New Data Letter

*Streptococcus pneumoniae* serotype 6D cross-reacting with serotypes 6A, 6B, and 6C factor sera

Running title: Pneumococcal serotype 6D

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We read the papers of Bratcher and Nahm (3) and Oftadeh et al. (9) with interest. Both papers reported that *Streptococcus pneumoniae* serotype 6D isolates react with factor antiserum specific to serotype 6C in addition to that to 6B. Pneumococcal serotype 6C, first reported in 2007, cross-reacts serologically with serotype 6A but is differentiated by a change in the *weIN* gene (*weINβ*) among the *cps* loci (8). In addition to serotype 6C, the novel serotype 6D has recently been reported in several countries (2, 4, 6, 7). In serotype 6D, it is assumed that *weINβ* is inserted into serotype 6B *cps* loci (2). Thus, it can be speculated that serotype 6D isolates react simultaneously with the factor sera 6c and 6d, which are bound to the serotypes 6B and 6C, respectively, which was confirmed by recent papers (3, 9).

Recently, we found serotype 6D pneumococcal isolates reacting with factor sera 6b (both absorbed and unabsorbed), which is specific to serotype 6A. As a part of a multinational study on invasive pneumococcal infections, we have collected 244 *S. pneumoniae* serogroup 6 isolates from 11 Asian countries including Korea, India, Japan, Hong Kong, Malaysia, the Philippines, Saudi Arabia, Sri Lanka, Taiwan, Thailand, and Vietnam during 2008 and 2009. Serotypes were determined using a conventional Quellung method (Statens Serum Institute, Copenhagen, Denmark). In addition, serotype-specific PCR method of Jacobs et al. (4) was used to identify serotypes 6C and 6D.

Among the 244 invasive *S. pneumoniae* serogroup 6 isolates, 73, 144, and 13 pneumococcal isolates were identified as serotypes 6A, 6B, and 6C, respectively (Table 1). Fourteen isolates could be identified as serotype 6D because they reacted with the factor sera 6c and 6d, and produced 1.8 kb products with the 5106-3101
primer pair set in PCR. This product indicates that the wciN gene of serotype 6B was
substituted by wciNβ gene. These S. pneumoniae serotype 6D isolates (13 from Korea
and one from Taiwan) were isolated from sputum, tracheal aspirates, and blood.
However, these isolates also reacted with factor serum 6b specific to serotype 6A
(Table 1). The 14 pneumococcal isolates reacted simultaneously with factor sera 6b, 6c,
and 6d.

To our knowledge, no pneumococcal isolates reactive with all the factor sera have
hitherto been reported. Oftadeh et al. (9) reported that serotype 6C isolates reacted with
unabsorbed factor serum 6b and did not with absorbed serum and that serotype 6D
isolates did not react with both absorbed and unabsorbed sera. However, our serotype
6D isolates reacted with both absorbed and unabsorbed factor sera 6b. It has been
suggested that serotype 6D emerged by substitution of the wciN gene by the wciNβ gene
from serotype 6B isolates (1, 2). However, the present result may indicate that serotype
6D did not arise simply by such a substitution. Even cps loci other than wciNβ gene of
serotype 6D may differ from those of serotype 6B, which is supported by the finding
that the results of PCR using the primer set 5101-3101, targets wchA and wciO,
differed between two serotypes. The structure of the cps locus in serotype 6D is now
under investigation.

In addition, the finding concerning the presence of S. pneumoniae isolates cross-
reactive with serotypes 6A, 6B, and 6C factor sera indicates that serotype 6D isolates
may exist in pneumococcal isolates originally identified as serotype 6A. Although it is
unknown if there is cross-protection for serotype 6D from serotype 6B which is in the
7-valent, 10-valent and 13-valent vaccines (PCV7, PCV10 and PCV13, respectively),
or for serotype 6D from serotype 6A which is in PCV13, an exact determination of the serotypes of clinical isolates is important with respect to vaccination.
References


Table 1. Results of Quellung reactions and PCR.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. of isolates (n=244)</th>
<th>Reaction with factor serum&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PCR product&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6b  6c  6d</td>
<td>5101-3101  5106-3101</td>
</tr>
<tr>
<td>6A</td>
<td>73 (29.9%)</td>
<td>+  -  -</td>
<td>958 or 1,267 bp</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.0 or 2.3 kb</td>
</tr>
<tr>
<td>6B</td>
<td>144 (59.0%)</td>
<td>-  +  -</td>
<td>958 or 1,267 bp</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.0 or 2.3 kb</td>
</tr>
<tr>
<td>6C</td>
<td>13 (5.3%)</td>
<td>+  -  +</td>
<td>No product</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.8 kb</td>
</tr>
<tr>
<td>6D</td>
<td>14 (5.7%)</td>
<td>+  +  +</td>
<td>No product</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.8 kb</td>
</tr>
</tbody>
</table>

<sup>a</sup>+, positive reaction; -, negative reaction.

<sup>b</sup>Method of Jacobs et al. (5)