patients with Empyema


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A total of 120 pleural fluid specimens from 113 pediatric patients were tested using two rapid antigen detection assays for *Streptococcus pyogenes*. Results were compared to culture, Gram stain, and PCR results. Each rapid antigen assay detected 9 out of 10 (90%) PCR-positive samples, with 100% specificity. These antigen detection assays are useful to provide microbiological diagnosis of empyema caused by *S. pyogenes*.

Empyema is a common complication of bacterial pneumonia in children. Based on culture, *Streptococcus pneumoniae* is the leading cause of empyema, followed by other Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus pyogenes*, with the latter often reported to be the second leading bacterium (2, 5, 7).

Since bacterial culture positivity rates for pediatric pleural fluid samples are low (1, 7, 10), probably because of antibiotic therapy prior to thoracentesis, non-culture-based rapid laboratory methods may assist in the establishment of an etiological diagnosis to help direct patient management. Rapid antigen assays are widely used for detection of *S. pyogenes* in throat specimens. We have tested pleural fluid specimens from children with complicated pneumonia with two rapid antigen tests, culture, Gram stain, and PCR for *S. pyogenes*.

A total of 120 pleural fluid specimens from 113 pediatric patients with empyema that were submitted for routine bacterial culture were included in the study. Gram stain, bacterial culture, and organism identification were performed using routine methods in the clinical microbiology laboratory. Specimens were stored at −70°C for further testing.

*S. pyogenes* antigen detection was performed using two rapid antigen assays: the QuickVue + streptococcus A test (Quidel Corporation, San Diego, CA) and the Directigen EZ group A streptococcus test (Becton, Dickinson and Company, Sparks, MD). To perform the assay with liquid specimens, swabs (included in each test kit) were dipped in pleural fluid. Swabs were then processed in the same way as recommended for throat swabs.

Nucleic acids were extracted with the MagNA Pure instrument (Roche Applied Science, Indianapolis, IN). Real-time PCR was performed using analyte-specific reagents (ASRs) from Roche Diagnostics (Indianapolis, IN) targeting *S. pyogenes* *ptsI* (encoding phosphotransferase system enzyme I) and the Roche LightCycler instrument (11). The analytical sensitivity is 20 copies per reaction.

Overall, 18 of the 120 specimens (15%) tested were positive for culture by a likely pathogen. Organisms identified included *S. pneumoniae* (7), *S. aureus* (2), *S. aureus* and *Haemophilus influenzae* (1), *H. influenzae* (1), *S. pyogenes* (1), *Moraxella catarrhalis* (1), and *Pseudomonas aeruginosa* (1). In addition, *Staphylococcus epidermidis* (1), *Streptococcus mitis* (1), *Propionibacterium acnes* (1), and *Peptostreptococcus* species (1) were isolated.

Gram stain was positive for bacteria in 11 specimens, including 9 that were culture positive: *Streptococcus pneumoniae* (6), *Hae-

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TABLE 1 Isolation, Gram stain, S. pyogenes PCR, and rapid antigen test results for 120 pleural fluids from pediatric patients

<table>
<thead>
<tr>
<th>Test result</th>
<th>No. of samples</th>
<th>Bacterial isolation</th>
<th>Gram stain</th>
<th>PCR for S. pyogenes</th>
<th>Antigen detection by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>QuickVue&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>streptococcus A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Directigen EZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>group A streptococcus</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>1</td>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Others&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>102</td>
<td>109</td>
<td>110</td>
<td>111</td>
<td>111</td>
</tr>
</tbody>
</table>

<sup>a</sup> Gram-positive cocci. This sample was negative by culture but positive by both antigen and PCR for S. pyogenes.

<sup>b</sup> Two S. aureus isolates, one each of H. influenzae, a mixture of H. influenzae and S. aureus, Pseudomonas aeruginosa, M. catarrhalis, Staphylococcus epidermidis, Streptococcus mitis, Propionibacterium acnes, and Peptostreptococcus species.

<sup>c</sup> H. influenzae, M. catarrhalis, and Peptostreptococcus species. One was culture negative.

and storage conditions. In a recent report from Canada, only 32% of patients with empyema had a microbiologic diagnosis, even after combining results of culture from pleural fluid, blood, and lung tissue (5). Another study reported similar data in that only 35% of blood and/or pleural fluid specimens of patients with empyema were positive by culture (1). Overall, bacterial culture positivity of patients with empyema ranges from 20 to 60% depending on study settings (6). In the present study, only 15% of specimens were positive by culture.

S. pyogenes is an important cause of empyema. Despite the relatively insensitive nature of culture methods used in most studies, S. pyogenes has been isolated as often or more often than S. aureus, making it the second leading bacterial pathogen following S. pneumoniae (1, 4, 5). In addition to having relatively low recovery rates, culture requires at least 1 to 2 days to achieve results. More-sensitive and more-rapid non-culture-based assays are needed for this sample type. Molecular assays have been shown to be helpful (1, 6). Although there is a lack of standardization for most bacterial targets, the S. pyogenes PCR assay used in this study appears to be useful for detecting S. pyogenes in pleural fluid. Rapid antigen assays, including latex agglutination and immunochromatographic testing, have been used for detecting S. pneumoniae (4, 6, 7, 9). To date, there is no report describing the utility of rapid antigen assays for diagnosis of empyema caused by S. pyogenes. In the present study, using PCR as a reference method, the two antigen assays for S. pyogenes demonstrated substantially higher assay sensitivity (90% for each) than the 10% by culture and Gram stain. Due to the relatively small sample size of the current study and the fact that most rapid antigen assays for S. pyogenes on throat samples have sensitivities of about 80%, a study of a larger number of samples would likely help verify the antigen assay’s sensitivity.

Since the antigen assays are rapid and very specific with high positive predictive value and S. pyogenes antimicrobial susceptibility is relatively predictable, a positive result can be helpful to assist treating physicians in selection of appropriate antibiotics. Since the assays are easy to perform and results can be obtained in less than 15 min, they are suitable for most laboratories to perform.

In summary, we found that a greater proportion of pediatric empyema cases are caused by S. pyogenes than indicated by culture and/or Gram stain; non-culture-based assays, i.e., PCR and rapid antigen tests, are much more sensitive and may provide useful etiological information for diagnosis.

REFERENCES


