Distribution of biotypes of *Haemophilus influenzae* and *H parainfluenzae* in patients with cystic fibrosis

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SUMMARY One hundred and eighty eight isolates of *Haemophilus influenzae* and 187 isolates of *H parainfluenzae* from patients with cystic fibrosis, patients with respiratory infections but without cystic fibrosis, and patients with neither cystic fibrosis nor respiratory infections were biotyped. Biotype I of *H influenzae* were found significantly more often in patients with cystic fibrosis compared with those with normal respiratory tracts. On the other hand, biotype II strains of *H influenzae* were found less often in the cystic fibrosis group. Half of the biotype V strains produced β -lactamase.

Biotyping of strains of the genus *Haemophilus* has been carried out extensively in recent years according to a scheme devised by Kilian.¹ The method has been applied as an epidemiological tool² and correlations have been established between biotypes, serotypes, antibiotic sensitivity, and site of isolation.^{3,4}

In view of the importance of *Haemophilus influenzae* as a respiratory pathogen in cystic fibrosis we undertook biotyping of strains of *H influenzae* and *H parainfluenzae* in a group of such patients and compared the results with isolates from patients with respiratory tract infections but without cystic fibrosis and a further group of patients in hospital with neither respiratory infections nor cystic fibrosis.

Patients and methods

ORGANISMS

Gram negative organisms corresponding to the general description of the genus *Haemophilus* were isolated on standard chocolate agar plates supplemented with 10 IU/ml of bacitracin.

PATIENTS

There were 179 patients in the cystic fibrosis group (group 1), 110 in the respiratory infection group (group 2), and 73 in the group with no respiratory infection (group 3). A total of 183 separate isolates were tested from group 1, 118 from group 2, and 74

from group 3. Groups 1 and 2 isolates were all from the respiratory tract. Group 3 isolates included 61 from throat swabs; two each from cerebrospinal fluid, umbilical swabs, ear swabs, and urine; four isolates from eye swabs; and one from a blood culture.

X AND V FACTOR

X and V factor requirements were determined with discs on Columbia agar base medium according to standard techniques. In addition, the requirement for X factor was tested by the ability of isolates to synthesise porphyrins from δ -amino-laevulinic acid.⁵ Strains designated as *H influenzae* in the text are those that were V factor dependent and incapable of converting δ -amino-laevulinic acid to porphyrins. On the basis of these criteria there were 188 isolates of *H influenzae*, with 82, 64, and 42 in groups 1, 2, and 3, respectively. There were 187 strains of *H parainfluenzae*, comprising 101, 54, and 32 in groups 1, 2, and 3, respectively.

BIOTYPES

Biotyping was carried out with API 10S strips to test for indole production, ability to split urea, and production of ornithine decarboxylase and β -galactosidase. The organisms were emulsified in 3.0 ml of peptone water supplemented with 0.3 ml of 1.0% haemin and 0.3 ml of β -nicotinamide adenine dinucleotide. Strips were incubated overnight at 37.0°C after addition of liquid paraffin to the indole cupule. Indole was detected with Erlich's reagent. The purity of the inoculums was checked by plating on to standard chocolate agar plates.

Table 1 Biotypes of 188 strains of *Haemophilus influenzae*

Biotype	Patient group (no and (%))			
	1	2	3	Total
I	24 (29)	15 (23)	8 (19)	47
II	19 (23)	20 (31)	18 (43)	57
III	10 (12)	11 (17)	8 (19)	29
IV	8 (10)	4 (6)	2 (5)	14
V	13 (16)	11 (17)	6 (14)	30
NT	8 (10)	3 (5)	0 (0)	11
Total	82	64	42	188

Group 1 = patients with cystic fibrosis.

Group 2 = patients with respiratory infections.

Group 3 = control group.

% figures refer to percentage of that biotype related to total of all biotypes for that group.

NT = not typeable.

Table 2 Biotypes of 187 strains of *Haemophilus parainfluenzae*

Biotype	Patient group* (no and (%))			
	1	2	3	Total
I	26 (26)	20 (37)	12 (37)	58
II	43 (42)	23 (43)	12 (37)	78
III	20 (20)	3 (5)	6 (19)	29
IV	10 (10)	8 (15)	1 (3)	19
NT	2 (2)	0 (0)	1 (3)	3
Total	101	54	32	187

*See footnote to Table 1.

SEROTYPING

Capsulated strains were detected by standard methods using rabbit antisera (Wellcome Reagents).

Results

DISTRIBUTION OF BIOTYPES

Table 1 shows that within the cystic fibrosis group *H influenzae* biotype 1 predominated and occurred significantly more often than similar isolates from the normal respiratory tracts of group 3 patients ($p = <0.05$). Biotype II is also of interest in that there appears to be a negative correlation between cystic fibrosis and the occurrence of this biotype when compared with similar isolates from normal respiratory tracts ($p = <0.01$). No significant differences in biotype distributions were noted in the case of *H parainfluenzae* strains in the three groups (Table 2).

COMPARISON OF PORPHYRIN SYNTHESIS AND X AND V TESTS

Of the total of 375 strains of *Haemophilus* examined, 336 gave agreement between X and V tests and the ability to synthesise porphyrins from δ -amino-laevulinic acid. Thirty four strains, however, would have been designated as *H parainfluenzae* on the basis of X and V results but failed to synthesise porphyrins, representing 18% of all true

H influenzae isolates. Table 3 shows distribution of the biotypes of these 34 *H influenzae* strains.

Only five strains which were found to synthesise porphyrins would have been wrongly classified as *H influenzae* on the results of X and V tests. If the figures for each biotype in Table 3 are considered as a percentage of the total numbers in each biotype as indicated in Table 1 they represent 14.8, 22.8, 10.3, 28.5, and 20% for biotypes I to V respectively.

β -LACTAMASE PRODUCTION AND BIOTYPES

Table 4 shows the relation between biotype and β -lactamase production. No fewer than 23.5% of *H influenzae* strains were β -lactamase producers compared with 7.4% for *H influenzae* isolates. Half of the β -lactamase positive strains of *H influenzae* were

Table 3 Distribution of *Haemophilus influenzae* strains giving anomalous X and V test results

Patient group*	No in each biotype					
	I	II	III	IV	V	NT
1	1	5	1	2	2	0
2	4	3	1	2	3	1
3	2	5	1	0	1	0

Figures represent the number of isolates that gave negative porphyrin tests but which have been classified as *Haemophilus parainfluenzae* on the basis of X and V test results.

*See footnote to Table 1.

Table 4 β -lactamase production of biotypes of *Haemophilus influenzae* and *H parainfluenzae*

Patient group*	Isolates	β -lactamase positive (%)	No in each biotype					
			I	II	III	IV	V	NT
<i>H influenzae</i>								
1	82	5 (6)	1	1	0	0	3	0
2	64	6 (9.3)	1	1	1	1	2	0
3	42	3 (7.1)	0	1	0	0	2	0
<i>H parainfluenzae</i>								
1	101	30 (30)	13	12	3	2	—	0
2	54	8 (14.8)	3	3	1	1	—	0
3	32	6 (18.7)	3	2	1	0	—	0

*See footnote to Table 1.

of biotype V. These seven strains represent 23.3% of all biotype V isolates. No differences were noted in the incidence of positive isolates in the three groups of patients.

DISTRIBUTION OF SEROTYPES AND BIOTYPES OF *H INFLUENZAE*

Capsule production was tested for in 152 of the 188 strains of *H influenzae*. Table 5 shows the distribution of the biotypes and serotypes of the 31 capsulated strains. Fifteen of the 41 (36.5%) biotype I strains were capsulated, most being of type b. Only 15.4% of biotype II strains were capsulated, with corresponding small percentages for the remaining biotypes.

Discussion

H influenzae of biotypes I and II are those most often isolated from septicaemic and meningitic illnesses, although geographical differences probably exist. Biotype III strains are the most common in Taiwan,⁸ and biotype V comprised 9% of isolates from cerebrospinal fluid and 25% of blood isolates in New York State.³ Biotypes II and III predominated in conjunctival isolates and also occurred among urinary tract strains.⁴ Among respiratory tract strains biotype II predominated in Sweden⁹ and in London.² Our own experience is similar for strains isolated from groups 2 and 3, but biotype II

strains were significantly less common among the cystic fibrosis group.

Three of the total of 11 strains of *H influenzae* that did not fall into biotypes I-V (Table 1) fitted the group that has tentatively been designated biotype VI.¹ Four strains gave positive reactions with ornithine decarboxylase, indole, and β -galactosidase but were urease negative. Whether these should be classified as *H influenzae* and designated biotype VII is debatable since *H influenzae* is usually considered to be β -galactosidase negative. A problem also arises with the remaining four strains that were negative in all four tests. These are being investigated further.

β -lactamase production has been recorded as being more common among biotype I strains of *H influenzae* compared with other biotypes.^{3,10} Similarly, a significantly higher incidence of β -lactamase production has been noted among capsulated compared with non-capsulated strains.⁹ In the present series, however, three of 31 (9.6%) capsulated strains were β -lactamase producers compared with 11 of 121 (9.0%) non-capsulated strains. Of more importance is the finding that of our 14 β -lactamase positive strains of *H influenzae* no fewer than seven were of biotype V. Further investigation is required to determine whether there is any relation between particular biochemical properties and ability to produce β -lactamase. Relevant to this may be the evidence that suggests an inverse relation between urease and ornithine decarboxylase production and β -lactamase production among non-capsulated strains. This has been taken to indicate that non-capsulated strains producing both enzymes may, for some reason, be unable to acquire transposon A determining lactamase production.⁹

β -lactamase production is much more common among strains of *H parainfluenzae*. No fewer than 43% of positive strains were of biotype I, which represents 32.7% of all biotype I strains. The 17 biotype II strains of *H parainfluenzae* represents 38.6% of all positive strains and 21.7% of all

Table 5 Relation between biotypes and serotypes of *Haemophilus influenzae*

Biotype	Serotype						No tested
	a	b	c	d	e	f	
I	0	13	1	1	0	0	41
II	0	3	3	1	0	0	46
III	0	2	0	0	0	0	22
IV	0	0	0	0	0	0	11
V	0	4	0	0	0	0	22
NT	0	2	0	0	0	1	10

NT = not typeable.

biotype II strains. Within the cystic fibrosis group 13 of the 26 (50%) biotype I strains were β -lactamase producers, the figure for biotype II being 27.9% of all biotype II strains. These are significantly greater than the percentage for biotypes I and II in the respiratory and control groups ($p < 0.01$). This may reflect selective antibiotic pressures within the cystic fibrosis group.

Capsulation was reported in 52 of 71 (73%) biotype I strains, of which 45 (63%) were type b, compared with only 12% for biotype II strains.¹ Similarly, 13 of 15 biotype I strains were of type b in the present series (Table 5). Significantly more capsulated strains were found in biotype I isolates compared with other biotypes. The relation between the presence or absence of capsulation and biotype is difficult to determine from published series since isolates reported have often been from cerebrospinal fluid and blood, where most are type b biotype I strains.

Since *H influenzae* is one of the major pathogenic organisms in children with cystic fibrosis biotyping procedures may have an important part to play in epidemiological investigations. In particular, certain biotypes may have increased pathogenic potential irrespective of serotype and this may correlate with certain of the phenotypically expressed biochemical properties of the organisms. Such properties may reflect the presence of virulence factors other than capsule. It will also be of interest to determine whether any increase in the incidence of β -lactamase production among *H influenzae* isolates is associated with a change in biotype distributions.

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