

SCIENTIFIC CORRESPONDENCE

Biotypes and serotypes of *Haemophilus influenzae* ocular isolates

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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 analysed 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 bacterium that appear to be associated with more severe infection. Biotype I, serotype b, for instance, is often associated with severe meningitis in children.¹ 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.²⁻⁴

The relation between *H influenzae* and specific ophthalmological 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 correla-

tion 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.⁵ 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 Moller. 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 χ^2 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 ($p = 0.22$).

Table 1 The frequency of various *Haemophilus influenzae* biotypes by site of infection

Site of infection (n = 62)	Biotype II (n = 38; 61%)	Biotype III (n = 23; 37%)	Biotype VII (n = 1; 2%)
Conjunctivitis (n = 48; 77%)	28 (58%)	20 (42%)	0
Keratitis (n = 8; 13%)	5 (63%)	2 (25%)	1 (13%)
Blepharitis (n = 3; 5%)	3 (100%)	0	0
Endophthalmitis (n = 2; 3%)	2 (100%)	0	0
Dacryocystitis (n = 1; 2%)	0	1 (100%)	0

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.⁶ The most common manifestation of ocular *H influenzae* infection was conjunctivitis by 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 cellulitis⁷ and haemorrhagic conjunctivitis,⁸ our study failed to demonstrate any cases of ocular infection with encapsulated organisms. This observation may be related to the introduction of the *H influenzae* type b (HiB) vaccine in 1985. In fact, recent studies have clearly demonstrated a decrease in the prevalence of serotype b systemic infection as well as a decrease in serotype b *H influenzae* preseptal and orbital cellulitis in children.⁷⁻⁹ While few of the patients from whom *H influenzae* was isolated in our series were young enough to have been vaccinated with HiB routinely, and the vaccination status of adult patients in the study was unknown, routine childhood vaccination with HiB would be expected to decrease the overall prevalence of type b *H influenzae* in the population.

The source of infection by *H influenzae* in our patients with ocular disease remains unknown. All but one patient in our study were found to be infected with *H influenzae* strains belonging to biotypes II and III. These two biotypes comprise the majority of commensal *H influenzae* organisms of the upper respiratory tract, excluding the oral cavity.¹ This observation suggests that the upper respiratory tract may be the source of most ocular *H influenzae* infections. In general, specific ocular infections were unrelated to *H influenzae* biotype.

As demonstrated by the present study, *H influenzae* is associated with a number of ocular infections ranging in severity from mild to sight threatening. Most previous investigations of ocular *H influenzae* infection have focused on childhood conjunctivitis, which is usually acute in nature.³⁻⁴ In our study, however, a sizeable proportion of the patients, approximately

one third, developed subacute *H influenzae* conjunctivitis, a fairly indolent disease that can be easily missed or misdiagnosed if conjunctival cultures are not obtained.¹⁰ *H influenzae* keratitis was also more common than expected given how infrequently it is reported in the literature. A 30 year retrospective study of the causes of bacterial keratitis reported a prevalence of less than 1%, for example.¹¹ 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.¹²⁻¹⁴ Conversely, *H influenzae* has been implicated as a fairly common cause of preseptal and orbital cellulites.⁷ 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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