Biotypes of *Haemophilus influenzae* That Are Associated with Noninvasive Infections

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In this study, we examined the biotypes of *Haemophilus influenzae* strains associated with noninvasive infections in hospitalized patients. Over an 18-month period, a total of 388 strains were isolated from patients of various ages (neonates to the elderly), and the biotypes of the strains were determined. Strains of biotype II accounted for 48% of the isolates; this was followed by strains of biotypes III and I (26 and 16%, respectively). The remaining 10% of the isolates were made up of strains of biotypes IV, V, VI, and VII. A total of 6% of strains were capsulated. The distribution of biotypes in specimens from the respiratory tract and associated sites was comparable to that obtained in similar investigations, but examination of isolates from neonatal and genital specimens did not support the concept that *H. influenzae* biotype IV is a major urogenital pathogen. Conflicting results regarding the incidence of certain biotypes in specimens, particularly those from the urogenital tract, may be due to the selection of different subpopulations of patients. Data relating to the specimens were used to evaluate the association between biotype and clinical diagnosis, the presence of other potential bacterial pathogens in the specimens, and the presence of viruses in the specimens. None of the differences in the distribution of biotypes which were examined was statistically significant.

The species *Haemophilus influenzae* comprises a very mixed group of organisms. They range from those that are part of the normal flora to those that are associated with conditions such as sinusitis, otitis media, and conjunctivitis to those that cause invasive disease. *H. influenzae* is indigenous to humans, and it is found as part of the normal bacterial flora mainly on the mucous membranes of the upper respiratory tract (14). Approximately 60 to 80% of healthy young children carry *H. influenzae* in the nasopharynx or throat. Rates of isolation from older children and adults are lower (14, 28).

Infections caused by *H. influenzae* can be divided into two groups: (i) acute, pyogenic, and usually invasive infections in which *H. influenzae* is the primary pathogen and (ii) noninvasive infections in which *H. influenzae* may play a secondary role. The invasive infections include meningitis and other septicemic conditions with local implications such as epiglottitis, cellulitis, arthritis, and osteomyelitis (8). These infections are invariably caused by capsulated strains which can be divided serologically into six types, a to f (22). Strains possessing a type b capsule are by far the most common. Noncapsulated strains are frequently implicated in noninvasive infections, and these strains are reportedly often chronic in nature (14). In 1976, Kilian (13) made an extensive taxonomic study of the genus *Haemophilus*. The results showed that the strains of *H. influenzae* can be divided into biotypes on the basis of certain enzymatic and biochemical properties, specifically, the ability to produce indole, urease, and ornithine decarboxylase. Eight biotypes of *H. influenzae* are currently recognized (12, 13, 21, 26). Studies conducted in various parts of the world have provided data from different populations, and these data have been used to assess the association between biotype and pathogenicity. The relationship between biotype I and, to a lesser extent, biotype II strains and invasive disease is now well documented, with the majority of isolates from cases of meningitis and septicemia being biotype I and serotype b (2, 11, 13, 15, 21). Both Albritton et al. (2) and Turk (27) recognized that it may be the possession of a capsule which is the important virulence determinant for these strains and that the correlation between biotype I strains and invasive infection may simply reflect an uneven distribution of type b strains among the biotypes.

Further studies into various aspects of the relationship between biotype and pathogenicity have produced some conflicting conclusions. Discrepant results have been obtained, for example, in different studies of biotypes involved in maternal, genital, and neonatal infections. Albritton et al. (1) compared the distributions of biotypes in specimens taken from the genitourinary and respiratory tracts of patients in Canada. Significant differences were found, the major difference being the more frequent isolation of biotype IV strains from the genitourinary specimens. Wallace et al. (29), in a study done in the United States, reported a strong association between noncapsulated biotype IV strains and strains of genital, maternal, or neonatal origin. Similarly, Casin et al. (5) reported a predominance of biotype IV strains from the genitourinary tracts of patients in France. In contrast to these findings, neither Kleiman et al. (16), in the United States, nor Milne et al. (19), in England, could attribute any significance to biotype IV strains as a cause of neonatal sepsis. A possible explanation for such variations is that, as suggested by Granato et al. (11), the distributions of frequencies of certain biotypes of clinical isolates of *H. influenzae* may vary significantly when comparisons are made worldwide.

Studies of the relationship between biotype distribution and antibiotic resistance have produced various results (2, 23, 25). Other reports on infections caused by *H. influenzae* have shown that age does not significantly alter the distribution of biotypes involved in noninvasive infections (2) and that, in patients with cystic fibrosis, biotype I strains are isolated significantly more often and biotype II strains are

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isolated less often in comparison with the frequency of isolation of strains from the normal respiratory tract (30, 31).

The present study was undertaken to evaluate certain aspects of the relationship between biotype and infection in the local population in Brisbane, Australia. The strains of H. influenzae examined were isolated from specimens collected from hospitalized patients with noninvasive infections. The patients concerned were from the Mater Adult, Children’s and Mothers’ Public Hospitals. The strains were isolated from a wide variety of specimens from patients of various ages (neonates to the elderly). The aims of the study were (i) to compare the distribution of biotypes of H. influenzae that cause noninvasive infections in the local population with that reported overseas and (ii) to evaluate any association between biotypes that cause noninvasive infection and parameters such as clinical diagnosis, the presence of other potential bacterial pathogens, and the presence of viruses in specimens.

MATERIALS AND METHODS

Bacterial strains. The biotypes of all H. influenzae strains reported by the laboratory to be potentially significant isolates over an 18-month period were determined. A total of 388 isolates were obtained from 371 patients. Multiple isolates from the same patient were included if the strains were obtained from different sites or were obtained from specimens collected from the same site at least 1 month apart.

Isolation and identification procedures. Primary isolation of H. influenzae strains was carried out by using chocolate agar or chocolate agar plus bacitracin (10 IU/ml) incubated at 35°C in an atmosphere of air plus 10% carbon dioxide. Strains were identified as isolates of H. influenzae on the basis of colony morphology, Gram stain reaction, and X and V-factor requirements (14). These requirements were assessed by using Haemophilus identification agar (7) with Oxoid X and V disks and by the porphyrin test (14).

Biotyping. The biotypes of the isolates were determined by the method described by Kilian (14). Liquid media prepared in the laboratory were inoculated with subcultures which were 24 h old or less. Test cultures were incubated in air for 4 h at 35°C. This biotyping system uses the presence of preformed enzymes, and because growth is not required, the media do not have to be supplemented with X and V factors.

Serotyping. Capsule typing was carried out on 24-h subcultures by using the Phadebact Haemophilus Test 50 kit. Strains were reported as having a type b capsule or a non-type-b capsule or as nontypeable.

Virus identification. Virus isolation and identification were performed by the Queensland State Health Laboratories.

Statistical analysis. Probability values for differences in frequency distributions were determined by chi-square analysis.

RESULTS

The biotypes of 388 H. influenzae strains were determined. The results are presented in Table 1 according to specimen type. The 388 strains were also serotyped. A total of 25 strains were capsulated. Fifteen had a type b capsule and 10 had a non-type-b capsule. The specimen types from which capsulated strains were isolated and the biotypes involved are given in Table 2.

Strains were divided into two groups, respiratory and neonatal-genital, to determine whether distinctive patterns of biotypes were associated with specimens from these two areas of the body. The respiratory group contained isolates from the respiratory tract and associated sites (nasopharyngeal aspirates, sputum specimens, eye swabs, tracheal aspirates, ear swabs, nose swabs, and bronchial washings), and the neonatal-genital group contained isolates from vaginal swabs, gastric aspirates from neonates, swabs from Bartholin’s gland abscesses, urine, and a lung swab collected from a neonate postmortem. The biotypes of strains isolated from these two groups of specimens are given in Table 3. Differences in the distributions of biotypes between the two groups were not statistically significant. The H. influenzae strains isolated from nasopharyngeal aspirates were used to assess the relationship between biotype and clinical diagnosis. Information on clinical diagnosis was available for 122 of the 133 children from whom these strains were obtained. The three categories addressed were upper respiratory tract viral infection (37 cases), asthma (21 cases), and pneumonia (17 cases). A total of 47 children were diagnosed with conditions other than these. No significant differences between the distributions of biotypes were found.

Strains of H. influenzae isolated from nasopharyngeal aspirates were also used to assess the relationship between biotype and the presence of viruses in specimens. A total of 110 aspirates from which H. influenzae strains were isolated were also cultured for viruses, and 45 of these specimens Table 2. Biotypes and specimen types associated with isolates of H. influenzae with type b and non-type-b capsules

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>No. of isolates of the following biotype:</th>
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<tbody>
<tr>
<td></td>
<td>Type b</td>
</tr>
<tr>
<td>Nasopharyngeal aspirate</td>
<td>10</td>
</tr>
<tr>
<td>Sputum</td>
<td>5</td>
</tr>
<tr>
<td>Eye swab</td>
<td>4</td>
</tr>
<tr>
<td>Vaginal swab</td>
<td>2</td>
</tr>
<tr>
<td>Nasopharyngeal aspirate</td>
<td>3</td>
</tr>
<tr>
<td>Nose swab</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
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yields one or more viruses. The viruses isolated were respiratory syncytial virus (15 specimens), paramyxovirus type 3 (9 specimens), cytomegalovirus (7 specimens), adenovirus (7 specimens), enterovirus (4 specimens), rhinovirus (3 specimens), influenza virus type A (2 specimens), and unidentified viruses (2 specimens). Differences in the distributions of biotypes between the two groups were not statistically significant.

The presence of or absence of other potential bacterial pathogens in specimens was recorded. The organisms classified as potential bacterial pathogens were Streptococcus pneumoniae, Streptococcus pyogenes, Branhamella catarrhalis, and Staphylococcus aureus. One or more of these organisms was isolated from 87 specimens, while the remaining 301 specimens contained no other potential bacterial pathogen. Calculations indicated that there was no significant difference between the distributions of biotypes.

**DISCUSSION**

Biotype II strains were isolated most frequently, accounting for 48% of strains; this was followed by strains of biotypes III and I (26 and 16% of the isolates, respectively) (Table 1). Strains of biotypes IV, V, VI, and VII were isolated in comparatively small numbers, making up 10% of all isolates. No strains of biotype VIII were detected. These results are in agreement with studies of isolates of *H. influenzae* from patients with noninvasive disease from Canada and the United States (2, 21, 25). Capsulated strains accounted for 65% of isolates (Table 2). This value is within the range of published values for capsulated strains isolated from patients with noninvasive infections (2, 25).

Comparison of isolates from respiratory specimens with those from neonatal-genital specimens showed that there was no significant difference in the distributions of the biotypes (Table 3). Because only 1 of the 12 strains isolated from neonatal-genital sites was biotype IV, the results of the present study may support the conclusions of other investigators as Drouet et al. (6) and Quentin et al. (24), which indicate that *H. influenzae* biotype IV is not a major urogenital pathogen, in contrast to data reported from Canada, the United States, and France, which indicate that biotype IV strains are significantly associated with urogenital infections (1, 5, 29). The percentage of biotype IV strains from the respiratory tract and associated sites was low (approximately 2%) and comparable to that generally reported (2, 8, 17, 21). The percentage of these strains from neonatal-genital specimens was higher (8%), although this difference was not statistically significant. It is not possible to make generalizations on the basis of the isolation of one biotype IV strain. If this value were substantiated by using a larger sample size, however, it would support suggestions that biotype IV urogenital isolates may form a group of organisms which have unique characteristics (3, 20, 24) and which are specially adapted to that environment (24). The isolation rates for biotype IV strains could vary significantly, depending on the section of the population being sampled. Strains of *H. influenzae* associated with urogenital infections may be sexually transmitted (10, 24), and relatively high isolation rates may be reported in populations that exhibit high levels of sexual activity, as demonstrated by Martel et al. (17). The discrepancies surrounding the reported roles of biotype IV strains in neonatal-genital infections may be related, therefore, to the patient subpopulation under investigation rather than to factors such as geographical differences.

Reports of increasing rates of neonatal sepsis caused by *Haemophilus* species have come from a variety of sources, indicating a potential worldwide problem (4, 5, 9, 19, 24). Relevant information was not available during the present study to determine whether the observed rate of neonatal infection represented an increase over the rate in earlier years. No correlations were found between biotypes of *H. influenzae* causing noninvasive infections and clinical diagnosis, the presence of other bacterial pathogens in the specimens, or the presence of viruses in respiratory specimens from young children. In patients with cystic fibrosis, changes in biotype distribution have been correlated with clinical condition (30, 31). Results obtained in the present study indicate that those parameters which influence biotype distribution in the respiratory tracts of patients with cystic fibrosis were not operating in young children with asthma, pneumonia, or viral respiratory tract infections in the population sampled in this study. Another explanation for the observed results which must be considered is that changes in the flora of the lower respiratory tract which are not necessarily reflected in nasopharyngeal samples, like those used in the present study, may be significant in association with the clinical conditions investigated here. Of the 110 nasopharyngeal aspirate cultures for viruses, 45 grew one or more viruses. Experiments with animal models have demonstrated that prior nasopharyngeal infection with viruses potentiates subsequent infection with *H. influenzae* (18). No evidence was found in the present study to indicate that the presence of viruses in the respiratory tract influenced the distribution of biotypes of *H. influenzae* at the site. No correlation was found between the distribution of biotypes and the presence of other potential bacterial pathogens in specimens from patients with noninvasive infections examined in the present study.

In summary, biotype II strains were isolated most frequently; this was followed by strains of biotypes III and I. Strains of biotypes IV, V, VI, and VII accounted for only a small percentage of isolates. The distribution of biotypes from the respiratory tract and associated sites agreed with published data from similar investigations. The results obtained from the examination of isolates from neonatal-genital specimens did not appear to support the concept that *H. influenzae* biotype IV is a major urogenital pathogen. No correlation was found between the distribution of biotypes causing noninvasive infections and clinical diagnosis, the presence of other bacterial pathogens in specimens, or the presence of viruses in respiratory specimens from young children. The 388 isolates included in this study were not preselected in any way, and the results reflect the incidence of biotypes in a cross section of the local population. Conflicting conclusions reached by other authors, specifically in relation to the role of biotype IV strains in neonatal-genital infections, may be due to the fact that results were obtained in those studies by using specimens from selected subpopulations of patients.
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REFERENCES