MAJOR ARTICLE

Prevention of *Haemophilus influenzae* Type b (Hib) Meningitis and Emergence of Serotype Replacement with Type a Strains after Introduction of Hib Immunization in Brazil

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Surveillance for *Haemophilus influenzae* meningitis cases was performed in Salvador, Brazil, before and after introduction of *H. influenzae* type b (Hib) immunization. The incidence of Hib meningitis decreased 69% during the 1-year period after initiation of Hib immunization (from 2.62 to 0.81 cases/100,000 person-years; $P < .001$). In contrast, the incidence for *H. influenzae* type a meningitis increased 8-fold (from 0.02 to 0.16 cases/100,000 person-years; $P = .008$). Pulsed-field gel electrophoretic analysis demonstrated that *H. influenzae* type a isolates belonged to 2 clonally related groups, both of which were found before Hib immunization commenced. Therefore, Hib immunization contributed to an increased risk for *H. influenzae* type a meningitis through selection of circulating *H. influenzae* type a clones. The risk attributable to serotype replacement is small in comparison to the large reduction in Hib meningitis due to immunization. However, these findings highlight the need to maintain surveillance as the use of conjugate vaccines expands worldwide.

A major public health advance has been the development and widespread use of *Haemophilus influenzae* type b (Hib) polysaccharide conjugate vaccines. Hib is an important cause of meningitis, pneumonia, and epiglottitis in the pediatric population and is responsible each year for >2 million cases of invasive disease and 300,000 deaths worldwide among children aged <5 years [1]. Hib infection rates have been reduced dramatically in countries that have implemented Hib conjugate vaccine programs as part of routine infant immunization [1, 2]: in the United States, annual cases of Hib meningitis among children aged <5 years decreased from >10,000 cases to <200 cases within a 10-year period after licensure of Hib conjugate vaccines [3].

In addition to preventing Hib invasive disease, several studies have shown that conjugate vaccines are effective in reducing nasopharyngeal colonization [4–7] and therefore may confer protection to populations not targeted for immunization through herd immunity [8]. On the other hand, reduction of Hib carriage may open ecological niches for *H. influenzae* non–type b strains

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Informed consent was obtained from patients or guardians according to procedures approved by the institutional review boards of the Oswaldo Cruz Foundation, Brazilian Ministry of Health, and Weill Medical College of Cornell University, and human experimentation guidelines of the Brazilian Ministry of Health, New York–Presbyterian Hospital, and US Department of Health and Human Services were followed in the conduct of the clinical research.

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and therefore potentially increase the risk of colonization and invasive disease by these strains [9, 10]. *H. influenzae* non–type b strains are generally believed to be less pathogenic than Hib [11] and are an infrequent cause of severe invasive disease [12–14]. However, reports have shown that these strains can cause outbreaks of meningitis and bacteremia [11, 15]. Furthermore, in the conjugate vaccine era, the incidence of *H. influenzae* non–type b invasive disease has increased in certain geographical locations [16–18]. In Salvador, Brazil, through active surveillance for meningitis, we had the opportunity to examine the incidence of *H. influenzae* non–type b disease before and after introduction of routine Hib immunization. In the present study, we provide evidence for serotype replacement with *H. influenzae* type a associated with the use of the Hib conjugate vaccine.

METHODS

Study site. The metropolitan region of Salvador is comprised of 30 municipalities in Northeast Brazil, with a total population of 3,208,893 inhabitants [19]. State health secretary protocol requires that suspected cases of meningitis from metropolitan Salvador be referred to the state infectious disease hospital for diagnosis and assessment of the need for isolation procedures. Notification of a case of meningitis to state health officials is mandatory, and this hospital reports 98% of the cases among residents of metropolitan Salvador [20].

In August 1999, the Hib conjugate vaccine was introduced as part of the routine infant immunization program in Brazil. Children aged <1 year were scheduled to receive 3 vaccine doses given at 2-month intervals. Children aged 12–23 months were scheduled to receive a single vaccine dose. Between August and December 1999 in metropolitan Salvador, 71,213 vaccine doses (*Haemophilus* b CRM-197 protein conjugate vaccine [HbOC]; Wyeth-Lederle) were administered to a target population of 58,412 children aged <1 year, and 22,488 vaccine doses were administered to a target population of 60,051 children aged 12–23 months [21]. In 2000, 162,303 doses (*Haemophilus* b tetanus toxoid protein conjugate vaccine [PRP-T]; Pasteur-Merieux) were administered to a target population of 59,261 children aged <1 year, and 14,461 doses were administered to a target population of 60,486 children aged 12–23 months. On the basis of information obtained from immunization cards, 72% of the children aged <1 year completed the 3-dose schedule in 2000. Of the children aged 12–23 months, 24% received the 1-dose schedule in 2000.

Surveillance. Active surveillance for *H. influenzae* meningitis was performed at the state infectious disease hospital between 9 March 1996 and 8 September 2000. A case was defined by the isolation of *H. influenzae* from the blood or cerebrospinal fluid of a patient with clinical signs and symptoms of meningitis. Clinical laboratory records were reviewed during the 5 workdays of the week to identify culture-positive case patients. A standardized data entry form was used to obtain information about demographic characteristics, clinical presentation, and outcome after discharge from the medical rec-

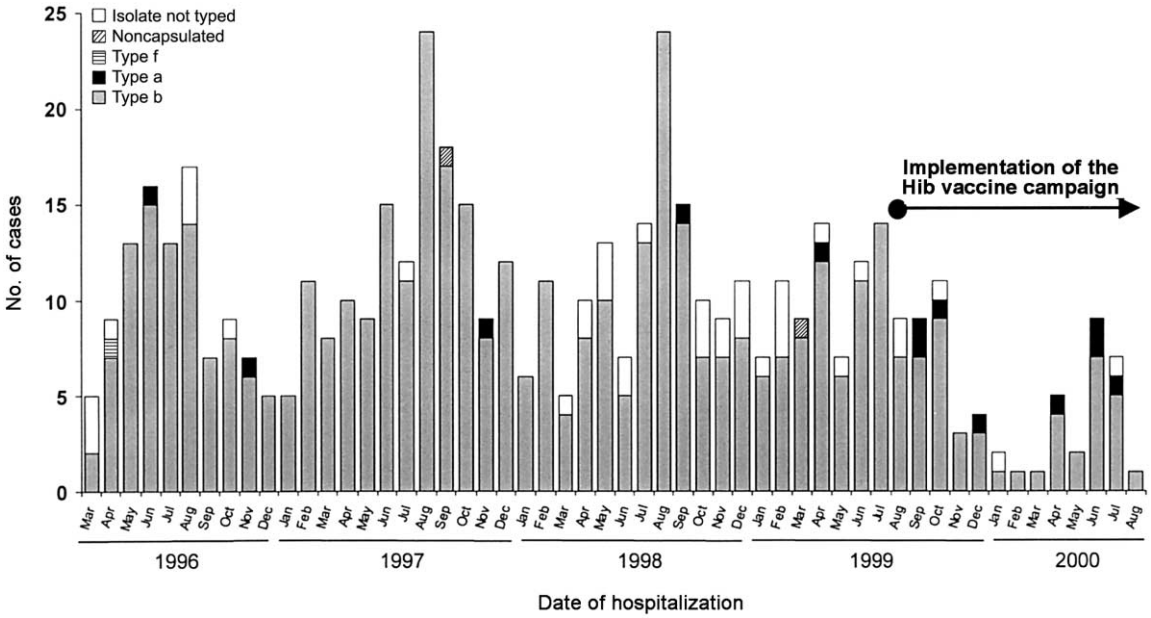

Figure 1. Monthly distribution of 522 *Haemophilus influenzae* meningitis cases identified during surveillance from March 1996 to August 2000 in Salvador, Brazil. Case patients were stratified according to the serotype status of the clinical isolate: *H. influenzae* type b *(gray bars),* type a *(black bars),* type f *(horizontal hatched bars),* noncapsulated *(cross-hatched bars),* and not typed, because the isolate was unavailable for serotyping *(white bars).*

ord. Immunization cards were reviewed to obtain information on the timing and number of Hib conjugate vaccine doses administered before hospitalization.

Laboratory investigation. *H. influenzae* was identified according to Gram-stain morphology and growth requirement for factors V and X. Biotyping was performed by use of the indole spot (Difco Laboratories) and ornithine decarboxylase and urease (BBL Microbiology Systems) tests. Commercial antiserum (Difco Laboratories) was used to determine capsular serotype. Each isolate was tested for slide agglutination with the complete panel of type a– to type f–specific antisera and a saline control. Isolates were serotyped at 2 laboratories in Brazil, and those identified as a *H. influenzae* non–type b serotype and those with discordant results were reanalyzed at the Centers for Disease Control and Prevention (CDC). A seminested polymerase chain reaction (PCR) method was used to amplify serotype-specific and nonspecific DNA sequences from the *H. influenzae* capsular loci [22]. Isolates were defined as noncapsulated if agglutination was not observed with the 6 type–specific antisera and if PCR capsular loci sequences conserved among serotypes were not detectable by PCR [22].

Pulsed-field gel electrophoresis (PFGE) was performed with *Sma*I-digested DNA [23, 24] of *H. influenzae* type a strains from Salvador and with those obtained during reference laboratory–based surveillance in Brazil and the United States. PFGE typing patterns were defined according to the criteria of Tenover et al. [25]. Closely related (1–3-band difference) and identical patterns were assigned a unique letter and number code, respectively.

Statistical analysis. Data entry and statistical analyses were performed with Epi Info version 6.04 software (CDC). Fisher's exact test or the χ^2 test was used to compare proportions, and the Kruskal-Wallis test was used to compare continuous data. Cumulative incidence was calculated on the basis of the number of case patients from metropolitan Salvador and population counts from the 1996 national census. The pre- and postvaccine periods were defined as the 3.5-year interval before and 1-year interval after 9 September 1999, respectively. Rates from the prevaccine period were used as the expected value to calculate the probability, according to the Poisson distribution, of observing postvaccine period rates.

RESULTS

Active surveillance identified 522 case patients with *H. influenzae* meningitis during the 4.5-year period between 9 March 1996 and 8 September 2000 (figures 1 and 2). In 483 (93%) of these case patients, isolates were serotyped by slide agglutination; 467 (96.7%) of the 483 isolates were Hib, 13 (2.7%) were *H. influenzae* type a, 2 (0.4%) were noncapsulated, and 1 (0.2%) was *H. influenzae* type f. PCR-based detection of

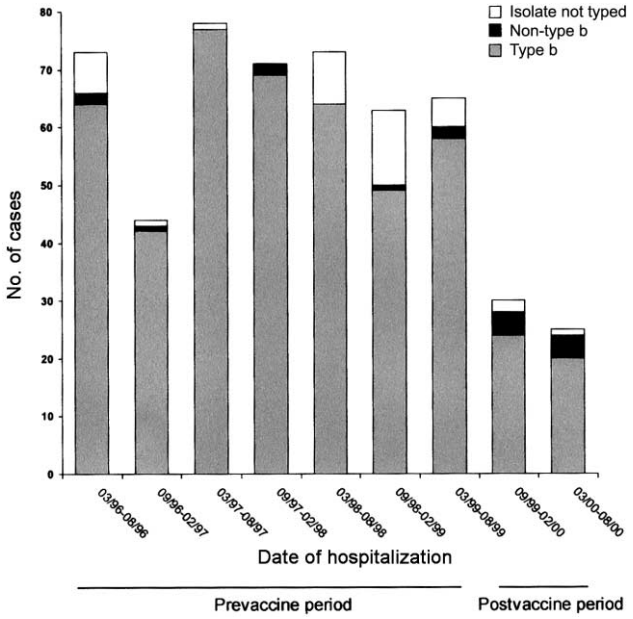

Figure 2. Distribution of 552 *Haemophilus influenzae* meningitis cases according to identification during 6-month surveillance periods, March 1996 to August 2000. Case patients were stratified according to the serotype status of the clinical isolate: *H. influenzae* type b *(gray bars),* non–*H. influenzae* type b (non-Hib; *black bars*), and not typed, because the isolate was unavailable for serotyping *(white bars).* Periods before (prevaccine) and after (postvaccine) initiation of the Hib immunization campaign are noted as lines below the figure. Dates are month/year.

capsular loci sequences confirmed the serotype of all *H. influenzae* non–type b isolates (results not shown). Isolates were serotyped from 431 (92%) of 467 cases identified during the prevaccine period and 52 (95%) of 55 of the cases identified during the postvaccine period. The proportion of *H. influenzae* type a cases increased from 5 (1.2%) of 431 to 8 (15.4%) of 52 ($P < .001$) after introduction of routine Hib immunization, but no significant increase was observed in the proportion of cases due to other *H. influenzae* non–type b isolates.

The incidence of *H. influenzae* meningitis among the 357 (68%) of the 522 patients who resided within metropolitan Salvador, decreased 64% during the 1-year period after the introduction of Hib immunization (pre- and postvaccine rates: 2.88 and 1.03 cases/100,000 person-years, respectively; $P < .001$; table 1). This decrease was a result of the significant reduction in the incidence of Hib meningitis among children aged <2 years (77%) and those aged 2–4 years (49%). However, the incidence of *H. influenzae* type a meningitis increased 8-fold after introduction of routine Hib immunization (pre- and postvaccine rates; 0.02 and 0.16 cases/100,000 person-years, respectively; $P = .008$). There was a significant difference between the rates of *H. influenzae* type a meningitis between pre- and postvaccine periods in the target population for Hib immunization (children aged <2 years; 0 and 1.77 cases/100,000

Table 1. Annual incidence of *Haemophilus influenzae* meningitis in Salvador, Brazil, before and after introduction of routine *H. influenzae* type b (Hib) immunization

Type of infection, age group	Prevaccine period	Postvaccine period	RR (95% CI)	*P*
All *H. influenzae* meningitis cases ($n = 357$)[a]				
0–4 years	30.08 (308)	9.91 (29)	0.33 (0.23–0.48)	<.001
<2 years	57.59 (228)	15.91 (18)	0.28 (0.17–0.45)	<.001
2–4 years	12.74 (80)	6.13 (11)	0.48 (0.26–0.90)	.022
5–9 years	1.02 (11)	0.98 (3)	0.95 (0.27–3.42)	1.00
Overall	2.88 (324)	1.03 (33)	0.36 (0.25–0.51)	<.001
With Hib isolates ($n = 320$)				
0–4 years	27.54 (272)	8.20 (24)	0.30 (0.20–0.45)	<.001
<2 years	53.80 (213)	12.38 (14)	0.23 (0.13–0.40)	<.001
2–4 years	10.98 (69)	5.57 (10)	0.51 (0.26–0.98)	.042
5–9 years	0.93 (10)	0.65 (2)	0.70 (0.15–3.19)	1.00
Overall	2.62 (294)	0.81 (26)	0.31 (0.21–0.46)	<.001
With *H. influenzae* type a isolates ($n = 7$)				
0–4 years	0.20 (2)	1.03 (3)	5.25 (0.88–31.42)	.08
<2 years	0.00 (0)	1.77 (2)	—	.049
2–4 years	0.32 (2)	0.56 (1)	1.75 (0.16–19.30)	.53
5–9 years	0.00 (0)	0.33 (1)	—	.22
Overall	0.02 (2)	0.16 (5)	8.75 (1.70–45.10)	.008

NOTE. Data are cases per 100,000 person-years (no. of cases), unless otherwise noted. CI, confidence interval; RR, relative risk; —, values could not be determined.

[a] Includes 2 case patients with noncapsulated isolates and 28 with isolates that were not serotyped.

person-years, respectively; $P = .049$), but not in the other age groups (table 1).

H. influenzae type a meningitis cases did not cluster spatially with respect to the neighborhood of residence during pre- and postvaccine periods. There were no significant differences with respect to age, sex, prior hospitalization, attendance at day care centers, and underlying chronic diseases between *H. influenzae* type a and *H. influenzae* non–type a meningitis cases (table 2).

Isolates from the 13 *H. influenzae* type a meningitis cases belonged to 2 distinct groups of closely related PFGE typing patterns (A [4 isolates] and B [9 isolates]; figure 3; table 3). Pattern A isolates were biotype I, and those with pattern B were biotypes II or III (table 3). *H. influenzae* type a patterns were unrelated to the 4 molecular typing patterns found in PFGE analyses of 15 of 44 Hib isolates obtained during the postvaccine period (results not shown). Two of 4 pattern A and 3 of 9 pattern B *H. influenzae* type a strains were isolated from case patients identified in Salvador before the introduction of Hib immunization. Furthermore, 7 of the 8 *H. influenzae* type a clinical isolates identified during national laboratory-based surveillance from other Brazilian cities during 1992–1998 had PFGE patterns (A [3 isolates] and B [4 isolates]) identical to those of *H. influenzae* type a isolates from Salvador (figure 3). PFGE patterns A and B found in *H. influenzae* type a strains from Brazil were unrelated to those of *H. influenzae* type a clinical isolates or reference strains from the United States.

Information on Hib immunization status was obtained from 14 (52%) of 27 *H. influenzae* meningitis case patients who were identified in the postvaccine period and were in the vaccine target population. Of the 4 case patients with *H. influenzae* type a meningitis interviewed, 2 had completed a 3-dose Hib immunization schedule, and 2 had received 2 doses of the vaccine (table 3). In contrast, of the 10 interviewed case patients with Hib meningitis, 4 had not received any doses of the conjugate vaccine, and 6 had received 1 dose before acquiring their illness.

The clinical manifestations seen in the 13 *H. influenzae* type a meningitis cases were similar in severity to those seen in the 549 *H. influenzae* non–type a cases (547 with Hib strains and 2 with noncapsulated strains) for whom clinical information and isolate serotype were obtained (table 2). Significant differences were not observed in mortality from meningitis due to *H. influenzae* type a and *H. influenzae* non–type a strains (case-fatality ratios, 23% vs. 16%, respectively; $P > .05$) or disease caused by these strains during the periods before or after the initiation of the Hib immunization campaign (results not shown). Neurological sequelae, such as hydrocephalus and auditory deficits, were observed at similar frequencies among survivors with *H. influenzae* type a (20%) and *H. influenzae* non–

Table 2. Characteristics of case patients with *Haemophilus influenzae* type a and *H. influenzae* non–type a meningitis identified between 1996 and 2000.

Characteristic	*H. influenzae* type a	*H. influenzae* non–type a
Median age in years (range)	1 (0–15)	1 (0–53)
Male sex	7 (54)	260 (57)
Underlying disease[a]	1 (10)	26 (6)
Seizures	4 (31)	127 (28)
Focal neurological signs	4 (31)	110 (24)
CSF examination[b]		
Leukocyte count, median cells $\times 10^3/\mu$L (range)	7.8 (0.8–10.0)	5.8 (0.03–31.0)
Glucose level, median mg/dL (range)	20 (20–39)	20 (20–60)
Protein level, median mg/dL (range)	260 (150–500)	300 (30–500)
ICU admission	3 (23)	96 (21)
Days in ICU,[c] median (range)	7 (3–8)	2 (1–33)
Case-fatality rate	3 (23)	75 (16)
Days of hospitalization, median (range)		
Among those who died	2 (1–3)	2 (1–16)
Among survivors	16 (12–40)	16 (10–75)
Neurological sequelae on discharge[d]	2 (20)	96 (25)

NOTE. Data are no. (%) of case patients, unless otherwise noted. Information on isolate serotype and clinical characteristics was obtained from 472 (90%) of 522 patients with *H. influenzae* meningitis identified during surveillance. Of these 472 patients, 13, 457, and 2 patients had *H. influenzae* type a, *H. influenzae* type b, and noncapsulated isolates. CSF, cerebrospinal fluid; ICU, intensive care unit.

[a] Percentages were calculated on the basis of 10 patients with *H. influenzae* type a isolates and 442 patients with *H. influenzae* non–type a isolates for which information on underlying disease was available.

[b] Results of CSF examination are shown for 13 patients with *H. influenzae* type a isolates and for 454 patients with *H. influenzae* non–type a isolates.

[c] Median days were calculated for patients admitted to the ICU. There was a significant difference ($P < .05$) between the 2 groups for this but not other characteristics.

[d] Sequelae among 394 survivors included ataxia (48), motor deficit (20), auditory deficit (15), and hydrocephalus (15).

type a (25%) meningitis (tables 2 and 3). *H. influenzae* type a case patients did not have outcomes different from those of *H. influenzae* non–type a case patients with respect to admission to the intensive care unit (ICU) or duration of hospitalization, although those who were admitted to the ICU did have a longer duration of stay than did *H. influenzae* non–type a cases (7 vs. 2 days; $P = .02$).

DISCUSSION

The present study's findings demonstrate the major public health impact of Hib immunization 1 year after its introduction in Brazil. The benefits are similar to those observed previously in countries, mostly in the developed world, that have adopted Hib conjugate vaccines [1]. Within the first year of the campaign in Salvador, overall Hib meningitis rates decreased 77% among children aged <2 years. Serious neurological sequelae were identified during hospitalization in >20% of children with *H. influenzae* meningitis. In addition to decreased meningitis and mortality rates, a major impact of Hib immunization in Salvador was the prevention of long-term morbidity and social burdens due to neurological sequelae.

In parallel, surveillance in Salvador identified a small but significant increase in *H. influenzae* type a meningitis rates. *H. influenzae* non–type b has been described to be the cause of invasive disease [11, 14–18, 26, 27]. However, strong evidence of serotype replacement has not been detected anywhere since Hib conjugate vaccines were introduced in the late 1980s [10]. In the present study, the evidence that serotype replacement occurred after introduction of routine Hib immunization in Salvador, Brazil is as follows: (1) a significant increase in the incidence of *H. influenzae* type a meningitis cases was observed after the initiation of the Hib conjugate vaccine campaign; (2) all *H. influenzae* type a strains belonged to 2 clonal groups present in Salvador before the introduction of the Hib conjugate vaccine; and (3) *H. influenzae* type a meningitis was documented in subjects who had previously been immunized with the conjugate vaccine. Because surveillance was limited to those with meningitis, the study's findings may not apply to the other forms of invasive *H. influenzae* disease.

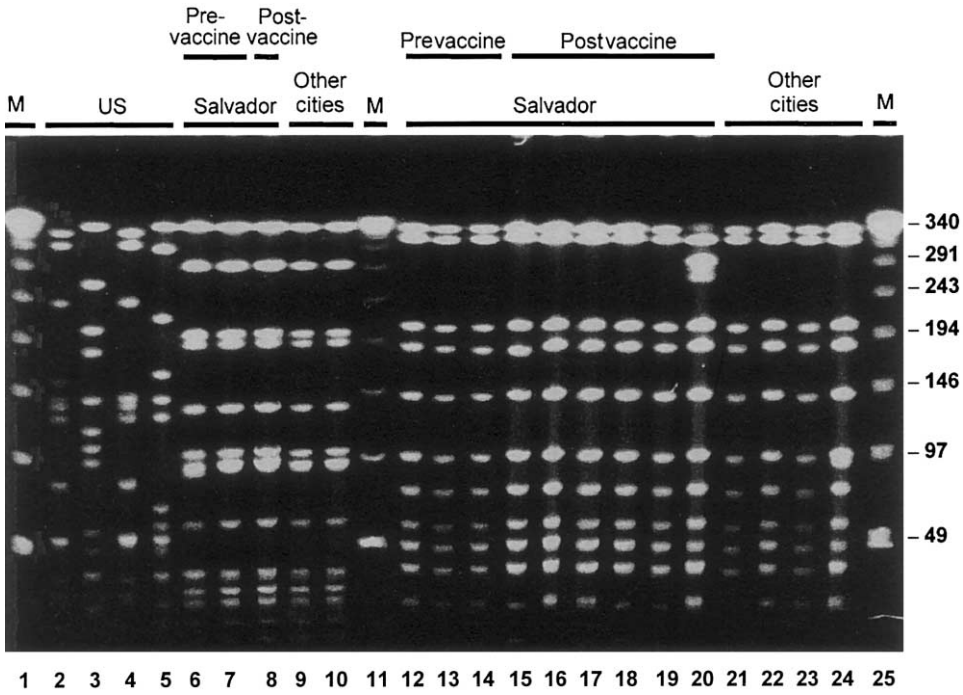

Figure 3. Pulsed-field gel electrophoresis (PFGE) analysis of *Sma*I-digested DNA from *Haemophilus influenzae* type a isolates. *H. influenzae* type a strains were isolated from meningitis case patients that were identified before *(lanes 6–7* and *12–14)* and after *(lanes 8* and *15–20)* introduction of routine *H. influenzae* type b (Hib) immunization. These isolates belonged to 2 closely related PFGE patterns (A1, *lanes 6–8;* B1 and B2, *lanes 12–19* and *20*, respectively). *H. influenzae* type a clinical isolates from other Brazilian cities had PFGE patterns identical to the A1 and B1 pattern (A1, Curitiba, *lane 9,* and São Paulo, *lane 10;* B1, São Paulo, *lanes 21, 23,* and *24;* and Recife, *lane 22*). The 2 closely related patterns observed for *H. influenzae* type a isolates from Salvador were unrelated to those for Hib isolates obtained during surveillance in Salvador (not shown), the *H. influenzae* type a reference strain (ATCC 9006), and isolates from the United States (US) (*lanes 2* and *3–5,* respectively). The position and size (kb) of fragments in the molecular mass standards (M; *lanes 1, 11* and *25*) are shown on the right.

Improved surveillance is an alternative explanation for the serotype shift identified after the introduction of the vaccine. However, increases in the rate of disease due to non-Hib other than *H. influenzae* type a were not observed. Moreover, differential case ascertainment for *H. influenzae* type a and non–type a meningitis cases is unlikely, given the similarity between patient groups and clinical presentations (table 2). Outbreaks of *H. influenzae* type a disease have been reported [11, 15], but *H. influenzae* type a cases identified in this study were not clustered in space or time and did not occur in specific risk groups, other than the pediatric population targeted by the immunization campaign. In addition, the similarity of host characteristics between *H. influenzae* type a and *H. influenzae* non–type a cases indicates that the increase in *H. influenzae* type a disease was not associated with a change in the population at risk for *H. influenzae* meningitis. Introduction of a new hypervirulent strain was not observed, because *H. influenzae* type a isolates from the postvaccine period had PFGE patterns identical to those of isolates from the prevaccine period.

Serotype replacement has not been previously detected for *H. influenzae* nasopharyngeal carriage [4, 6, 7] or invasive disease [12, 28] in the postvaccine era. Few reports have described increased rates of *H. influenzae* non–type b invasive disease in regions where Hib conjugate vaccines have been used [16–18], but molecular typing studies were not performed to determine whether increased rates were due to serotype replacement. Furthermore, long-term surveillance in countries such as the United States, which have used conjugate vaccines for >10 years, has not found sustained increases in the rates of *H. influenzae* non–type b invasive disease [29]. We demonstrated that 2 clonally related groups of strains were responsible for transmission of *H. influenzae* type a meningitis in Salvador. Identification of serotype replacement in meningitis cases, therefore, appears to be due in part to the presence of circulating virulent *H. influenzae* type a clones not found in other locations. The presence or lack of circulating virulent *H. influenzae* non–type b clones may be an explanation why increased rates of *H. influenzae* non–type b disease after Hib immunization have been observed in few epidemiological settings. However, we found that *H. influenzae* type a strains from geographically disparate regions of Brazil had PFGE patterns identical to those in Salvador, which indicates that dissemination of these clonal groups is not a local phenomenon that is restricted to our surveillance region. With increasing reports

Table 3. Characteristics of 13 patients with *Haemophilus influenzae* type a meningitis identified during surveillance in Salvador, Brazil.

Patient	Month/year of hospitalization	Age	Sex	No. of Hib vaccine doses[a]	Isolate biotype (PFGE type)	Days in ICU	Outcome (days of hospitalization)	Neurological sequelae on discharge
1	07/1996	4 years	M	0	II (B1)	0	Discharged (21)	Auditory deficit
2	11/1996	3 years	M	0	II (B1)	0	Discharged (12)	None
3	12/1997	9 months	F	0	I (A1)	0	Death (2)	—[b]
4	09/1998	2 years	M	0	II (B1)	0	Discharged (17)	None
5	04/1999	4 months	F	0	I (A1)	3	Death (3)	—[b]
6	09/1999	3 years	F	0	II (B1)	0	Discharged (16)	None
7	09/1999	5 months	F	ND	II (B1)	8	Discharged (40)	Hydrocephalus
8	10/1999	15 years	M	0	I (A1)	0	Discharged (13)	None
9	12/1999	18 months	F	2	I (A1)	0	Discharged (12)	None
10	04/2000	5 months	M	2	II (B1)	7	Discharged (31)	None
11	06/2000	12 months	M	3	III (B1)	0	Discharged (16)	None
12	07/2000	9 years	M	0	II (B1)	0	Death (1)	—[b]
13	07/2000	8 months	F	3	II (B2)	0	Discharged (12)	None

NOTE. Hib, *Haemophilus influenzae* type b; ICU, intensive care unit; ND, not determined; PFGE, pulsed-field gel electrophoresis.

[a] Hib immunization campaign was initiated on 9 August 1999.

[b] Sequelae were not recorded since the patient died during hospitalization.

of virulent *H. influenzae* type a [11, 15, 30] and *H. influenzae* type f [26, 27] strains isolated from different regions of the world and with the expanding global use of Hib conjugate vaccines, serotype replacement may become an emerging and more widespread possibility.

Clinically, the virulence of *H. influenzae* type a strains was indistinguishable from that of Hib: the case-fatality ratios among *H. influenzae* type a and *H. influenzae* non–type a meningitis patients were 23% and 16%, respectively (table 2). Increased virulence among *H. influenzae* type a, which was generally considered to be a rare cause of invasive disease in the prevaccine era [11, 15, 31], appears to be associated with partial deletion of 1 of 2 *bexA* copies within duplicated *cap* loci [15] and/or amplification of *cap* loci [30]. Virulent *H. influenzae* type a strains identified in this study were responsible for sporadic meningitis cases in the prevaccine period. Introduction of the Hib immunization contributed to an increase in the rates of meningitis due to these strains. Our study found that cases of *H. influenzae* type a meningitis occurred among infants who previously had received $\geq$2 vaccine doses. We propose that, in Salvador, the use of Hib conjugate vaccines provided circulating virulent type a strains an increased opportunity to replace Hib during nasopharyngeal colonization.

Although surveillance in Salvador identified a significant increase in the rate of *H. influenzae* type a meningitis that resulted from serotype replacement, the impact of this finding is small in comparison to substantial and large reduction in the burden of Hib meningitis attributable to the use of the conjugate vaccine (table 1). Without question, the public health priority for *H. influenzae* disease is the widespread introduction of the Hib conjugate vaccine in developing countries, where the cost of conjugate vaccines has thus far precluded their use. More than 10 years after the introduction of Hib conjugate vaccines, immunization schedules contributed to <2% reduction in the global burden of Hib disease [1]. This situation is expected to improve as efforts progress to reduce vaccine costs, as was done recently in Brazil [32], and as more developing countries, such as those in Latin America, adopt Hib immunization programs. However, the finding of this study suggests that, as global immunization coverage expands, continued surveillance for *H. influenzae* will be needed to monitor potential increases in disease due to serotype replacement.

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