Complicated Meningitis caused by a rare serotype of *Haemophilus influenzae* in Portugal

Rita Calado\(^a\), Célia Betencourt\(^b\), Helder Gonçalves\(^a\), Nuno Cristino\(^c\), Paulo Calhau\(^c\), Paula Bajanca Lavado\(^b\),∗

\(^a\)Hospital do Espírito Santo de Évora, EPE, Pediatrics, 7000-811 Évora, Portugal
\(^b\)National Institute of Health, Department of Infectious Diseases, 1649-016 Lisbon, Portugal
\(^c\)Hospital Garcia de Orta, EPE, 2805-267 Almada, Portugal

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**Abstract**

We report a case of meningitis due to *Haemophilus influenzae* serotype d strain in an infant. As far as we know, this is the first report of a serotype d strain, responsible for childhood invasive disease in Europe, demonstrating an emerging of *H. influenzae* non-b serotype, in the post-vaccination era.

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1. Introduction

*Haemophilus influenzae* was, in the pre-vaccine era, a frequent cause of bacterial meningitis in children, with more than 95% of the cases due to serotype b strains. The *H. influenzae* type b (Hib) conjugate vaccine was introduced in Portugal in 1994, firstly in a voluntary basis and then included the National Vaccination Plan (NVP) in the year 2000. Despite the lack of data for ethnic minorities, all children are now immunized with four doses of Hib vaccine by the age of 18 months, which conducted to a significant decrease of invasive infections among children. However, we are increasingly characterizing non-capsulated strains (NC) as well as non-b serotypes, responsible for invasive disease (Bajanca et al., 2004), as have been also described in other countries (Campos et al., 2003; Cerquetti et al., 2000; Kalies et al., 2009; Kunze et al., 2008; Ribeiro et al., 2003; Ulanova and Tsang, 2009; Urwin et al., 1996).

2. Case report

A five-month-old boy, with craniosynostosis of coronal cranial sutures, and no other dismory or significant past medical history, was admitted to Pediatric Emergency Department of Hospital Espírito Santo, in Évora, after two days of fever, vomiting and deteriorating general condition. There was no diarrhea, cough, wheezing, rash or preceding upper respiratory tract symptoms. His immunizations were up to date for his age. According to the Portuguese NVP the child took two doses of Hib vaccine, at two and four months of age, and still lacks the other two doses, at 6 and 18 months. On admission the child was febrile, pale and lethargic, but interacting with his parents. Vital signs were stable with mild tachycardia and his fontanelle was non palpable. No purpura, focal signs or sensory and motor deficits were observed. Development was within normal limits. Blood analysis showed white blood cell count of 11.8 × 10⁹/L (73.6 % neutrophile), normal platelet count, serum glucose of 92 mg/dL and elevated C-reactive protein. Blood cultures were negative.Turbid cerebrospinal fluid (CSF) was obtained. CSF parameters were as follows: leukocytes (uncountable with PMN predominance), glucose 20 mg/dL and protein 204 mg/dL. The Gram staining
revealed very rare Gram-negative bacilli. Based on these first results empirical therapy was established, consisting of intravenous ceftriaxone (100 mg/kg/day) and vancomycin (60 mg/kg/day). On the cultural exam of CSF a H. influenzae was isolated. The following methods were used to characterize the strain: determination of biotype (AphiNH, BioMérieux, Marcy l’Etoile, France), serotype characterization by slide agglutination method (Difco, BD, Madrid, Spain) and confirmed by Polymerase Chain Reaction, with specific primers to all known H. influenzae serotypes (a to f) (Falla et al., 1994), detection of β-lactamase production by a chromogenic cephalosporin assay (Oxoid, Madrid, Spain) and determination of the minimum inhibitory concentrations (MIC) to thirteen antibiotics: ampicillin, amoxicillin-clavulanate, cefotaxime, cefaclor, cefuroxime, cefépine, meropenem, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, rifampin, ciprofloxacin and azithromycin, by a microdilution assay (Siemens Diagnostic, Deerfield, IL). Susceptibility to antibiotics was categorized according to CLSI breakpoints (Clinical and Laboratory Standards Institute (CLSI), 2009).

We characterized a H. influenzae strain, biotype IV, serotype d, β-lactamase negative and fully susceptible to all antibiotics tested. Mild fever persisted and a focal seizure was noted on the 3rd day after admission. Computed tomography of the head was performed but no changes were found. Two days after the child continued to be febrile and seizures recurred. Magnetic resonance imaging (MRI) revealed left subdural empyema so he was transferred to Pediatric Intensive Care Unit of Hospital Garcia de Orta in Almada, where craniotomy was performed and subdural collection was drained. Purulent fluid was collected but no bacteria were isolated. Antibiotic therapy was readjusted, adding metronidazol and sodium penicillin to ceftriaxone and vancomycin, for a total of five weeks, after which the child was discharged. We assisted to a completely recover, apparently without neurologic sequelae or hearing loss. The following methods were used to characterize the strain: determination of biotype (AphiNH, BioMérieux, Marcy l’Etoile, France), serotype characterization by slide agglutination method (Difco, BD, Madrid, Spain) and confirmed by Polymerase Chain Reaction, with specific primers to all known H. influenzae serotypes (a to f) (Falla et al., 1994), detection of β-lactamase production by a chromogenic cephalosporin assay (Oxoid, Madrid, Spain) and determination of the minimum inhibitory concentrations (MIC) to thirteen antibiotics: ampicillin, amoxicillin-clavulanate, cefotaxime, cefaclor, cefuroxime, cefépine, meropenem, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, rifampin, ciprofloxacin and azithromycin, by a microdilution assay (Siemens Diagnostic, Deerfield, IL). Susceptibility to antibiotics was categorized according to CLSI breakpoints (Clinical and Laboratory Standards Institute (CLSI), 2009).

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3. Discussion

We report here an unusual case of meningitis, in an infant, caused by H. influenzae serotype d, which has rarely been involved in invasive disease (Holmes and Kozinn, 1983; Viner and Massanari, 1979; Warren et al. 2010) and to our best knowledge it had never been reported before in the post-vaccine era in Europe. Although routine worldwide immunization with conjugate Hib vaccines caused a dramatic decrease in the incidence of the invasive disease due to this serotype, we would like to emphasize that disease due to non-b type strains, as well as NC strains is becoming more frequent than before. This change in the disease epidemiology has been expected, since many years have passed after the introduction of the Hib vaccine in most of the countries (Adderson et al., 2001; Cerquetti et al., 2003; O’Neill et al., 2003; McConnell et al., 2007; Rogers and Manninge, 2010). It is important to alert clinicians and microbiologists for the emerging of non-b serotypes as well as NC strains of H. influenzae being responsible for severe invasive disease, especially in vaccinated children.

References


