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Changes in serotype distribution of *Haemophilus influenzae* meningitis isolates identified through laboratory-based surveillance following routine childhood vaccination against *H. influenzae* type b in Brazil

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ABSTRACT

Following routine childhood vaccination against *Haemophilus influenzae* type b (Hib) disease in Brazil in 1999, passive laboratory surveillance reported increasing numbers of non-b serotypes and nontypeable *H. influenzae* (NTHi) from meningitis cases. To characterize this increase, we analyzed data on 3910 *H. influenzae* isolated from cerebrospinal fluid or blood from meningitis cases that were sent to the national reference laboratory for serotyping from 1990 to 2008. Hib accounted for 98% of *H. influenzae* meningitis isolates received during 1990–1999 versus 59% during 2000–2008, while non-b serotypes increased from 1% to 19% and NTHi increased from 2% to 22% of *H. influenzae* isolates received during the two periods. Higher proportions of non-b serotypes and NTHi than Hib were isolated from blood rather than cerebrospinal fluid. Estimated incidence rates for *H. influenzae* meningitis for Sao Paulo state remained below 1 case per million population during 2000–2008, although annual incidence of NTHi meningitis (mean, 0.03 cases per 100,000 population) increased in several age groups. Changes in surveillance for *H. influenzae* following introduction of Hib conjugate vaccine likely contributed to increased numbers of non-b and nontypeable *H. influenzae* meningitis isolates received at the national reference laboratory.

1. Introduction

Prior to the introduction of *Haemophilus influenzae* type b (Hib) conjugate vaccines, Hib was the leading bacterial cause of meningitis in children <5 years worldwide, accounting for over 90% of invasive *H. influenzae* disease [1,2]. In countries that have introduced Hib conjugate vaccines into national immunization programs, incidence of invasive Hib disease has fallen dramatically in all age groups [1–5]. As Hib became less common, invasive disease caused by serotypes other than b (serotypes a, c, d, e and f) and nontypeable *H. influenzae* (NTHi) were increasingly reported [6–10]. Although type b was the predominant serotype among *H. influenzae* meningitis cases prior to Hib vaccine introduction, non-b serotypes and NTHi may invade the central nervous system and cause meningitis. Despite reports of NTHi as an emerging pathogen in some settings [6–8], population-based studies have not reported increased incidence of meningitis due to NTHi [11,12].

Brazil conducts passive surveillance for bacterial meningitis and reporting of *H. influenzae* meningitis has been mandatory since 1977. *H. influenzae* isolates are sent to the national reference laboratory for serotyping. Following introduction of routine childhood immunization against Hib disease in Brazil's national immunization program in 1999, the Brazilian Ministry of Health recommended that all *H. influenzae* isolates be serotyped. During 2000–2008, the national reference laboratory reported increased numbers of non-b serotypes and NTHi among *H. influenzae* isolates from meningitis cases. To investigate possible explanations for this increase, we analyzed the age, serotype distribution and source of isolates for *H. influenzae* meningitis cases with isolates sent to the national reference laboratory for serotyping from 1990 to 2008.

2. Methods

In 1999, Hib conjugate vaccine was introduced through Brazil's national immunization program with three doses recommended at 2, 4 and 6 months of age without a booster dose. In the first year, catch up vaccination was offered for children up to 5 years of

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age. For the period 2000–2008, reported coverage with three doses of Hib vaccine was 92% among children less than 1 year of age, based on administrative data from Brazil's national immunization program [13].

2.1. Surveillance for H. influenzae meningitis

In Brazil, epidemiological surveillance for meningitis was established following outbreaks of meningococcal meningitis in the 1970s. In 1977, *H. influenzae* meningitis was added to the list of diseases subject to mandatory notification. Since 1998, hospitals and health facilities have notified cases directly to the Ministry of Health using an electronic system (SINAN) [14]. According to the Brazilian Ministry of Health, a confirmed case of *H. influenzae* meningitis is defined as isolation or detection of the bacteria in blood or cerebrospinal fluid [CSF] from a patient with a clinical diagnosis of meningitis; serotyping of isolates is not mandatory. Beginning in 2000, the Brazilian Ministry of Health recommended blood cultures in suspected cases of bacterial meningitis and their use increased in hospitals throughout Brazil. At the same time, the Brazilian Ministry of Health recommended that all *H. influenzae* isolates be sent to the national reference laboratory for serotyping.

2.2. Characterization of H. influenzae isolates

H. influenzae isolates were sent for serotyping and antibiotic susceptibility testing to the national reference laboratory at the Adolfo Lutz Institute (IAL) in Sao Paulo, which is also the public health laboratory for the Sao Paulo state health department. Hospitals and state public health laboratories submitted isolates with name and age of case-patient, clinical diagnosis (meningitis, pneumonia, sepsis, other) and source of isolate (CSF, blood or other). Isolates were lyophilized and stored at IAL.

H. influenzae isolates were confirmed by Gram-stain morphology, growth requirement for factors V, X, and porphyrin and biochemical tests according to standard methods [15]. Capsular typing was performed by slide agglutination with antisera to types a–f (Difco) [16]. We refer to isolates that did not react with type-specific antiserum as 'nontypeable' (NTHi). All nontypeable *H. influenzae*, as well as isolates that demonstrated cross-reactivity in slide agglutination, were tested by PCR for the presence of capsule-specific genes [17]. Hib strains from which capsule expression genes have been deleted are referred to as "b"-strains.

All *H. influenzae* isolates were tested for β-lactamase production by the chromogenic cephalosporin method [18]. Minimum inhibitory concentrations (MIC) of ampicillin, chloramphenicol, ceftriaxone and rifampicin were determined by broth microdilution according to guidelines of the Clinical and Laboratory Standards Institute [19]. For *H. influenzae* meningitis isolates, resistance to each antibiotic was defined as MIC $\geq$2 μg/ml for ampicillin, MIC $\geq$4 μg/ml for chloramphenicol, MIC >2 μg/ml for ceftriaxone and MIC $\geq$2 μg/ml for rifampicin. *H. influenzae* ATCC 49247 and *Staphylococcus aureus* ATCC 29213 were used as controls for β-lactamase production and MIC determination. *H. influenzae* serotyping and antimicrobial susceptibility testing performed at the national reference laboratory were validated annually as part of an external quality assurance program for *Haemophilus* sp. characterization in the SIREVA network in Latin American countries [20]. From 2001 to 2003, the quality assurance program was coordinated by the *Haemophilus* Reference Unit of the United Kingdom Public Health Laboratory Service in Oxford, England, and from 2004 to 2008, by the Centro Nacional de Microbiologia, Instituto de Salud Carlos III in Madrid, Spain.

2.3. Data analysis

Data were entered and analyzed using EpiInfo version 6.04 (Centers for Diseases Control and Prevention, Atlanta, GA) and SPSS version 16 (SPSS Inc, Chicago, IL). For this analysis, only *H. influenzae* isolates from mengitigis cases were included. In the case of receipt of multiple *H. influenzae* isolates from the same episode, only one isolate was included. Isolates from blood were excluded if an isolate from CSF was provided for the same episode. There were no episodes in which more than one type of *H. influenzae* was isolated. Chi-squared tests were used to compare proportions. *P* values less than 5% were considered statistically significant.

Because isolate referral patterns were more consistent in the state of Sao Paulo than in other states during the period, we used isolates from meningitis cases in Sao Paulo to calculate annual incidence of *H. influenzae* meningitis in the state. We divided the number of *H. influenzae* meningitis isolates received each year by the corresponding population obtained from the Brazilian Institute for Geography and Statistics (IBGE) [21]. Chi-squared tests for trend were used to compare cumulative, age-specific incidence during three-year time intervals, according to *H. influenzae* type.

3. Results

From 1990 to 2008, a total of 3910 *H. influenzae* isolates from patients with a clinical diagnosis of meningitis were sent to the national reference laboratory; 3509 (90%) isolates were from cerebrospinal fluid and 401 (10%) from blood. Case-patient age was provided for 3115 (80%) isolates. Among 3115 *H. influenzae* isolates for which patient age was reported, children younger than 5 years accounted for 2158 (91%) of 2362 *H. influenzae* meningitis isolates received from 1990 to 1999, versus 507 (67%) of 753 during 2000–2008 ($p < 0.05$). The number of *H. influenzae* isolates received annually according to age of case-patient is shown in Fig. 1. Comparing the pre-vaccine period (1990–1999) to the post-vaccine period (2000–2008), percentages of isolates from patients aged 5–14 years increased from 6% (147/2362) to 14% (107/753), from patients 15 to 59 years from 2% (50/2362) to 15% (112/753) and from those 60 years and older from <1% (7/2362) to 4% (27/753) of *H. influenzae* meningitis isolates.

Following Hib vaccine introduction, the annual mean number of *H. influenzae* isolates received by the national reference laboratory fell from 305 to 96. Yearly distribution of the three major types of *H. influenzae* isolates (Hib, serotype a [Hia] and NTHi) is shown in Fig. 2. Hib accounted for 2975 (98%) of 3050 *H. influenzae* meningitis isolates during the pre-vaccine period versus 513 (59%) of 860 isolates received during the post-vaccine period. During the pre-vaccine period, 24 (1%) isolates belonged to serotype a, 3 serotype f, 2 serotype d and 46 (2%) NTHi. During the post-vaccine period, 118 (14%) were serotype a, 193 (22%) NTHi and 36 (5%) other encapsulated types (21 type f, 8 type d, 5 type e and 2 type c). Of 239 NTHi isolates received from 1990 to 2008, only 3 were b-mutants. During the post-vaccine period, 87% of Hib, 76% of Hia, 61% of non-b serotypes and 63% of NTHi isolates were obtained from CSF. After 2003, Hib no longer accounted for the majority of *H. influenzae* meningitis isolates received. The source of Hib isolates was significantly more likely to be CSF than isolates of Hia ($p = 0.001$), NTHi ($p < 0.001$) or other encapsulated types ($p < 0.001$), while similar proportions of NTHi and non-b serotypes were isolated from blood and CSF ($p = 0.07$). Increased numbers of NTHi isolates from blood specimens were received during the post-vaccine period (Fig. 3).

During the Hib vaccination era, meningitis cases among residents of Sao Paulo state accounted for 331 (39%) of 860 *H. influenzae* isolates received, including 45% of Hia and 61% of NTHi isolates versus just 28% Hib isolates. Of 189 *H. influenzae* meningitis isolates