Nasopharyngeal carriage and antibiotic resistance of *Haemophilus influenzae* in healthy children

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SUMMARY

An investigation was undertaken to determine the isolation rate and antibiotic resistance of *Haemophilus influenzae* from the nasopharynx of young children. The 996 subjects studied were up to 6 years of age. *H. influenzae* was isolated from 304 (30·5 %) and strains of capsular type b from 11 (1·1 %). Age, sibling status, season, respiratory infection and antibiotic therapy all influenced isolation rates. The overall prevalence of antibiotic resistance in the strains isolated was ampicillin 5·4 % (all β-lactamase producers), cefaclor 0·3 %, chloramphenicol 1·3 %, erythromycin 38·2 %, tetracycline 1·3 %, trimethoprim 5·4 % and sulphamethoxazole 0 %. Ampicillin resistance was more common in type b than non-capsulated strains.

INTRODUCTION

Haemophilus influenzae is a major cause of bacterial infection in young children. Capsulated strains of Pittman type b are responsible for a variety of invasive infections, the commonest of which is meningitis. Other manifestations include epiglottitis, septic arthritis, pneumonia, cellulitis and osteomyelitis. Non-capsulate strains are a frequent cause of acute otitis media. Risk factors associated with systemic disease have included age (Committee on Infectious Diseases, 1985), season of the year (Feldman, Fraser & Koehler, 1973), family size (Ounsted, 1950), low socioeconomic status (Michaels & Schultz, 1973), day-care attendance (Istre *et al.* 1985), household contact with a case (Ward *et al.* 1979), race (Coulehan, Michaels & Williams, 1976; Ward *et al.* 1981; Fraser, 1982), immunosuppression (Weitzman *et al.* 1977), asplenia (Snyder & Brunjes, 1965) and agamma-globulinaemia (Domz & Dickson, 1957). How some of these predisposing factors act to increase the incidence of infection is unclear. In attempts to elucidate this, several investigations have measured pharyngeal and nasopharyngeal carriage rates of *H. influenzae* and marked variations in results have been encountered. In open child populations overall isolation rates between 25 % (Sell, Turner & Federspiel, 1973) and 82 % (Dawson & Zinnemann, 1952) have been recorded and for type b strains between 2 % (Lerman, Kucera & Brunken, 1979) and 15 % (Stephenson *et al.* 1985). In those living together in closed communities type b carriage rates up to 70 % (Turk, 1963) have been described.

Numerous factors have been reported to influence isolation rates. These have included age (Sell, Turner & Federspiel, 1973), racial group (Turk, 1963), nursery attendance (Turk, 1963), institutional care (Turk, 1963; Mpaire, 1970), season of

the year (Henderson *et al.* 1982), presence of respiratory infection (Masters *et al.* 1958), antibiotic therapy (Scheifele & Fussell, 1981), sampling techniques (Michaels *et al.* 1976) and laboratory methodology (Chapin & Doern, 1983). It is presumably differences in study design reflecting these many variables that has led to the diversity of findings.

In general *H. influenzae* has been considered as one of the more antibiotic susceptible of bacterial species. In 1977 a survey in England and Scotland indicated that resistance had arisen to most of the antibiotics commonly used to treat haemophilus infections although the prevalence was low (Howard, Hince & Williams, 1978). When the study was repeated in 1981 an increase in resistance was observed particularly with regard to ampicillin (Philpott-Howard & Williams, 1982). Recently higher rates of resistance have been recorded in other parts of the World (Doern *et al.* 1986). Multiply resistant type b strains have also been encountered including those resistant to both ampicillin and chloramphenicol. Isolation of these latter strains has in general been sporadic (Uchiyama *et al.* 1980; Kenny, Isburg & Michaels, 1980; Simasathien, Duangmani & Echeverria, 1980; Garvey & McMullin, 1983), however, a high prevalance has been observed in Spain (Campos *et al.* 1986).

It has been suggested that the incidence of invasive haemophilus infections may be increasing in the UK (Howard, 1987). In view of a general increase in antibiotic resistance and the possibility that the epidemiology might be changing it was decided to examine in our own area the nasopharyngeal carriage rates of *H. influenzae*, the prevalence of antibiotic resistance in the isolates recovered and the relationship between these observations and several epidemiological factors.

MATERIALS AND METHODS

The study population

Children aged up to 5 years of age attending child welfare clinics for routine examinations or immunizations and reception classes of primary schools in Gwynedd, North Wales were chosen for study. The study was undertaken between October 1984 and January 1986. Approval for the study was obtained from the Gwynedd Health Authority ethical committee. Parental consent was obtained and a questionnaire completed for each participant providing information regarding a subject's age, sex, family size and history of recent respiratory infection or antibiotic therapy.

Sampling

Samples were obtained by passing a cotton tipped, thin wire (Medical Wire and Equipment Co. Ltd) gently back from one nostril, along the floor of the nasal cavity until it touched the posterior wall of the nasopharynx. It was withdrawn after remaining in place for a few seconds. Two of the authors carried out all of the swabbing.

Immediately after they had been obtained, swabs were inoculated onto chocolate agar supplemented with 10 mg/l bacitracin. Plates were spread and then returned to the laboratory in a sealed cannister containing an atmosphere enriched with carbon dioxide (produced using Whitley Gaskit CO_2 System, Don

Whitley Scientific Ltd). Culture plates were then incubated at 37 °C for 48 h prior to further study.

Microbial identification

Organisms were accepted as *H. influenzae* if they were non-haemolytic on horse blood agar, exhibited satellitism around an XV disc and were unable to produce porphyrin from a substrate of aminolaevulinic acid (Kilian, 1976). Strains exhibiting iridescence on nutrient agar supplemented with 5% Filde's extract (Oxoid Ltd) were serotyped using agglutinating antisera from Wellcome Reagents Ltd.

Antibiotic sensitivity tests

Minimum inhibitory concentrations of the following antibiotics were determined for all strains isolated using an agar dilution method: ampicillin, cefaclor, chloramphenicol, erythromycin, tetracycline, sulphamethoxazole and trimethoprim. Doubling dilutions of antibiotics were incorporated into Oxoid DST agar supplemented with 0·25% lysed horse blood and nicotinamide adenine dinucleotide (10 mg/l, Sigma Chemicals Ltd). Inocula were prepared by diluting overnight broth cultures (Oxoid nutrient broth no. 2 supplemented with Filde's extract) to give an estimated 10^4 (10^2 in the case of sulphamethoxazole) c.f.u. per 3μl drop when applied to the surface of a plate with a multipoint inoculator (Denley Ltd). Inoculated plates were incubated in air at 37 °C for 18 h. The end point was interpreted as the lowest concentration inhibiting growth: a faint haze was disregarded. All strains were tested for β-lactamase production using chromogenic cephalosporin (O'Callaghan, Morris & Kirby, 1972).

Organisms requiring more than 2 mg/l of chloramphenicol for inhibition were tested for *in vitro* inactivation of the drug using the method of Howard, Williams & Poole (1986).

Statistical methods

Differences were statistically analysed using the χ^2 method with the Yates correction for small numbers. A P value of less than 0·05 was considered significant.

RESULTS

Nasopharyngeal colonization

H. influenzae was isolated from 304 of 996 subjects studied (30·5%). Twenty-three of the isolates were en-capsulated. These represented 7·5% of the strains of *H. influenzae* isolated and an overall carriage rate of 2·3%. The distribution of serotypes is listed in Table 1.

The isolation rates of *H. influenzae* by sex, age group and family size are presented in Table 2. There were no differences in the colonization rates between the sexes; however, age markedly influenced isolation. With increasing age the overall carriage rate increased from 21·7% in children under 1 year to 57·5% in those aged between 5 and 6 years.The carriage rate of type b strains was highest in children aged between 2 and 3 years (7·2%). This was significantly higher than the prevalence in children above ($P < 0.05$) or below ($P < 0.001$) this age group.

Table 1. *The nasopharyngeal colonization of children with* H. influenzae – *serotype distribution*

Serotype	Number	Percent of *H. influenzae* isolated	Carriage rate (%)
a	0		
b	11	3·6	1·1
c	0		
d	2	0·65	0·2
e	2	0·65	0·2
f	8	2·6	0·8
Non-typable	281	92·4	28·2
Total	304	100	30·5

Table 2. *The nasopharyngeal colonization of children with* H. influenzae – *influence of sex, and family size*

	Total sampled	No. (%) carrying *H. influenzae*	No. (%) carrying *H. influenzae* type b
Sex			
Male	487	152 (31·2)	4 (0·8)
Female	509	152 (29·8)	7 (1·3)
Age (months)			
⩽ 12	585	127 (21·7)	3 (0·5)
13–24	107	26 (29)	0
25–36	69	22 (32)	5 (7·2)
37–48	57	24 (42·1)	1 (1·7)
49–60	102	55 (53·9)	1 (1·0)
61–72	66	38 (57·5)	1 (1·5)
No. of siblings			
0	338	41 (12·1)	0
1	396	135 (34)	6 (1·5)
⩾ 2	252	122 (48·4)	5 (1·9)

Table 3. *The nasopharyngeal colonization of children with* H. influenzae – *interaction of age and family size*

No. of siblings	Age (months)	No. in group	No. (%) *H. influenzae*	No. (%) type b
0	0–24	320	31 (9·7)	0
	25–48	6	0	0
	49–72	16	10 (62·5)	0
1	0–24	237	66 (27·8)	2 (0·8)
	25–48	80	24 (30·0)	3 (3·7)
	49–72	76	40 (52·6)	2 (2·6)
⩾ 2	0–24	137	56 (40·9)	1 (0·7)
	25–48	38	21 (55·2)	2 (7·9)
	49–72	71	42 (59·1)	1 (1·4)

Table 4. *The nasopharyngeal colonization of children with* H. influenzae –
influence of respiratory infection

		No. % carrying	
	No. of samples	H. influenzae	H. influenzae type b
Current symptoms			
None	666	167 (25)	6 (0·9)
Runny nose/coryza/snuffles	215	80 (37)	4 (1·8)
Other*	102	52 (51)	1 (0·9)
Symptoms in past 2 weeks			
None	506	114 (22·5)	4 (0·8)
Runny nose/coryza/snuffles	324	110 (34)	6 (1·8)
Other*	156	74 (47)	1 (0·6)
Symptoms in family within past 2 weeks			
None	675	177 (26)	6 (0·9)
Runny nose/coryza/snuffles	158	64 (40·5)	2 (1·3)
Other*	109	48 (44)	3 (2·7)

* Includes pharyngitis, otitis media, sinusitis and lower respiratory infection.

The carriage rate also varied with family size. Both the overall and type b isolation rates rose as sibling numbers increased. The difference in overall carriage rates between children with no siblings and those with one, and with one compared with two were both significant ($P < 0.001$). The difference in carriage rates of type b strains in children without siblings compared with those with two or more was significant ($P < 0.05$).

Table 3 demonstrates the combined effect of family size and age. Children under 2 years of age had the lowest isolation rates of *H. influenzae* in each sibling category; however, the rate increased as sibling numbers rose for each age group under 4 years. Above that age there were no significant differences in the isolation rate when the two siblings groups were compared.

Table 4 illustrates the influence of symptoms of respiratory infection on the isolation rate. The significance of snuffles, runny noses or other coryzal symptoms were often difficult to assess and the results for this category have been listed separately from those where there was a more definite history of infection. The overall isolation rate was increased when symptoms were either current, or had been present in the subject or the family within the preceeding 2 weeks. This was most marked in the category where the occurrence of infection was most certain. The presence of respiratory infection did not influence the isolation of type b strains.

There were 60 instances where there was no evidence of infection in a subject where there had been symptoms in the family. In this group, 26 (43 %) carried *H. influenzae* compared with 26 % where there were no family symptoms ($P < 0.01$).

The influence of antibiotic therapy is shown in Table 5. A higher isolation rate was obtained from children who had received antibiotics within 2 weeks of sampling compared with those who had not ($P < 0.001$). The increased isolation rate of type b strains seen in the former group was not significant.

The percentage isolation of *H. influenzae* in subjects sampled at different times

Table 5. *The nasopharyngeal colonization of children with* H. influenzae *– influence of antibiotic therapy*

		No. (%) carrying	
	No. of samples	H. influenzae	H. influenzae type b
No antibiotics	743	179 (24)	7 (0·9)
Antibiotics within 2 weeks of sampling	131	62 (47)	3 (2·3)

Table 6. *The nasopharyngeal colonization of children with* H. influenzae *– influence of season*

Period	No. tested	No. positive for H. influenzae (%)
1984		
October–December	142	37 (26)
1985		
January–March	206	79 (38)
April–June	148	43 (29)
July–September	223	41 (18)
October–December	196	77 (39)
1986		
January	81	27 (33)

Table 7. *The antibiotic susceptibility of 298 strains of* H. influenzae

Antibiotic	$\leqslant 0\cdot06$	0·12	0·25	0·5	1	2	4	8	16	$\geqslant 32$
Ampicillin	—	16	136	126	4	—	3	6	7	—
Cefaclor	—	2	3	11	56	171	54	1	—	—
Chloramphenicol	3	84	130	66	11	—	3	1	—	—
Erythromycin	—	1	5	31	147	105	9	—	—	—
Tetracycline	—	43	212	38	1	—	4	—	—	—
Trimethoprim	164	84	25	5	2	1	1	7	2	7
Sulphamethoxazole	14	60	55	104	12	26	16	11	—	—

No. of strains conforming to each minimum inhibitory concentration (mg/l)

of the year is shown in Table 6. The figure obtained for the July to September period was lower than that seen at other times ($P < 0\cdot05$).

Antibiotic resistance

The distribution of the minimum inhibitory concentrations (MIC's) of seven antibiotics for 298 strains is presented in Table 7. Organisms were considered resistant if the MIC's were greater than 2 mg/l for ampicillin, 4 mg/l for cefaclor, 2 mg/l for chloramphenicol, 1 mg/l for erythromycin, 2 mg/l for tetracycline, 4 mg/l for trimethoprim and 8 mg/l for sulphamethoxazole. Using these criteria the overall prevalence of resistance was ampicillin 5·4 % (all were β-lactamase producers); cefaclor 0·3 %; chloramphenicol 1·3 %; erythromycin 38·2 %; tetracycline 1·3 %; trimethoprim 5·4 % and sulphamethoxazole 0 %.

All four chloramphenicol resistant strains inactivated the drug *in vitro* and in addition were resistant to tetracycline; two were also resistant to trimethoprim.

Ampicillin resistance was present in 3·9 % of non-typable strains compared with 27 % of type b ($P < 0·01$). There were no significant differences in the prevalence of antibiotic resistance for any of the epidemiological factors investigated including a history of antibiotic therapy within 2 weeks of sampling.

DISCUSSION

In the present study predominantly healthy children were selected for study who were attending either welfare clinics or primary schools. In previous investigations populations have been studied using either throat or naso-pharyngeal swabs or both. It has been suggested that throat swabs are associated with higher isolation rates (Michaels *et al.* 1976), however, Masters *et al.* (1958) were unable to demonstrate appreciable differences for the age group studied here. It was felt unreasonable to submit infants to sampling from two anatomical sites for this exercise. Nasopharyngeal swabs were found to be easier to maintain consistency of sampling and were chosen as the preferred technique.

A number of reports have indicated that there is a male predominance in the incidence of invasive haemophilus disease. This has been noted for meningitis (e.g. Goldacre, 1976), and particularly for epiglottitis (e.g. Bass, Steele & Wiebe, 1974; Jones, 1970). In accordance with previous findings the results of the present study did not indicate any relationship between sex and carriage rate.

The occurrence of invasive haemophilus disease is also affected by age and sibling status. The majority of cases of meningitis occur in children under 3 years of age and the peak incidence is seen in those less than 1 year of age (Goldacre, 1976). Epiglottitis tends to occur in a slightly older age group (Turk & May, 1967). Haemophilus menigitis is also related to numbers of siblings, a disproportionate number of cases occurring as family size increases (Ounsted, 1950; Michaels & Schultz, 1973). Correlations between carriage rates, age and sibling status have provided inconsistent results. In the present study there was a steady increase in the overall recovery rate of *H. influenzae* up to 4 years of age, type b carriage being most prevalent between 25 to 48 months. This relationship between age and isolation of *H. influenzae* was similar to that previously observed by other authors (e.g. Kilian, Heine-Jensen & Bulow, 1972; Sell, Turner & Federspiel, 1973). Increasing family size was associated with both an increased overall isolation rate and increased isolation of type b. Lerman, Kucera & Brunken(1979) and Stephenson *et al.* (1985) were both unable to demonstrate differences in carriage rates for either age or sibling status; however, all the subjects in the former study, and the majority in the latter were older than 3 years of age. Age and sibling status had no effect on isolation rates in this age group in the present investigation.

It would appear that in our area the carrige rate of type b strains is lowest during the period of maximum susceptibility to haemophilus meningitis. Increasing family size is associated with a higher incidence of disease and the findings indicate that this may act by increasing contact with the organisms

during this period. Further studies examining the dynamics of carriage in this population are required to elucidate this point.

Early reports in the literature suggested that *H. influenzae* becomes more prevalent in the upper respiratory tract several days after the onset of a coryzal illness (Straker, Hill & Lovell, 1939). Masters *et al.* (1958) confirmed that nasal carriage was higher in the presence of a nasal discharge and postulated that this was due to a combination of both increased numbers and acquisition from a secondary source. Sell, Turner & Federspiel (1973) demonstrated a marked increase in isolation of both nontypable and type b strains in the presence of respiratory illness and noted that this was more marked the greater the severity of the illness. Our results were similar although type b carriage was not affected. The increased prevalence in children who had been well but for whom there was a history of respiratory infection in the family, provided indirect evidence to suggest that the increase in *H. influenzae* associated with respiratory infection may lead to increased transmissibility.

Further evidence supporting an association between carriage and respiratory infection is provided by Henderson *et al.* (1982) who demonstrated that there was a seasonal variation in upper respiratory tract colonization with *H. influenzae* which correlated with the prevalence of circulating respiratory viruses and acute otitis media. Consistent with this was the finding that isolation was significantly lower in subjects examined between the July and September period in the present study. Other authors (e.g. Lerman, Kucera & Brunken, 1979; Schiefele & Fussell, 1981; Stephenson *et al.* 1985) have failed to link respiratory infection with carriage. These inconsistencies are difficult to explain.

Recent administration of antibiotics was associated with an increased isolation of *H. influenzae*. This may have related to respiratory infection or to the suppression of more sensitive pharyngeal flora. Stephenson *et al.* (1985) and Schiefele & Fussell (1981) demonstrated an increase in ampicillin resistance if either ampicillin or amoxicillin had been given in the preceding 3 or 6 months. This was not seen in the present study but detailed antibiotic histories were not available and information regarding prescription was limited to 2 weeks prior to sampling.

Although there was variation in carriage rates in relation to several of the categories studied, the type b isolation rate, particularly in relation to the younger age groups, was lower than many of those previously recorded. Gwynedd is a rural county in North Wales with a low population density. It has previously been noted (Turk, 1975) that the prevalence of antibody levels to *H. influenzae* type b in industrial areas in the United Kingdom may be higher than those encountered in rural districts. Most studies of haemophilus carriage rates have been undertaken in urban situations and the finding of a lower *H. influenzae* type b prevalence in Gwynedd is compatible with the possibility that contact with the organism is less frequent in this setting.

The antibiotic susceptibility results provided an estimate of the prevalence of resistance in a community, unbiased by hospital populations. It is thus of interest to compare the similarity between these results and those recorded in the multi-centre survey carried out in the UK in 1981 (Philpott-Howard & Williams, 1982), of which a large percentage of strains would have been derived from hospital

patients. Ampicillin resistance was present in 5·4 % of strains compared with 6·2 % (5·8 % β-lactamase positive) in the UK study, tetracycline 1·3 % compared with 3·1 %, chloramphenicol 1·3 % compared with 1·03 % and sulphamethoxazole 0 % compared with 1·5 %. Trimethoprim resistance was markedly more common (5·4 %) than in the 1981 survey (1·4 %). In accordance with previous UK studies (Howard, Hince & Williams, 1978; Philpott-Howard & Williams, 1982) there was significantly higher prevalence of β-lactamase production in type b compared with non-capsulate strains. No β-lactamase negative, ampicillin-resistant organisms were encountered. Cefaclor has previously been shown to be active against β-lactamase producers (Neu & Fu, 1978) and this was confirmed in the present study.

Erythromycin resistance was recorded in 38·2 % of strains examined; however, it is difficult to ascribe with certainty a cut-off point on the basis of the sensitivities recorded. High-level resistance to erythromycin in *H. influenzae* was not present and the strains represented a continuous population with regard to susceptibility to this agent. The highest concentration accepted to represent sensitivity was equivalent to the peak serum levels achieved after 500 mg oral doses of erythromycin stearate and base in fasting subjects (Malmborg, 1979).

The results of this study indicate that several epidemiological factors which have been shown to affect the incidence of haemophilus disease may also influence the nasopharyngeal isolation of *H. influenzae* in a childhood population. Antibiotic resistance in this species is seen to be well established in our community, although for most antibiotics it remains at a low level.

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