Molecular Discrimination between Neisseria meningitidis Serogroups W-135 and Y Based on the Nucleotide Recognition Domain Sequence of the Capsule Polymerases

Pathogenic Neisseria meningitidis isolates in most cases express one of five capsular polysaccharide-defined serogroups, i.e., A, B, C, W-135, and Y. The capsular polysaccharides of serogroups W-135 and Y are composed of repetitive disaccharide units of sialic acid linked to galactose and glucosamine, respectively (1). The capsule polymerases and the encoding genes synF and synG (also referred to as siaD<sub>W-135</sub> and siaD<sub>Y</sub>, respectively) are closely related (5). We recently demonstrated that a single amino acid at position 310 in the N-terminal glycosyltransferase domain of the capsule polymerases is responsible for substrate specificity toward UDP-galactose or UDP-glucose (4). Amino acid 310 is part of the EX<sub>4</sub>E motif characteristic for the nucleotide recognition domain within glycosyltransferases (6). 310P determines the serogroup W-135 capsule, and 310G determines the serogroup Y capsule. Others furthermore demonstrated that the capsule polymerase of strains that expressed a mixed galactose/glucose-sialic acid polysaccharide were associated with 310S (11).

As part of disease surveillance, nonculture diagnosis is necessary in patients who received antimicrobial agents prior to admission. The DNA sequences of synF and synG provide the basis for several diagnostic assays (2, 7–10). However, most of these assays are hampered by the fact that they rely on polymorphic sites that are functionally irrelevant.

Here, we describe the discrimination of serogroups W-135 and Y based on amino acid 310 within the capsule polymerases. The test was validated using 300 meningococcal isolates derived from invasive disease (<i>n</i> = 160), pneumonia or meningococcal meningitis (<i>n</i> = 7), and asymptomatic carriage (<i>n</i> = 133). Invasive strains were obtained from the German reference laboratory for meningococci. Carriage strains were isolated during the Bavarian meningococcal carriage study (3). By slide agglutination with antisera for identification of Neisseria meningitidis W-135 and Y (Oxoid, Wesel, Germany), 87 serogroup W-135 strains and 210 serogroup Y strains were identified. Three strains reacted with both antisera. For nonculture diagnosis, a 700-bp DNA fragment was amplified from each strain by PCR and the amino acid sequence of the EX<sub>7</sub>E motif was deduced from the DNA sequence of the PCR product. EX<sub>7</sub>G<sub>X</sub>E and EX<sub>7</sub>P<sub>X</sub>E were found in 210 of 210 serogroup Y strains and in 86 of 87 W-135 strains, respectively, without false positives (100% specificity for both motifs) (Table 1). In one strain, which was identified as serogroup W-135 by slide agglutination, and in the three strains (DE8946, DE9555, and DE10572) that reacted with both antisera, EX<sub>S</sub>X<sub>E</sub> was found. The serogroups of these strains should be reported as “W-135,” “Y,” or “mixed W-135/Y.” Of note, in an enzyme-linked immunosorbent assay (ELISA) employing monoclonal antibodies (12), DE8946, DE9555, and DE10572 reacted only with the W-135-specific reagent. Nevertheless, taking into account the recent report providing data on mixed-serogroup W-135/Y polysaccharides in some strains (11), we suggest that a component analysis is necessary to definitively resolve the composition of the capsular polysaccharides expressed by these strains. In summary, the determination of amino acid 310 of Neisseria meningitidis serogroup W-135 and Y capsule polymerases turned out to be a reliable marker with the exception of only 1.3% of strains, which harbored an ambiguous serine at this site.

TABLE 1. Correlation between serogroups and the EX<sub>7</sub>E motif within the capsule polymerases of serogroup W-135 and Y meningococci

<table>
<thead>
<tr>
<th>Serogroup&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of strains with the motif:</th>
<th>EX&lt;sub&gt;S&lt;/sub&gt;X&lt;sub&gt;E&lt;/sub&gt;</th>
<th>EX&lt;sub&gt;7&lt;/sub&gt;G&lt;sub&gt;X&lt;/sub&gt;E</th>
<th>EX&lt;sub&gt;7&lt;/sub&gt;P&lt;sub&gt;X&lt;/sub&gt;E</th>
</tr>
</thead>
<tbody>
<tr>
<td>W-135</td>
<td>86</td>
<td>0</td>
<td>210</td>
<td>0</td>
</tr>
<tr>
<td>Y</td>
<td>0</td>
<td>210</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Ambiguous</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Serogroup as determined by slide agglutination.

REFERENCES


Heike Claus*
Wataru Matsunaga
Ulrich Vogel
University of Würzburg
Institute for Hygiene and Microbiology
German Reference Laboratory for Meningococci
Josef-Schneider-Str. 2 (E1)
97080 Würzburg, Germany

*Phone: 49(931)20146936
Fax: 49(931)20146445
E-mail: hclaus@hygiene.uni-wuerzburg.de

*Published ahead of print on 14 July 2010.