Biotypes and serotypes of *Haemophilus influenzae* ocular isolates

A M Alrawi, K C Chern, V Cevallos, T Lietman, J P Whitcher, T P Margolis, E T Cunningham, Jr

**Aim:** To determine which subtypes of *Haemophilus influenzae* are most commonly associated with ocular disease, and whether the site of ocular *H influenzae* infection is correlated with specific subtypes of the organism.

**Methods:** The biotypes and serotypes of ocular *H influenzae* isolates collected at the Francis I Proctor Foundation between March 1989 and January 2000 were examined. A total of 62 ocular isolates were retrieved from frozen storage and plated on chocolate agar. Biotypes were assigned based upon the ability of the isolates to produce indole, urease, and ornithine decarboxylase. Capsular subtypes a–f were determined by slide agglutination using commercially available subtype specific antisera. Identified biotypes and serotypes were then analyzed with regard to site of infection.

**Results:** Patient age ranged from 1 to 92 years with a median age of 45 years. 38 (61%) of the isolates were biotype II, 23 (37%) were biotype III, and one (2%) was biotype VII. All of the isolates were non-encapsulated and thus serologically non-typable. *H influenzae* biotype II was found in 28 of 48 (58%) conjunctivitis cases, five of eight (63%) keratitis cases, and two of two (100%) endophthalmitis cases. Biotype III was found in 20 of 48 (42%) conjunctivitis cases, two of eight (25%) keratitis cases, and a single case of dacryocystitis. Biotype VII was associated with one of eight (13%) keratitis cases.

**Conclusion:** Most ocular *H influenzae* isolates appear to be serologically non-typable strains from biotypes II and III, less virulent subtypes that frequently colonise the nasopharynx. In addition, the site of ocular *H influenzae* infections appears to be largely independent of species subtype.

*Haemophilus influenzae* is responsible for a number of human diseases ranging from chronic respiratory infection to meningitis. Eight biotypes and six serotypes of *H influenzae* have been identified. Biotyping and serotyping have been used to investigate patterns of colonisation of *H influenzae*, as well as to identify strains of the bacteria that appear to be associated with more severe infection. Biotype I, serotype b, for instance, is often associated with severe meningitis in children. In contrast, non-serotypable strains of *H influenzae*, particularly biotypes II and III, are frequently commensal to the upper respiratory tract. While colonisation with biotypes II and III usually does not progress to disease, these same biotypes have been implicated in the pathogenesis of sinusitis, otitis media, acute and chronic exacerbations of lower respiratory tract infection, and acute and chronic conjunctivitis.

The relation between *H influenzae* and specific ophthalmologic diagnoses has not been studied. The purpose of this study was to define what subtypes of *H influenzae* are common in ocular disease, and to determine whether there is a correlation between various *H influenzae* subtypes and the site of ophthalmic *H influenzae* infection.

**METHODS**

The organisms included in this study were isolated from clinical samples submitted to the microbiology laboratory at the Francis I Proctor Foundation over an 11 year period from March 1989 to January 2000. Clinical diagnoses were provided by the ophthalmologists who submitted the specimens for culture. Cultures were considered positive if any colonies of *H influenzae* were grown from the specimen. A total of 62 ocular *H influenzae* isolates from 62 patients with ocular *H influenzae* infections were recovered and classified according to methods described previously.

In brief, isolates were retrieved from frozen storage, plated on chocolate agar, and incubated at 35°C in an atmosphere of air plus 5–7% carbon dioxide. Isolates were identified on the basis of haemolysis and X and V factor requirements. Biotypes were assigned based upon the ability of isolates to produce indole, urease, and ornithine decarboxylase. Indole production was demonstrated with dry slide indole (Difco). Urease activity was tested using Christensen's urea agar slant. Ornithine decarboxylase activity was demonstrated in 1 ml of the medium of Møller. Isolates of biotype II were ornithine negative, but indole and urease positive. Isolates belonging to biotype III were ornithine and indole negative and urease positive. A single biotype VII isolate was ornithine and urease negative, and indole positive. Biotypes I, IV, V, and VI were not isolated in this study. Serotyped testing was performed using Bacto- *H influenzae* antisera to types a–f (Burroughs-Wellcome). The first six negative results obtained on isolates were confirmed by the Centers for Disease Control and Prevention, Atlanta, Georgia, to validate our assays.

The spectrum of diagnoses for each biotype were compared using a χ² test. If a cell’s value was less than or equal to 5, a Fisher’s exact test was performed. P Values of less than 0.05 were accepted as statistically significant.

**RESULTS**

The results of this study are summarised in Table 1. Patient age ranged from 1 to 92 years with a median age of 45 years and a mean age of 42 years. All isolates were serologically non-typable. Biotype II comprised 38 of 62 (61%) isolates, while biotype III accounted for 23 of 62 (37%), and biotype VII for one of 62 (2%) cases. Conjunctivitis was the most common diagnosis (48 of 62, or 77%), with acute conjunctivitis (21 of 33, or 64%) identified more often than subacute conjunctivitis (12 of 33, or 36%). Biotype II represented 28 of 48 (58%) conjunctivitis cases, whereas biotype III represented 20 of 48 (42%) being biotype III. Although organisms of biotype II tended to be a more common cause of conjunctivitis than organisms of biotype III, this difference did not achieve statistical significance (p = 0.22).
Keratitis accounted for eight of 62 (13%) cases, with biotype II (five of eight, or 63%) being most common, followed by biotype III (two of eight, or 25%) and then biotype VII (one of eight, or 13%; p = 0.94). Other diagnoses included three cases of blepharitis (5%) and two cases of endophthalmitis (3%) due to biotype II, and a single case of dacryocystitis (1.5%) due to biotype III.

DISCUSSION

No evidence exists on the relation between H influenzae subtype and site of ocular infection. The present study demonstrated that non-encapsulated H influenzae organisms belonging to biotypes II and III comprised the vast majority of ocular H influenzae isolates. The most common manifestation of ocular H influenzae infection was conjunctivitis with a 6:1 margin over bacterial keratitis, and biotype II was by far the most common subtype. In addition to conjunctivitis and keratitis, other ocular presentations of H influenzae included blepharitis, endophthalmitis, and dacryocystitis.

While ocular infections by encapsulated H influenzae have been previously described, including preseptal and orbital cellulitis and haemorrhagic conjunctivitis, our study failed to demonstrate any cases of ocular infection with encapsulated strains of H influenzae. The most prevalent site of infection for encapsulated H influenzae was the nasopharynx, and in some cases severe H influenzae conjunctivitis was the most frequent ocular H influenzae infection and was subacute in nature in about one third of patients. Individual commensal strains appeared to bear little relation to the site of ocular infection.

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REFERENCES


Table 1

<table>
<thead>
<tr>
<th>Site of infection</th>
<th>Biotype II (n = 38; 61%)</th>
<th>Biotype III (n = 23; 37%)</th>
<th>Biotype VII (n = 1; 2%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctivitis</td>
<td>28 (58%)</td>
<td>20 (42%)</td>
<td>0</td>
</tr>
<tr>
<td>Keratitis</td>
<td>5 (63%)</td>
<td>2 (25%)</td>
<td>1 (13%)</td>
</tr>
<tr>
<td>Blepharitis</td>
<td>3 (100%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Endophthalmitis</td>
<td>2 (100%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dacryocystis</td>
<td>0</td>
<td>1 (100%)</td>
<td>0</td>
</tr>
</tbody>
</table>

This figure has been more or less confirmed by the relatively few case reports of contact lens associated H influenzae corneal ulcers that have appeared in the literature over the past quarter century.

Conversely, H influenzae has been implicated as a fairly common cause of preseptal and orbital cellulitis. The fact that no cases of cellulitis were identified in our study was unexpected but may have been related to referral bias at our institution, or perhaps to changes in prevalence of type b infection in the HIB vaccine era as mentioned previously.

In conclusion, while more virulent, encapsulated subtypes of H influenzae were not identified in this study, infection by commensal strains of H influenzae was common, and in some cases severe. H influenzae conjunctivitis was the most frequent ocular H influenzae infection and was subacute in nature in nearly one third of patients. Individual commensal strains appeared to bear little relation to the site of ocular infection.
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