Evolving epidemiology of invasive *Haemophilus* infections in the post-vaccination era: results from a long-term population-based study

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Abstract

Historically, *Haemophilus influenzae* (Hi) serotype b (Hib) caused most invasive *Haemophilus* infections worldwide, mainly in children. In 1989 routine childhood vaccination against Hib was initiated in Iceland. We conducted a population-based study of all patients in the country with *Haemophilus* spp. isolated from sterile sites (n = 202), from 1983 to 2008. Epidemiology, clinical characteristics of the infections and serotypes of the isolates were compared during the pre-vaccination (1983–1989) and post-vaccination era (1990–2008). Following the vaccination, the overall incidence of Hib decreased from 6.4 to 0.3/100 000 per year ($p < 0.05$) whereas the incidence did not change significantly for infections caused by Haemophilus sensu lato not serotype b, hereafter referred to as non-type b Hi (0.9 vs 1.2, respectively). The most frequent diagnosis prior to 1990 was meningitis caused by Hib, which was subsequently replaced by pneumonia and bacteraemia caused by non-type b Hi. Most commonly, non-type b Hi were non-typeable (NTHi; 40/59), followed by Hi serotype f (14/59) and Hi serotype a (3/59). Pregnancy was associated with a markedly increased susceptibility to invasive *Haemophilus* infections (RR 25.7; 95% CI 8.0–95.9, $p < 0.0001$) compared with non-pregnant women. The case fatality rate for Hib was 2.4% but 14% for non-type b Hi, highest at the extremes of age. Hib vaccination gives young children excellent protection and decreases incidence in the elderly due to herd effect in the community. Replacement with other species or serotypes has not been noted. Pregnant women are an overlooked risk group.

Keywords: *Haemophilus influenzae*, Hib, mortality, NTHi, pregnancy, vaccination
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Introduction

Haemophilus influenzae (Hi) are classified according to their capsular polysaccharides into six serotypes (a–f) and non-capsular strains [1]. Encapsulated strains, especially Hi type b (Hib), are aggressive pathogens that primarily cause infections in children <5 years of age because of their lack of T-cell independent response to polysaccharides [2]. This capsular type caused more than 95% of invasive *H. influenzae* infections in Scandinavian children before routine vaccination was initiated against Hib [3]. On the other hand, Hi carrying other capsular types and non-capsular strains (Haemophilus sensu lato not serotype b, hereafter referred to as non-type b Hi) commonly cause invasive infections in persons with comorbidities [4,5]. In May 1989, routine vaccination against Hib was started in Iceland with the vaccine PRP-D (ProHIBiT®), followed by a dramatic decrease in Hib disease [6].

Despite the early success of vaccination against Hib, the concern remains that during longer follow-up, non-type b Hi with other capsular types or non-typeable Hi (NTHi) strains may replace Hib. Recent studies have been inconclusive regarding this matter [5,7,8]. Furthermore, with reduced pharyngeal carriage a natural booster to antibody production may be lost, resulting in waning immunity [9]. The purpose of this study was to analyse trends in invasive disease caused by *H. influenzae* in Iceland for 26 years, from 1983 to 2008, at the 20-year anniversary of nationwide childhood vaccination against Hib in Iceland.

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Materials and Methods

Setting and clinical data

In May 1989, routine vaccination against Hib was started in Iceland with the vaccine PRP-D (ProHIBiT®). The vaccine was administered at the age of 3, 4, 6 and 14 months, and during the first year of immunisation children at the age of 1–3 years were offered one dose [6]. From 2000 to 2005 Pentavac® was used, followed by Infanrix-Polio+Hib® at the age of 3, 5 and 12 months (http://www.landlaeknir.is). This nationwide long-term comparative study of invasive infections caused by *Haemophilus* spp., included 6 years in the pre-vaccine era (1983–1989) and 20 years following implementation of Hib vaccination in 1989 (1990–2008). All patients with *Haemophilus* spp. isolated from cerebrospinal fluid (CSF), blood or joint fluid from 1 January 1983 to 31 December 2008 were identified retrospectively by a nationwide search in microbiology databases. For the sake of completeness, all serogroups of *H. influenzae* were included, in addition to NTHi as well as infections caused by *H. parainfluenzae*. Information regarding age, sex, date of diagnosis, source of isolate (blood, CSF or joint fluid) and vital status 30 days from diagnosis was available for all patients. Permission to access more detailed clinical information concerning the 202 cases was received from all hospitals and health clinics in Iceland. Of these, 191 charts were retrieved, thus giving a yield of 95%. Clinical information was reviewed and registered, including clinical presentation, signs and symptoms, past medical history, laboratory results, antibiotic therapy and outcomes. The National Bioethics Committee of Iceland and the Data Protection Authority of Iceland approved the study.

Microbiology

Information was collected regarding blood culture results and cultures of CSF, serotypes of the isolates and beta-lactamase production. Isolates were serotyped using Difco Haemophilus influenza antiserum (Becton Dickinson, Sparks, MD, USA). Beta-lactamase production was tested for with the clover leaf test until the year 2000 and thereafter with the Nitrocephin test (Oxoid, Cambridge, UK).

Statistics

For calculations of age-specific incidence, population data were obtained from Statistics Iceland (http://www.statice.is). Data from this source were also used to calculate the average total fertility rate of Icelandic women, as well as mean live births and late foetal deaths per year in 1983–2008. The total fertility rate was used to calculate the annual number of pregnancy-years, assuming 9 months of gestation for the cohort. Data on induced abortions in Iceland during 1982–2009 and their timing during gestation were obtained from the Directorate of Health (http://www.landlaeknir.is/Heilbrigdistolfraedi/Fostureydingar) and used to calculate the number of additional pregnancy-years that were not associated with live births or late foetal deaths. The number of unregistered, spontaneous abortions was assumed to be 16% of live births and follow the same timeline [10]. These numbers were combined to calculate the average annual number of pregnancy-years of the nationwide cohort. Incidence of invasive *Haemophilus* disease in pregnant women was then calculated as the ratio between the number of annual pregnancy-associated infections and pregnancy-years and expressed as the number of infections for every 100 000 pregnancy-years. The incidence rates in pregnant and non-pregnant women of childbearing age were compared using 'Comparing time person-time rates', which gave rate ratios and 95% confidence intervals (95% CI). Statistical analysis was performed by using OpenEpi (http://www.openepi.com). Proportions were compared by using χ^2 and Fisher exact tests. A p value of <0.05 was considered significant. All comparisons were two-tailed.

Results

During the 26 years of study, 202 cases of invasive *Haemophilus* infections were diagnosed in Iceland. During 1983–1989, before routine childhood vaccination against Hib, 123 infections were identified, 104 (85%) of them in children <16 years of age. In the post-vaccination era 1990–2008, 79 cases were diagnosed, 25 (32%) of them children. The annual numbers of Hib and non-type b Hi infections in Iceland, 1983–2008, are shown in Fig. 1. Age-specific incidence rates

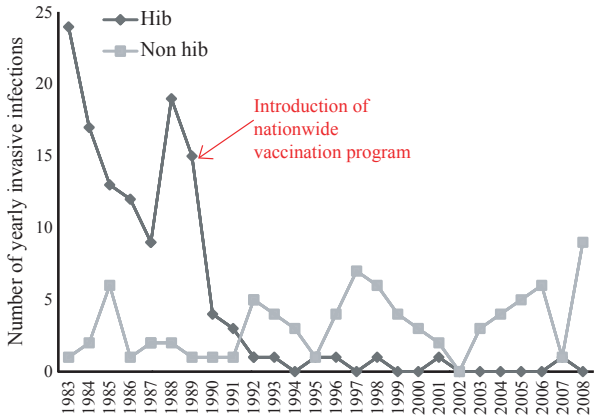

FIG. 1 Yearly number of invasive Hib and non-type b Hi infections in Iceland.

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TABLE 1. Age-specific incidence for infections caused by *Haemophilus influenzae* type b (Hib) and other (non b Hi)

	1983–1989				1990–2008			
	Hib		Non-b Hi		Hib		Non-b Hi	
Age	*n*	Incidence	*n*	Incidence	*n*	Incidence	*n*	Incidence
<1	32	109.0	2	6.8	3	3.8	7	8.9
1–5	6	41.1	1	0.7	3	0.7	9	2.2
6–20	5	1.1	2	0.5	0	0	4	0.2
21–40	1	0.2	1	0.2	5	0.3	13	0.8
41–60	4	1.3	2	0.6	1	0.1	8	0.7
>60	5	2.2	6	2.7	2	0.3	23	2.7

All incidence values represent number of cases/100 000 population/year.

for invasive Hib and non-type b Hi disease, before and after Hib vaccination, are presented in Table 1. The yearly incidence of Hib dropped by 96% (95% CI, 92.8–97.6), from 6.4/100 000/year before 1990, to 0.3/100 000/year following adoption of routine childhood immunisation ($p < 0.0001$). The incidence of non-type b Hi during 1983–1989 had a yearly incidence of 0.9/100 000/year and increased to 1.2/100 000/year post-Hib vaccination, but this was not a significant increase (p 0.16). The reduction in incidence of invasive Hib infections was most pronounced among children aged <1 and 1–5 years ($p < 0.05$).

About one in five of all Hib isolates tested for beta-lactamase production were positive (24 of 115 isolates tested, 21%), whereas 16% of non-type b Hi isolates tested were positive. The association between clinical diagnosis and species or serotypes, as well as timing of the infection, is summarised in Table 2. As shown, NTHi were most commonly isolated from patients with pneumonia or bacteraemia in the post-vaccine era, followed by *H. influenzae* type f (Hif), which primarily caused bacteraemic pneumonia. Only seven cases (3.5%) of all invasive disease were caused by *H. parainfluenzae* and the epidemiology of this species remained stable. Meningitis was the most frequent diagnosis (63/202, 31%) in the cohort, most commonly caused by Hib in children in the pre-vaccination period. Almost one-quarter (13/57, 23%) of the children with meningitis suffered from long-term sequelae, most commonly hearing loss. In the post-vaccination era bacteraemic pneumonia caused by non-type b Hi became the most frequent clinical presentation of invasive disease.

Predisposing conditions of children and adults with Hib and non-type b Hi are summarized in Table 3. As shown, severe underlying disease was more frequent among both children and adults with non-type b Hi infections compared with those with Hib. Antecedent viral infection diagnosed clinically was commonly reported in children with Hib, whereas children with non-type b Hi were more likely to suffer from chronic conditions.

Among 18–40-year-old women, 13 were diagnosed with *Haemophilus* disease, nine of whom were pregnant, and five of them lost their foetus (median gestational age 12 weeks compared with 40 weeks for those who gave birth). According to nationwide data on fertility, late foetal deaths and induced abortions, combined with previously published data on frequency of spontaneous abortions [10], the incidence of invasive *Haemophilus* disease in pregnant women was 9.2 infections/100 000/year (nine infections during 98 014 person-years), whereas non-pregnant women in the same age group had an incidence of 0.4/100 000/year (four infections during 1 118 167 person-years). The rate ratio between the two groups is 25.7 (95% CI, 8.0–95.9; $p < 0.0001$). Non-type b Hi caused 6/9 of the infections in pregnancy and 8/11 of the neonatal infections, where the majority was caused by NTHi (5/6 and 4/8, respectively). The infection was fatal in two neonates (NTHi and non-type b Hi) while all the pregnant women survived.

Of the 14 fatal cases, three were caused by Hib; one severely disabled child had not received vaccination, in the post-vaccine era, and two adults in the pre-vaccine era

TABLE 2. Clinical diagnosis according to species, serotype and time interval

	Serotype/species										1983–1989		1990–2008	
Diagnosis	a	b	c	d	e	f	NTHi	*Parainfluenzae*	Non b	Uncertain	Hib	Non-Hib	Hib	Non-Hib
Pneumonia	1	12	1			6	16			4	9	4	3	24
Meningitis		58				3				1	55	2	3	2
Bacteremia	2	16			1	2	16	3			14	5	2	19
Epiglottitis		4				1					4			1
Cellulitis		10				2	1	1		1	10	1		4
OM/SA		10					1	1			9		1	2
Other		5		1			6	2	3		1	2	4	10
NA		8	1							2	7		1	3
All cases	3	123	2	1	1	14	40	7	3	8	109	14	14	65

NR, diagnosis not available.
For diagnosis according to serotype all cases were included. OM/SA, osteomyelitis/septic arthritis.

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TABLE 3. Predisposing conditions in children and adults with Hib infections compared with non b Hi (*n* = 191)

	Children				Adults			
Predisposing condition	**Hib, *n* = 101 (%)**	**Non b Hi, *n* = 24 (%)**	**OR (95% CI)**	**p value**	**Hib, *n* = 14 (%)**	**Non b Hi, *n* = 52 (%)**	**OR (95% CI)**	**p value**
Smoking					6 (43)	19 (37)	1.3 (0.4–4.4)	0.7
Alcoholism					1 (7)	4 (8)	1.1 (0.1–29)	0.9
Viral symptoms	43 (43)	6 (25)	2.2 (0.8–6.5)	0.12		4 (8)		
Pregnancy					3 (21)	6 (12)	2.1 (0.4–9.7)	0.4
Otitis media	36 (36)	2 (8)	6.0 (1.5–39.9)	0.01	1 (7)	1 (2)	3.8 (0.1–155)	0.4
CV disease	2 (2)	2 (8)	4.4 (0.4–44.4)	0.2	2 (14)	22 (42)	4.3 (1.0–31)	0.06
Lung disease	3 (3)	3 (13)	4.5 (0.7–28.4)	0.09	3 (21)	12 (23)	1.1 (0.3–5.6)	0.9
Cancer	1 (1)	2 (13)	8.9 (0.6–270)	0.1	2 (14)	14 (27)	2.2 (0.5–15)	0.4

OR, odds ratio; CV disease, cardiovascular disease.
If patients had more than one predisposing condition they were all included.

died from septicaemia and pneumonia. Thus, the case fatality rate for Hib was 2.4%. The case fatality rate for non-type b Hi was 14%. The highest fatality rates were observed at the extremes of age, <1 year (2/10, 20%), 41–60 years (2/9, 22%) and >60 years (7/33, 21%). Of the 11 non-type b Hi strains associated with fatal infections, two were encapsulated, serogroups c and f, six were NTHi and three were only classified as non-type b Hi. The most common causes of death were pneumonia (7/11) and bactaeremia (3/11).

Discussion

This study confirms a remarkable and sustained success of Hib conjugate vaccination in Iceland. During the two decades of follow-up where all species of invasive *Haemophilus* spp. were included, an increase in Hib infections was not noted. This contrasts with the early experience from the United Kingdom [11,12]. In Iceland a booster dose was used from the beginning, which may explain this difference [6]. During the last 5 years of our study, Hib was all but eliminated, occurring in only one of 24 (4%) invasive infections. Similar to the results of others, the reduction in incidence of Hib was most pronounced among young children, and from 1990 onwards non-type b Hi infections became more than twice as common as Hib in young children [9,12]. Interestingly, the incidence of invasive Hib infections also decreased markedly in the elderly after childhood vaccination was initiated. This reduction is most likely caused by the herd effect, where the unvaccinated elderly benefit from the reduction in nasopharyngeal carriage of Hib in vaccinated children, leading to reduced exposure and subsequent infections [13,14]. Most studies have concentrated on the age-group 0–4 years although Perdue *et al.* and Peltola *et al.* [3,15] also demonstrated a reduction in incidence of invasive disease among adults in Alaska and Scandinavia.

The age group 21–40 is of particular interest, childbearing women in particular. It has been proposed that the immunosuppressive effects of pregnancy increase the susceptibility of the host to *H. influenzae* infections [16]. We found a significant increase in incidence among pregnant women compared with other women of the same age. A prospective study found that child-bearing women in the age group 18–39 years had a six-fold increased risk of *H. influenzae* bacteraemia compared with adults of the same age [17]. Our results show that the relative risk associated with pregnancy may be even higher. Carriage studies have shown that only 1.8/1000 parturient women carry non-encapsulated Hi in their genital tract [18], but they were found in 8/110 women with preterm rupture of membranes [19]. It has been suggested that unusually virulent NTHi biotype II or IV strains may preferentially cause invasive infections among neonates and pregnant women [19–21]. Further characterization of the strains in our study may therefore be of interest. The incidence of non-type b Hi infections among infants younger than 1 year from 1990 to 2008 was 8.9/100 000/year, which is similar to the results of Ladhani *et al.* [9].

Currently, NTHi are the most common types of Hi causing invasive disease in Iceland, similar to results of large-scale studies from Australia, Canada and Denmark [22]. Hif are currently the most common encapsulated invasive *H. influenzae* isolates in Iceland. Hif infections are similar to NTHi in that 10/14 (72%) cases had severe predisposing conditions and the most common clinical diagnosis was pneumonia. Other studies have reported a high prevalence (60–80%) of predisposing conditions among patients with Hif infections [12,23] and they are most frequent in young individuals and the elderly [4,23]. Resman *et al.* [24] recently reported a significant increase in serious invasive infections ascribed to Hif, mainly in individuals >60 years of age in Sweden. This resonates well with our study, as 9/14 (64%) Hif patients were >60 years of age. A recent study from Utah shows the emergence of *H. influenzae* type a as the most common in

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children, leading to a disease similar to Hib [25]. A similar increase was not noted in our study.

Viral infections of the upper airway make patients more prone to colonization and infection with both Hib and non-type b Hi [26,27]. Interestingly, a higher proportion of patients with Hib reported a history of preceding viral infection, which may be explained in part by the different age composition of the two groups. However, a similar difference was also noted with respect to otitis media, which was the second most frequent predisposing condition among children with Hib infections, 36/101 (36%), while children with NTHi infections had a history of otitis media in only 2/24 (8%) cases (p 0.01). This is somewhat surprising, because NTHi frequently cause otitis media in children [7], but this may be indirect evidence of the relative lack of invasive potential of these strains. Similarly, children who were infected with non-type b Hi were more likely to have severe predisposing conditions. This is in agreement with other studies and reinforces that non-type b Hi are opportunistic pathogens with less virulence compared with Hib [4,7,12]. The most common predisposing condition among adults with Hib infections was smoking, which has been linked to increased susceptibility to infections [28]. As other authors have concluded, adults with non-type b Hi infections frequently had serious predisposing conditions, most commonly coronary artery disease, lung disease and malignant disease [8,12,29].

The age-related fatality rate for patients with Hib was 2.4% and for non-type b Hi infections it was 14%, being highest at the extremes of age, which is in accordance with data from Scandinavia, the United Kingdom and the USA [5,11,30]. With the availability of a conjugated vaccine for NTHi, it may therefore be prudent to study the effectiveness of this vaccine in select groups of adult patients with predisposing conditions, including young women. The strength of our study is its population-based design, giving a clear picture of the epidemiology of invasive *Haemophilus* disease before and after vaccination for Hib. Despite the long time-frame, clinical information was still available for 95% of the cases. The main limitation of our study is the limited power in statistical analyses of small subgroups.

In conclusion, we present a population-based study of invasive *Haemophilus* disease in Iceland, spanning 26 years. Following vaccination, which started in 1989, invasive infections caused by Hib were reduced by 96%, in part by the herd effect. The concern that other non-type b Hi species might replace Hib has not materialized after 20 years of follow-up. Pregnancy is an important risk factor, which may be amenable to prevention, either by selective screening and antibiotic therapy or by vaccination. The high case fatality rate of non-type b Hi infections in the elderly with underlying conditions also raises questions regarding the potential efficacy of vaccination for NTHi in this risk group.

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Transparency Declaration

The authors have no conflict of interests to disclose.

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