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Vaccination with *Haemophilus influenzae* type b meningococcal protein conjugate vaccine reduces oropharyngeal carriage of *Haemophilus influenzae* type b among American Indian children

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The effect of *Haemophilus influenzae* type b (Hib) meningococcal protein conjugate vaccine (Hib-OMPC; Merck, Sharp & Dohme) on oropharyngeal (OP) carriage of Hib was evaluated in Navajo and Apache Indian children, who are known to be at high risk for invasive Hib disease. We obtained 1423 OP swabs at well child visits from 1321 children 3 months to 4 years of age: 293 of the swabs were obtained from children before the administration of any Hib-OMPC; 1119 were taken after the primary vaccination series; and 11 after the booster dose. Swabs were tested for the presence of Hib capsular polysaccharide antigen by enzyme-linked immunosorbent assay. Forty of 1423 swabs were positive for Hib. Among the 40 positive swabs 5 (13%) were obtained from children who

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had received Hib-OMPC vaccine appropriate for age at swabbing, compared with 500 of 1383 (36%) of negative swabs. Children who were OP carriers of Hib were older than noncarriers (mean age, 13 and 9 months, respectively) and a greater proportion of carriers (48%) had symptoms of respiratory infection at the time of swabbing than noncarriers (30%). These variables were significantly related to increased risk of OP carriage of Hib when incorporated jointly in a logistic regression model: not vaccinated according to age (odds ratio 2.7, 95% confidence interval 1.00 to 7.05); increase of age in months (odds ratio 1.1, 95% confidence interval 1.02–1.10); and respiratory infection symptoms present (odds ratio 2.0, 95% confidence interval 1.06–3.77). Thus besides preventing invasive Hib disease, appropriate vaccination with Hib-OMPC appears to reduce OP carriage of Hib.

INTRODUCTION

The natural reservoir for *Haemophilus influenzae* type b (Hib) bacteria is the human oro- and nasopharynx. Hib bacteria may reside in the pharynx and cause asymptomatic carriage that may continue up to 6 months.[1, 2] Oropharyngeal (OP) carriage of Hib is rare during the first months of life, but averages 3 to 5% among healthy preschool- and school age children; carriage is again rare among adults.[2–5]

OP carriage may proceed to invasive infection either directly (epiglottitis, pneumonia) or through a bacteremic phase (meningitis, cellulitis, arthritis) via mechanisms that are still poorly understood. The epidemiology of invasive Hib disease varies between populations; an annual incidence rate of Hib meningitis from 19 to 69 of 100 000 among children under 5 years of age has been reported in the United States general population compared with 10 times higher rates among American Indians and Alaskan Eskimo children.[6–9] In populations with high incidence rates the age distribution is shifted towards younger ages. In studies conducted among Alaskan Eskimo children the OP carriage rate of Hib was similar to that reported in Caucasian populations.[3–5, 10] However, studies conducted among White Mountain Apache Indian children suggest that carriage begins earlier, with 2 to 5% rates from 2 months on (M. Santosham, personal communication), which would be in accordance with the high incidence rate and earlier onset of invasive Hib disease in this population.

The "first generation" Hib vaccine, consisting of Hib capsular polysaccharide (PRP) has been shown to be protective against invasive Hib disease among children older than 18 months of age.[11] This vaccine has also been shown to induce mucosal IgA antibody responses in saliva after parenteral immunization of adults and children.[12] However, PRP has not affected OP carriage of Hib.[11, 13] The "second generation" Hib vaccines (conjugates of PRP to protein) differ from the PRP vaccine in several aspects; they are immunogenic and protective in infants and induce a booster response to repeated vaccinations indicating their T cell-dependent character. One of the Hib conjugate vaccines (PRP-D; Connaught Laboratories, Inc., Swiftwater, PA) has recently been shown to reduce or prevent acquisition of OP carriage of Hib among Caucasian children in Finland and in Dallas, Texas.[5, 13] The Hib meningococcal protein conjugate vaccine (Hib-OMPC; Merck, Sharp & Dohme Research Laboratories) seems to be a better immunogen: it induces a significant serum Hib anticapsular antibody response after the first dose given at 2 months of age and was shown to protect against invasive Hib disease among American Indian infants after the first dose.[14]

The purpose of the present study was to evaluate whether Hib-OMPC would reduce OP carriage of Hib in a population at high risk of invasive Hib disease, the Native American infants and children.

MATERIAL AND METHODS

Study population. Enrollment for the study was initiated in April, 1991, after the licensure of Hib-OMPC and ended in December 1991. Navajo, San Carlos Apache and White Mountain Apache Indian infants and children living in their respective reservations who came for any of their Hib-OMPC vaccinations at the Indian Health Service clinics or the Johns Hopkins Project study clinics (eight sites on the Navajo reservation and one each on the two Apache reservations) were enrolled in the study after written informed consent was obtained from the parent(s)/guardian(s). The study was approved by the Committee on Human Volunteers at the Johns Hopkins University School of Hygiene and Public Health, the Indian Health Service and respective Institutional Review Boards for the three Indian populations.

Collection of OP swabs and data on study participants. Study nurses and Navajo and Apache fieldworkers were trained to obtain OP swabs from infants and children before the start of the study. The swabs were taken using a cotton-tipped applicator that was swept over the oropharynx of the child. The swabs were immediately placed in Stuart transport medium (Transpocult®; kindly provided by Orion Diagnostica, Espoo, Finland) and frozen in −70°C. At one Navajo and one Apache study site swabs (a total of 421) were simultaneously cultured for Hib bacteria by selective methods (see below) before freezing.

At the time of swabbing data were collected regarding the child's Hib vaccination status before swabbing,

any signs and symptoms of respiratory infection and the use of antimicrobial treatment during 4 weeks before swabbing. Data were recorded both from the child's medical record and by observations done by the nurse at the time of swabbing.

Vaccine and vaccination schedule. The vaccine used for the immunization of children in the present study was Hib-OMPC, which contained 15 μg of PRP and 131 to 272 μg of a Group B meningococcal OMPC. The recommended schedule for vaccination was at 2 and 4 months of age (primary series) and a booster dose at 12 to 15 months of age.[15] However, the present study started shortly after the licensure of Hib-OMPC, which had been shown to be 95% efficacious in preventing invasive Hib disease in the Navajo population.[14] Thus after licensure (and during the time of the present study) there was a period when Hib-OMPC was offered to all unvaccinated or incompletely vaccinated children up to 5 years of age.

Detection of OP carriage of Hib. *Antigen detection methods.* The frozen swabs were transported to the National Public Health Institute in Helsinki, Finland, for detection of Hib capsular polysaccharide antigen by enzyme-linked immunosorbent assay (ELISA): Polystyrene microtiter plates were coated with ammonium sulfate precipitation-purified burro anti-Hib antibodies (Burro 132; provided by John B. Robbins, National Institutes of Health, Bethesda, MD). The liquid formed in the Transpocult® tubes after freezing was boiled and used as a sample. Each sample was pipeted in quadruplicates into the wells. The bound Hib capsular polysaccha-ride (PS) was detected by adding in the next steps rabbit anti-Hib antiserum (Wellcome Diagnostics, Dartford, United Kingdom) and peroxidase-conjugated anti-rabbit IgG (Bio-Rad Laboratories, Richmond, CA). All antisera were diluted in phosphate-buffered saline containing 10% fetal bovine serum (Life Technologies, Ltd., Paisley, Scotland). Washings were done with phosphate-buffered saline containing 0.05% Tween 20®. Control wells containing 0, 0.1, 1 and 10 ng of Hib PS (kindly provided by Dr. P. McVerry, Connaught Laboratories) were included into each plate. The sample was considered positive if the optical density reading was above the optical density reading of the control containing 0.1 ng of Hib PS/ml. The specificity of the assay was checked by assaying a number of samples to which differing amounts of *H. influenzae* bacteria of serotypes a and c to f were added. These samples remained negative. Some positive and negative samples were also retested by using monoclonal anti-Hib antibodies (EII7-5, kindly provided by Dr. Dace Madore, Praxis Biologics, Inc., Rochester, NY) as the first or second antibody. The results from these assays were consistent with those received from the original assay using polyclonal antiserum (manuscript in preparation).

Culture methods. A subset of 421 swabs (30% of all swabs obtained) was cultured for Hib before freezing using the Levinthal-bacitracin antiserum agar plates (ASA plates)[16] to compare the ELISA method with the bacterial culture method. Burro anti-Hib antiserum for the preparation of the ASA plates was provided by Dr. Mimi P. Glode, Denver, CO. Culture and identification of Hib isolates were performed as described previously.[5] Colonies that were found to produce a precipitation halo on the ASA plate were confirmed to be Hib by latex agglutination and growth requirements. In addition the swabs were inoculated on chocolate agar-bacitracin plates for detection of *H. influenzae*. Of probable *H. influenzae* colonies detected on the chocolate agar-bacitracin plate 2 of each morphology were streaked on an ASA plate to determine whether they were Hib. This was confirmed, as above, with the use of the latex agglutination method and growth requirements. All Hib isolates as well as possible cross-reactive bacteria (i.e. isolates that presented a precipitation halo on the ASA plate and were found not to be Hib) were stored in skim milk at −70°C.

Definitions. A child was considered to be "vaccinated according to age at swabbing" if she/he had received Hib vaccinations according to recommendations of the Department of Health and Human Services, Navajo Area Indian Health Service, December, 1990, and recommendations of the vaccine manufacturer.[15] Children in different age groups were considered "vaccinated according to age at swabbing" if they had received the following number of doses of Hib-OMPC before obtaining of the OP swab: (1) age 3 to 5 months, at least 1 dose; (2) age 6 to 11 months, at least 2 doses; (3) age 12 to 14 months (a) 1 dose at ≥12 months of age, or (b) 2 doses with the first dose at ≥7 months of age and the second dose at ≥9 months of age, or (c) 3 doses with the third dose at ≥12 months of age, or (d) 3 doses, with the first at ≥7 months of age and the second ≥9 months of age and the third at any time thereafter; (4) age 15 months old or older (a) 1 dose at ≥15 months of age, or (b) 2 doses with the second dose at ≥12 months of age, or (c) 3 doses with the third dose at ≥12 months of age.

Statistical methods. Data were analyzed using logistic regression[17, 18] with type 1 error level of 0.05 (two-tailed). A model was tested, with Hib OP carriage (yes/no) as the dependent variable. The following independent variables were included stepwise in the model: Hib-OMPC vaccination status (vaccinated according to age at swabbing, yes/no); symptoms of respiratory infection at swabbing (yes/no); age at swabbing (continuous, in months); and antimicrobial treatment before swabbing (yes/no).

The design of the study allowed children to be swabbed more than once, which was the case for 99

(8%) of the study participants (2 swabs from 96 children and 3 swabs from 3 children). Repeat swabbings occurred with a mean 91-day interval (range, 35 to 203 days). In none of the cases was Hib detected from more than 1 swab per child. Controlling for repeated swabbing or the exclusion of a second swab did not alter the results; thus data from all swabs were included in the analyses and treated as independent observations.

RESULTS

The ELISA used in the present study was found to be 412 of 413 (99.9%) specific and 5 of 6 (83%) sensitive in detecting Hib from the swabs compared with bacterial culture by the ASA plate method, with a 5 of 8 (63%) positive predictive value. Two additional isolates were detected by the ASA plate method that produced halos around the colonies on the ASA plate but were found not to be Hib (i.e. bacteria that may cross-react with Hib antibodies). Both of these swabs were negative in the ELISA assay and thus did not cause false positive results.

A total of 1950 swabs were collected from April through December, 1991; 1423 of these were obtained from 1321 children who were at least 3 months of age at the time of swabbing and could thus have been vaccinated with Hib-OMPC before swabbing. 81% of the study participants were Navajo, 15% Apache, 2% Hopi and 2% other Native American Indian children. The age of the children at swabbing ranged from 3 months to 4 years (median, 8.7 months). Two hundred ninety-three of the children had not received any Hib-OMPC vaccine before swabbing, 1119 had received the primary series of vaccinations (i.e. ≥1 dose before age 15 months) and 11 had received the primary series and a booster dose at ≥12 months of age.

Forty children (2.8%) were found to be OP carriers of Hib (Table 1). Fourteen of the carriers had not received any Hib-OMPC (or other Hib vaccine) before swabbing, whereas 26 had received at least one dose of Hib-OMPC. However, only 5 of the 40 carriers (13%) *vs.* 36% of noncarriers had been vaccinated with Hib-OMPC according to age at swabbing. A greater proportion of carriers had symptoms of respiratory infection present at time of swabbing (48%) than did noncarriers (30%). This was especially true for conditions affecting the oro- and nasopharynx, i.e. pharyngitis, rhinorrhea, cough and otitis media, but not for conjunctivitis. In addition carriers were older (median age, 13.2 months) than noncarriers (8.4 months). Furthermore among children who had received at least one dose of Hib-OMPC before swabbing (26 carriers and 1104 noncarriers) the time between the last vaccination with Hib-OMPC and the time of swabbing was longer among carriers (median, 5.7 months; range, 59 days to 3 years) than among non-

TABLE 1. Characteristics of Navajo and Apache Indian infants and children according to their *Haemophilus influenzae* type b oropharyngeal carriage status

	No. of Swabs*	M/F (%)	Race (%)			Age (Months)			≥1 Dose of Hib-OMPC before Swabbing	Vaccinated According to Age at Swabbing (%)	Respiratory Infection Present at Swabbing (%)							Time since Last Vaccination (Months)‡		Any Anti-microbial Used before Swabbing		
			Navajo	Apache	Other	Range (months–years)	Mean ± SD	Median			Total	Rhinorrhea	Cough	Pharyngitis	Conjunctivitis	Otitis-media	Mean	Median	0–7 days before	8–14 days before	15–28 days before
Total§	1423	49/51	81	15	4.0	3.0–4.7	9.7 ± 5.7	8.7	1130	35	30	14	12	1.7	4.0	19	4.4	3.0	17	16	15
Carriage present	40	38/62	83	15	2.5	3.3–3.8	13.6 ± 8.8	13.2	26 (65)§	13	48	25	20	5.5	5.0	30	7.0	5.7	17	15	15
Carriage absent	1383	49/51	81	15	4.5	3.0–4.7	9.6 ± 5.5	8.4	1104 (80)	36	30	14	11	1.6	4.0	19	4.4	3.0	17	16	15

* We obtained 1423 swabs from 1321 children; 2 swabs were obtained from 96 and 3 swabs from 3 children, respectively. In all cases only 1 of the swabs was positive for Hib.
† No difference in the male:female ratio between carriers and noncarriers (chi square 2.0, 1 df; $P = 0.15$).
‡ Recorded for children who received at least one dose of Hib-OMPC before swabbing (total number of children 1130, of which 26 were carriers and 1104 noncarriers).
§ Numbers in parentheses, percent. The total number of swabs collected was 1950; 527 of these came from children younger than 3 months of age, who are not included in the analyses.
Among these there were 2 of 527 (0.4%) swabs that were positive for Hib antigen (i.e. OP carriage of Hib). Only 58 of 527 (11%) had symptoms of respiratory infection present at swabbing. From 6 to 8% had used antimicrobial treatment before swabbing. The race and sex distributions among the 527 were similar to those of the rest of the study population presented.

carriers (median, 3.0 months; range, 26 days to 1.7 years). The race and sex distribution was similar among carriers and noncarriers. There was no difference in the frequency of antimicrobial treatment usage among carriers and noncarriers or in the types of antimicrobials used.

The reduction of OP carriage of Hib after each dose of Hib-OMPC is presented in Table 2. Among children 3 months to 14 months of age at swabbing the frequency of carriage was reduced in those who had received 1, 2 or 3 doses of Hib-OMPC before swabbing compared with those who did not receive any Hib-OMPC before swabbing. Among children 15 months old or older, no reduction was apparent for those who had received 1 or 2 doses of Hib-OMPC before swabbing. In this age group, however, as a result of the recommended vaccination schedule, the time between last dose of Hib-OMPC and swabbing was also longest: median duration since the last dose of Hib-OMPC 160 and 280 days, respectively, after one or two prior doses compared with a median of 72 and 150 days, respectively, for those 3 to 14 months old at swabbing.

The effect of appropriate Hib-OMPC vaccination at the time of swabbing on OP carriage of Hib was evaluated in a logistic regression model. The following variables showed an association in a single variable model and were entered stepwise into the model: not vaccinated according to age at swabbing; age at swabbing; and presence of respiratory infection at swabbing (Table 3). The model that best described the data was the one incorporating all three variables jointly. These variables were independently associated with increased risk of OP carriage of Hib. Inadequate vaccinations with Hib-OMPC increased the risk of carriage 2.7-fold controlling for the effect of age and respiratory infections. The presence of respiratory infection symptoms increased the risk of carriage 2-fold, after controlling for the effect of age and Hib-OMPC vaccination status.

TABLE 3. Factors associated with risk of oropharyngeal carriage of *Haemophilus influenzae* type b among Navajo and Apache Indian infants and children

Risk Factor	OR*	95% CI	*P*
Not vaccinated according to age	2.66	1.00–7.05	0.05
Age (months)	1.06	1.02–1.10	0.003
Respiratory infection present	2.00	1.06–3.77	0.03

* Estimates for OR from the full model incorporating all three variables simultaneously into the logistic regression model.

OR, odds ratio as an estimate of risk for OP carriage of Hib; 95% CI, 95% confidence interval for the odds ratio.

DISCUSSION

The present study demonstrated that appropriate vaccination with Hib-OMPC reduced OP carriage of Hib among Navajo and Apache Indian children, populations known to be at high risk for invasive Hib disease. Thus the Hib-OMPC conjugate vaccine seems to have an effect similar to that of PRP-D which has been shown to reduce OP carriage of Hib among Caucasian children in Finland and Dallas, TX.[5, 13]

The ELISA was found to be highly specific and sensitive compared with the ASA plate method in the detection of OP carriage of Hib. Three swabs were positive with ELISA and negative with culture. These may represent true positive findings caused by, e.g., detection of Hib strains that are capable of producing Hib capsular polysaccharide but not excreting it to the extracellular space[19] or antimicrobial use before swabbing. If this were the case the positive predictive value of the ELISA test, now relatively low, would increase accordingly.

The effect of Hib-OMPC on OP carriage of Hib among Indian children was not as dramatic as that observed in Finland where none of the children who had received PRP-D before swabbing carried Hib.[5] However, in that study all children were 3 years of age at swabbing and had received either the primary series of PRP-D in infancy at 3, 4 and 6 months of age and a booster dose at 14 to 18 months of age or one dose of PRP-D at 24 months of age. These two vaccine regimens have been shown to induce similar serum Hib anticapsular antibody concentrations at 3 years of age (geometric mean titer (GMT) levels of >3 μg/ml)[20] (own unpublished data). In addition PRP-D had been used nationwide in Finland since 1986 and this

TABLE 2. Oropharyngeal carriage of *Haemophilus influenzae* type b among Navajo and Apache Indian infants and children according to Hib-OMPC vaccination status before swabbing

Age at Swabbing (Months)	No. of Swabs/Total*					Carriage Rate (%)
	No. of Hib-OMPC doses before swabbing					
	0	1	2	3	Total	
3–5	3/85 (3.5)†	4/388 (1.0)	0/6 (0)		479	1.5
6–11	3/95 (3.2)	2/181 (1.1)	1/73 (1.4)	0/12 (0)	361	1.8
12–14	4/33 (12)	2/54 (3.7)	2/132 (1.5)	4/87‡ (4.5)	307	3.9
≥15	4/80 (5.0)	2/43 (4.6)	9/140 (6.4)	0/13 (0)	276	5.4
All ages	14/293 (4.8)	10/666 (1.5)	12/345 (3.4)	4/112 (3.5)	1423	2.8

* Number of swabs that were positive for Hib OP divided by the total number of swabs in each cell of the table.

† Numbers in parentheses, carriage rate (percent).

‡ Of these 87 children 76 had received 3 doses of Hib-OMPC at 2, 4 and 6 months of age, because they were participants of an immunogenicity study and only 11 had received 3 doses of Hib-OMPC according to the recommendations, i.e. at 2, 4 and ≥12 months of age. Among these 11 there was no OP carriage of Hib.

may have interfered with the natural transmission of Hib.[21] Similarly one dose of PRP-D given at >18 months of age to primarily Caucasian children in Dallas, TX, reduced OP carriage of Hib significantly, although not as dramatically as among Finnish children.[13]

It appeared that the ability of Hib-OMPC to reduce OP carriage of Hib might wane with time; i.e. the longer the time elapsed since last vaccination with Hib-OMPC, the smaller the reduction/prevention would be. This would be in accordance with the decline in Hib serum antibodies after primary vaccination with Hib-OMPC.[14, 22] However, it was not possible to analyze this variable further because of the high correlation of this variable and the age of the child, which was significantly associated with the risk of OP carriage of Hib, and the relatively small number of OP carriers observed (total of 40).

Based on antibody data from the Navajo and Apache Indian infants the present results were not surprising. Primary doses of Hib-OMPC at 2 and 4 months of age induced significant serum antibody responses.[14, 22, 23] However, these concentrations dropped within the following 2 months to GMT antibody titers of 0.97 and 1.35 μg/ml, respectively, after the first and second doses. Eight months after the second dose (i.e. at 12 months of age), the titers had dropped further to 0.40 μg/ml. However, measured 1 month after the booster dose given at 12 to 15 months of age, the concentrations were at 8.4 μg/ml.[14, 22, 23] Antibody data from studies conducted in Finland and Dallas, TX, show considerably higher antibody concentrations for children at the time when OP carriage of Hib was analyzed[20, 24] (own unpublished data). Such high concentrations were not found among children who received PRP vaccine[25] and among whom no effect on OP carriage of Hib was detected.[11, 13] In a recent study evaluating a third Hib conjugate vaccine, Hib oligosaccharide-CRM_{197} protein vaccine (HbOC; Praxis Biologics, Inc.) given at 3, 5 and 9 months of age, no overall effect on OP carriage of Hib was observed.[26] However, the intensity of colonization was reduced among vaccinees. Children were swabbed at 4.3 years of age, approximately 3.5 years after the third dose of HbOC. The GMT serum Hib antibody titer of the vaccinated children who were not colonized with Hib was low, 0.97 μg/ml, but a booster type response was observed among those who were colonized with Hib (GMT 53.69 μg/ml).[26] This indicates that vaccination with HbOC might prime the vaccinees, so that upon mucosal challenge with Hib bacteria there would be a rapid booster response in the serum antibody concentration.

It has been proposed that a high serum Hib anticapsular antibody concentration might lead to passive transudation of primarily IgG antibodies to mucosal surfaces. Based on animal studies using the infant rat model, the serum antibody concentration needed for this effect has been suggested to be approximately 10 μg/ml.[27] This would be in accordance with the studies published thus far and the present study. Recent studies among Finnish infants have, in addition, indicated that there may be a component of local production of IgA at the mucosal surface after vaccination with Hib conjugate vaccine.[28] For this component the immunogenic differences between Hib conjugate vaccines, e.g. differences in the priming capacity,[29] may be of importance.

The OP carriage of Hib among unvaccinated children in the present study was higher and carriage started earlier in infancy compared with that reported previously among Caucasian children.[1, 3, 30] Carriage rates greater than 3% were observed among infants as young as 3 to 5 months old. Among British infants 0 to 12 months of age carriage was rarely detected (0.5%) before the start of Hib vaccinations; a similarly low rate of carriage was observed among United States infants, 0.7% among 0- to 5-month-olds, and 2.8% among 6- to 1-month-olds.[1, 30] The high rate of carriage that starts early in infancy is accordance with the high incidence of invasive Hib disease among Navajo and Apache Indian children.[7, 8, 22]

The present study also demonstrated that the presence of symptoms of an upper respiratory infection was associated with the risk of OP carriage of Hib. A similar finding has been reported by Howard et al.[30] for OP carriage of nontypable *H. influenzae* but was not found significant by Michaels et al.[3] for OP carriage of Hib, although carriage rates were somewhat higher among those with upper respiratory tract symptoms (5.7% *vs.* 3.6%). Influenza A and other major respiratory viruses have been demonstrated to induce changes in the epithelium of pharyngeal cells[31] and in human volunteers influenza A infection has also been shown to enhance attachment of Hib to pharyngeal epithelium.[32] Because attachment of Hib to the oropharyngeal mucosa is believed to be the initial step in the development of invasive Hib disease, any condition that enhances this step may increase the risk for invasive Hib disease. Among Navajo and Apache Indian children the rate of upper respiratory infection is high; in the present study 30% of children who presented for well child visit to be vaccinated were found to have symptoms related to an upper respiratory infection.

The present study indicates that vaccination with Hib-OMPC, similar to that with PRP-D, considerably reduces colonization rates among vaccinees and thus reduces the opportunity for transmission in the population at large. This is expected to further enhance the effectiveness of Hib-OMPC in the prevention of invasive Hib infections.

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