Haemophilus influenzae Activity in a Single Medical Center in Israel in the Post-Vaccine Era

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ABSTRACT: **Background:** The incidence of invasive disease due to *Haemophilus influenzae* has decreased since the implementation of vaccination against serotype B.

Objectives: To describe the epidemiological, clinical and microbiological characteristics of patients with *H. influenzae* meningitis or bacteremia in the vaccine era in Israel.

Methods: We reviewed the medical records of all patients admitted to Shaare Zedek Medical Center between 1997 and 2010 who had blood or cerebrospinal fluid culture positive for *H. influenzae*.

Results: The study group comprised 104 patients – 57 children and 47 adults. Overall, 21 (20%) of the infections were due to serotype b. The children had shorter hospitalizations (6 vs. 12 days, $P = 0.005$) and lower mortality rate (5% vs. 28%, $P = 0.003$) as compared to the adults. Bacteremic pneumonia was the most common diagnosis in adults (45% vs. 28% in children, $P = 0.08$) while meningitis was more common in children (17% vs. 3.5%, $P = 0.09$). There was a seasonal pattern, with infections being more common during the winter and spring.

Conclusions: Invasive *H. influenzae* disease is uncommon but still exists in both children and adults. The disease course tends to be more severe in adults. Even in the global vaccination era, serotype b constitutes a significant portion of invasive disease.

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KEY WORDS: *Haemophilus influenzae*, conjugate vaccine, meningitis, bacteremia

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Haemophilus influenzae is a respiratory pathogen causing a broad spectrum of infections, ranging from otitis media to life-threatening infections. Routine vaccination was implemented in Israel in 1996 and is given at 2, 4, 6, and 12 months of age. Prior to the implementation of the vaccine, *H. influenzae* serotype b was a leading cause of childhood meningitis. Since then, the incidence of invasive Hib disease has fallen dramatically; nevertheless, cases are still reported every year. The incidence of Hib disease in adults decreased as well since the implementation of vaccination due to herd immunity [1], as has been the case with pneumococcal infection [2]. Other serotypes, including a, c, d, e, f and non-typable, cause invasive disease in both children and older age groups. In some countries, Hib immunization has led to the emergence of non-typable isolates as the leading cause of *H. influenzae* invasive disease [3].

The aim of this study was to describe the epidemiological, clinical and microbiological characteristics of patients with *H. influenzae* meningitis or bacteremia in the vaccine era.

PATIENTS AND METHODS

PATIENTS

The computerized database of Shaare Zedek Medical Center, a 550 bed university-affiliated general hospital, was reviewed for all patients who had blood or cerebrospinal fluid cultures positive for *H. influenzae* between January 1997 and June 2010. The following data were retrieved from the medical records: patient age and gender, underlying diseases, date of admission, hospitalization length, number of previous Hib vaccinations, temperature at admission, complete blood count, liver function tests, CSF parameters, antibiotic treatment, discharge diagnosis, need for intensive care, and outcome. The microbiological data for all *H. influenzae* bacterial isolates were reviewed for serotype and antibiotic susceptibility. Patients with Hib disease were compared with those who had non-serotype b disease. We also compared the clinical and microbiological features of children (age 0–16 years) and adults.

Vaccine failure was defined as invasive Hib disease occurring more than 2 weeks after one dose given at 1 year of age or older, or more than 1 week after ≥ 2 doses given at < 1 year of age [4].

MICROBIOLOGY

All *H. influenzae* isolates recovered from blood or CSF at Shaare Zedek Medical Center's microbiology laboratory were grown on chocolate II agar (Becton Dickinson, ref 221169 - BD BBL™, USA) and incubated at 35°C for 24 hours

Hib = *H. influenzae* serotype b

CSF = cerebrospinal fluid

in 5% CO_2. Isolates were routinely identified by API-NH strips (BioMerieux Sa, 69280 Marcy l'Etoile, France) and serotyped by slide agglutination using specific *H. influenzae* antisera (Difco™, Becton Dickinson). Bacterial susceptibility testing was performed by disk diffusion method following the recommendations of the Clinical Laboratory Standards Institute [5]. Beta-lactamase production was detected using a Cefinase™ disk (BBL, Becton Dickinson).

STATISTICS

Statistical analysis was performed using SPSS version 12. Categorical parameters were compared by Fisher's exact test. Continuous variables were compared by *t*-test.

RESULTS

During the study period there were 104 cases of *H. influenzae* meningitis and bacteremia in 57 children and 47 adults. Nine cases of bacteremia were nosocomial. Patients ranged in age from 1 day to 93 years. Forty-five patients (43%) were females. Nineteen patients were admitted to the intensive care unit, 18 needed mechanical ventilation and 16 (15%) died. The most common discharge diagnosis were pneumonia (n=37, 36%), followed by bacteremia without focus (n=26, 25%) and meningitis (n=11, 11%). Seven percent of infections had an otogenic focus. Discharge diagnoses are shown in Table 1. Ninety-three (89%) of the patients were treated with beta-lactam antibiotics, 3 patients with quinolones, and 1 patient with aminoglycosides. Three patients who arrived while being resuscitated were not able to receive antibiotics, and in four patients the data regarding antibiotic treatment were not available.

TYPE B VS. NON TYPE B DISEASE

There were 21 cases of Hib disease (20%), of which 73 (70%) were non-type b, and in 11 cases serotyping was not available. Hib disease was more common in children, representing 25% of the cases compared to only 15% of adult cases ($P = 0.2$). There was no difference in the age of patients with type b disease as compared to those with non-type b disease, in both children and adults. The main diagnoses associated with Hib infection were pneumonia (n=8, 38%), buccal cellulitis (n=5, 24%) and meningitis (n=4, 19%), while non-type b infection was more commonly associated with pneumonia (n=40, 55%) and bacteremia without source (n=23, 32%). Death occurred in 8 of the 73 patients (11%) in whom non-serotype b disease was identified, and in 3 of the 21 patients (14%) with serotype b disease ($P = 0.64$). More patients with Hib infection required management in the ICU (13% vs. 30%, $P = 0.07$), but there was no difference in hospitalization length (7 vs. 8 days, P = NS). Ampicillin resistance was identified in 60% of serotype b isolates vs. 29% of non-serotype b ($P = 0.01$). There were only three *H. influenzae* strains (two type b and one non-typable) resistant to amoxicillin-clavulonate.

ICU = intensive care unit

Table 1. *H. influenzae* discharge diagnoses in adults and children

	Children n (%)	Adults n (%)	Total n (%)
Gastrointestinal	3 (5)	0	3 (3)
No focus	15 (26)	11 (24)	26 (25)
Bronchiolitis	3 (5)	0	3 (3)
Cellulitis	6 (11)	0	6 (6)
Cholangitis	0	2 (4)	2 (2)
Gynecological	0	8 (17)	8 (8)
Meningitis	9 (16)	2 (4)	11 (11)
Pneumonia	16 (28)	21 (45)	37 (36)
Otitis media	4 (7)	0	4 (4)
Other	1 (2)	3 (6)	4, (4)
Total	57 (100)	47 (100)	104 (100)

Table 2. Demographic, clinical and laboratory characteristics of children vs. adults with *H. influenzae* infection

	Children	Adults	*P*
Males, n (%)	35 (61)	24 (50)	0.29
Age (yrs, mean ± SD)	1.72 ± 2.6	66 ± 21	NA
Hospitalization length (days) *	6 ± 5.8	12 ± 13.2	0.005
Underlying conditions, n (%)	15 (32)	37 (79)	< 0.001
Diagnosis, n (%)			
Pneumonia	16 (28)	21 (45)	0.08
Bacteremia	16 (28)	11 (23)	0.59
Meningitis	8 (17)	2 (3.5)	0.09
Gynecological	0	8 (17)	NA
Cellulitis	6, (10.5)	0	0.005
Fever (max) (ºC)	39.05 (1.1)	38.25 (1.06)	0.001
WBC (x 10^3/µl, mean ± SD)	17.74 (9.3)	15.65 (14.1)	0.38
PMNs (%, mean ± SD)	63.21 (20.2)	81.25 (19.7)	< 0.001
HgB (g/dl, mean ± SD)	11.46 (1.82)	12.05 (2.07)	0.14
PLT (x 10^3/µl, mean ± SD)	406 (180)	198 (180)	< 0.001
Type-B n (%)	13 (23)	7 (15)	0.31
Mortality, n (%)	3 (5)	13 (28)	0.001

* Preterm babies were excluded (three patients) because their hospitalization length was not determined by the infection
NA = not applicable, WBC = white blood count, PMN = polymorphonuclear cells, HgB = hemoglobin, PLT = platelets

Figure 1. Age distribution of patients with *H. influenzae* infections by serotype

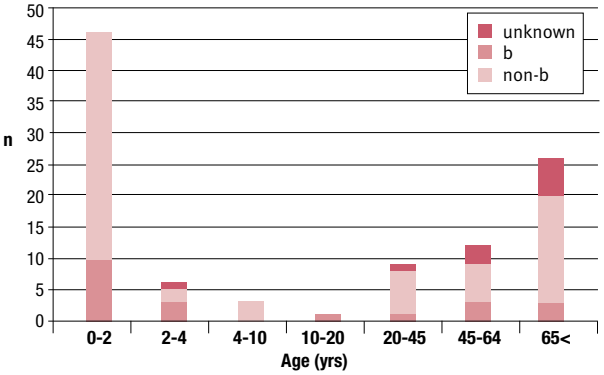
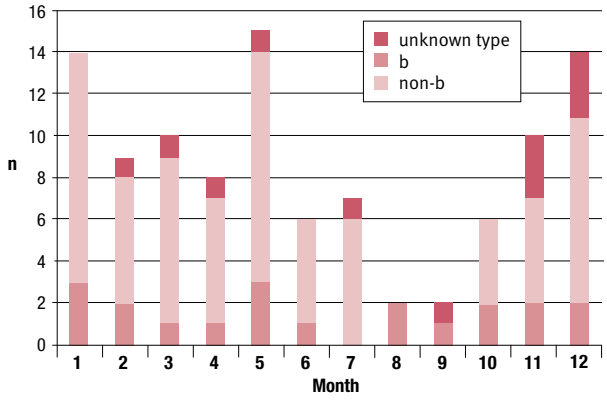


Figure 2. *H. influenzae* infection by month



CHILDREN VS. ADULTS [TABLE 2]

The mean age of the patients was 1.7 years for children and 66 for adults. Age distribution is shown in Figure 1. Most cases occurred among children younger than one year and adults over 65 years old. Adults had longer hospitalizations than children (mean 12 days vs. 6 days, $P = 0.005$) and a higher mortality rate (28% vs. 5%, $P = 0.001$). Thirty-seven (79%) of the adults and 18 (32%) of the children had underlying conditions. Prematurity (n=6) and trisomy 21 (n=3) were the most common underlying conditions in children, whereas ischemic heart disease (n=10), diabetes mellitus (n=10) and malignancy (n=9) were the most common underlying conditions in adults. Pneumonia was more common in adults (45% vs. 28%, $P = 0.08$), whereas meningitis was more common in children (17% vs. 3.5%). Facial cellulitis occurred in six children but in none of the adults.

GYNECOLOGICAL INFECTIONS

Eight women had gynecological infections: septic abortion in six and chorioamnionitis in two. One case of chorioamnionitis resulted in preterm labor at 33 weeks. One woman with septic abortion required intensive care due to septic shock. Only one of the cases of gynecological infections was due to serotype b. There were no cases of mortality.

NEONATAL DISEASE

During the study period there were five cases of neonatal infection (occurring during the first 30 days of life): three occurred on the first day of life, one on the fifth day of life and one at 18 days. Three of the neonates were born prematurely (< 37 weeks). All four early-onset cases (occurring in the first week of life) presented as sepsis and required intensive care, while the only case of late-onset infection presented as mild upper respiratory tract infection. All neonates had non-type b disease and none of them died.

VACCINE FAILURES

Five cases of Hib bacteremia occurred, all in fully immunized children who received four doses of the conjugated vaccine and thus were considered vaccine failures. The mean age of children with vaccine failure was 5.1 years (range 1.9–15.5 years). Three of these children had underlying conditions: trisomy 21, ectodermal dysplasia and Leigh disease; the first two are known to be associated with humoral immunodeficiency [6,7]. The rest of the HIB infections occurred in unvaccinated children whose parents ideologically objected to vaccinations, or in adults with underlying diseases.

SEASONALITY

The number of infections ranged from 4 to 12 cases per year, with no specific pattern over the years (data not shown). This high rate of Haemophilus invasive infections may be partially explained by relatively low HIB vaccine coverage in some subpopulations in Jerusalem. Low socioeconomic status and crowded housing conditions might also contribute to an increase in carrier state of all serotypes of Haemophilus, leading to invasive infections under certain conditions. We found a seasonal pattern, with infections being more common during the winter and spring [Figure 2]. The rate of Hib infection was stable throughout the year. Non-b strains showed a seasonal pattern, with no cases during the summer and the highest number of cases occurring in January and May.

DISCUSSION

We report the results of 13 years surveillance of *H. influenzae* meningitis and bacteremia in the era of the conjugated Hib vaccine. Routine surveillance of *H. influenzae* type b infections is conducted in Israel, but the epidemiology of non-b serotypes has not been well studied. A few studies have suggested that the implementation of Hib vaccination has resulted in the emergence of non-b invasive infec-

tions [8,9]. However, our study did not demonstrate a rise in non-b serotype invasive disease throughout the years. Non-HIB bacteremia comprised 0.03–0.07% of total blood cultures taken, with no rise throughout the years (data not shown), and thus our study does not support the serotype replacement theory. Comparison of type b vs. non-b disease revealed some clinical and microbiological differences. Underlying associated medical conditions were more common in adults and in children with non-type b disease, which is consistent with previous reports [10,11].

Comparison of infections in children and adults showed that adult patients had longer hospitalizations and worse outcome, which is in agreement with previous studies of *H. influenzae* infections [12] as well as other invasive infections [13]. In accordance with previous studies, we found that non-Hib infection was relatively more common in adults [12] and that the highest incidence was in young infants and adults over 65 years [14].

Gynecologic and neonatal infections caused by *H. influenzae* are uncommon, probably because it is an infrequent colonizer of the genital tract (less than 1%) [15,16]. Gynecologic infections are sometimes associated with considerable morbidity and even mortality in both the mother and the newborn [17]. Non-typable *H. influenzae* is the main serotype that has been associated with obstetric and gynecologic infections and has been reported to cause early-onset neonatal disease with a high rate of mortality [18]. We found five cases of neonatal infection. The number of neonatal cases was too small to draw conclusions regarding mortality rate, which was lower than previously described [13]. The difference between early- and late-onset infection might be explained by horizontal infection by family members in the latter cases vs. acquisition of the bacteria from the maternal genital tract in early-onset disease. The neonatal rate of infection was stable in our hospital throughout the years, in contrast to a few reports suggesting an increase in the incidence of neonatal infection [19].

H. influenzae infections were more common during the winter and spring, especially for non-b strains. We assume that the rate of invasive infections is in direct relationship to nasopharyngeal carriage of the bacteria, but data regarding *H. influenzae* carriage are limited. A few studies found high carriage rates in the winter [20,21], but one study did not find a difference between spring and autumn carriage in children [22]. There seems to be a similar pattern of pneumococcal invasive disease in our institute (unpublished data). Studies to assess the nasopharyngeal carriage are required to explain the seasonality of invasive disease caused by these organisms. Intercurrent viral infections could also act as a trigger for Haemophilus invasive infections, possibly by airway modifications that enhance bacterial adherence and invasion.

We encountered five cases of Hib vaccine failure, three of them occurring in children with underlying conditions. A study of Hib vaccine failure in Israel estimated the risk to be 0.15 per 100,000 child years at risk [23]. Hib vaccine failure is rare, but it can occur with any immunization schedule.

The present study had some limitations. First, patients were identified only by a positive blood or CSF culture. Thus, patients with severe *H. influenzae* infections, such as non-bacteremic pneumonia, were not included. It is therefore possible that the results of this study do not reflect the entire spectrum of invasive *H. influenzae* infection. Second, because of the retrospective nature of the study, some data were not available for all study patients, thus limiting the power of the analysis for those variables.

CONCLUSIONS

Invasive *H. influenzae* disease is uncommon in both children and adults, but a few cases are seen every year. Invasive disease remains an important concern even in the vaccine era. Therefore, it should be considered in patients with meningitis, pneumonia or sepsis, especially in unvaccinated children and patients with underlying conditions. Replacement of Hib invasive disease by non-B Haemophilus strains was not found in this study. Even in the global vaccination era, serotype b constitutes a significant portion of invasive *H. influenzae* disease, emphasizing the importance of continuous surveillance.

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Capsule

IFITM3 restricts the morbidity and mortality associated with influenza

The 2009 H1N1 influenza pandemic showed the speed with which a novel respiratory virus can spread and the ability of a generally mild infection to induce severe morbidity and mortality in a subset of the population. Recent in vitro studies show that the interferon-inducible transmembrane (IFITM) protein family members potently restrict the replication of multiple pathogenic viruses. Both the magnitude and breadth of the IFITM proteins' in vitro effects suggest that they are critical for intrinsic resistance to such viruses, including influenza viruses. Using a knockout mouse model, Everitt and colleagues tested this hypothesis directly and found that IFITM3 is essential for defending the host against influenza A virus in vivo. Mice lacking *Ifitm3* display fulminant viral pneumonia when challenged with a normally low-pathogenicity influenza virus, mirroring the destruction inflicted by the highly pathogenic 1918 "Spanish" influenza. Similar increased viral replication is seen in vitro, with protection rescued by the re-introduction of *Ifitm3*. To test the role of IFITM3 in human influenza virus infection, the authors assessed the *IFITM3* alleles of individuals hospitalized with seasonal or pandemic influenza H1N1/09 viruses. They found that a statistically significant number of hospitalized subjects show enrichment for a minor *IFITM3* allele (SNP rs12252-C) that alters a splice acceptor site, and functional assays show the minor CC genotype IFITM3 has reduced influenza virus restriction in vitro. Together these data reveal that the action of a single intrinsic immune effector, IFITM3, profoundly alters the course of influenza virus infection in mice and humans.

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Eitan Israeli

I am only one
But still I am one
I cannot do everything
But still I can do something
And because I cannot do everything
I will not refuse to do the something that I can do

Edward Everett Hale (1822-1909), American author, historian and clergyman

May my silences become more accurate

Theodore Roethke (1908-1963), American poet and laureate of the Pulitzer Prize for Poetry