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Case Report

Maternal and neonatal sepsis caused by *Haemophilus influenzae* type d

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A 29-year-old pregnant woman was admitted to hospital with signs of sepsis and threatened preterm labour. The premature neonate also showed signs of sepsis. *Haemophilus influenzae* biotype III was cultured from a midstream urine sample taken from the mother, maternal placental swabs and neonatal blood cultures. The placental and neonatal isolates were both found to be serotype d by PCR, and were indistinguishable by PFGE.

Introduction

The species *Haemophilus influenzae* encompasses a diverse group of organisms that can be part of the normal flora of humans but can be opportunistic and primary pathogens (Harper & Tilse, 1991). Some strains possess a polysaccharide capsule and are associated with invasive diseases such as meningitis, pneumonia and septicaemia (Peltola, 2000; Ulanova & Tsang, 2009). There are six capsular serotypes (a–f), of which type b (Hib) is by far the most common (Peltola, 2000; Ulanova & Tsang, 2009). However, the majority of strains have no capsule and are termed non-typable (NTHi). These are more commonly part of the normal flora, less invasive and frequently involved in opportunistic respiratory tract infections (Kalies *et al.*, 2009; McConnell *et al.*, 2007; Erwin & Smith, 2007; Heath *et al.*, 2001). Both encapsulated and NTHi strains can be differentiated into biotypes (I–VIII), and although some studies have shown an association between some biotypes and serotypes, and between some biotypes and the anatomical location of the organism or infection, the associations are relatively weak and inconsistently observed (Kilian, 1976; Harper & Tilse, 1991).

Since the introduction of a compulsory or recommended childhood conjugate vaccine for Hib in most developed countries, cases of infection with serotype b have dropped dramatically although cases of invasive disease have not declined to an equal extent (Kalies *et al.*, 2009; McConnell *et al.*, 2007; Tsang *et al.*, 2007). Some replacement with non-type b serotypes has been observed, but in general serotype replacement has not occurred with *H. influenzae* as it has with *Streptococcus pneumoniae* (Ladhani *et al.*, 2008; McConnell *et al.*, 2007; Sill *et al.*, 2007; Lipsitch, 1999; Hargreaves *et al.*, 1996). The majority of this increase in non-type b invasive disease is due to non-encapsulated strains, and of the invasive disease cases caused by non-type b encapsulated strains, those caused by types a and f are the most prevalent and cases due to type d are rare (McConnell *et al.*, 2007; Sill *et al.*, 2007; Ribeiro *et al.*, 2003; Heath *et al.*, 2001; Kalies *et al.*, 2009). Although *H. influenzae* type d has been shown to cause disease at a similar frequency to type b in an animal model (Roberts *et al.*, 1981), few cases of *H. influenzae* type d invasive disease have been reported in the scientific literature. Of those which have been described, clinical presentations have been traumatic implantation meningitis in an adult (Viner & Massanari, 1979); mild respiratory disease in a neonate (Hershckowitz & Mordechai, 2004); two cases of pneumonia; one case of empyema and shock; and one case of meningitis in a child (McConnell *et al.*, 2007).

H. influenzae represents a significant cause of antepartum and post-partum sepsis, neonatal meningitis, fetal death *in utero* and premature rupture of membranes, and in the majority of cases these infections are associated with NTHi (Hershckowitz & Mordechai, 2004; Martinez *et al.*, 1999; Quentin *et al.*, 1993). Contrary to earlier beliefs, characterization of these strains does not show a predominance of a particular 'genital biotype', with the exception of a specific subset of strains of biotype IV, which have now been identified as a genetically distinct cryptic genospecies with a strong association with urogenital, neonatal and mother–infant infections (Quentin *et al.*, 1996).

We describe a case of neonatal and maternal sepsis caused by *H. influenzae* capsular type d, biotype III.

Abbreviations: Hib, *Haemophilus influenzae* type b; NTHi, non-typable *Haemophilus influenzae*.

Case report

A 29-year-old pregnant woman (gravida 4, para 3) presented with initial Braxton Hicks contractions progressing to regular contractions with no per-vaginal loss but decreased fetal movements at 30 weeks and 5 days of gestation. At initial presentation she was febrile at 39.5 °C but otherwise haemodynamically stable. She was treated with three doses of nifedipine and intramuscular celestone for threatened pre-term labour. Initial investigations included a low vaginal swab with moderate growth of *Candida albicans* and normal vaginal flora. Midstream urine examination revealed no evidence of infection. At admission there was a leukocytosis of 17.3 nl^{-1} with a predominant neutrophilia of 15.2 nl^{-1} and the C-reactive protein was elevated at 35 mg l^{-1}. Due to a severe hypersensitivity reaction to penicillin, she received initial empirical treatment with vancomycin. The fetal heart rate remained relatively tachycardic (180–185 beats min^{-1}), and as there were no decelerations, the patient progressed to normal vaginal delivery with a diagnosis of presumed chorioamnionitis. Approximately 11 h after presentation, she delivered a living baby boy. Antibiotic therapy was initially ceased; however, 6 h after delivery she was again febrile with associated rigors, headache and lethargy. Blood cultures taken at this time were negative; however, *H. influenzae* biotype III (10^7–10^8 organisms l^{-1}) was cultured from a midstream urine sample on this occasion. Two placental swabs were also positive for *H. influenzae* biotype III. Placental histopathology was consistent with a pre-term placenta with florid changes of chorioamnionitis with umbilical cord phlebitis and arteritis. The mother had gentamicin added to the existing empirical regime of vancomycin immediately post delivery. Antibiotics were ceased after 4 days as there had been significant clinical improvement and inflammatory markers were improved. Culture results were unfortunately not available until the final day of intravenous therapy and hence the empirical antibiotic regime was not adjusted. The mother completed her treatment course with 5 days of oral erythromycin and remained well post discharge.

The 1900 g male infant on delivery made no respiratory effort, requiring immediate resuscitation and intubation. APGARs were 4 and 8 at 1 and 5 min, respectively. Surfactant was administered for hyaline membrane disease and amoxicillin and gentamicin were commenced for presumed sepsis. The initial 24 h of life were also complicated by anaemia requiring transfusion, hypoglycaemia and variable ventilatory requirements, all consistent with sepsis. Blood cultures taken at the time of initial resuscitation also isolated *H. influenzae* biotype III. Gentamicin was withheld due to poor urine output at day 2 and ceased at day 3 with the availability of culture results. Treatment of *H. influenzae* sepsis continued with amoxicillin for a total of 10 days. Follow-up blood cultures at day 6 were negative. The baby was ventilated for a total of 4 days, although there was no evidence of pneumonia either radiologically or microbiologically. He was discharged at day 40 of life with no sequelae of sepsis or prematurity.

Laboratory analysis

Both the urine and placental swab isolates from the mother and the blood culture isolate from the child were noticed to produce large, mucoid colonies unlike those produced by NTHi. The isolates were oxidase-positive, catalase-positive, Gram-negative coccobacilli and were nutritionally dependent on X and V factors (bioMérieux). Each isolate was identified as *H. influenzae* biotype III (indole-negative, urease-positive and ornithine decarboxylase-negative) using the API NH system (bioMérieux). All three isolates were sensitive to amoxicillin, amoxicillin–clavulanate, cefaclor, moxifloxacin and ceftriaxone as determined by disc diffusion testing (CLSI, 2006).

The serotypes of the maternal placental isolate and the child's blood culture isolate were determined by PCR as previously described (Falla *et al.*, 1994). Both were found to belong to capsular type d. Both strains were tested for possible identity to the genital *Haemophilus* cryptic genospecies using PCR to a specific 16S rRNA fragment (Quentin *et al.*, 1996) and both were negative. Strain relatedness of the isolates was then determined by PFGE using the restriction enzyme *Sma*I (Roche) as previously described (Saito *et al.*, 1999). Both isolates yielded indistinguishable macrorestriction patterns, with no band differences observed. The maternal urine isolate was not available for further investigation.

Discussion

This case represents an example of maternal and neonatal sepsis caused by *H. influenzae* type d, biotype III. Although the demonstration of biotype III in the isolates suggested that they were not the genital cryptic genospecies, the problems with specificity of biotyping have been demonstrated as biotypes I, II and IV have been identified as the genital cryptic genospecies by PCR (Quentin *et al.*, 1993; Martinez *et al.*, 1999). The exclusion of the genital cryptic genospecies by PCR in this study is consistent with the strains being serotype d, as all previous strains identified as genital cryptic genospecies have been NTHi (Martinez *et al.*, 1999).

Given the increasing prevalence of non-type b *H. influenzae* invasive disease, it is important for both clinicians and laboratory staff to be aware of the potential for such infections to occur. In particular, it is recommended that growth of *H. influenzae* in maternal vaginal swabs should always be reported by the laboratory to the requesting clinician, and should not be disregarded as normal flora. Due to its nutritional requirements, *H. influenzae* will not grow well on blood agar, and it is also recommended that specimens collected from the placenta or vagina of pregnant mothers showing signs of premature rupture of

membranes, chorioamnionitis and antepartum or postpartum sepsis should be inoculated onto agar selective for *H. influenzae*, in order to ensure recovery of this important maternal and neonatal pathogen.

In summary, we report a case of invasive maternal and neonatal infection with a strain of the clinically rare serotype d of *H. influenzae*, adding to the spectrum of disease reported for this capsular type.

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