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Factors Affecting Pharyngeal *Haemophilus influenzae* Type b Colonization Rates in Children

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Over 1,300 children were studied in an analysis of factors that might affect pharyngeal colonization with *Haemophilus influenzae* type b. Our semiquantitative methods for the culture of *H. influenzae* type b, consisting of inoculation of 0.001 ml of throat swab fluid on antiserum agar plates and division of the results into three grades of intensity, showed agreement as to intensity of colonization in over 80% of repeat throat cultures. Our data also suggest that throat swabs are more efficient than nasopharyngeal swabs for detecting colonization, particularly for older children. All 17 *H. influenzae* type b carriers found with either method were detected with throat swabs, but six had negative nasopharyngeal cultures; four of these six were lightly colonized older children. Furthermore, colony counts were apt to be higher on plates inoculated with throat swab fluids. The frequency of pharyngeal *H. influenzae* type b colonization in children visiting health department clinics and pediatricians' offices was low during the first 6 months of life (0.7%) but averaged 3 to 5% throughout the rest of childhood. Approximately two-thirds of the carriers were colonized at an intensity too low to be detected by standard laboratory techniques. No influence on colonization rates was found for sex, race, season, economic status, or common childhood infectious diseases such as coryza or otitis media.

Earlier studies demonstrated that an antiserum agar medium provides a rapid, sensitive, and highly specific technique for the detection and quantitation of *Haemophilus influenzae* type b in the pharynx (8). The main purpose of the present investigation was to analyze factors that might affect pharyngeal *H. influenzae* type b colonization rates. Initially there was an effort to determine the relative efficiency of nasopharyngeal and oropharyngeal cultures and the reliability of a semiquantitative grading system for intensity of colonization. The importance of such factors as age, sex, race, season, and economic status was evaluated. There was also an attempt to ascertain whether *H. influenzae* type b colonization rates were higher in children with otitis media, coryza, or other respiratory infections.

MATERIALS AND METHODS

Population. Eighty-nine children were included in a comparative study of nasopharyngeal and oropharyngeal culture and in an analysis of a system of grading intensity of colonization. For these studies, a population with an expected high *H. influenzae* type b carrier rate was desired. Siblings of patients at Children's Hospital of Pittsburgh with *H. influenzae* type b meningitis or epiglottitis were therefore chosen.

The rest of the study subjects consisted of 1,307 children cultured in six Allegheny County Health Department clinics or in the private offices of eight pediatricians in the Pittsburgh metropolitan area. Two clinics and five of the pediatricians were located outside the city of Pittsburgh but within Allegheny County. Thirty percent of the children came from clinics, 15% were black and 47% were female. Children of all ages were represented but most were very young. About half were less than 3 years old, and almost as many were less than 1 year (26%) than were of school age (28% were 5 to 16 years old). All seasons were represented, but 76% of the cultures were obtained during the winter and spring months. The study covered a period of 3.5 years, ending in December 1975.

Study plan. Children used for the comparison of nasopharyngeal and oropharyngeal cultures had both swabbings on the same occasion. Two groups were used for evaluation of the semiquantitative grading system. One group was cultured on two occasions several days apart and the other group was cultured twice on the same occasion but separated by a 10- to 15-min interval.

For the rest of the study, one of us (C.S.P. or F.E.S.) spent most of a day once a week for several weeks at a time in health department clinics or

pediatricians' offices. These were visited on a rotating basis in an effort to obtain a representative sample of the population in the Pittsburgh metropolitan area. An attempt was made to culture all children visiting the facility on this day, with the exception of those with apparent otitis or respiratory infections and those who had been cultured previously. During the last year of the study, children with otitis media and respiratory infections of various kinds were cultured and analyzed separately from others cultured at the same time and place. Age, sex, race, date, and facility were recorded.

Swabbing technique. Throat and nasopharyngeal swabbings were all performed by four specially trained individuals. The throat was cultured with a cotton-tipped swab which was swept laterally across the oral pharynx to extend over at least one of the tonsillar regions. The nasopharyngeal swab (cotton-tipped wire) was passed completely through the nose to the nasopharynx, where it was left for about 10 s before removal. All swabs were immediately immersed in 1.0 ml of Trypticase soy broth (BBL) and inoculated onto culture medium 1 to 4 h later.

Culture medium. An antiserum agar medium was used which utilizes hyperimmune *H. influenzae* type b burro serum and bacitracin incorporated into a clear agar with a Levinthal base (8). Plates (100-mm petri dishes) were inoculated by radial streaking of swab fluids with a platinum loop calibrated to deliver 0.001 ml. The same plate was then more heavily inoculated by swabbing the periphery of the agar to demonstrate colonization too light to be detected by testing 0.001 ml of swab fluid. Plates were incubated at 37°C for 40 to 44 h, and identification was achieved by observing for halos of precipitation surrounding *H. influenzae* type b colonies.

System for grading intensity of colonization. A three-point grading system was developed. A "light" category included those carriers who probably would not have been detected with standard laboratory media. Previous work demonstrated that *H. influenzae* type b cannot be isolated from sheep blood agar, rabbit blood agar, or chocolate human blood agar with Fildes supplement when less than 20 to 30 colonies are present on simultaneously inoculated antiserum agar (8). Light colonization was therefore considered to be present when there were less than 25 colonies on an antiserum agar plate which had been inoculated by the aforementioned procedure. The "heavy" category included all plates with over 75 colonies (generally too numerous to count accurately), and the "moderate" category included the remaining positive cultures, i.e., those with 25 to 75 *H. influenzae* type b colonies on a plate.

Statistical methods. Differences were statistically analyzed by using the chi-square method with the Yates correction for small numbers. A P value of less than 0.05 was considered significant.

RESULTS

Comparison of nasopharyngeal and oropharyngeal culture. Thirty-one childhood siblings of patients with *H. influenzae* type b infection were studied. The age range was 6 months to 10 years, with a mean of 3.2 years. Table 1 shows that oropharyngeal (throat) culture was more efficient than nasopharyngeal culture in detecting *H. influenzae* type b colonization. The older childhood carriers were not apt to be colonized at the nasopharyngeal site. Only four *H. influenzae* type b carriers were over 8 years of age; the throat was lightly colonized in each (4 to 16 colonies per plate) and *H. influenzae* type b was not detected in the nasopharynx of any of them. Besides the six children who were throat positive and nasopharynx negative, there were 11 positive at both sites. Five of these had at least twice as many organisms detected at one site as the other. In one, more organisms were found in the nasopharynx, but in the other four more organisms were found in the throat.

Reliability of grading intensity of colonization. Fifty-eight siblings of patients with *H. influenzae* type b infection were studied. Throat swabbing of 23 siblings was repeated after an average interval of 3.6 days. There was considered to be agreement if both cultures in the pair fit into the same category (negative, light, moderate, or heavy, as defined earlier). In this experiment, there was agreement as to intensity of colonization in 19 pairs (83%). Agreement may have been somewhat better than this figure implies, since the culture results for two "disagreeing" pairs included a "negative" on one occasion and two or six colonies, respectively, on the other occasion.

A second experiment using 35 siblings swabbed 10 to 15 min apart resulted in agreement for 28 pairs (80%). Again this figure might be misleading since one pair of "disagreeing" cultures yielded 22 and 34 colonies, respectively, and another yielded first 60 and later over 75 colonies. The number of colonies did not appear to be influenced by the order of the swabbing or the amount of experience of the swabber.

Colonization of children visiting clinics and doctors' offices. A total of 1,110 children without apparent otitis or respiratory infection were tested. The results for 543 children under the

TABLE 1. *Comparison of throat and nasopharyngeal swabbing in the detection of H. influenzae type b carriers during the study of 31 children from families of patients with Haemophilus meningitis or epiglottitis*[a]

Nasopharynx	Throat	
	+	−
+	11	0
−	6	14

[a] Chi-square = 11.4; $P < 0.001$.

age of 5 years have been published (8). Table 2 summarizes the final results of this phase of the study, providing data on both frequency and intensity of colonization for children through age 16 years. It can be seen that the frequency is low during the first 6 months of life (0.7%) but averages 3 to 5% throughout the rest of childhood. There is also a suggestion of higher intensity of colonization in the younger children, but it is not statistically significant. Approximately half of those studied were less than 3 years of age and six of the 15 carriers in this age group were heavily colonized. In contrast, only 2 of 21 older childhood carriers were heavily colonized.

During a later phase of the study, an additional 197 children with otitis media, upper-respiratory infection (coryza), and other respiratory infections were compared to 393 children without otitis or respiratory infection seen concurrently in pediatricians' offices. Table 3 shows that the *H. influenzae* type b carrier rates for children with these illnesses were not significantly higher than the rate for the uninfected children. There was also no relationship between illness and intensity of *H. influenzae* type b colonization.

Finally, the entire group of 1,307 children cultured in clinics and doctors' offices was analyzed to determine whether other factors might have influenced the *H. influenzae* type b colonization rate. Table 4 shows the apparent lack of effect of sex, race, and season. Moreover, the rate was not significantly different for the 385 children cultured in the six health department clinics (2.6%), as compared with that for the 922 children cultured in the offices of the eight pediatricians (3.8%).

TABLE 2. *Frequency and intensity[a] of pharyngeal H. influenzae type b colonization of 1,110 children without apparent respiratory infection cultured at doctors' offices and health department clinics*

Age	No. colonized with *H. influenzae* type b			
	Light	Moderate	Heavy	Total (%)
0-5 mo	0	0	1	1/140 (0.7)
6-11 mo	2	0	2	4/144 (2.8)
1 yr	3	1	1	5/172 (2.9)
2 yr	3	0	2	5/137 (3.6)
3 yr	3	1	0	4/103 (3.9)
4 yr	4	0	0	4/108 (3.7)
5-8 yr	5	2	2	9/170 (5.3)
9-12 yr	1	1	0	2/87 (2.3)
13-16 yr	1	1	0	2/49 (4.1)
Total	22	6	8	36/1,110 (3.2)

[a] Intensity of colonization considered light if less than 25 colonies, moderate if 25 to 75 colonies, and heavy if more than 75 colonies (on antiserum agar culture plate).

DISCUSSION

Nearly all publications including information on *H. influenzae* type b colonization have been based on nasopharyngeal rather than oropharyngeal cultures (1-4, 9, 10, 12, 15, 16). Our data suggesting the superiority of throat culture for *H. influenzae* type b were unexpected, since Masters et al. (7) had reported that throat and nasopharyngeal cultures were about equally efficacious for isolation of encapsulated *H. influenzae*, most of which were type b.

The present investigation has demonstrated that our method of culture can provide reliable data on the intensity of pharyngeal *H. influenzae* type b colonization. The use of this semiquantitative technique resulted in information suggesting that older children are less likely to be heavily colonized than younger children, as well as more likely to be throat positive and nasopharynx negative for *H. influenzae* type b.

TABLE 3. *Pharyngeal H. influenzae type b colonization rates for children with otitis media and respiration infection*

Health status of child	Proportion[a] (%)
Acute otitis media	4/84 (4.8)
Upper-respiratory infection	3/53 (5.7)
Other respiratory infection[b]	2/60 (3.3)
Total with otitis or respiratory infection	9/197 (4.6)
Neither otitis nor respiratory infection	14/393 (3.6)

[a] Proportion, number colonized/number tested.
[b] Includes pharyngitis, laryngitis, bronchitis, and infection at multiple respiratory sites.

TABLE 4. *Lack of effect of sex, race, and season on the frequency of pharyngeal H. influenzae type b colonization of 1,307 children visiting clinics and doctors' offices*

Category	No. colonized/no. tested (% colonized)
Sex	
Male	23/678 (3.4)
Female	22/629 (3.5)
Race	
White	39/1,108 (3.5)
Black	6/192 (3.1)
Other	0/7
Season	
Fall	8/197 (4.1)
Winter	18/411 (4.4)
Spring	13/577 (2.3)
Summer	6/122 (5.0)

A somewhat similar observation has recently been made by Hendley et al. (6) for the pneumococcus; throat culture was reported to be significantly more efficient than nasopharyngeal culture in detecting colonization in adults. In that study pneumococcal colonization in children was detected more often with throat swabs than with nasopharyngeal swabs, but the difference was not a significant one. Masters et al. (7) showed little difference between nasopharyngeal and throat culture for the pneumococcus in adults, but they found nasopharyngeal sampling to be slightly more sensitive in children.

Little is known about circumstances that affect the frequency of *H. influenzae* type b colonization. Families including a child with *H. influenzae* type b meningitis or epiglottitis are apt to contain asymptomatic carriers (3, 15, 16). Sibling carrier rates of over 50% are to be expected in such families. Other influences on *H. influenzae* type b colonization are less well studied, although some have reported lower carrier rates with increasing age (3, 7, 9, 16). In a large study from London (7), *H. influenzae* type b carrier rates were found to be about 3% for children under 5 years, 0.8% for older children, and 0.4% for adults. A study from Uganda (9) also reported higher rates in preschool than in school-age children. The present study in Pittsburgh clinics and doctors' offices revealed a fairly constant rate throughout childhood after the first six months of life. Similar results were reported from Jamaica (15), where *H. influenzae* type b was found in 2% of 100 infants 3 months to 2 years old, and in 3.5% of 200 school-age children.

Part of the explanation for discrepancies in colonization rates among various studies might be the small number of older children studied. For example, there were only 17 children over 5 years old included in the study from Uganda (9). There were only 49 children above the age of 12 years in the Pittsburgh study. Another reason for discrepancies might be differences in sensitivity of the cultural methods used. It is conceivable that our use of antiserum agar medium and throat rather than nasopharyngeal swabs allowed detection of appreciably more lightly colonized older children than were found in some of the earlier investigations.

The present study failed to demonstrate higher pharyngeal *H. influenzae* type b colonization rates during various common childhood infections. This finding contrasts with those of Sell et al. (12), who found both typable and nontypable (unencapsulated) *H. influenzae* present more often when children were ill than when they were not. We found that children with upper-respiratory infection did not have a significantly increased rate of *H. influenzae* type b colonization, although reports in the older literature suggest that *H. influenzae* (untyped) becomes more prevalent several days after the onset of coryza (13, 14). The carrier rate for Pittsburgh children with otitis media was also not increased, a finding which is consistent with extensive data indicating that most otitis media associated with *H. influenzae* is due to unencapsulated strains (5, 11, 12).

No support could be found in our data for an influence on *H. influenzae* type b colonization for sex, race, season, or economic status, as indicated by type of health care facility utilized. Groups of young children in residential facilities may occasionally have high carrier rates (2, 9, 15), as may families including children with serious *H. influenzae* type b infections (3, 15, 16). For most open populations, however, the *H. influenzae* type b carrier rate is low, usually less than 5% (1, 7, 9, 12, 15). We have found that about two-thirds of the childhood carriers have too few organisms in the pharynx to be detected by standard laboratory media. If these children with light colonization are excluded, the *H. influenzae* type b carrier rate approaches 1%. This figure should be kept in mind when considering the possible significance of the pharyngeal isolation of *H. influenzae* type b from a child with a condition which might be caused by this organism.

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