Emergence of non-serotype b encapsulated *Haemophilus influenzae* as a cause of pediatric meningitis in northwestern Ontario

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Before the introduction of the conjugate vaccine, *Haemophilus influenzae* serotype b (Hib) was the leading cause of bacterial meningitis in children. Although successful in reducing Hib cases, the vaccine confers no protection against other serotypes of *H influenzae*, such as a (Hia), or f (Hif). The emergence of invasive disease caused by non-Hib in northwestern Ontario (38 cases between 2002 and 2008) with predominance of Hia was previously reported by the authors. At that time, no cases of pediatric meningitis caused by *H influenzae* were recorded in the region. Continued surveillance identified 12 new cases of invasive non-Hib between January 2009 and July 2011. Among these cases, three young children developed meningitis with severe complications caused by Hia or Hif. The present article describes these cases along with the characteristics of recent *H influenzae* isolates from the region, (ie, their genetic background and antibiotic sensitivity). The findings point to the clonal nature of circulating Hia strains as well as to an increase in frequency and severity of pediatric invasive *H influenzae* infections in northwestern Ontario.

Key Words: Case series; *Haemophilus influenzae*; Meningitis; Serotype a; Serotype f

Despite modern medical advances, bacterial meningitis continues to be a major cause of morbidity and mortality in infants and children (1). *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* serotype b (Hib) are the primary pathogens that cause this disease in children younger than five years of age (1). Over the past two decades, cases of Hib meningitis have significantly decreased due to the introduction of Hib conjugate vaccines in the late 1980s (2). However, recent evidence suggests the emergence of serious invasive disease caused by non-Hib (3-5).

Previously, we reported 38 cases of non-Hib invasive disease in northwestern Ontario between 2002 and 2008 (6). Sixteen cases (42%) were in children younger than five years of age with infections caused by *H influenzae* serotype a (Hia), *H influenzae* serotype f (Hif) and non-typeable strains of *H influenzae*. The clinical presentations of these children included sepsis, pneumonia and septic arthritis, but no cases of meningitis were recorded (6). During continued surveillance of the region, we identified 12 new cases of invasive *H influenzae* disease due to non-Hib strains, including three cases of pediatric meningitis with serious complications. In the present study, we report our observations of these 12 cases of invasive *H influenzae* disease between January 2009 and July 2011, with specific focus on the clinical course of meningitis. Because we are now observing a more severe course of invasive non-Hib disease

L'émergence d'un *Haemophilus influenzae* de sérotype non-b encapsulé comme cause de méningite pédiatrique au nord-ouest de l'Ontario

Avant l'adoption du vaccin conjugué, l'*Haemophilus influenzae* de sérotype b (Hib) était la principale cause de méningite bactérienne chez les enfants. Le vaccin a réussi à réduire les cas de Hib, mais il ne confère aucune protection contre les autres sérotypes du *H influenzae*, tels que le sérotype a (Hia) ou le sérotype f (Hif). Les auteurs ont déjà signalé l'émergence d'une maladie invasive de sérotype non-b au nord-ouest de l'Ontario (38 cas entre 2002 et 2008), à prédominance de Hia. À l'époque, aucun cas de méningite pédiatrique causée par le *H influenzae* n'avait été déclaré dans la région. La surveillance continue a permis de dépister 12 nouveaux cas de *H influenzae* invasif de sérotype non-b entre janvier 2009 et juillet 2011. Parmi ces cas, trois jeunes enfants ont contracté une méningite accompagnée de graves complications causées par le Hia ou le Hif. Le présent article décrit ces cas ainsi que les caractéristiques des récents isolats de *H influenzae* dans la région (c'est-à-dire leurs antécédents génétiques et leur sensibilité aux antibiotiques). Les observations font croire à la nature clonale des souches de Hia en circulation ainsi qu'à une augmentation de la fréquence et de la gravité des infections invasives à *H influenzae* en pédiatrie au nord-ouest de l'Ontario.

in the region than before, using laboratory analyses, we addressed the question of whether specific genetic or phenotypic characteristics of the isolates could be responsible for their high virulence.

METHODS

Clinical data were collected from the Thunder Bay Regional Health Sciences Centre in northwestern Ontario. The hospital charts of the cases were retrospectively reviewed. The present study was approved by the Research Ethics Boards of all the involved institutions. Identification and biotyping of *H influenzae* was performed using standard biochemical tests (7) and confirmed by 16S ribosomal RNA sequencing (8). Serotyping was performed by both bacterial agglutination test, using antisera from Difco Diagnostics (Canada), and polymerase chain reaction to detect *bexA* and the serotype-specific genes according to the procedure described by Falla et al (9). Clonal analysis of the *H influenzae* isolates was done by multilocus sequencing typing (MLST) (10). For Hia isolates, detection of the IS1016-*bexA* partial deletion in their capsule loci was performed by polymerase chain reaction (11). Detection of β -lactamase production in *H influenzae* was conducted using BBL DrySlide Nitrocef (Becton Dickinson, Canada). Antibiotic susceptibility testing was performed using the disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines (12).

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TABLE 1
Characteristics of 12 cases of non-serotype b *Haemophilus influenzae* invasive disease from January 2009 to July 2011 in northwestern Ontario

Patient	Age	Sex	Characteristics of bacteria	Clinical presentation	Disease outcome	Underlying conditions
1*	18 months	M	Serotype a, biotype II ST-23	Meningitis, pneumonia	Infection cleared, persistent seizure activity	Unknown
2*	8 months	F	Serotype a, biotype II ST-929	Meningitis, pneumonia	Infection cleared	Multicystic dysplastic kidney
3*	23 months	M	Serotype f, biotype I ST-124	Meningitis, pneumonia	Infection cleared, persistent seizure activity	Unknown
4	29 months	F	Serotype a, biotype II ST-23	Pneumonia	Infection cleared	Unknown
5	15 years	M	Serotype a, biotype II ST-23	Pneumonia	Infection cleared	Unknown
6	45 years	F	Serotype a, biotype II ST-56	Pneumonia	Unknown	Unknown
7	45 years	M	Serotype e, biotype IV ST-69	Pneumonia	Infection cleared	Drug and alcohol abuse
8	46 years	M	Nontypeable, biotype IV	Pneumonia	Infection cleared	Diabetes, acute renal failure
9	54 years	M	Not typed	Pneumonia	Unknown	Multiple myeloma
10	60 years	F	Nontypeable, biotype V	Pneumonia, sepsis	Infection cleared	Huntington's chorea
11	63 years	F	Not typed	Pneumonia	Unknown	Hypertension, dyslipidemia, diabetes, coronary artery disease
12	87 years	F	Not typed	Pneumonia	Infection cleared	Hypertension, osteoporosis, osteoarthritis

*Meningitis cases. All typed strains were negative for β -lactamase and sensitive to amoxicillin, chloramphenicol, cefaclor, ceftriaxone, tetracycline, clarithromycin, azithromycin, cotrimoxazole, ciprofloxacin, moxifloxacin, levofloxacin, imipenem and meropenem. All *H influenzae* type a isolates were negative for the IS1016-bexA partial deletion. All isolates were cultured from venous blood and in patients 1 and 2 also from cerebrospinal fluid. F Female; M Male; ST Sequence type

Case 1

An 18-month-old boy presented to the emergency department (ED) with a three-day history of drowsiness, irritability and vomiting. He was born via spontaneous vaginal delivery induced secondary to a fall. His medical history was significant for neonatal jaundice, two episodes of viral upper respiratory infections and viral gastroenteritis. On physical examination, he appeared lethargic and irritable. He had a temperature of 35.6°C, heart rate of 80 beats/min, blood pressure of 99/51 mmHg, respiratory rate of 20 breaths/min and a blood oxygen saturation of 93% in room air. Auscultation of his chest revealed no adventitious sounds. Neck stiffness was evident and he tested positive for Kernig's sign. His cerebrospinal fluid (CSF) was moderately turbid and slightly xanthochromic. The CSF had a white blood cell (WBC) count of $4.35 \times 10^9/L$ (94% neutrophils, 3% lymphocytes and 3% monocytes), glucose concentration of <1.1 mmol/L (normal 2.2 mmol/L to 3.9 mmol/L) and a protein level of 3.52 g/L (normal 0.12 g/L to 0.60 g/L). His initial chest x-ray showed increased density in the right upper lobe suggesting a small area of atelectasis or pneumonia. Based on these findings, he was started on intravenous (IV) ceftriaxone and vancomycin and was admitted to the pediatric ward for treatment of his meningitis and possible pneumonia. On admission, his WBC was $12.9 \times 10^9/L$. This increased to $27.6 \times 10^9/L$ on day 4 of hospitalization. His hemoglobin levels dropped from 90 g/L to 73 g/L over the course of four days, for which he received packed red blood cells. On the same day, he developed an acute episode of staring and nonresponsiveness to sound and pain. He subsequently experienced absent movements of his left side. A head magnetic resonance imaging (MRI) scan revealed an abnormal signal in the right frontal lobe consistent with leptomeningeal meningitis along with cerebritis. Phenytoin was added to prevent seizure activity. He was then transferred to a children's hospital in southern Ontario for more advanced care. By this time, both his CSF and blood cultures had been tested by the provincial Public Health Laboratories (Toronto, Ontario) and had been found positive for Hia, biotype II, which is susceptible to a wide range of antimicrobials (Table 1). There were also concerns about decreased movement in his right hip; however, no evidence of congenital hip dysplasia or septic arthritis was found. He later experienced a few seizure episodes with positive electroencephalogram findings. He was started on carbamazepine shortly afterward. After approximately one month of hospitalization, he was sent home with a six-week course of oral ampicillin, carbamazepine and phenobarbital. In later visits, his right hip movement had improved but there were concerns about his hearing and speech development.

Case 2

An eight-month-old girl presented to the ED with a two-day history of fever and emesis. She was a twin female born via Caesarean section at 38 weeks' gestation. At birth, her hemoglobin and hematocrit levels were elevated at 226 g/L and 0.7, respectively. This was resolved in repeat blood work. She was also diagnosed with a left multicystic dysplastic kidney. On admission, her WBC count was $39.5 \times 10^9/L$ with 36.5% neutrophils, and her hemoglobin, platelets, sodium and potassium levels were 112 g/L, $617 \times 10^9/L$, 138 mmol/L and 3.6 mmol/L, respectively. Her chest x-ray revealed perihilar lymphadenopathy and an area of infiltrate in the right middle lobe. A lumbar puncture was performed, which was consistent with bacterial meningitis (the absolute values of the CSF components were not available at the time of data extraction). She was started on IV ceftriaxone and oral azithromycin. Both her CSF and blood cultures were positive for Hia, biotype II, which was susceptible to all tested antimicrobials (Table 1). Three days after her admission, she developed a transient right-sided seizure activity. A MRI scan of her head revealed a 5 mm subdural fluid collection on the left side. This abnormality had completely resolved in a follow-up MRI scan a week later. She was treated with IV antibiotics for 14 days and sent home with plans for future follow-up.

Case 3

A 23-month-old boy presented to the ED with a two-day history of fever, rhinorrhea, cough and irritability. On presentation, he experienced a generalized seizure lasting 20 min. He was born at 39 weeks' and six days' gestation via spontaneous vaginal delivery. His family history was significant for epilepsy and schizophrenia. The patient was intubated and moved to the intensive care unit. He had an initial WBC count of $11.7 \times 10^9/L$ and a sodium level of 127 mmol/L. His initial chest x-ray showed a highly dense area in the apex of the right lung, suggestive of pneumonia. His head computed tomography scan was unremarkable. He was started on ceftriaxone and vancomycin while awaiting transfer to a children's hospital in London, Ontario. While in London, a lumbar puncture was performed, which was negative for any pathogens, but this was likely due to the treatment with antibiotics. His hyponatremia was treated with 3% saline and gradually improved to 139 mmol/L; his hypokalemia and hypophosphatemia were corrected with potassium chloride and sodium phosphate, respectively. He was transferred back with the same antibiotics and phenobarbital for seizure control. Later, his blood cultures proved to be positive for Hif, biotype I, which were fully susceptible to

all antimicrobials (Table 1). The patient recovered well, with ongoing concerns about his hearing.

RESULTS

During 19 months of observation (between January 2009 and July 2011), 12 cases of invasive *H influenzae* disease due to non-Hib (Table 1) were identified. All *H influenzae* isolates were from blood and CSF cultures. Children younger than five years of age accounted for 33% of the cases. Three of the four infected children had developed meningitis with serious complications. Of the nine serotyped isolates, there were five Hia, one Hif, one *H influenzae* serotype e (Hie) and two nontypeable *H influenzae*. Both nontypeable *H influenzae* isolates and Hie were found in adults. All five Hia strains were related to one another by MLST and belonged to the clonal complex defined by the sequence type (ST)-23 and two related STs (ST-56 and ST-929) with one or two housekeeping gene alleles different from ST-23. None of the Hia strains harboured the *IS1016-bexA* deletion (Table 1). All nine serotyped *H influenzae* isolates in the present study did not produce β -lactamases and were fully susceptible to different classes of antibiotics commonly used for treatment of *H influenzae* infections, which include the β -lactams (ampicillin, amoxicillin, cefaclor, ceftriaxone, imipenem, meropenem), macrolides (clarithromycin, azithromycin), fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin), chloramphenicol, cotrimoxazole and tetracycline.

DISCUSSION

Acquisition of a polysaccharide capsule is a major contributor to the virulence of *H influenzae*; the capsule protects the bacteria against the host defense mechanisms (ie, complement-dependent killing and phagocytosis) (13). Of the six known capsular types, Hib is the most virulent (14). Before the introduction of the conjugate Hib vaccines, Hib was the leading cause of invasive bacterial disease in children younger than five years of age (15). In the United States and United Kingdom, Hib was the most common cause of pyogenic meningitis in three- to 18-month-old infants (16). In Canada, the incidence of invasive Hib disease has decreased from 1.89 cases per 100,000 persons in 1989 to 0.3 in 2004 (17). In the post-Hib vaccine era, non-serotype b strains have become the primary cause of invasive *H influenzae* disease. The majority of these new cases are caused by nontypeable strains followed by serotype f (3,5,18). The shift toward more virulent non-serotype b strains may be a result of capsule switching or replacement (19).

Encapsulated serotypeable *H influenzae* has been described as clonal (20), and each serotype of *H influenzae* contains strains with their own unique MLST profiles (20). Therefore, analysis of their genetic background by MLST can identify the phenomenon of capsule switching among *H influenzae* isolates. Unlike the Hib strains, the non-serotype b encapsulated *H influenzae* strains analyzed in the present study showed unique MLST profiles according to their serotype. For example, the five Hia strains were all related to one another by MLST and belonged to the clonal complex defined by ST-23 and two related STs (ST-56 and ST-929) with one or two housekeeping gene alleles different from ST-23 (Table 1). ST-23 is the predominant ST found among Hia isolates in Manitoba (20), and three of the five Hia isolates found in the present study belonged to this ST. Hia isolates belonging to ST-56 have been identified in British Columbia (21), while ST-929 has not yet been encountered. In line with the clonal nature of the ST-23 clonal complex isolates, none of the Hia isolates in the present study harboured the *IS1016-bexA* deletion, as we have previously reported for ST-23 isolates in northern Ontario and Manitoba (22,23).

The single Hie and Hif isolates were also unrelated to Hib strains in terms of their genetic background as revealed by MLST. The Hif clone of ST-124 has also been detected in both Manitoba (20) and British Columbia (21), and is the predominant ST among Hif strains found in Canada (National Microbiology Laboratory, unpublished data). The Hie clone identified by ST-69 has been detected before in Manitoba (20). Therefore, the genetic background of the non-serotype b encapsulated *H influenzae* strains analyzed in the present

study confirmed that they were not capsule-switched serotype b strains or serotype b strains that have lost their capsule export genes.

In the current study, 42% of cases were caused by Hia, 8% by Hif, 8% by Hie and 25% by nontypeable strains (Table 1). All adult cases with complete history (patients 7 to 12, Table 1) experienced underlying conditions that could cause reduced immunity. Prevalence of immunocompromised and elderly individuals among patients with invasive *H influenzae* disease in the postvaccine era have been reported by others (18). The predominance of Hia cases in the present study was consistent with our previous findings in the region (6,22). What was remarkable about our current findings was the shift toward more serious disease in the pediatric population. In the span of approximately two years, in a region with a population of approximately 250,000, four children younger than five years of age were infected with non-Hib strains, three of whom developed meningitis. The emergence of Hia meningitis in the postvaccine era has been reported in the North American Arctic (4), but to the best of our knowledge, the two cases discussed in the current report are without precedence in northwestern Ontario.

A large study from the Canadian Immunization Monitoring Program, ACTive (IMPACT) identified 25 pediatric cases of invasive Hia disease from 1996 to 2001, with 52% of cases presenting as meningitis (24). However, although IMPACT encompasses nearly 90% of the pediatric tertiary care beds in Canada, this program does not cover northern Ontario, and 24 of 25 cases of Hia disease were from the western Canadian provinces (Manitoba, Saskatchewan, Alberta and British Columbia) (24).

Both cases of Hia meningitis reported in the present study required hospitalization and long-term antibiotic therapy; furthermore, both children cleared the infection with some persistent seizure activity and developmental issues. Lima et al (25) reported poorer outcomes in Hia meningitis caused by strains having the *IS1016-bexA* partial deletion. Both isolates lacked this deletion and were also negative for β -lactamase and that may explain the success in treatment. The third case of meningitis was caused by Hif. Among non-serotype b encapsulated *H influenzae* causing invasive disease, Hif is now becoming the most prevalent in North America and Europe (3,5). However, it mainly causes disease in the elderly and immunocompromised individuals. For example, Fickweiler et al (26) reported a case of meningitis caused by Hif in an eight-year-old immunodeficient girl. In our case series, the 23-month-old boy did not exhibit any obvious immune defects and it is possible that this strain of Hif was highly virulent. Socioeconomic status is recognized as a key risk factor for developing bacterial meningitis (1). It should be noted that all of the children in the present study were of poor socioeconomic status and from remote rural areas of northwestern Ontario.

The emergence of invasive disease caused by non-Hib is of great concern worldwide. In Canada, some evidence of an increasing prevalence of invasive disease caused by non-Hib in the post-Hib vaccine era has been accumulated during the past five to six years (3,4,6,21,23,24). However, until recently, the national surveillance of invasive *H influenzae* disease was limited by Hib impeding the longitudinal epidemiological analysis (3). Although invasive Hib disease has been a reportable infection in Canada since 1979, invasive *H influenzae* disease caused by non-Hib strains was only included in the revised national notifiable disease list in 2007 (27), and not all provinces implemented the policy at the same time. Despite these limitations, our findings point to a change in severity of disease in the pediatric population of northwestern Ontario. This emphasizes the need for continued surveillance of non-Hib strains in the post-Hib vaccine era and for research on host and microbial factors responsible for the development of severe disease.

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