Validation of Factor 6d Antiserum for Serotyping \textit{Streptococcus pneumoniae} Serotype 6C$^\dag$

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Factor 6d antiserum reacts with the new \textit{Streptococcus pneumoniae} serotype 6C. Serogroup 6 isolates, consisting of 49 6A, 42 6B and 98 6C strains from the United States and Israel, serotyped in parallel by PCR and capsular swelling methods, were all identified correctly. The new factor 6d antiserum accurately identifies serotype 6C.

Until recently there were 90 recognized distinct pneumococcal serotypes, with serogroup 6 represented by two serotypes, 6A and 6B. The serogroup 6 capsular \textit{wciP} gene loci differ from each other by only 1 nucleotide, and this difference produces 6A \textit{WciP} with a serine at residue 195, while 6B \textit{WciP} has an asparagine at the same residue (2). In 2007, however, a 91st serotype was described: serotype 6C (15, 16). Serotype 6C cross-reacts serologically with serotype 6A and was initially differentiated from serotype 6A by using monoclonal antibodies and shown to have a different capsular polysaccharide from that of serotype 6A (16). The same investigators then characterized the genetic basis of the new serotype as having a new \textit{wciN} gene region of the capsular locus encoding glucosyltransferase, replacing the original galactosyltransferase (15). Furthermore, they found that this change was present in strains isolated up to 27 years ago and postulated that the 6C capsular type originated more than 27 years ago from a single recombination event in a 6A locus, in which 6A \textit{wciN} was replaced by a gene of unknown origin. Serotype 6C has subsequently been recognized in several countries and is an important replacement serotype, following the introduction of a conjugate vaccine (3–10, 12–14). Recent U.S. surveillance showed a significant 164% increase in the prevalence of invasive disease due to serotype 6C, increasing from 0.22 cases per 100,000 in 1999 to 0.57 and 0.58 cases per 100,000 in 2006 and 2007, respectively, while rates of invasive disease due to serotypes 6A and 6B markedly decreased (3).

The investigators who described serotype 6C also postulated that the same change that transformed serotype 6A into serotype 6C would transform serotype 6B into a new serotype (2). Although they were initially unable to detect this change among 264 serotype 6B strains examined, a strain with this change was produced experimentally. Naturally occurring serogroup 6 strains with this change have recently been detected in 14 of 34 nasopharyngeal isolates collected from Fijian children between 2004 and 2007, as well as in 2 of 14 nasopharyngeal isolates collected from Korean children in 2008; these strains have been designated the new putative serotype 6D (1, 9). Initial findings with monoclonal antisera indicate that serotype 6D can be differentiated from the other serogroup 6 serotypes serologically (1, 2). Reactions of serotype 6D with commercially available polyvalent antiserum have not yet been characterized.

The absence of commercially available serotyping reagents has limited the detection of serotypes 6C and 6D. Recently, however, a serotype 6C-specific antiserum, factor 6d, was developed by Statens Serum Institut, Copenhagen, Denmark. This report describes the validation of the new factor serum 6d in two laboratories. Isolates of serogroup 6 from various strain collections were recovered from frozen storage and tested by PCR, as described by Park et al. (15), and the standard serotyping method using the capsular swelling reaction (11).

Serotyping by the capsular swelling method was performed using the standard antisera for serogroup 6, consisting of group 6, factor 6b and factor 6c antisera, as well as the newly introduced factor 6d antiserum (Statens Serum Institut). All serogroup 6 isolates are identified by the group 6 antiserum, with serotype 6A being additionally positive with factor 6b, serotype 6B with factor 6c, and serotype 6C with factors 6b and 6d. Serotyping by PCR of DNA extracts was performed using two forward primers, 5101, which attaches to nucleotides 6949 to 6966 in the gene \textit{wciN}, and 5106, which attaches to nucleotides 5897 to 5916 in gene \textit{wchA}, and a common reverse primer, 3101, which attaches to nucleotides 7888 to 7905 in gene \textit{wciO} (15). Primer pair 5101-3101 produces products of 958 or 1,267 bp with serotypes 6A and 6B, while no product is produced with serotypes 6C or 6D. Primer pair 5106-3101 produces \textit{wciN} PCR products of 2.0 or 2.3 kb with serotypes 6A and 6B and 1.8 kb with serotypes 6C and 6D (2, 9). PCR was performed as a simplex reaction with the two primer pairs or as a multiplex reaction with all three primers.

One-hundred eighty-nine serogroup 6 isolates, originating from several surveillance collections in the United States and Israel, were tested (Table 1). Serotypes identified by capsular swelling reactions with serogroup 6 factor antisera were 49 serotype 6A, 42 serotype 6B, and 98 serotype 6C. PCR ampli-
TABLE 1. Results of PCR amplification and capsular swelling reactions with serogroup 6 factor antisera for 189 isolates of S. pneumoniae

<table>
<thead>
<tr>
<th>PCR primer set (product size [bp])</th>
<th>No. of isolates with capsular swelling reaction with factor antiseruma</th>
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<tbody>
<tr>
<td></td>
<td>6b only</td>
</tr>
<tr>
<td>5101-3101 (958)</td>
<td>48</td>
</tr>
<tr>
<td>5101-3101 (1,267)</td>
<td>1</td>
</tr>
<tr>
<td>5106-3101 (1,800)</td>
<td>—</td>
</tr>
</tbody>
</table>

a All isolates reacted with serogroup 6 antiserum, while additional reaction to factor antiserum indicate the following: 6b, serotype 6A; 6c, serotype 6B; 6b and 6d, serotype 6C. —, no isolates had these characteristics.

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REFERENCES