Distribution and Relationship to Serotype of *Haemophilus influenzae* Biotypes Isolated from Upper Respiratory Tracts of Children and Adults in Papua New Guinea

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The relationship between serotypes and biotypes of 505 carriage strains of *Haemophilus influenzae* isolated from the upper respiratory tracts of well children, children with pneumonia, and healthy adults was studied. All except serotype c were significantly associated with one or two specific biotypes (*P* < 0.001). No encapsulated organisms belonging to biotypes V, VI, or VII were encountered. No significant difference in the interaction of biotypes and serotypes isolated from well and sick children was present. Both encapsulated and nonserotytable biotype I *H. influenzae* strains were commonly carried in the upper respiratory tracts of healthy Melanesian children. The distribution of nonserotytable *H. influenzae* strains occurred throughout all biotypes, and the frequency of nonencapsulated biotype III and IV strains differed significantly from serotytable organisms with the same biotype (*P* < 0.001).

Few data are available on either the relationship between biotypes and serotypes of *Haemophilus influenzae* isolated from the upper respiratory tracts of children and adults or the distribution of biotypes among nonserotytable strains by source of isolation. In reports in which biochemical profiles and capsular antigens have been compared, the sample size, apart from serotype b, has been too small to provide statistically meaningful results (1, 9, 15, 18, 19). Kilian (10) and Kilian and Frederiksen (11) have biotyped 296 encapsulated clinical isolates, but type b strains predominated and constituted over 80% of the total sample.

Systemic infections caused by nonencapsulated *H. influenzae* strains and serotypes other than b are common in both children and adults (3, 4, 6, 8, 13, 14, 17, 20, 21). Biochemical profiling developed by Kilian (10) has provided a useful epidemiological marker which has enabled invasive, nonserotytable *H. influenzae* strains to be both differentiated and related to coexisting carriage in the upper respiratory tract and elsewhere (3, 6, 13, 14, 20, 21).

The object of the present study was to clarify the relationships among *H. influenzae* biotypes and serotypes and the distribution of biotypes among nonencapsulated carriage organisms isolated from the upper respiratory tract. Because beta-lactamase-producing variants are uncommon in Papua New Guinea (16), no attempt could be made to relate penicillin resistance to antigenic or biochemical properties.

**MATERIALS AND METHODS**

A total of 505 *H. influenzae* strains isolated from the noses or nasopharynxes of 410 children aged 2 weeks to 5 years and 95 healthy adults were studied between March 1980 and January 1983. A total of 343 isolates, including 169 serotypable organisms, were cultured from well children, and a further 66 isolates, of which 35 were type specific, were recovered from children with mild or moderate pneumonia. (The classification of acute lower respiratory tract infection within the Pneumonia Research Programme in Goroka is based on International Classification of Diseases, 9th revision, World Health Organization, Geneva, 1977.) All strains from adults were nonserotypable.

Primary cultures were made on chocolate (horse blood) agar with and without bacitracin (300 μg/ml) and incubated at 36°C in a 10% carbon dioxide-enriched atmosphere. *H. influenzae* isolates were identified by Gram stain, colony morphology on chocolate agar, and dependence for growth on hemin and NAD. Encapsulated strains were serotyped by slide agglutination with *H. influenzae* antisera a through f (Wellcome Reagents Ltd., Beckenham, England). The substrates urea, ornithine, and tryptophane, in conjunction with the Minitek system (BBL Microbiology Systems, Cockeysville, Md.) were used to biochemically type all strains by the method of Kilian (10) as modified by Back and Oberhofer (2).

Statistical analysis was carried out with the biomedical data processing software package developed at the University of California, Los Angeles in conjunction with a PDP 11/34A minicomputer (Digital Equipment Corp., Marlboro, Mass.).

**RESULTS**

All isolates were biotyped as types I through VII, and all encapsulated strains were serotype specific. For statistical analysis, encapsulated biotype III strains were omitted because of their infrequency. No encapsulated biotype V, VI, or VII organisms were isolated. Of the serotypable strains, 97% belonged to biotypes I, II, and IV. A comparison of serotypes a through f with biotypes I, II, and IV (Table 1) revealed a significant association of all serotypes except c with one or two specific biotypes (*χ²* = 181.384; 10 df; *P* < 0.001); i.e., serotype f strains were biotype I specific, 98% of type b strains belonged to biotypes I and II, biotype IV contained most (89%) serotype d isolates, serotype e organisms were distributed exclusively in biotypes I and IV, and 58% of serotype a strains were found in biotype II.

Nonserotypable *H. influenzae* isolates from children and adults were distributed throughout all biotypes, with those of I, II, and III containing more than 70% of all isolates from either group. No significant age-related difference in the number of strains in any biotype was present in either group. However, the distribution of encapsulated biotypable organisms differed significantly from the biotype distribution of nonserotypable organisms within biotypes III through VI (*χ²*...
TABLE 1. Relationship between biotypes and serotypes of H. influenzae isolated from the upper respiratory tracts of Melanesian children

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. of isolates</th>
<th>No. of strains with following biotype:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>a</td>
<td>43</td>
<td>10</td>
</tr>
<tr>
<td>b</td>
<td>45</td>
<td>17</td>
</tr>
<tr>
<td>c</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>d</td>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td>e</td>
<td>36</td>
<td>13</td>
</tr>
<tr>
<td>f</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>NT</td>
<td>301</td>
<td>72</td>
</tr>
</tbody>
</table>

Note: NT, Non-serotypable.

= 130.85; 5 df; P < 0.001). Types IV, VI, and VII were the least common biotypes among nonencapsulated strains. Biotype VII has only recently been described (7).

Table 2 shows the relationship between biotypes and serotypes isolated from well and sick children. The distribution of nonserotypable organisms in each group did not differ significantly. This was also true for the biotype and serotype interaction among type-specific strains in the same groups (χ² = 24.74; 15 df; 0.10 > P > 0.05), although here the chi-square value borders on significance.

DISCUSSION

This study has shown that the biotypes of encapsulated strains of H. influenzae isolated from the upper respiratory tracts of children in Papua New Guinea are confined to four of seven biotypes within which a marked biotype specificity is apparent. The report of Kilian and Frederiksen (11) which describes the serotype-biotype relationship of 423 clinical isolates of H. influenzae is the most substantive to date. Although the source of their strains is not clear, several findings that closely parallel those in the current investigation include the rarity of encapsulated biotype III and V organisms and the biotype specificity of serotypes d and f. The association of type e isolates with biotype IV appears to be exclusive, according to Kilian and Frederiksen (11) and Kamme (9), but respiratory strains of this serotype in Papua New Guinea are found in both biotypes I and IV. The biotypes of smaller numbers of types d, e, and f strains published earlier by Kilian (10) are similar to more recent findings. Oberhofer and Back (15) have noted the specificity of type f variants for biotype I. Type c H. influenzae strains are unusual elsewhere (11) but comparatively common in Papua New Guinea, although less so than other serotypes. Of 16 serotype c strains reported in this study, 50% were biotype I, and the remainder were distributed among biotypes II, III, and IV. None of 17 biotype VI isolates were encapsulated.

Frederiksen and Kilian (5) have reported the close association of biotype I H. influenzae strains with serotype b strains and pathogenicity. Long et al. (12) have also found that biotype I strains, regardless of encapsulation, are significantly related to disease. In well carriers, most H. influenzae strains belong to biotypes other than I, with those of biotypes II and III being predominant (11). In the current study, however, the carriage of nonserotypable biotype II and III organisms in well and sick children does not differ significantly from biotype I strains (χ² = 0.715, Yates correction) and the latter, regardless of capsules, are present in the upper respiratory tracts of 30% of well children in Papua New Guinea.

Although the findings in this study differ in part from those published elsewhere, a number of reports of H. influenzae biotype and serotype interactions have either studied few non-type b encapsulated strains or have failed to clearly define the source of clinical isolates or relate biotype distribution to serotypable isolates. Different regional or ethnographic serotype-biotype patterns of carriage and invasion by H. influenzae may exist, particularly between developed and developing countries. Further studies are required to clarify these differences.

ACKNOWLEDGMENTS

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LITERATURE CITED


TABLE 2. Relationship between biotypes and serotypes of H. influenzae isolated from well (n = 344) and sick (n = 66) children

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. of isolates from:</th>
<th>No. of strains from well (no. from sick) children with following biotype:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Well children</td>
<td>Sick children</td>
</tr>
<tr>
<td>a</td>
<td>38</td>
<td>5</td>
</tr>
<tr>
<td>b</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td>c</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>d</td>
<td>31</td>
<td>5</td>
</tr>
<tr>
<td>e</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>f</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>NT</td>
<td>175</td>
<td>31</td>
</tr>
</tbody>
</table>

Note: NT, Non-serotypable.


