School-Aged Children: A Reservoir for Continued Circulation of Haemophilus influenzae Type b in the United Kingdom

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Background. A resurgence of Haemophilus influenzae type b (Hib) disease occurred in the United Kingdom between 1999 and 2003 and was partially attributed to lower immunogenicity of combination vaccines. The reservoir for Hib that led to transmission in this period is unknown.

Methods. We estimated the point prevalence of Hib carriage in school-aged children and adults, using oropharyngeal swabbing and selective media. We characterized the Hib isolates by multilocus sequence typing (MLST) and measured Hib antibody concentrations in adults by enzyme-linked immunosorbent assay.

Results. Point prevalence for Hib carriage in 855 children aged 6–16 years was 4.2% (95% confidence interval [CI], 2.5%–5.9%). Five clonal groups of Hib were identified by MLST, 86% from the lineage of sequence type 6. No Hib was isolated in 385 adults (upper limit of 95% CI, 0.95%). The geometric mean concentration of serum antibody to polyribosylribitol phosphate was 0.47 μg/mL (95% CI, 0.37–0.59 μg/mL) in adults.

Conclusions. Hib carriage is common in school-aged children, who are a significant reservoir for ongoing transmission of Hib to susceptible individuals in the United Kingdom. Surveillance of transmission and immunity across all ages of the population is essential to monitor the evolution of Hib epidemiology.

Haemophilus influenzae type b (Hib) conjugate vaccines have been widely used around the world since the late 1980s to protect against invasive Hib disease in early childhood. Conjugation of the Hib surface polysaccharide polyribosylribitol phosphate (PRP) to a carrier protein results in a vaccine that not only stimulates specific anti-PRP antibody production but also primes for immunological memory and inhibits acquisition of carriage [1].

Routine immunization of infants against Hib was established in the United Kingdom in 1992, with a 3-dose schedule of Hib conjugate vaccine at 2, 3, and 4 months of age. Disease rates fell in all ages and remained very low for the first 6 years but began to increase again from 1998, principally affecting children aged 1–4 years [2]. By 2002, it was apparent that the increased rate of vaccine failures could, in part, be attributed to interaction of the Hib component of an acellular pertussis–containing combination vaccine (diphtheria and tetanus toxoids [DT] and acellular pertussis [DTaP]–Hib) with the other components, resulting in a reduced primary antibody response [3]. A booster campaign for children under the age of 4 years was conducted in 2003 to raise population immunity once more, and subsequently disease rates have fallen [4].

Other countries have reported the reemergence of Hib disease despite immunization programs. The Netherlands has used an infant schedule that includes a

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booster dose at 11 months, but investigators have documented an increase in disease several years after the introduction of the vaccine [5]. A resurgence of disease was reported in a native Alaskan population after a change to the conjugate vaccine, which was partly attributed to the persisting circulation of Hib (9% colonization in preschool children) [6].

The reason for the reemergence of Hib disease in the United Kingdom is multifactorial. Numerous contributing factors have been suggested [5, 7], but 3 key issues have not been clarified. First, for Hib disease rates to rise so rapidly after a reduction in vaccine efficacy, Hib must have been circulating in the population, but there were few data to indicate ongoing colonization. Before the introduction of Hib vaccine, the rate of colonization in preschool children was 8%–12% [8, 9], but a series of samples from preschool children attending day care after the vaccine was introduced demonstrated a clear fall in carriage: 4% in 1992, 1% in 1994, and 0% in both 1997 and 2002 [10]. Another study from 1994 found a point prevalence of 0%–2% in 3–4-year-olds from whom swabs were obtained on 5 occasions during 1 year [11]. After the booster campaign in 2003, which offered reimmunization to all children <4 years old, disease rates fell in all age groups, including unvaccinated adults. This suggests Hib circulation in preschool children, despite the lack of documented carriage, and there are no data for other age groups. Where is the reservoir for Hib?

Second, the role in maintaining immunity of repeated exposure to Hib antigens through colonization, natural boosting, has not been estimated. In unvaccinated populations, repeated encounters with Hib throughout childhood are believed to lead to protective levels of anti-PRP antibody. The production of anti-PRP antibodies by cross-reacting antigens from other organisms has been reported, but the clinical significance is not well understood [12]. Unvaccinated children in the United Kingdom from the prevaccine era showed a rise in anti-PRP antibody from nonprotective levels at 1 year of age to >1 μg/mL at age 15 years [13]. Similarly, vaccinated cohorts also eventually develop similar antibody levels irrespective of their vaccination schedules. The association of higher anti-PRP antibody concentrations after colonization with Hib also seems to confirm this finding [11, 14, 15]. Anti-PRP antibodies have been reported to plateau at ~1 μg/mL throughout adulthood [16–18].

Finally, an important factor to consider with the reemergence of any disease is the emergence of new clones, as has been observed with pertussis [19]. A number of techniques have been used to characterize Hib isolates, including pulse-field gel electrophoresis [20], multilocus enzyme electrophoresis [21], and multilocus sequence typing (MLST) [22]. The latter is currently considered the most appropriate tool for molecular epidemiology, because of its reproducibility and access to the international database at the MLST Web site (http://www.mlst.net) for verification of strains [23, 24]. What are the colonizing strains in the United Kingdom? The present study was undertaken to estimate the point prevalence of Hib carriage in school-aged children and adults, to survey the seroprevalence of Hib antibody in adults, and to document the diversity of colonizing Hib strains in the United Kingdom.

METHODS

Subject Recruitment

School-aged children. Healthy children 6–16 years of age were recruited from schools in Oxfordshire in 2005. Informed consent was given, and information was collected on risk factors associated with invasive disease, including age, sex, household size, number and age of siblings, and cigarette smoking. Oxfordshire has a low level of deprivation compared with other areas of the United Kingdom but is close to average for income and levels of employment. Vaccination status was not available, but immunization rates have remained >95% during the past decade and mainly >97% in the region.

Adults. Mothers of preschool children and 20–40-year-old working adults were recruited through childcare/toddler group organizations or through workplaces, respectively, with informed consent provided. Volunteers were excluded if they had a history of known or likely immunodeficiency or antibiotic use within the past 4 weeks. Ethical approval was obtained from Oxfordshire’s research ethics committee.

Sample Collection and Analysis

Oropharyngeal swabs. A single throat swab was collected from each participant with a sterile cotton-tipped wooden swab. The swab was wiped across the rear of the oropharynx and tonsils, with care taken not to touch the teeth, gums, or tongue. Swabs from adults were inoculated directly onto the Hib antisera agar plates (Columbia base with yeast extract, NAD, hemin, bacitracin, and sheep anti-Hib antisera) within 6 h of swab collection [14]. Swabs from children were stored in a 2-mL bijou bottles containing Haemophilus transport medium (tryptone soya broth, nicotinamide adenine dinucleotide [NAD], and hemin) and inoculated onto Hib antisera agar plates (Columbia base with yeast extract, NAD, hemin, bacitracin, and sheep anti-Hib antisera) within 6 h of swab collection [14]. Swabs from adults were inoculated directly onto the Hib antisera agar. Controlled experiments with swabs plated directly and swabs inoculated in Haemophilus transport medium before plating out did not show differences in colony growth or ability to detect Hib in artificially positive samples.

Microbiological analysis. All samples were incubated at 37°C in an atmosphere of 5% carbon dioxide and checked at 24 and 48 h for iridescent or precipitating colonies. After storage at 4°C for another 5 days, samples were discarded if no further precipitation was found. Colonies identified by iridescence and/or precipitation were picked onto chocolate agar plates and incubated at 37°C in an atmosphere of 5% carbon dioxide overnight. X and V dependence (Mast Group) and slide agglutination (polyvalent [Becton Dickinson] and type b and e antisera [Bio-Stat]) were tested to confirm the identity of the organism as...
H. influenzae and type b or e, respectively. Slide agglutination with serotype e antiserum was used as the negative control, because type e is the next most common strain of encapsulated H. influenzae after type b. All H. influenzae isolates identified by X and V dependence were emulsified in a mixture of tryptone soya broth and 10% glycerol solution and stored at −80°C.

Confirmation of Hib by polymerase chain reaction. Isolates were confirmed as H. influenzae type b by polymerase chain reaction, as described by Falla et al. [25], using Hib primers b1 and b2 (Operon Biotechnologies). Gel electrophoresis was used to identify amplified DNA.

Multilocus sequence typing (MLST). The allelic profile of the Hib isolates was obtained by sequencing internal fragments of the 7 housekeeping genes, as described elsewhere by Meats et al. [22]. Samples were sequenced at the Department of Zoology, University of Oxford, with a 3730xl DNA analyzer (Applied Biosystems). The forward and reverse sequences were edited and trimmed using the Sequence Typing Analysis and Retrieval System (STARS) and submitted to the MLST Web site (http://www.mlst.net) for assignment of allele numbers and sequence type.

Serum sampling and Hib ELISA analysis. A 5-mL blood sample was collected from the first 100 adults participating in the swabbing study who provided informed consent (50 from the mothers of toddlers and 50 from the workplace group). Samples were centrifuged, and the serum was stored at −80°C. The anti-PRP antibody concentration was quantified using a standardized ELISA technique, as described elsewhere [26].

Statistics
With no previous point prevalence figures available for these age groups in the United Kingdom, an estimated prevalence of 1% was used to calculate a sample size of 500 required for each study group, giving a 95% confidence interval (CI) of 0.3%–2.3% with the binomial exact method. Point prevalence was estimated by dividing the number of observed cases by the number of subjects swabbed. For groups of school-aged children, CIs were calculated to account for the fact that subjects were recruited within schools. The SAS procedure Surveyfreq was used to compute variance estimates based on the clustering by school in the study design, with equal weighting for each individual (i.e., adjusting for clustering effect). Associations between Hib carriage and other factors were assessed by χ² or Fisher’s exact test. The amount of clustering in schools was assessed by simulation. The 36 positive results were assigned to participants randomly and under assumptions of clustering within schools. The outcome was compared to our observation of cases in 12 schools. All analysis was performed using SAS (version 8.2; SAS Institute).

RESULTS

Subject Recruitment

School-aged children. Of the 83 schools approached between January and May 2005, 24% took part in the study. The schools were from both the state and independent sectors, distributed throughout urban, suburban, and rural areas of Oxfordshire. A total of 1010 subjects were recruited, with a recruitment rate of 21% (range, 3%–62%).

Of 1010 subjects, 155 (15%) were excluded from the analysis. One child was too young, 4 children had taken antibiotics before participation, and 150 subjects were excluded because of an error in the preparation of a batch of Hib antiserum agar. There were no significant differences in the demographic profiles between the excluded subjects and those included in the study, as summarized in table 1.

Adult subjects. Among adults, 17% (16 of 94 subjects) in nursery/toddler groups and 15% (16 of 105 subjects) in workplaces participated. A total of 388 adults (200 mothers of preschool child and 188 working adults) were recruited between March 2004 and May 2005. The proportion of subjects recruited from any organization ranged from 2% to 11%. Three subjects were excluded for recent antibiotic use. Demographic information is summarized in table 2.

Hib Isolation

Hib was isolated from 36 of the 855 oropharyngeal swabs collected from children, giving a carriage rate of 4.2% (95% CI, 2.5%–5.9%). The carriage rate was higher in the 6–12-year-olds (prevalence, 4.8% [95% CI, 2.7%–7.0%]) than in the 13–16-
year-olds (prevalence, 3.3% [95% CI, 0.3%–6.4%]), although the CIs are wide and overlap. The carriage rate by age group ranged from 2.7% to 8.2% (figure 1). There was a nonsignificant difference in Hib carriage between female and male subjects (2.5% for female vs. 5.5% for male subjects; \( P = .08 \)). Identification of Hib carriage was not affected by household size, number of siblings, or history of cigarette smoking. Hib was isolated in 60% of the schools visited, and the rate of carriage within schools varied from 0% to 12% of the subjects recruited. The carriage rates observed in 12 schools gave no evidence of clustering in schools above that expected by chance.

MLST was performed on the 36 isolates collected. Sequence type (ST) 6 was found in 31 samples (86%). Five STs were found, although none were new to the Hib MLST database, and there was no evidence of clustering in schools. Allelic profiles are shown in table 3.

Hib was not isolated from any of the 385 adult subjects (upper limit of 95% CI, 0.95%). The slightly different method used to obtain throat swabs from adults before incubation may have affected the growth of the colonies. However, other encapsulated \( H.\ influenzae \) were detected, and no differences between adults and children were observed in the general growth of organisms. Encapsulated \( H.\ influenzae \) other than type b were isolated from 1.9% of children (16 of 855 [95% CI, 0.9%–2.8%]) and 0.8% of adults (3/385). \( H.\ influenzae \) type e was identified by slide agglutination in 1.2% of children (10/855) and 0.5% of adults (2/385).

### Serum Analysis

The geometric mean concentration of anti-PRP IgG antibody in blood obtained from a sample of adult subjects was 0.47 \( \mu g/mL \) (95% CI, 0.37–0.59 \( \mu g/mL \); <0.15 \( \mu g/mL \) in 20% and <1 \( \mu g/mL \) in 77%). There were no significant differences in antibody levels between the 2 recruitment groups.

### DISCUSSION

**Carriage of Hib.** We have found that >4% of healthy school-aged children are colonized with Hib, indicating that school-aged children may be an important reservoir for ongoing transmission of this organism in the United Kingdom. This cohort of children received the DT–whole-cell pertussis–Hib combination vaccine as 3 primary doses in infancy but would not have received any subsequent booster doses of vaccine. Hib immunization in infancy without a booster, although highly effective in preventing disease in the preschool age, does not provide complete protection against colonization into the school years. We know of no similar studies performed previously in the United Kingdom with which these data can be compared, but a clinic-based study conducted in Pittsburgh, Pennsylvania, before the introduction of routine vaccination found carriage rates of 3%–5% in 5–16-year-old children [27]. Thus, these “fully” immunized children studied in the United Kingdom had rates of

![Figure 1](image_url)

**Figure 1.** Isolation rate of \( Haemophilus influenzae \) Type b (Hib) in school-aged children, by age.
carriage comparable to those in an unvaccinated group in the United States. We also note that our county has low levels of deprivation compared with other regions of the United Kingdom and that our data may be an underestimate.

We propose the following model to explain the dynamics of Hib carriage and disease over the past 15 years in the United Kingdom. The introduction of Hib conjugate vaccine for infants in 1992 with a catch-up campaign to 4 years of age markedly reduced the circulation of Hib in the population. The dramatic fall in disease rates resulted from a combination of direct protection of individuals through vaccine-induced immunity and a reduction in the exposure of susceptible young children to Hib as a result of herd protection. Because no booster immunization was used in the United Kingdom, the transmission-blocking effect of the catch-up campaign presumably wore off during the 1990s, resulting in the modest increase in disease rates observed at the end of the 1990s and before the use of DTaP in the United Kingdom in 1999 and 2000 [2]. Not only did the reduction in immunogenicity of Hib in the DTaP-Hib combination vaccine used in 1999/2000 provide less protection, but lower anti-PRP antibody levels may also have been less effective at preventing colonization [28]. This possibility is supported by a recently published study that found colonization in 2.1% (95% CI, 0.7%–6%) of vaccinated 2–4-year-old children in 2003 [29].

We found a higher carriage rate in 6–12-year-old children than in 13–16-year-old teenagers, a difference that corresponds to their vaccination status: the former received 3 doses in infancy, whereas the latter received a single dose between the ages of 1 and 4 years. We did not detect any Hib in the 385 adults from whom swabs were obtained, which is in keeping with the low carriage rates generally assumed in adults.

Diversity of Hib strains. These data are the first to show the genetic diversity of Hib strains colonizing healthy children in the United Kingdom. The predominance of ST6 (86%) is similar to that reported for invasive strains in vaccinated populations in the United Kingdom (87%) [30], The Netherlands (76%) [31] and other countries, such as the United States, Poland, and the Czech Republic [22, 32, 33].

MLST of colonizing isolates collected from poorly vaccinated Pennsylvanian communities and of disease isolates did not always find the same strain [22]. In one community, ST44 was found in all 5 carriage isolates and 1 disease isolate. In another, ST6 was the strain for all 13 carried isolates, and ST44 was the strain for 1 disease isolate. Further studies are needed in well-vaccinated communities to determine whether disease strains are merely a reflection of colonizing strains or whether certain strains may be more invasive.

ST190 was the second most prevalent strain in our study (5%) and in the Dutch study (4%) [31]. The latter noted that ST190 (a single-locus variant of ST6 in the midh locus) was the only ST not found in the prevaccine era; it emerged as a prominent strain in the postvaccine era. In the United Kingdom, there was no other ST that occurred frequently in the postvaccine era disease isolates; however, in the prevaccine era, ST44 (15%) was second to ST6 (64%) [30]. The molecular epidemiology of disease isolates before and after the introduction of routine vaccination has recently been described by means of MLST in the United Kingdom and The Netherlands, with significant increases in genetic diversity reported [30, 31, 34].

Carriage and immunity. Carriage is required for the transmission of Hib disease, but it may have an equally important role in the maintenance of immunity. The duration of immunity provided by Hib conjugate vaccines has been shown to vary depending on the carrier protein [11, 35, 36]. The level of anti-PRP antibody wanes with time after immunization, influenced by age at vaccination and the number and timing of doses. Hib antibody levels may wane more rapidly in situations where there is little transmission of Hib and less opportunity for natural boosting.

In our study of healthy adults, 80% had Hib antibody concentrations above the estimated short-term protective threshold of 0.15 μg/mL [37]. However, the mean antibody level for our samples (0.47 μg/mL [95% CI, 0.37–0.59 μg/mL]) is significantly below the estimated long-term threshold for protection, 1 μg/mL [37]. The mean antibody levels might have been even lower if the adults sampled had included more men than women (our sample was 91% female). A survey of stored blood samples from adults in their fourth decade showed a significant decrease in Hib antibody concentrations, from 1.29 μg/mL (95% CI, 0.90–1.64 μg/mL) in 1991 to 0.53 μg/mL (95% CI, 0.35–0.73 μg/mL) in 1997 [38]. A subsequent increase to 0.77 μg/mL (95% CI, 0.56–0.97 μg/mL) in 2002 may be a reflection of increasing exposure. Both studies have shown adult Hib antibody concentrations <1 μg/mL, consistent with our finding of low Hib carriage in adults. Data from The Netherlands show anti-PRP antibody concentrations of 1.46 μg/mL in 20–24-year-olds, which diminish to 0.73 μg/mL in 75–79-year-olds [17]. This trend of waning antibody in later life may be further accentuated in an environment of low natural boosting, making the elderly population increasingly at risk of disease when exposed.

Ongoing changes to the routine vaccination schedule in the United Kingdom are likely to result in further changes in the effectiveness of the Hib vaccine. In 2005, the infant doses were changed from DTaP-Hib with oral polio vaccine to DTaP-Hib with inactivated polio vaccine. In September 2006, a booster dose of Hib vaccine to be given at 12 months of age was introduced, and further efforts are under way to ensure that children <5 years of age who have not received a booster since their infant doses of Hib do receive a booster at preschool age. However, if natural boosting is essential to sustain immunity, the absence of any natural or vaccine-induced boosting among cohorts older than preschool age may lead to an increasing cohort of individuals whose immunity wanes during the subsequent decades and who become susceptible to infection.
We have provided evidence of Hib carriage in children throughout the school years and have therefore demonstrated a reservoir of Hib in the United Kingdom that could be transmitted to the unimmunized and to those (including adults) among whom protective antibody levels have waned. Hib has not been eliminated in the United Kingdom, and ongoing surveillance for invasive Hib disease and monitoring of immunity and colonization rates across all age groups will continue to provide important insights into the impact of vaccination on the ecology of this important pathogen.

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**References**


