Invasive disease caused by *Haemophilus influenzae* type a in Northern Ontario First Nations communities

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Seven epidemiologically unrelated cases of invasive *Haemophilus influenzae* type a (Hia) disease were identified in First Nations communities of Northwestern Ontario, Canada, in 2004–2008. In all cases, Hia was isolated from blood. The clinical presentation in most of the cases was moderately severe and all patients responded to antibiotic therapy. Laboratory analysis of Hia isolates from Northwestern Ontario indicated striking similarities in their phenotypic and genotypic characteristics. The findings are discussed in the context of current epidemiology of invasive Hia disease. Our data along with some published studies by others suggest an increased susceptibility to this infection among North American indigenous populations.

**Introduction**

*Haemophilus influenzae* is a Gram-negative coccobacillus which colonizes the nasopharynx of healthy individuals, but can also cause severe invasive diseases, such as meningitis, epiglottitis, septic arthritis and septicaemia (Peltola, 1993; Morris *et al.*, 2008). Most invasive infections are caused by encapsulated *H. influenzae*. Based on antigenic properties of their capsular polysaccharides, six (a, b, c, d, e, f) serotypes have been identified (Pittman, 1931). Before the late 1980s, *H. influenzae* type b (Hib), characterized by a polyribosyl ribitol phosphate capsule, was the most common cause of meningitis, epiglottitis and other invasive bacterial infections in children (Murphy, 2005). Introduction of Hib conjugate vaccines in the early 1990s dramatically decreased the incidence of invasive Hib disease among children in many industrialized countries (Peltola, 1993). In Canada, a rapid decline in Hib-associated morbidity and mortality has occurred since the conjugate Hib vaccine became part of the routine childhood immunization schedule in 1991 (Public Health Agency of Canada, 2006). Vaccination also significantly decreases the carriage rate of Hib in young children (Barbour, 1996). There is concern that following widespread vaccination against Hib, *H. influenzae* strains may undergo capsule switching or replacement to fill the ecological niche previously occupied by Hib (Tsang, 2007). Indeed, the emergence of invasive non-type b *H. influenzae* disease has been reported in several countries (Heath *et al.*, 2001; Ribeiro *et al.*, 2003; Bajanca *et al.*, 2004; Degani *et al.*, 2008; Tsang *et al.*, 2007; Brown *et al.*, 2009). Interestingly, there are apparent geographical disparities in the prevalence of different types of *H. influenzae*, i.e. between Europe and America. The role of the genetic background as a predisposing factor to invasive disease caused by certain capsular types of *H. influenzae* remains unexplored, although some studies suggest an increased incidence of invasive *H. influenzae* type a (Hia) disease among indigenous people of North America (Millar *et al.*, 2005; McConnell *et al.*, 2007; Bruce *et al.*, 2008). We describe seven epidemiologically unrelated cases of invasive Hia disease that occurred in First Nations communities of Northwestern Ontario (Canada) during 2004–2008.

**Case reports**

**Case 1**

Patient 1 was a 15-month-old aboriginal male who presented with persistent fever, cough and irritability. He was diagnosed with chest X-ray-confirmed left-sided pneumonia and empyema (Fig. 1) and was treated with
intravenous (i.v.) cefuroxime for 3 days. He was transferred to a tertiary care paediatric hospital due to worsening symptoms, and treated with i.v. cefuroxime for a further 14 days and subsequently with oral amoxicillin for 1 month. Blood cultures grew Hia, sensitive to ampicillin. A follow-up chest X-ray at 21 months showed some residual atelectasis but had cleared at 22 months.

Case 2
Patient 2 was a 33-month-old previously well aboriginal male who presented with fever. An outpatient throat swab was positive for *Streptococcus pyogenes* and he was started on erythromycin. He presented 2 days later with a recurrent fever of 40 °C and subsequently developed a swollen, erythematous, painful right ankle. An X-ray from admission was normal. Blood cultures grew Hia, which was β-lactamase-negative and sensitive to ampicillin, chloramphenicol and cefotaxime. The patient was transferred to a tertiary care paediatric hospital and was diagnosed with possible osteomyelitis/septic arthritis of his right ankle. Blood cultures again grew Hia (β-lactamase-negative). His bone scan, X-ray and ankle ultrasound were normal. His erythrocyte sedimentation rate (ESR) was very elevated at 104 mm h⁻¹ (normal values=20–30) and white blood cell (WBC) count was 20.9 × 10⁹ l⁻¹ with 80 % neutrophils. He was initially treated with i.v. cefuroxime for 10 days, and then he was discharged on oral amoxicillin for 25 days, for a total of 5 weeks of antibiotics for a presumed osteomyelitis or septic arthritis. Upon discharge, his ESR was 29 and a repeat ankle X-ray did not show any bone destruction. He fully recovered.

Case 3
Patient 3 was a 4-year-old aboriginal morbidly obese male with a BMI of 32 and a history of mild reactive airway disease who presented with severe abdominal pain and vomiting. He had an occasional cough for the preceding month and had been exposed to a cousin with pneumonia. His abdominal examination was benign, but a chest X-ray revealed a left lower lobe infiltrate. He was afebrile on admission, but the next morning developed a fever of 39.1 °C. His WBC count was elevated at 28.8 × 10⁹ l⁻¹ and blood cultures grew Hia. He was treated with i.v. cefuroxime and oral azithromycin. His WBC count had normalized by day 4. He was discharged on oral cefuroxime and fully recovered.

Case 4
Patient 4 was a 34-year-old aboriginal female who presented with cough, shortness of breath and fever of 38.9 °C. Underlying medical conditions and risk factors included type 2 diabetes mellitus, reactive airway disease, hypercholesterolaemia and smoking. Her WBC count was elevated at 19.6 × 10⁹ l⁻¹. A chest X-ray demonstrated a lingular and left lower lobe pneumonia and she was treated with i.v. ceftriaxone and doxycycline for 3 days. Blood cultures grew Hia, which was β-lactamase-negative and sensitive to ampicillin, chloramphenicol, ceftriaxone and meropenem. She was discharged on oral cefuroxime and recovered completely.

Case 5
Patient 5 was a 65-year-old aboriginal female who presented with a fever of 38.6 °C, nausea, vomiting and severe left elbow pain for 2 days. Her past medical history included tuberculous spondylitis at age 11, pulmonary and spinal tuberculosis at age 14 and Ménière's disease at age 42. She was being treated for congestive heart failure, chronic obstructive pulmonary disease, kyphoscoliosis and depression. She was diagnosed with upper respiratory illness and suspected elbow cellulitis. On admission, she had tachypnoea (respiratory rate=60) with a slightly elevated WBC count of 11.7 × 10⁹ l⁻¹ with 92 % neutrophils. Her chest X-ray appeared grossly normal but was difficult to interpret due to the scoliosis. Elbow X-ray was normal. Elbow aspiration was unsuccessful, but blood cultures grew Hia, β-lactamase-negative, sensitive to ampicillin, chloramphenicol, ceftriaxone and meropenem. She was treated with levofloxacin for 4 days i.v. and 1 week orally. Her respiratory and joint symptoms resolved.

Case 6
Patient 6 was a 6.5-year-old aboriginal female who presented with tonsillitis, fever of 38.3 °C and appeared unwell. The previous year she had a surgical correction of her atrial septal defect. At admission, the WBC count was elevated (18.2 × 10⁹ l⁻¹) and blood cultures were positive...
for Hia. She was treated with clindamycin and ampicillin for 10 days and completely recovered. Her other medical conditions included iron deficiency anaemia, for which she took ferrous gluconate. She had completed the series of Hib conjugate vaccine according to the immunization schedule prior to her illness.

**Case 7**

Patient 7 was a 47-year-old aboriginal male who presented with tachypnoea (respiratory rate=40), haemoptysis and severe pleuritic chest pain. His past medical history included rheumatic fever at age 6, appendectomy at age 16 and abdominal hernia repair at age 22. Upon hospital admission, the patient had a dramatic chest X-ray with right middle and lower consolidations as well as a lingular infiltrate (Fig. 2). His WBC count was elevated at 25.6 × 10^9 l^-1 and blood cultures were positive for Hia. Sputum smears for acid-fast bacilli, fungi and cytological abnormalities were negative. He was treated with i.v. cefuroxime and oral azithromycin. After 6 days of i.v. antibiotic therapy, he was feeling well and discharged home on oral amoxicillin.

**Comment**

We could confirm with certainty complete vaccination status in only one of the four paediatric cases (patient no. 6). However, the other children have likely been vaccinated due to the presence of a regional vaccination programme.

**Methodology and laboratory results**

Eight individual Hia isolates from Northwestern Ontario obtained during 2004–2008, including four isolates recovered from the seven cases described in this study, were analysed for their phenotypic and genotypic characteristics. The four isolates unrelated to the cases were included for comparison purposes and to examine the genetic diversity of invasive Hia isolates recovered from the Northwestern Ontario region. All isolates were from blood cultures performed in clinical laboratories in Sioux Lookout and Thunder Bay hospitals. The hospital charts were retrospectively reviewed for the demographical and clinical information. The study was approved by the Research Review Committee, Meno-Ya-Win Health Centre (Sioux Lookout, Ontario), and the Research Ethics Board of Thunder Bay Regional Health Sciences Centre.

Identification of *H. influenzae* was carried out using standard methods (Kilian, 2007) and confirmed by 16S rRNA gene sequencing (Lau *et al.*, 2004). Serotyping was accomplished using both a bacterial agglutination test and a PCR assay (Sill *et al.*, 2007a). Biotyping, multilocus sequence typing, PFGE and detection of the IS1016-bexA partial deletion were performed as previously described (Tsang *et al.*, 2006).

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**Fig. 2.** (a) Posteroanterior chest radiograph of the 47-year-old patient in case 7 showing extensive consolidation in the right middle lobe and basal segments of the right lower lobe with some infiltrates in the lingula of the left lung. (b) Lateral chest radiograph of the same patient.
All eight isolates were positive for the capsular transport gene bexA, and confirmed to be serotype a by PCR. All eight isolates belonged to biotype II and to the clonal group of sequence type (ST)-23. Identical PFGE patterns (pattern a1) with Smal enzyme were found in all eight isolates. None of the eight Hia isolates were found to contain the IS1016-bexA deletion in their capsule locus, cap. All eight isolates were also β-lactamase-negative and were susceptible to ampicillin, amoxicillin–clavulanic acid, cefaclor, ceftriaxone, chloramphenicol, sulfamethoxazole–trimethoprim, ciprofloxacin, moxifloxacin and clarithromycin.

Discussion

In the post-Hib vaccine era, non-type b H. influenzae (including other non-b serotypes and non-typable strains) causes most of the invasive H. influenzae disease, with the highest prevalence of disease due to non-typable H. influenzae. Among non-type b encapsulated H. influenzae causing invasive disease, serotype f is becoming the most prevalent in both North America and Europe (Dworkin et al., 2007; Tsang et al., 2007; Adam et al., 2010; Ladhani et al., 2010). In the province of Ontario, Canada, serotype a comprised a small percentage (2.1%) of all the invasive H. influenzae isolates (n=1455) both before and after the introduction of the Hib conjugate vaccine (Adam et al., 2010). In contrast, in Northwestern Ontario, 13 out of 31 (42%) invasive H. influenzae isolates serotyped in 2002–2008 were Hia (Brown et al., 2009). Of note, this region has a significant proportion of aboriginal people, i.e. 19.6% of the population (Statistics Canada, 2006).

Previous studies found a high incidence of invasive Hia disease among North American aboriginal people, including Canadian First Nations (Hammitt et al., 2005; Millar et al., 2005; McConnell et al., 2007; Bruce et al., 2008). According to Bruce et al. (2008), the incidence rate of invasive Hia disease in the North American Arctic was 2.9/100 000 for indigenous and 0.2/100 000 for non-indigenous people. The highest reported incidence rate of invasive Hia disease was among indigenous children <2 years of age, i.e. 52.6/100 000 (Bruce et al., 2008). Although severe cases of invasive Hia disease have been reported in non-aboriginal populations (Adderson et al., 2001; Kapogiannis et al., 2005; de Pádua et al., 2009), this disease is rare in the general population (ABC surveillance data quoted from Kapogiannis et al., 2005; Adam et al., 2010). In our study, we describe seven epidemiologically unrelated cases of invasive Hia disease in.aboriginal individuals that have occurred over a period of 4 years in a region with population of 25 000, i.e. with a mean annual incidence rate of 7/100 000. The aboriginal population in the area is 82% including 28 First Nations communities (Walker et al., 2009).

The severity of some previously reported Hia disease cases, i.e. presenting as meningitis and septic arthritis, was reminiscent of the invasive disease caused by Hib (Adderson et al., 2001; Kapogiannis et al., 2005). Multiple copies of the capsule locus as well as a partial deletion of the IS1016-bexA gene, which stabilizes the capsule locus causing an increase in the production of capsule polysaccharides, have been reported in both Hib and Hia as a mechanism for their enhanced virulence (Kroll et al., 1993, 1994). However, not all Hia isolates from invasive disease have the IS1016-bexA partial deletion (Hammitt et al., 2005; Tsang et al., 2006; Bruce et al., 2008). According to Bruce et al. (2008), this mutation was absent from 28 Hia isolates from the North American Arctic, including those involved in fatal cases. Indeed, according to Leaves et al. (1995), Hia strains usually contain intact tandemly repeated copies of cap. It remains unknown whether any additional virulence factors, besides the capsule, may contribute to the virulence of Hia. In our study, clinical presentation of all the cases was moderately severe and all patients completely recovered although they required hospitalization and prolonged antibiotic therapy. The most common presentation was pneumonia (four out of seven cases); in two cases Hia disease involved joints. Of note, five out of the seven patients (aged 4–65 years) had significant underlying medical conditions, which may have contributed to reduced immunity, resulting in invasive Hia disease.

Although Hia has the potential to cause outbreaks, so far only one outbreak has been reported in the literature (Hammitt et al., 2005). Very few studies have examined Hia carriage rates, but in two studies performed in Alaska, rates of about 16–43% were found among close contacts of a culture-confirmed Hia invasive disease case patient (Hammitt et al., 2005, 2006). The cases presented in our study appeared to be epidemiologically unrelated, as they occurred in isolated communities accessible to one another by air only, although all the Hia isolates from these cases showed remarkable phenotypic and genotypic similarities. This implies that Hia circulates in Northern Ontario, in particular, in First Nations communities, and may represent a significant risk for susceptible individuals, such as young children and adults with underlying co-morbidities contributing to decreased immunity, e.g. diabetes mellitus. Invasive Hia disease has also been reported to be common among the Navajo and White Mountain Apache American Indians, i.e. with an annual incidence rate of 20.2/100 000 for children under the age of 5 years (Millar et al., 2005). In the Keewatin Region of Nunavut (northern Canada), the estimated incidence of invasive Hia disease was 418.7/100 000 for Inuit children under 5 years old (McConnell et al., 2007). In four Canadian western provinces (British Columbia, Alberta, Saskatchewan and Manitoba), the rate of invasive Hia disease among aboriginal children under 5 years of age was 3.7/100 000 (McConnell et al., 2007). Previous studies found that similar risk factors can predispose to invasive Hia as to Hib disease, i.e. young age, exposure to other children in child care centres and overcrowding (Adderson et al., 2001).

We compared our findings to previously published reports describing invasive Hia disease and/or the bacteria isolated...
from the cases (Table 1). Of the 10 studies, six provided DNA fingerprinting results (Adderson et al., 2001; Ribeiro et al., 2003; Millar et al., 2005; Kapogiannis et al., 2005; Hammitt et al., 2005; Bruce et al., 2008) and used the restriction enzyme Smal for digestion of the Hia genomic DNA. In two of these studies (Hammitt et al., 2005; Bruce et al., 2008), an additional enzyme, Apal, was also used, while in the study reported by Millar et al. (2005), the restriction enzyme Blal was also employed. While it was impossible to compare the DNA fingerprints reported in these six studies, more than one DNA fingerprinting pattern has been observed. However, the DNA fingerprinting in our study using multilocus sequence typing and PFGE analysis of Hia isolates from Manitoba and Northwestern Ontario suggested clonality of the Hia isolates collected from these two neighbouring regions in Canada.

<table>
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<th>Reference</th>
<th>Nature of study</th>
<th>Study cases/isolates*</th>
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<tr>
<td>Rutherford &amp; Wilfert</td>
<td>Report of two cases and review of literature (North</td>
<td>Female (2 years 5 months), fever and respiratory distress; pleural effusion yielded Hia</td>
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<td>(1984)</td>
<td>Carolina, USA)</td>
<td>Male (45 years), history of alcoholism, gastrointestinal bleeding; blood culture Hia-positive</td>
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<td>Kroll et al. (1994)</td>
<td>Detection of IS1016-bexA deletion in the encapsulation</td>
<td>Five Hia meningitis and pneumonia cases in Gambia were analysed; isolates from three cases showed the IS1016-bexA deletion</td>
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<td>(cap) locus of Hia (Africa)</td>
<td>White female (6 months); white female (1 year); female (7 months); male (13 months). All four cases with meningitis and bacteraemia and with Hia isolated from blood and CSF cultures. Male (4 years), with flash burn to the face, fever and lower lobe infiltration; endotracheal secretions yielded pure culture of Hia</td>
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<td>Adderson et al. (2001)</td>
<td>Described five cases of severe Hia invasive disease</td>
<td>Thirteen Hia case isolates were divided into two closely related PFGE groups, each with distinct biotype</td>
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<td>(Utah, USA)</td>
<td>Severity and mortality of Hia meningitis cases were similar to those of non-type a cases including Hib</td>
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<td>Ribeiro et al. (2003)</td>
<td>Reported eightfold increase in Hia meningitis in</td>
<td>Female (6 months), with history of respiratory illness, developed pneumonia and blood culture yielded Hia; 4 months later developed a separate episode of Hia meningitis</td>
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<td>Brazil after introduction of Hib immunization,</td>
<td>Male (8 months), with pyelonephritis, developed fever and refusal to move left leg; joint fluid grew Hia, successfully treated with ceftriaxone; 3 months later fever and pain returned to his left leg and left arm; blood and joint fluid grew Hia</td>
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<td>characterized Hia cases and case isolates</td>
<td>Male (4 months), with history of neurodegenerative disease, developed bilateral pneumonia; blood cultures yielded Hia</td>
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<td>Hammitt et al. (2005)</td>
<td>Outbreak of invasive Hia disease among Alaska natives</td>
<td>Male (14 months) of Middle Eastern descent, developed fever and swelling of right hand; blood culture grew Hia</td>
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<td>Kapogiannis et al. (2005)</td>
<td>Reported two cases of invasive Hia disease with</td>
<td>Seventy-six cases of invasive Hia disease were studied, median age of cases was 12 months; most Hia isolates were from blood, or blood and CSF; most common presentation was meningitis, followed by pneumonia; others presented as cellulitis and septic arthritis; unrelated Hia isolates were 90% similar when analysed by PFGE</td>
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<td>isolates showing IS1016-bexA deletion (ABC surveillance</td>
<td>Four random isolates, three from blood (2-year-old female, 1-year-old male and 57-year-old female) and one from ear (10-month-old male) Three blood isolates belonged to unrelated clonal groups of ST-4, ST-23 and ST-62; ear isolate belonged to another clonal group of ST-403</td>
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<td>Millar et al. (2005)</td>
<td>study, USA)</td>
<td>Fourty-two Hia cases were identified among 132 cases with serotype information; 30 of the Hia cases occurred in children 2–5 years of age, 8 in adults aged 21–73 years; 35 of the 38 Hia cases with ethnicity data were aboriginal; the most common clinical presentations were meningitis and pneumonia, followed by septic arthritis</td>
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<td>Sill et al. (2007a)</td>
<td>Characterized four Hia isolates recovered from</td>
<td>White female (5 months), CSF culture Hia-positive</td>
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<td>Bruce et al. (2008)</td>
<td>Described epidemiology of Hia in North American</td>
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<td>de Pádua et al. (2009)</td>
<td>Case report of Hia meningitis in Brazil</td>
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*CSF, Cerebrospinal fluid.
In the present study, the eight isolates from Northwestern Ontario gave a DNA fingerprint identical to one of the three related fingerprint types found among Hia isolates recovered in Manitoba (Tsang et al., 2006). In addition, the single ST of Northwestern Ontario Hia isolates is also the predominant ST found among Hia isolates in Manitoba. This is not surprising as the region of Northwestern Ontario is adjacent to the province of Manitoba, where many aboriginal communities are found. Similarity of the Hia isolates found in these two neighbouring provinces is further supported by our findings that invasive Hia isolates from Northwestern Ontario did not have the IS1016-bexA partial deletion. The lack of this mutation in the Hia cap locus may explain the apparently less virulent nature of the Hia disease observed in this series, i.e. lack of meningitis and mortality among the cases.

Despite the apparent similarity of invasive Hia isolates in Manitoba and Northwestern Ontario, other reports suggested some degree of Hia genetic diversity. In a study of four Hia isolates collected from patients in the province of Quebec, Canada (Sill et al., 2007b), three distinct groups were identified: two groups were characterized by unrelated ST-4 and ST-62, and the third group consisted of two isolates of related STs (ST-23 and ST-403). Partial deletion of the IS1016-bexA gene was found in an isolate identified as ST-4 while this deletion was absent from the other three strains. In another study of Hia isolates recovered from meningitis patients in Salvador, Brazil, two clonal groups of Hia were identified, one represented by ST-4 with partial deletion of the IS1016-bexA gene while the other was represented by ST-23 without the partial deletion of the cap genes (Lima et al., 2010).

In agreement with some previously published reports (Hammitt et al., 2005; Millar et al., 2005; McConnell et al., 2007; Bruce et al., 2008), this study suggests that North American aboriginal people may have an increased susceptibility to Hia invasive disease. Importantly, before the introduction of Hib conjugate vaccines, some North American indigenous populations experienced the highest rates of invasive Hib disease worldwide (reviewed by Ulanova & Tsang, 2009). Whether genetic or environmental factors (or both) determine an increased susceptibility of aboriginal populations to invasive infections caused by encapsulated bacterial pathogens is uncertain and needs further study. Although the development of a new conjugate Hia vaccine appears feasible (Jin et al., 2007), more research is warranted towards a better understanding of immunoepidemiology of invasive Hia disease. In particular, continuing surveillance of all non-type b H. influenzae invasive infections is needed to establish whether or not an increase in the incidence of invasive Hia occurs in the post-Hib vaccine era. Conjugated Hib vaccine is not effective against non-b serotypes or non-typable strains of H. influenzae. Therefore, as Hib disease is eliminated in the vaccinated populations, non-b serotypes or non-typable H. influenzae strains may replace Hib to become the major invasive bacterial pathogens if they are of significant pathogenic potential. However, if such strains are considerably less virulent than Hib, they may only cause occasional disease in an opportunistic manner.

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References


