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 invasive non-Hib disease caused by non-Hib (3-5).

In the late 1980s (2), however, recent evidence suggests the emergence of more severe course of invasive non-Hib disease caused by non-Hib (3-5). 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 invasive non-Hib disease caused by non-Hib (3-5).

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 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 hexA 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-hexA partial deletion in their capsule loci was done by multilocus sequencing typing (MLST) (10). For Hia isolates, detection of the IS1016-hexA partial deletion in their capsule loci was done by multilocus sequencing typing (MLST) (10). For Hia isolates, detection of the IS1016-hexA partial deletion in their capsule loci was done by multilocus sequencing typing (MLST) (10). For Hia isolates, detection of the IS1016-hexA partial deletion in their capsule loci was done by multilocus sequencing typing (MLST) (10). For Hia isolates, detection of the IS1016-hexA partial deletion in their capsule loci was done by multilocus sequencing typing (MLST) (10).

The clinical presen-
**TABLE 1**

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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Characteristics of bacteria</th>
<th>Clinical presentation</th>
<th>Disease outcome</th>
<th>Underlying conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>18 months</td>
<td>M</td>
<td>Serotype a, biotype II ST-23</td>
<td>Meningitis, pneumonia</td>
<td>Infection cleared, persistent</td>
<td>Unknown</td>
</tr>
<tr>
<td>2*</td>
<td>8 months</td>
<td>F</td>
<td>Serotype a, biotype II ST-929</td>
<td>Meningitis, pneumonia</td>
<td>Infection cleared</td>
<td>Multicytic dysplastic kidney</td>
</tr>
<tr>
<td>3*</td>
<td>23 months</td>
<td>M</td>
<td>Serotype f, biotype I ST-124</td>
<td>Meningitis, pneumonia</td>
<td>Infection cleared, persistent</td>
<td>Unknown</td>
</tr>
<tr>
<td>4</td>
<td>29 months</td>
<td>F</td>
<td>Serotype a, biotype II ST-23</td>
<td>Pneumonia</td>
<td>Infection cleared</td>
<td>Unknown</td>
</tr>
<tr>
<td>5</td>
<td>15 years</td>
<td>M</td>
<td>Serotype a, biotype II ST-23</td>
<td>Pneumonia</td>
<td>Infection cleared</td>
<td>Unknown</td>
</tr>
<tr>
<td>6</td>
<td>45 years</td>
<td>F</td>
<td>Serotype a, biotype II ST-56</td>
<td>Pneumonia</td>
<td>Infection cleared</td>
<td>Unknown</td>
</tr>
<tr>
<td>7</td>
<td>45 years</td>
<td>M</td>
<td>Serotype e, biotype IV ST-69</td>
<td>Pneumonia</td>
<td>Infection cleared</td>
<td>Drug and alcohol abuse</td>
</tr>
<tr>
<td>8</td>
<td>48 years</td>
<td>M</td>
<td>Nontypeable, biotype IV</td>
<td>Pneumonia</td>
<td>Infection cleared</td>
<td>Diabetes, acute renal failure</td>
</tr>
<tr>
<td>9</td>
<td>54 years</td>
<td>M</td>
<td>Not typed</td>
<td>Pneumonia</td>
<td>Infection cleared</td>
<td>Unknown</td>
</tr>
<tr>
<td>10</td>
<td>60 years</td>
<td>F</td>
<td>Nontypeable, biotype V</td>
<td>Pneumonia, sepsis</td>
<td>Infection cleared</td>
<td>Huntington’s chorea</td>
</tr>
<tr>
<td>11</td>
<td>63 years</td>
<td>F</td>
<td>Not typed</td>
<td>Pneumonia</td>
<td>Infection cleared</td>
<td>Hypertension, dyslipidemia, diabetes, coronary artery disease</td>
</tr>
<tr>
<td>12</td>
<td>87 years</td>
<td>F</td>
<td>Not typed</td>
<td>Pneumonia</td>
<td>Infection cleared</td>
<td>Hypertension, osteoporosis, osteoarthritis</td>
</tr>
</tbody>
</table>

* 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
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 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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