Direct Identification of *Streptococcus pneumoniae* Capsular Types in Pleural Fluids by Using Multiplex PCR Combined with Automated Fluorescence-Based Capillary Electrophoresis

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Methods for rapid and reliable serotyping of *Streptococcus pneumoniae* not based on bacterial culture are needed in the epidemiological surveillance of pneumococcal infection. Recently, we developed a multiplex PCR scheme combined with fragment analysis and automated fluorescent capillary electrophoresis (FAF-mPCR) to identify 40 serotypes/serogroups from clinical isolates (1). In the present study, we report the performance of this assay directly on clinical samples from culture-negative patients. The development of PCR-based serotyping assays performed directly on clinical samples that are not required to contain viable bacteria has the potential to overcome some of the difficulties associated with these microbiological diagnostic procedures that can perform poorly, especially in pediatric patients (2).

This study retrospectively evaluated 98 consecutive pleural fluid (PF) samples from pediatric patients who had been diagnosed with invasive pneumococcal disease (IPD) from January 2010 to December 2011 at the University Hospital Sant Joan de Deu (Barcelona, Spain) and University Children’s Hospital of Zürich (Zürich, Switzerland). DNA had been extracted from 200 µL of PF by using a MagNA Pure compact instrument (Roche Applied Science) according to the manufacturer’s instructions. Detection of pneumococcal DNA in PF had been performed as described previously based on PCR of pneumolysin (*ply*) and pneumococcal capsular *wzg* genes (both had to be simultaneously positive to confirm any case as a positive pneumococcal infection) (2) and subsequent capsular typing of *S. pneumoniae* DNA-positive samples (3). High bacterial loads had been detected in 71 samples (threshold cycle \( C_T \) value for the *ply* gene, \( \leq 30 \)), while the remaining 27 had lower bacterial loads (\( C_T \) value for the *ply* gene, \( >30 \)) (Table 1). The remaining DNA extracts were stored at \( \pm 80^\circ \text{C} \). Informed consent was obtained by researchers from the two hospitals who were in charge of routine bacteriological surveillance of pneumonia.

FAF-mPCR is composed of only three multiplex fluorescent PCRs for the specific detection of 40 serotypes (1), and each reaction includes detection of the *cpsA* gene common to all pneumococcal serotypes, which was used as the internal control. The PCR amplification protocol and fluorescent fragment size analysis were as described in the published assay (1), except for the number of amplification cycles, which was increased to 35.

Among the 98 PF samples positive for *S. pneumoniae* DNA, the *cpsA* gene (internal control) was detected in 74 samples and all of them were correctly serotyped with the FAF-mPCR. Among the remaining 24 samples, no *cpsA* amplification was detected after 35 cycles of amplification; of these 24 samples, 23 (95.8%) had \( C_T \) values of \( >30 \) in the previous diagnostic real-time PCR assay and only one sample had a \( C_T \) value of \( \leq 30 \) (this sample had been serotyped as 9V/A/N/L with the multiplex real-time PCR). The congruence between FAF-mPCR and multiplex real-time PCR results based on the Wallace coefficient was 0.998 (0.995 to 1.000) in those samples with \( C_T \) values of \( \leq 30 \). The results of FAF-mPCR and multiplex real-time PCR were in complete agreement for the 24 capsular types detected by both methods in the 74 samples that had positive detection of *cpsA*. Moreover, two additional capsular types were identified by FAM-mPCR in two samples in which the capsular type had not been identified with the multiplex real-time PCR assay (Table 1).

In conclusion, these results indicate that FAF-mPCR could be used for pneumococcal capsular typing directly on clinical samples with high bacterial loads (\( C_T \) value for *ply* by real-time PCR, \( \leq 30 \)), even in culture-negative patients.

### Table 1

<table>
<thead>
<tr>
<th>( C_T ) for <em>ply</em> (no. of <em>ply</em>-positive samples/total no.)</th>
<th>Serotype detected (no. of samples) by:</th>
<th>Multiplex real-time PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \leq 30 ) (71/98)</td>
<td>1 (19), 3 (28), 4 (1), 5 (2), 14 (2), 19A (9), 7F/A (5), 9V/A (1), unknown (1)</td>
<td>1 (19), 3 (28), 4 (1), 5 (2), 14 (2), 19A (9), 7F/A (5), 9V/A/N/L (2), unknown (3)</td>
</tr>
<tr>
<td>( &gt;30 ) (27/98)</td>
<td>3 (3), 7F/A (1)</td>
<td>1 (1), 3 (8), 19A (2), 6A (1), 7F/A (3), unknown (12)</td>
</tr>
</tbody>
</table>

* a unknown, serotype could not be identified by FAF-mPCR as one of the 40 serotypes or by multiplex real-time PCR as one of the 24 serotypes, respectively.

b Serotype detected by FAF-mPCR but not present in the multiplex real-time PCR.
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REFERENCES

