Case Report

Haemophilus influenzae serotype b and a capsule-deficient type mutant (b−) invasive disease in a partially vaccinated child in Brazil

Nathalia G. S. Caldeira,1 Ivano de Filippis,1 Tânia Catão Arruda,2 Maria Eulália Córte Real,3 Alice Batalha de Jesus1 and Antonio Eugenio C. C. de Almeida1

1Instituto Nacional de Controle de Qualidade em Saúde, INCQS/FIOCRUZ, Rio de Janeiro, Brazil
2Laboratório Central/LACEN-PE, Recife, Pernambuco, Brazil
3Hospital C. Picanço, Recife, Pernambuco, Brazil

We report a rare case of infection by two different types of Haemophilus influenzae strains in a child who received only one dose of the H. influenzae serotype b (Hib) conjugate vaccine (DTwP + Hib). The strains were recovered from blood and cerebrospinal fluid (CSF) and were phenotypically identified as Hib and non-typable H. influenzae, respectively, after serological tests. The two strains were characterized by PCR capsular typing, multilocus sequence typing and PFGE. Our results suggest that the infection was caused by the bloodstream invasion by a single Hib strain, followed by the diffusion of the bacteria across the blood–brain barrier and into the CSF. The strain recovered from the CSF, however, was identified as a capsule-deficient type mutant (b−) strain. Despite the high efficacy of the Hib conjugate vaccine, the increase in the numbers of strains able to escape the immune system of the vaccinated population advocates continued surveillance.

Introduction

Haemophilus influenzae serotype b (Hib) has been an important cause of paediatric morbidity and mortality. The routine use of the conjugate Hib vaccine has produced a dramatic decline in the incidence of invasive Hib disease over a very short period of time (Slack et al., 1998; Peltola, 2000; Heath et al., 2001; Kriz et al., 2005). As a consequence, non-b and non-typable H. influenzae (NTHi) isolates are nowadays the leading cause of invasive H. influenzae disease in countries with ongoing Hib vaccination programmes (Sarangi et al., 2000; Bajanca et al., 2004; Ito et al., 2011).

In encapsulated H. influenzae strains, the genes for the production of the polysaccharide capsule are organized in a capsulation (cap) locus, formed by three different functional regions. Regions I (bexABCD) and III are common to all capsular types and contain the genes necessary for transport and processing of the capsular structures, while region II contains serotype-specific biosynthesis genes (Kroll et al., 1991; Van Eldere et al., 1995). Since the implementation of routine immunization against Hib just over a decade ago, non-encapsulated strains have taken on greater importance as a cause of bacteraemia, meningitis and other forms of invasive disease (Ito et al., 2011). Non-encapsulated strains are now commonly found to cause sepsis in invasive H. influenzae infection in the elderly. Nonetheless, strain replacement of Hib with serotype f and non-typable strains in children under 5 years has been reported (Adam et al., 2010).

This study describes a rare case of septicaemia and meningitis due to a Hib strain and a capsule-deficient type mutant (b−) strain isolated simultaneously from blood and cerebrospinal fluid (CSF) in a child.

Case report

A 5-month-old male was admitted to the Municipal Hospital of the Recife, Pernambuco State, in the northeastern region of Brazil after falling from a swing 2 days earlier. He had symptoms and signs of meningitis such as lethargy, convulsion, bulged fontanelle, neck rigidity and a 39 °C fever. Meningitis infection was considered as a possible cause and a spinal tap was performed. The child's past medical history was notable for frequent upper respiratory tract infections but there was no evidence of any immunocompromising condition. The patient had

Abbreviations: CSF, cerebrospinal fluid; Hib, H. influenzae serotype b; MLST, multilocus sequence typing; NTHi, non-typable H. influenzae; SAT, slide agglutination test.

The GenBank/EMBL/DDBJ accession numbers for partial bcs3 gene sequences of H. influenzae strains are JN411086 and JN411087.
received only one dose of the Hib conjugate vaccine (DTwP + Hib). Two weeks before admission the child had whooping cough. Samples of CSF and blood were collected and submitted for laboratory analysis. Blood tests revealed leukocytosis and the CSF results showed abundant white cells (1024 µl⁻¹) and a protein level of 40 mg dl⁻¹, with 90 % neutrophils. The glucose level was 10 mg dl⁻¹. A Gram stain revealed Gram-negative bacilli. Bacterial meningitis was diagnosed based on clinical and laboratory findings, and empirical treatment with ceftriaxone sodium hydrate was promptly initiated (120 mg kg⁻¹ day⁻¹, every 12 h). After culture confirmation, the antibiotic therapy with ceftriaxone was continued for 7 days with 100 mg kg⁻¹. Blood culture for aerobic and anaerobic microorganisms was carried out by inoculating 1 ml blood sample into 20 ml trypticase soy broth containing sodium polyanthol sulfonate (Becton Dickinson). Blood culture sample into 20 ml trypticase soy broth containing sodium polyanthol sulfonate (Becton Dickinson). Blood culture was positive after incubation at 35 °C for 5 days. CSF was cultured on brain heart infusion chocolate agar (Difco) enriched with 10 % defibrinated horse blood at 37 °C for 24 h, and bacterial growth was detected after 24 h. Gram staining of bacterial colonies recovered from blood and CSF samples was performed, showing abundant pleomorphic Gram-negative species. The suspected cultures were sent to the National Institute for Quality Control in Health (INQCS) for additional identification and serotypes tests, such as the slide agglutination test (SAT), biotyping, antimicrobial sensitivity and PCR capsular typing tests, as previously described by Falla et al. (1994) and Ledeboer & Doern (2011). The serotyping tests were repeated and five individual colonies of the blood culture and CSF isolates were tested and were identified by the SAT as Hib and NTHi, respectively. The results of the biotyping test indicated that both isolates were biotype II. After antibiotic therapy, the patient showed a complete resolution of symptoms, return of normal function and normalization of blood parameters.

The negative serology result combined with the negative PCR result for the bexABCD (region I), but positive result for type b polysaccharide capsule gene bcs3 (region II), confirms that strain P3271 isolated from the CSF was actually a type b capsule-deficient mutant (b⁻) lacking the bexABCD or bexA gene (region I) – a strain with the capsulation locus but with a 1.2 kb deletion within the bexA gene that is necessary for polysaccharide export (Falla et al., 1994; Van Eldere et al., 1995). On the other hand, strain P3271/A isolated from the blood showed a positive serology result for type b and positive PCR result for capsule determination and capsular type, which confirms that this isolate was a regular Hib strain. MLST analysis was performed as previously described by Meats et al. (2003) and typing based on the bacterial genomic DNA fingerprinting pattern obtained by PFGE has been reported to determine whether the two isolates were the same strain (Smith & Cantor, 1987; Tenover et al., 1995; Saito et al., 1999; Qin et al., 2012). Both strains were classified as ST-6 by MLST analysis and PFGE analysis confirms the MLST result, since the two strains showed identical fingerprinting patterns (Fig. 1), suggesting that they could be the same isolate. Partial sequences of the bcs3 gene were deposited in GenBank under accession numbers JN411086 and JN411087.

**Discussion**

Trends in the changing epidemiology of invasive *H. influenzae* disease should alert health authorities to increase the surveillance of this important bacterial disease. In the past, surveillance of invasive *H. influenzae* disease had been restricted to monitoring only Hib cases, but with the success of the Hib immunization programme, most countries have focused their attention on the invasive non-type b type disease, caused most commonly by NTHi strains (van Belkum et al., 1994; Ito et al., 2011).

The results of our study show clearly that a b⁻ strain caused meningitis in a vaccinated child. It is likely that a Hib strain invaded the patient’s bloodstream, rapidly reaching the meninges and causing meningitis. We cannot confirm whether part of the Hib strain population lost or modified part of the cap genetic machinery, changing to Hib⁻ while in the blood or after crossing the blood–brain barrier, but it is likely that the immune system played an important role in this event as the strain was circulating in

![Fig. 1. PFGE (agarose). Lanes: M, lambda DNA PFGE ladder; Hib⁻, isolate from CSF; Hib, isolate from blood.](image-url)
the bloodstream. Considering that the child had one dose of vaccine, we could even speculate about a possible positive selection caused by the immune barrier, allowing the escape of the Hib⁻ strain, considering that the child showed only meningeal signs. This hypothesis would be of high concern since it could occur in vaccinated children. In any case, these findings suggest that Hib⁻ strains may have the same pathogenic potential as Hib strains. (Rahman et al., 2008; Ito et al., 2011).

Considering that serotyping by SAT is less accurate, being unable to detect Hib⁻, and invasive *H. influenzae* infections have become rare, we recommend a combination of culture and strain replacement due to Hib vaccination. In Ontario, Canada: evidence for herd effects in invasive *H. influenzae* type b disease following introduction of Hib vaccination. J Infect Chemother 17, 63–68.


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**References**


