**Streptococcus pneumoniae** serogroups and colony morphology: a look back

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**SUMMARY**

From 1985 to 1987, **Streptococcus pneumoniae** isolates were collected from children under 5 years of age in the Asaro Valley, Papua New Guinea as part of a study on bacterial colonization and respiratory tract infections. Data on serogroup and colony morphology were collected to survey serogroups and associated colony morphologies present in the area and to assess whether colony morphology can be indicative of serogroup. In total, 5989 colonies were examined; serogroups 6, 10, 14, 15, 19, 23, 33, 34, 35 and non-serotypeable strains were the most common and accounted for 77% of all the colonies, with serogroups 6, 19 and 23 accounting for 48%. The majority of colonies displayed the typical draughtsman morphology, though serogroups 10 and non-serotypeable isolates most often displayed a raised colony morphology. Of the 15 mucoid colonies identified 73% were serotype 3, though only 29% of serotype 3 isolates were mucoid. Thus colony morphology is of limited value in identifying the pneumococcal serogroup/serotype apart from mucoid colonies, which are likely to be serotype 3.

**Introduction**

Pneumonia is the leading killer of children under the age of five worldwide, and the Gram-positive bacterium **Streptococcus pneumoniae** (the pneumococcus) is the most common aetiological agent (1). **S. pneumoniae** is frequently carried in the nasopharynx of children and though colonization is generally asymptomatic, it is a prerequisite for diseases such as pneumonia and otitis media. Furthermore, carriage serves as a reservoir for maintaining strains of **S. pneumoniae** in human populations. Rates of colonization and subsequent disease are particularly high in developing countries. Studies on **S. pneumoniae** acquisition and carriage in Papua New Guinea (PNG) found that 100% of infants are colonized with **S. pneumoniae** by the age of three months (2,3). More recently, carriage rates of greater than 80% at the age of three months have been reported (4).

**S. pneumoniae** is a diverse species that is classified into immunologically distinct serotypes based upon capsular polysaccharides present on the bacterial surface. Over 90 different serotypes have been identified. Some are related to each other and belong to a single serogroup such as serotypes

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15B and 15C within serogroup 15, whereas some serogroups consist of a single serotype, such as serotype 3. *S. pneumoniae* is typically grown on blood agar and identified using phenotypic tests such as the presence of α-haemolysis, bile solubility and optochin sensitivity (5). The Quellung reaction, performed by incubation of bacteria with specific antisera, is considered the gold standard serotyping method (6). *S. pneumoniae* colony morphology can vary, although colonies typically display raised edges and a concave centre, referred to as a ‘draughtsman’ shape. Serotypes 3, 8 and 37 are generally associated with a mucoid colony morphology (7, 8). To our knowledge, a systematic examination of colony morphology of different pneumococcal serotypes identified in the nasopharynx has not been reported previously. Between 1985 and 1987 nasal swabs were collected monthly from children in highland communities located in the Asaro Valley near Goroka in the Eastern Highlands Province (3). *S. pneumoniae* isolates obtained from these samples were examined for colony morphology and serogroup in order to assess the variety of pneumococcal strains present in this population and determine if colony morphology can be used to identify particular serogroups.

### Materials and Methods

A total of 1449 nasal swabs were collected from 158 study participants aged <5 years (3). Swabs were placed in Amies transport medium before plating on 5% horse blood agar and pathogens were identified as described by Montgomery and colleagues (3). In brief, *S. pneumoniae* was identified by standard methods using selective media. Four colonies were picked from each primary culture that grew and were subcultured; morphologically different colonies were selected if present. Colonies were then serogrouped with Statens Serum Institut antisera and colony morphology recorded. At the time of this study we were unable to do factor typing to establish serotypes within the same serogroup. We therefore report all results as serogroups. Each colony was recorded as having a single phenotype, either the typical or atypical draughtsman morphology or one of the common variants, such as flat, raised, irregular, mucoid or elongated. In total, 5989 colonies were examined.

### Results and Discussion

A total of 37 different serogroups were identified from the samples. Table 1 lists the ten most common serogroups found in the 5989 colonies examined and, for each, the percentage of colonies that displayed the described morphology. Serogroups 6, 10, 14, 15, 19, 23, 33, 34, 35 and non-serotypeable strains were the most common and accounted for 77% of all the colonies, with serogroups 6, 19 and 23 accounting for 48% of colonies. The draughtsman morphology was the most common morphology for all of these serogroups with the exception of serogroup 10 and non-serotypeable strains, both of which most often displayed a raised colony morphology. The data suggest that colony morphology cannot be used to predict serogroup, as the draughtsman morphology dominated the majority of serogroups. The exception was serotype 3 (not included in table), which was the only serotype to frequently display a mucoid phenotype. Of the 15 mucoid colonies identified in this large dataset, 11 (73%) were serotype 3. Hence if a mucoid colony is present, it is likely to be a serotype 3. However, the utility of using the mucoid morphology to identify serogroup 3 is limited as it was only observed in 29% of the 38 colonies that were serotype 3. While serogroups 8 and 37 are known to be associated with the mucoid morphology (8), no mucoid colonies were observed for the 24 isolated serogroup 8 pneumococci, and we did not isolate serogroup 37 in this extensive carriage study. Colony morphology can be useful for identifying the presence of multiple serotypes in the same sample, although the same serogroup can display different morphologies (Table 1). Carriage of multiple serogroups is common in Papua New Guinean children: in this study, one-third of specimens contained 2 to 4 serogroups (3). Factor typing would no doubt have identified even more samples with simultaneous carriage of multiple capsular types.

In summary, these data provide a detailed evaluation of pneumococcal serogroups and associated colony morphologies carried by Papua New Guinean children living near Goroka during the mid-1980s. Although serotyping technologies have advanced since then, it is interesting to note that the most common serogroups at that time are still among the most prevalent in a period before
the introduction of pneumococcal conjugate vaccine in PNG (4). Although colony morphology is insufficient for identifying most serotypes, it can be a useful tool for identifying the presence of multiple serotypes in a single sample and can indicate the presence of mucoid serotypes. In more recent years, examination of the morphology of \textit{S. pneumoniae} has also assessed opacity, since a transparent colony phenotype is associated with increased adherence and colonization compared to opaque phase variants of the same strain (9).

**REFERENCES**

8. Waite RD, Penfold DW, Struthers JK, Dowson CG. Spontaneous sequence duplications within capsule genes \textit{cap8E} and \textit{tts} control phase variation in \textit{Streptococcus pneumoniae} serotypes 8 and 37. \textit{Microbiology} 2003;149:497-504.

**TABLE 1**

<table>
<thead>
<tr>
<th>Serogroup*</th>
<th>6</th>
<th>10</th>
<th>14</th>
<th>15</th>
<th>19</th>
<th>23</th>
<th>33</th>
<th>34</th>
<th>35</th>
<th>NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of isolates</td>
<td>1246</td>
<td>158</td>
<td>297</td>
<td>251</td>
<td>1024</td>
<td>634</td>
<td>244</td>
<td>134</td>
<td>222</td>
<td>394</td>
</tr>
<tr>
<td>% of total</td>
<td>20.8</td>
<td>2.6</td>
<td>5.0</td>
<td>4.2</td>
<td>17.1</td>
<td>10.6</td>
<td>4.1</td>
<td>2.2</td>
<td>3.7</td>
<td>6.6</td>
</tr>
<tr>
<td>Draughtsman</td>
<td>44.4</td>
<td>19.0</td>
<td>41.4</td>
<td>43.4</td>
<td>38.7</td>
<td>37.1</td>
<td>42.6</td>
<td>55.2</td>
<td>31.1</td>
<td>32.0</td>
</tr>
<tr>
<td>Atypical draughtsman</td>
<td>12.0</td>
<td>17.1</td>
<td>19.5</td>
<td>17.1</td>
<td>11.8</td>
<td>16.7</td>
<td>20.9</td>
<td>15.7</td>
<td>9.0</td>
<td>13.2</td>
</tr>
<tr>
<td>Irregular</td>
<td>6.0</td>
<td>0.6</td>
<td>4.7</td>
<td>6.4</td>
<td>6.0</td>
<td>7.6</td>
<td>4.1</td>
<td>3.0</td>
<td>8.1</td>
<td>3.8</td>
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<tr>
<td>Flat</td>
<td>18.9</td>
<td>22.8</td>
<td>17.5</td>
<td>15.1</td>
<td>21.0</td>
<td>18.9</td>
<td>12.7</td>
<td>11.9</td>
<td>22.5</td>
<td>10.2</td>
</tr>
<tr>
<td>Raised</td>
<td>11.2</td>
<td>40.5</td>
<td>13.5</td>
<td>15.1</td>
<td>19.0</td>
<td>15.5</td>
<td>17.2</td>
<td>13.4</td>
<td>18.9</td>
<td>37.8</td>
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<tr>
<td>Elongated</td>
<td>2.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.8</td>
<td>1.1</td>
<td>1.4</td>
<td>1.6</td>
<td>0.0</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Other</td>
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<td>2.0</td>
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<td>2.8</td>
<td>0.8</td>
<td>0.7</td>
<td>9.5</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*For each serogroup, the number of colonies identified and the overall percentage of the 5989 colonies examined are shown in bold; the percentages of colonies displaying a designated colony morphology are listed in italics. NT = non-serotypeable.