MAJOR ARTICLE

Epidemiology of Invasive *Haemophilus influenzae* Type A Disease among Navajo and White Mountain Apache Children, 1988–2003

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(See the editorial commentary by Butler on pages 831–3)

Background. Before the introduction of *Haemophilus influenzae* type b (Hib) conjugate vaccines, rates of *H. influenzae* disease among Navajo and White Mountain Apache (WMA) children were among the highest reported worldwide. Routine Hib vaccination has significantly reduced rates of Hib disease in these populations. As Hib disease rates decrease to very low levels, there are concerns that non–type b strains of *H. influenzae* may emerge as more prevalent causes of invasive disease in children.

Methods. We reviewed population-based, active laboratory surveillance data from the period of 1988–2003 for invasive *H. influenzae* type a (Hia) disease among Navajo and WMA children aged <5 years. Clinical information on cases was collected by chart review. A sample of Hia isolates from Navajo children was typed by pulsed-field gel electrophoresis (PFGE).

Results. During 1988–2003, a total of 76 reported cases of invasive Hia disease occurred among Navajo and WMA children. The overall annual incidence was 20.2 cases per 100,000 population aged <5 years. There was no increase in Hia disease rates after Hib vaccination was introduced. The median age of patients was 12 months. Meningitis (50% of cases) was the most common presentation, followed by pneumonia (27.6%). Two children with Hia disease died. PFGE analysis revealed a limited genetic diversity of Hia strains in this population.

Conclusions. Active surveillance data showed high rates of invasive Hia disease among Navajo and WMA children but no increase in the incidence after Hib vaccination was introduced. The presentation of Hia disease is similar to that of Hib disease in the prevaccine era.

Before the introduction of conjugate vaccines for *Haemophilus influenzae* type b (Hib), Navajo and White Mountain Apache (WMA) populations in the southwest United States experienced rates of *H. influenzae* meningitis that were 3–5-fold higher than the rates in the general US population (152–254 vs. 45–60 per 100,000 population aged <5 years, respectively) and that were among the highest reported worldwide [1–5]. Routine immunization for Hib in these American Indian populations has led to significant reductions in rates of Hib disease and oropharyngeal carriage in the past decade [6, 7]. The near-elimination of Hib disease in some populations [8] led to the speculation that other *H. influenzae* serotypes and nontypable *H. influenzae* strains may emerge as important causes of invasive disease [9].

Case reports from the pre–conjugate vaccine era recognized that *H. influenzae* type a (Hia) was an infrequent cause of meningitis and pneumonia [10, 11]. More recently, a cluster of cases of invasive Hia disease among children was described [12]. However, it is not known whether the incidence of invasive Hia disease has increased since the introduction and widespread use of Hib conjugate vaccines. To date, only 1 population-based study has described the trend of invasive

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Hia disease in the Hib vaccine era [13]. Ribeiro et al. [13] reported an 8-fold increase in the incidence of Hia meningitis after the introduction of routine Hib immunization in Salvador, Brazil. However, the incidence estimates from the prevaccine and vaccine periods were based on a small number of cases (i.e., 2 and 5 cases, respectively) identified over a 4.5-year period.

Since the introduction of Hib conjugate vaccines among Navajo and WMA populations in 1988 [14], the epidemiology of invasive disease due to non–type b *H. influenzae* has, to our knowledge, not been described. We reviewed population-based, active laboratory surveillance data for cases of invasive *H. influenzae* disease during the period of 1988–2003 and found that the majority of cases due to non–type b strains were due to Hia. Herein, we report the epidemiology of invasive Hia disease among Navajo and WMA children aged <5 years in an era of widespread immunization for Hib. In addition, we examine the clonal relationship of invasive Hia isolates among Navajo children, as determined by PFGE.

MATERIALS AND METHODS

Population and area. The Navajo Nation, located in the southwest United States, covers >65,000 km^2 (>25,000 sq mi) in northern Arizona, western New Mexico, and southern Utah. The Navajo Nation is one of the largest Native American tribes in the United States, consisting of >230,000 tribal members. The WMA tribe consists of ~14,000 enrolled members living on the Fort Apache Indian reservation, which is located in central Arizona and is ~6400 km^2 (~1.6 million acres) in size. The annual birth cohorts of the Navajo and WMA are ~4500 and ~300, respectively. Health care on both reservations is administered by the Indian Health Service (IHS; Rockville, MD), which operates a number of hospitals and health clinics located throughout the reservations.

Invasive disease surveillance. Cases of *H. influenzae* disease were identified through population-based, active laboratory surveillance conducted on the Navajo and WMA reservations. The surveillance system identifies cases of invasive *H. influenzae* disease among Navajo and WMA people of all ages who are seen at IHS facilities and private health facilities surrounding the reservations, as well as facilities that are distant from the reservations that serve as referral centers. Audits of the laboratory logs from these facilities are conducted to identify cases missed by the routine surveillance activities. We included cases of *H. influenzae* disease among Navajo or WMA children aged <5 years (we restricted our analysis to children aged <5 years, because the majority of patients with Hia identified by surveillance were in this age group). For this study, a child was considered to have invasive *H. influenzae* disease if he or she had *H. influenzae* cultured from a normally sterile site (e.g., blood, CSF, or joint fluid specimens) during the period from 1 January 1988 through 31 December 2003. Clinical information was collected by chart review.

Microbiology. Isolates were confirmed to be *H. influenzae* by colony morphology, appearance on a Gram stain, and growth on chocolate agar supplemented with factors X and V. Serotyping was performed by the Center for American Indian Health (Baltimore, MD) by slide agglutination with *H. influenzae* polyvalent and monovalent antisera (DIFCO Laboratories). All *H. influenzae* serotypes (i.e., types a–f) were tested for each isolate. Serotyping results for isolates collected during the period of 1996–2002 were verified with the laboratory surveillance systems of the Arizona and New Mexico state health depart-

Table 1. Cases of invasive *Haemophilus influenzae* (Hi) disease, by serotype, among Navajo and White Mountain Apache children aged <5 years, 1988–2003.

	Year																
Variable	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	Total
Hi infections	74	66	53	28	14	13	15	17	16	11	10	11	14	11	16	9	378
Percentage of typed Hi infections	44.6	37.9	37.7	42.9	57.1	69.2	73.3	82.4	81.3	90.9	90	90.9	92.9	100	100	88.9	58.7
Type																	
A	0	0	0	4	1	4	6	7	8	6	7	5	5	7	11	5	76
B	33	25	20	8	5	3	1	5	2	2	1	3	3	0	1	2	114
Non-a, non-b	0	0	0	0	2	2	4	2	3	2	1	2	5	4	4	1	32
Estimated type																	
A	0	0	0	9	2	6	8	9	10	7	8	6	5	7	11	6	94
B	74	66	53	18	9	4	1	6	2	2	1	3	3	0	1	2	245
Rate[a]																	
Type a	0	0	0	28.3	6	17.3	23.8	27.9	33.1	24.5	29.1	22.6	19.6	28.8	43.8	23.1	
Type b	269	228	174	56.6	27.1	11.5	2.9	18.6	6.6	6.9	3.6	11.3	11.8	0	3.9	7.7	

NOTE. Data are no. of cases, unless otherwise indicated. Other Hi include types c ($n = 7$), d ($n = 2$), f ($n = 6$), and nontypable ($n = 17$).

[a] Data are no. of cases per 100,000 children aged <5 years and are based on the estimated number of cases to account for incomplete serotype information.

ments (Phoenix and Sante Fe, respectively). Isolates with discordant serotype results were sent to the Centers for Disease Control and Prevention (CDC; Atlanta, GA) for serotyping by PCR. The serotyping results from the CDC were considered to be definitive.

Invasive* H. influenzae *disease rates. Annual age-specific user population estimates obtained from the IHS were used as the denominators to calculate rates of disease. Incidence rates (with 95% CIs) were calculated using Poisson regression. Invasive Hia disease rates over time were modeled by linear regression, and significance was evaluated by the F test.

PFGE. A convenience sample ($n = 32$) of Hia isolates from Navajo children with invasive Hia disease from the period of 1993–2002 was analyzed by PFGE. For control samples, we used 7 isolates that had been recovered from children with invasive Hia disease from Washington State (2 children); Nevada (2); Washington, D.C. (1); and British Columbia, Canada (1). Genomic DNA was prepared as described by Gautom [15], with slight modifications. Plugs were made without sodium dodecyl sulfate. Isolates collected from September 1993 through January 1999 and from June 1999 through March 2002, were digested with *Bln*I and *Sma*I. Gels were processed using a CHEF mapper XA system (Bio-Rad Laboratories) in 0.5× Tris-borate-EDTA at 14°C, 6 V/cm. Pulse conditions for *Bln*I and *Sma*I were 1.0–8.1 s for 18 h and 2.2–35.1 s for 14 h, respectively. TIFF files were analyzed with BioNumerics software (Applied Maths). Cluster analyses using the Dice correlation for band matching with a 1.0% position tolerance and a hierarchic unweighted-pair group method with averaging algorithm were used to generate a dendogram describing the relationship among Hia pulsotypes.

Statistical analysis. For the years for which IHS-user population figures were not available, denominators were estimated using an exponential equation ($y = y_o e^{kt}$) assuming a constant rate of population growth. For incidence rates, the estimated number of cases of Hia or Hib disease was calculated by applying the proportions of Hia or Hib among isolates with a known serotype to the total number of cases of *H. influenzae* disease. This method adjusts for the varying proportion of *H. influenzae* isolates for which the serotype is known and assumes that isolates available for serotyping are representative of all isolates. All analyses were performed using SAS, version 8.0 (SAS Institute), or Excel 2000 (Microsoft) software.

Ethics. The study was approved by the institutional review boards of the Johns Hopkins University, the Phoenix area, Navajo Nation, and National Indian Health Service; and the WMA tribe.

RESULTS

From 1 January 1988 through 31 December 2003, a total of 378 episodes of invasive *H. influenzae* disease among Navajo and WMA children aged <5 years were identified (table 1). Serotype information was available for 222 (58.7%) of the isolates; the proportion of *H. influenzae* isolates serotyped varied by year (table 1). Of the 89 *H. influenzae* isolates collected during 1996–2002, a total of 73 (82%) were serotyped by the Center for American Indian Health, and of these, 44 (60%) were also serotyped by either the New Mexico or Arizona state laboratories. Of these 44 cases, the serotyping results between the Center for American Indian Health laboratory and either of the state laboratories were discordant for 11 cases (25%). Isolates for 8 of these cases were still available for evaluation by the CDC. The serotype results from the Center for American Indian Health and CDC laboratories were in agreement for 5 of these isolates. The remaining 3 isolates, which were identified as Hib by the Center for American Indian Health, were identified as nontypeable (2 cases) and Hia (1 case) by the CDC.

Of the *H. influenzae* isolates with known serotypes, the age distribution of patients was as follows: 0–5 months, 22.1% of isolates; 6–11 months, 36%; 12–17 months, 22.9%; 18–23 months, 8.6%; and ⩾24 months, 10.4%. The age distribution

Table 2. Characteristics of 76 Navajo and White Mountain Apache children with invasive *Haemophilus influenzae* type a (Hia) Disease, 1988–2003.

Characteristic	Value
Age group, months	
0–5	7 (9.2)
6–11	28 (36.8)
12–17	24 (31.6)
18–23	8 (10.5)
⩾24	9 (11.8)
Age, median months (range)	12 (4–59)
Tribe	
Navajo	61 (80.3)
White Mountain Apache	15 (19.7)
Male sex	44 (57.9)
Specimen source	
Blood only	56 (73.7)
Blood and CSF	10 (13.2)
CSF only	8 (10.5)
Blood and joint fluid	2 (2.6)
Diagnosis[a]	
Meningitis	38 (50)
Pneumonia	21 (27.6)
Other[b]	13 (17.1)
Bacteremia without focus	7 (9.2)
Hospitalization	60 (78.9)
Duration of hospitalization, median days (range)	8 (1–28)
Death	2 (2.6)

NOTE. Data are no. (%) of children, unless otherwise indicated.

[a] Diagnosis categories are not mutually exclusive.

[b] Other diagnoses include cellulitis, seizures, septic arthritis, and other associated conditions.

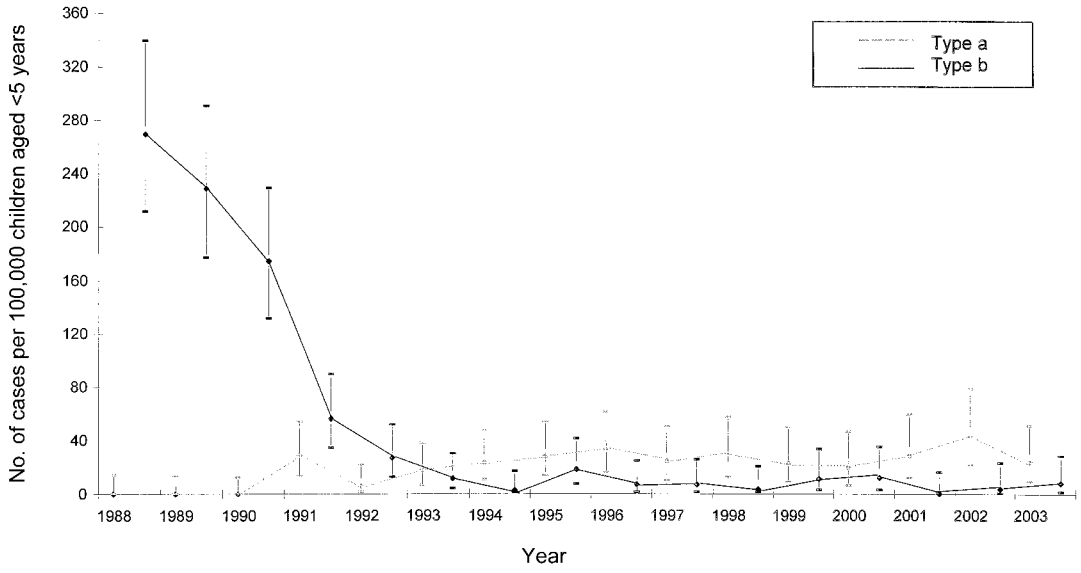

Figure 1. Invasive *Haemophilus influenzae* type a and type b disease among Navajo and White Mountain Apache children aged <5 years, 1988–2003

of all patients with invasive *H. influenzae* disease was similar. Seventy-six cases (34.2%) were due to Hia (table 2).

During the 16-year period, the overall incidence of invasive Hia disease among Navajo and WMA children was 20.2 cases per 100,000 population aged <5 years (95% CI, 16.3–24.7 cases per 100,000 population aged <5 years). Overall annual rates of invasive Hia disease were higher among WMA children than among Navajo children (65.9 vs. 17.4 cases per 100,000 population aged <5 years; $P < .005$). Annual rates varied from 0 cases per 100,000 population aged <5 years (95% CI, 0–14.5 cases per 100,000 population aged <5 years) to 43.8 cases per 100,000 population aged <5 years (95% CI, 14.6–66.8 cases per 100,000 population aged <5 years) (figure 1). However, since the introduction and routine use of Hib conjugate vaccines in these populations, we found no significant increase in the incidence of invasive Hia disease.

There was no clear seasonal pattern of invasive Hia disease. Patients were identified from geographically distinct areas of the Navajo and WMA reservations. No temporal or geographic clustering of cases was apparent. To our knowledge, none of the patients lived in the same household, attended the same day care center, or had any other close contact with another patient. There were no cases of recurrent Hia disease or cases that occurred in children with other invasive bacterial infections.

The majority of patients (80.3%) were Navajo (table 2), although disease rates among the WMA were higher because of the smaller population size. The median age at disease onset was 12 months. Hia meningitis occurred at a younger age (mean, 11.2 months) than did all other Hia disease (mean, 16.9 months) ($P < .0001$). Forty-four (57.9%) of the patients were male. Hia was most frequently isolated from blood cultures only (73.7%) or from both blood cultures and CSF cultures (13.2%). The most common presentation was meningitis (50%), followed by pneumonia (27.6%).

Sixty children (78.9%) were hospitalized. The median duration of hospitalization was 8 days (range, 1–28 days). At the time of discharge, 5 children had postmeningitis hearing loss diagnosed. Two children (2.6%) with Hia disease died; both were <1 year of age and had meningitis.

Hia isolates recovered from Navajo children with invasive disease during the period of 1993–2002 were typed by PFGE (figure 2*A*). *Bln*I- and *Sma*I-digested DNA of Hia isolates from geographically and temporally distinct cases on the Navajo reservation demonstrated 90% and 91% similarity, respectively. By contrast, Hia isolates recovered from children with invasive disease in the general population of the United States and Canada demonstrated only 63% and 36% similarity with *Bln* I- and *Sma*I-digested DNA, respectively (figure 2*B*). The genetic relatedness of Hia isolates recovered from children from the Navajo reservation suggests their origin from parental strains [16], the clonal pulsotypes we have designated AZNMB1 and AZNMS1 (figure 3).

DISCUSSION

These data document high rates of invasive Hia disease in Navajo and WMA populations known to be at high-risk for invasive Hib and pneumococcal disease. The overall annual incidence was 20.2 cases per 100,000 children aged <5 years. By contrast, the rate of all non–type b invasive *H. influenzae* disease among similarly aged US children was 0.83 cases per 100,000 children [17].

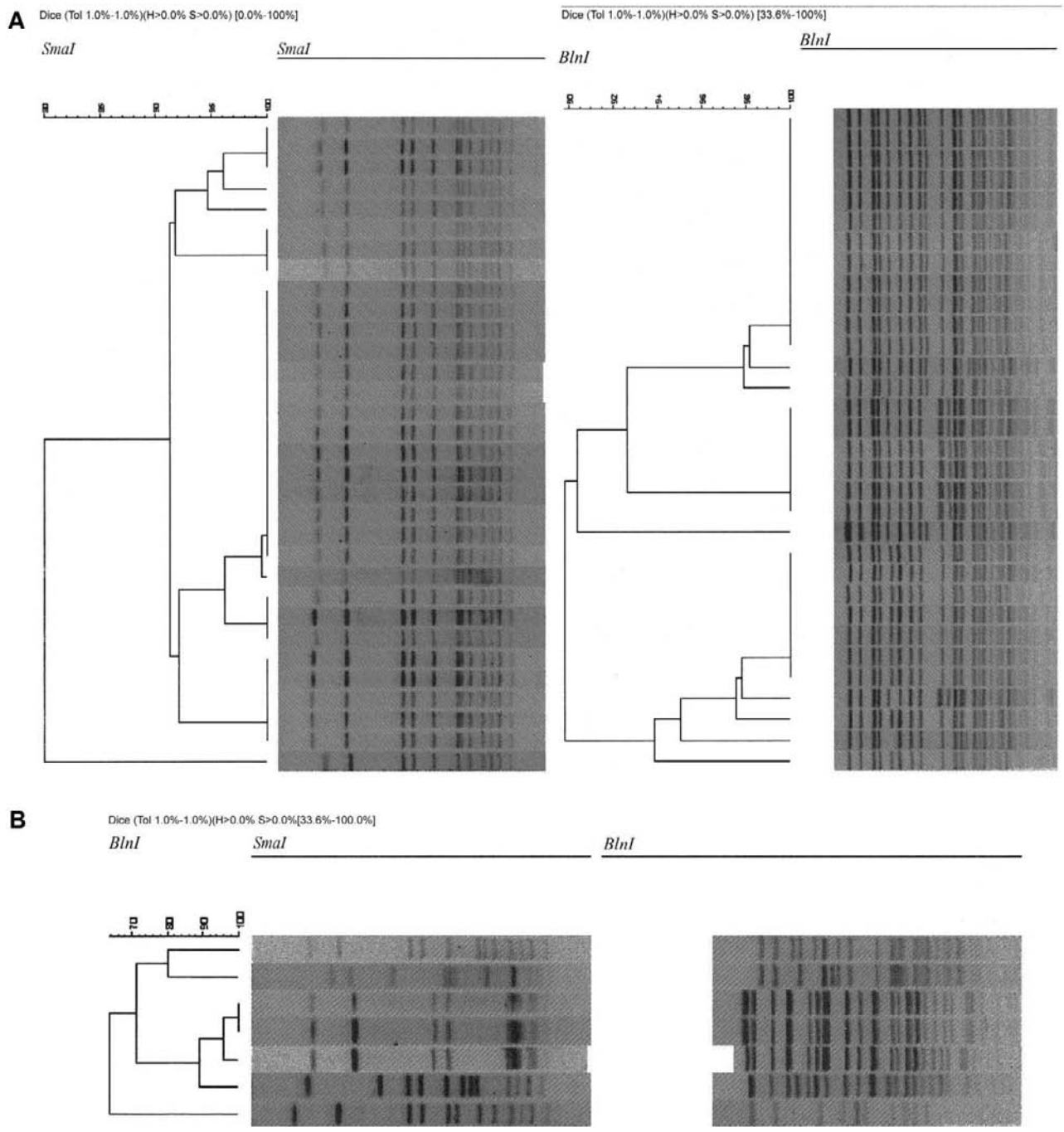

Figure 2. *Bln*I- and *Sma*I-digested DNA of *Haemophilus influenzae* type a (Hia) from Navajo children *(A)* and US and Canadian children *(B)* aged <5 years, 1993–2002. Dice correlation/unweighted-pair group method with averaging algorithm analyses of *Bln*I and *Sma*I pulsotypes yielded 90% and 91% similarity, respectively, for Navajo children and 63% and 36% similarity, respectively, for US and Canadian children.

The reasons for the increased risk for invasive Hia disease in these populations are unknown and are likely to be multifactorial.

Very limited data on Hia disease are available before 1988, when Hib conjugate vaccines were first used in these populations. In a study of WMA children, 3 (17%) of 18 cases of invasive *H. influenzae* disease during a 15-month period were due to Hia; the remainder were due to Hib [1]. No cases of Hia disease were reported among Navajo children in the pre–conjugate vaccine era, when the majority of cases of *H. influenzae* infection were assumed to be due to Hib [3]. In 1988, ~10% of Navajo children aged <24 months had received Hib conjugate vaccine; by 1992, this proportion was >90%, and to

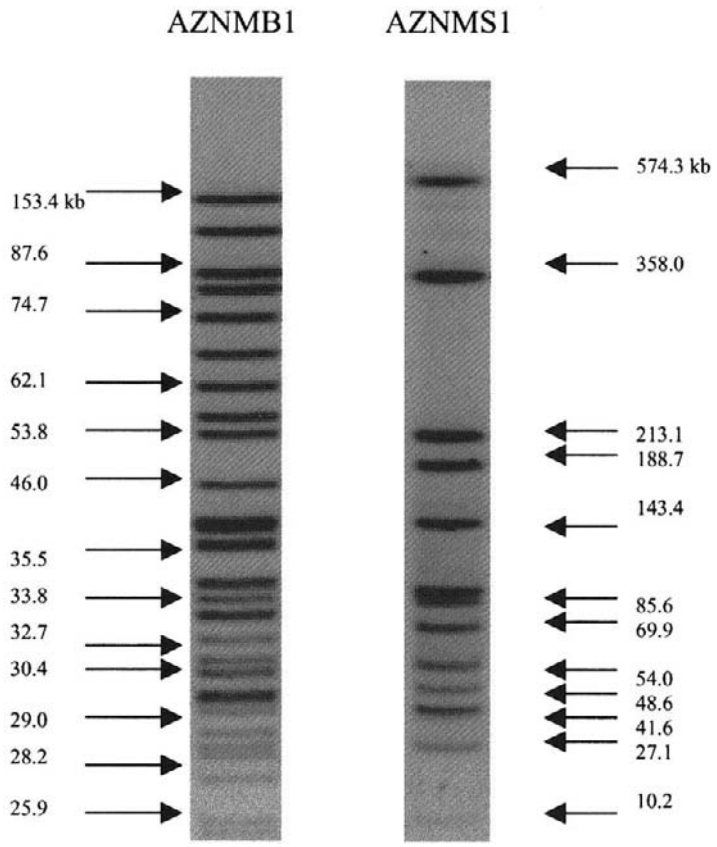

Figure 3. *Bln*I- and *Sma*I-digested clones (AZNMB1 and AZNMS1, respectively) of invasive *Haemophilus influenzae* type a (Hia) isolates recovered from Navajo children.

date, Hib immunization coverage remains high in both populations [6]. As the risk of Hib disease continues to decrease to very low levels, Hia has become the leading cause of invasive *H. influenzae* disease among Navajo and WMA children.

In the past decade, significant decreases in the rate of invasive Hib disease and oropharyngeal Hib carriage have been reported in the general US population and among the Navajo and WMA populations [6, 7, 17, 18]. Because Hib conjugate vaccines prevent disease caused specifically by type b strains, the potential for serotype replacement disease by non–type b strains has been a subject of concern [19]. Since the introduction of Hib vaccination in the United States, 2 population-based studies have reported increases in the incidence of non–type b *H. influenzae* disease [20, 21]; no increases in Hia disease were reported in these studies. In Brazil, an increased incidence of Hia meningitis was reported after the introduction of routine Hib immunization [13]; however, annual incidence estimates were based on a small number of cases identified over a brief period of observation.

The clinical characteristics of invasive Hia disease among Navajo and WMA children bear a striking resemblance to those of Hib disease in the vaccine era: meningitis was the most common presentation and the majority of children with meningitis were <1 year of age [1, 3]. Before Hib vaccines were available, the case-fatality rate for invasive Hib disease among Navajo children was 4% [3]. It is not known whether the high rates of invasive Hia disease among Navajo and WMA children are attributable to the circulation of particularly virulent strains of Hia. Genetic analysis of invasive Hia isolates in other settings have described the presence of a genetic mutation—the IS1016-*bexA* mutation—thought to be associated with the enhanced virulence of *H. influenzae* strains [12, 22]. It is not known whether any of the Hia isolates from Navajo and WMA children possessed the IS1016-*bexA* deletion or any other virulence factors that could cause enhanced pathogenicity.

Molecular methods, such as PFGE, are important tools for examining the relatedness of bacterial strains. By comparing patterns of chromosomal DNA fragments separated on an agarose gel, the genetic relatedness of multiple isolates can be assessed [16]. The molecular characterization of invasive Hia isolates from Navajo children suggests a limited genetic diversity of Hia strains on the Navajo reservation. Although these isolates originated from temporally and geographically distinct cases on the reservation, a high degree of relatedness was apparent when analyzed by PFGE. These findings are consistent with those of other studies of the molecular epidemiology of

invasive Hia isolates, which have suggested the common evolution of Hia from a limited number of clones [23, 24].

It is possible that cases of Hia disease from before 1991 may have been missed, because the proportion of *H. influenzae* isolates available for serotyping was low (<40% of the total). Given the small size of these populations, 1 or 2 missing cases could have a significant impact on the calculated rates. It is possible that cases occurred that were not captured by the surveillance system, either at facilities on or off the reservation; however, because system audits are conducted on a regular basis, this is unlikely. However, there was no known systematic selection of isolates for collection; therefore, we do not believe that there is a bias in the incidence estimates. Only a small number of isolates from both the Center for American Indian Health laboratory and either of the state laboratories were available for serotype comparison and confirmation by PCR. Although problems with the accuracy of serotyping results have been recognized [25], the high level of congruency between the serotyping results from 2 laboratories indicates that there were no systematic errors in serotyping.

We did not collect detailed clinical and demographic information on patients beyond that which was available on the patients' charts; thus, we were not able to assess the role that potential risk factors or risk markers (e.g., underlying illness, household crowding, and exposure to young children) played on the development of Hia disease in these children. Finally, Hib conjugate vaccine was already in use in this population by 1988, albeit not among the youngest children. It is possible that an increase in the rate of invasive Hia disease had already occurred by 1988 and, therefore, was not detected in this analysis.

Additional studies are needed to elucidate factors associated with the high rate of non–type b *H. influenzae* invasive disease in these populations in the Hib vaccine era. Rates of Hia carriage in these populations are not known. Molecular analysis of Hia isolates among the Navajo and WMA population to look for bacterial genetic markers associated with strain virulence may be helpful. Studies to identify risk factors or risk markers of invasive Hia disease may help to identify modifiable conditions that might reduce the risk of disease in these and other populations. Finally, our retrospective evaluation did not identify epidemiological links between persons with invasive Hia disease; this supports the current recommendation to not use chemoprophylaxis for contacts. However, a more detailed evaluation for epidemiological links and microbiological evidence of Hia colonization of close contacts of cases may help clarify this issue further.

The 5–8 annual cases of invasive Hia disease identified by our surveillance system need to be juxtaposed with the 40–60 cases of invasive Hib disease prevented annually through routine Hib immunization. Although we did not observe an increase in the rate of invasive Hia disease in these populations after the introduction of Hib conjugate vaccines, ongoing population-based active surveillance for invasive *H. influenzae* disease is needed to monitor temporal trends of type b and non–type b *H. influenzae* disease in the Hib vaccine era.

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