Invasive disease caused by *Haemophilus influenzae* in Sweden 1997–2009; evidence of increasing incidence and clinical burden of non-type b strains

F. Resman[1,2], M. Ristovski[1], J. Ahl[1,2], A. Forsgren[1], J. R. Gilsdorf[3], A. Jasir[4], B. Kaijser[5], G. Kronvall[6] and K. Riesbeck[1]

1) *Medical Microbiology, Department of Laboratory Medicine,* 2) *Infectious Diseases, Department of Clinical Sciences, Lund University, Skåne University Hospital, Malmö, Sweden,* 3) *Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, MI, USA,* 4) *Department of Clinical Microbiology and Immunology, Lund University, Skåne University Hospital, Lund,* 5) *Department of Clinical Bacteriology, Gothenburg University, Gothenburg and* 6) *Clinical Microbiology—MTC, Karolinska Institute, Karolinska Hospital, Stockholm, Sweden*

Abstract

Introduction of a conjugated vaccine against encapsulated *Haemophilus influenzae* type b (Hib) has led to a dramatic reduction of invasive Hib disease. However, an increasing incidence of invasive disease by *H. influenzae* non-type b has recently been reported. Non-type b strains have been suggested to be opportunists in an invasive context, but information on clinical consequences and related medical conditions is scarce. In this retrospective study, all *H. influenzae* isolates (n = 410) from blood and cerebrospinal fluid in three metropolitan Swedish regions between 1997 and 2009 from a population of approximately 3 million individuals were identified. All available isolates were serotyped by PCR (n = 250). We observed a statistically significant increase in the incidence of invasive *H. influenzae* disease, ascribed to non-typeable *H. influenzae* (NTHi) and encapsulated strains type f (Hif) in mainly individuals >60 years of age. The medical reports from a subset of 136 cases of invasive *Haemophilus* disease revealed that 48% of invasive NTHi cases and 59% of invasive Hif cases, respectively, met the criteria of severe sepsis or septic shock according to the ACCP/SCCM classification of sepsis grading. One-fifth of invasive NTHi cases and more than one-third of invasive Hif cases were admitted to intensive care units. Only 37% of patients with invasive non-type b disease had evidence of immunocompromise, of which conditions related to impaired humoral immunity was the most common. The clinical burden of invasive non-type b *H. influenzae* disease, measured as days of hospitalization/100 000 individuals at risk and year, increased significantly throughout the study period.

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Corresponding author: K. Riesbeck, Medical Microbiology, Department of Laboratory Medicine Malmö, Lund University, Skåne University Hospital, SE-205 02 Malmö, Sweden
E-mail: kristian.riesbeck@med.lu.se

Introduction

Haemophilus influenzae is a frequent colonizer of the human respiratory tract. The species is subdivided into non-encapsulated and encapsulated strains that are typed based upon the presence of one of six antigenically distinct polysaccharide capsules designated a to f [1]. Strains that do not have a capsule are denoted non-typeable *H. influenzae* (NTHi). The carriage rates of NTHi in healthy pre-school children are as high as 65% [2], suggesting that NTHi is usually a commensal. However, NTHi frequently causes otitis media and pneumonia in children [3], and is the most common bacterial finding in exacerbations of chronic obstructive pulmonary disease [4]. Invasive NTHi cases are suggested to be opportunistic infections [3], even though information on correlated medical conditions is scarce.

Invasive disease by *H. influenzae* has historically been analogous with disease by encapsulated strain type b (Hib), a feared cause of sepsis, epiglottitis and meningitis in children and occasionally in adults [5]. In the early 1990s, the conjugated Hib vaccine was introduced in most countries in the Western World, and a dramatically reduced incidence of invasive Hib disease occurred [6]. The incidence of

bacteraemia caused by Hib in Sweden in the late 1980s was approximately 30/100 000 children, and these numbers had decreased 10-fold by 1994 [7]. The current Swedish Hib vaccine coverage rate is 99%.

Several reports on the epidemiology of invasive non-type b *Haemophilus* disease in the post-Hib-vaccine era have been published in recent years [8–13]. Indications of a rising incidence of invasive non-type b disease have been observed in North America [8,9,13,14]. The status in Europe is less clear, but a recent study encompassing surveillance data from 14 European countries between 1996 and 2006 showed a small but significant increase in incidence of invasive NTHi disease [15]. In contrast, a German study covering 1998–2005 did not reveal any increase in invasive non-type b disease [16]. Even though invasive Hib disease has been successfully repressed following the widespread introduction of the Hib vaccine, invasive disease by *H. influenzae* non-type b remains a clinical challenge.

Most epidemiological reports on invasive non-type b *H. influenzae* disease lack information on disease severity. Furthermore, although suggested as an opportunistic disease, there is little information on underlying conditions associated with invasive non-type b cases. In the present retrospective study we show that invasive disease caused by non-type b strains of *H. influenzae* has increased in incidence in Sweden in the period 1997–2009, that it readily affects individuals who are otherwise in good health, and is often clinically severe.

Materials and Methods

Bacterial strains and culture conditions

The *H. influenzae* collection comprised clinical isolates from three densely populated regions in Sweden: Stockholm, Gothenburg and Skåne county (see Supplementary material Fig. S1). All saved isolates from blood and cerebrospinal fluid (CSF) had been stored at −70°C. All available isolates (n = 250) were grown on chocolate blood agar and incubated at 35°C in a humid atmosphere containing 5% CO_2.

DNA preparation and molecular typing

To amplify the capsule transport gene, a *bexA* colony PCR was performed on all available strains (n = 250) [17]. To increase the sensitivity, all strains were also screened for *bexB* using primers 5′-TTGTGCCTGTGCTGGAAGGT TATG-3′ and 5′-GGTGATTAACGCGTTGCTTATGCG-3′. Strains positive for *bexA* and/or *bexB* were further tested using specific primers against types b, a, d and f, c, and e *cap* loci in sequential order. Whenever a strain had previously been typed by PCR, the result was included in the analysis in case the strain was not available (n = 21) for contemporary PCR testing. Results from serotyping by agglutination with antisera were not used because this method is considered inferior in specificity [18]. The caspsule gene was assumed to be expressed in all isolates carrying the *bex* and *cap* loci. The strict commensal *Haemophilus haemolyticus* can be indistinguishable from *H. influenzae* by standard bacteriological techniques so all isolates were tested by a slightly modified version of the PCR described by Murphy *et al.* [19]. Instead of a nested PCR, an initial PCR with primers denoted as 16S3′ and 16SNor [19] was performed. If a product of the correct size was not obtained, isolates (n = 6) were subjected to 16S rRNA sequencing.

Patient data

Basic epidemiological data such as culture date, age and gender were available for all strains. Medical reports from all patients in the county of Skåne (n = 136) were studied, and information on immunocompromise, sepsis severity, duration of stay in hospital, intensive care treatment and mortality was registered. Sepsis severity was defined according to the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) consensus document [20]. As a result of the retrospective nature of the study, not all medical reports contained complete information on all ACCP/SCCM criteria. Only objective and registered parameters were included in the analysis. Meningitis/epiglottitis with sepsis are life-threatening conditions, so these cases were considered severe regardless of other criteria. Immunocompromise was defined according to a definition adapted from the Merck Manual [21]; see Supplementary material Table S1.

Data sorting and estimates of population at risk

All blood and CSF samples taken in the included geographical areas were processed by four central laboratories that kept complete back records of invasive isolates by a database (three laboratories) or by a written list (one laboratory). All recorded *H. influenzae* isolates from blood and CSF were included in the study. Because of variations in storage routines, not all isolates had been saved, and only a few had been previously serotyped by PCR. Therefore, two separate analyses were performed. The primary analysis was based on all recorded strains regardless of serotype, and the secondary analysis was based only on years when at least half of the isolates from each laboratory could be or had been serotyped by PCR (n = 285). The laboratories were Malmö (all years 1997–2009), Lund (2004–2009), Gothenburg Östra Laboratory (1999–2009), Gothenburg Sahlgrenska Laboratory (1997–2000, 2003–2004 and

2007–2009) and Karolinska Laboratory Solna, Stockholm (1998, 2000 and 2004–2008). Any recorded strain from an included year in the secondary analysis that could not be retrieved was registered as 'not analysed (N/A)' (n = 17) and included in the subsequent analysis. Each geographical area had tertiary-care units, where patients referred from outside the area were treated. The addresses of patients (n = 11) with invasive *Haemophilus* disease at tertiary units were checked, and six of these could be confirmed as residents of the included geographical areas. For the remaining five patients, no information was available on living addresses at the time of sepsis. For each year in the study, two estimates of population at risk were defined (Table 1); one estimate for each of the described analyses. Population data were collected from the Swedish central statistics agency (http://www.ssd.scb.se). The estimates of population considered the population by geographical area served by each laboratory unit and year. In Lund and Malmö, the areas of Helsingborg and Kristianstad were added during the course of the study. In Stockholm, the population base was the northern part (with the addition of St Görans hospital in 1997 and 1998) for adults and the greater area of Stockholm for children. In Gothenburg, the inner city was the population base with the addition of North Bohuslän from 2001. The numbers for Stockholm and Gothenburg were double-checked by comparing the numbers of visits to the emergency-care units of the included hospitals with the total emergency-care visits in the greater counties (from which an exact population estimate was known each year).

Statistical analysis

Assuming a linear relation of data, trend tests using linear regression analysis were performed on all data using PAWS statistic 18.0.

Ethical approval

This study was approved by the regional ethical committee for medical research in Lund, Sweden (2009/536).

Results

Increasing incidence of invasive *H. influenzae* disease

Back records revealed 410 cases of invasive disease caused by *H. influenzae* in the defined geographical areas during the years 1997–2009 (Table 1). The incidence varied from 0.5 (in 1998) to 1.7 (in 2007) cases per 100 000 individuals (Fig. 1a). In the primary analysis, which included all recorded isolates regardless of serotype, there was a significantly increased incidence (constant = 0.082, 95% CI 0.040–0.123, p ≤ 0.001). In the secondary analysis, only regions and years where >50% of isolates were typed by PCR (n = 285) were included (Table 1). As shown in Fig. 1(b), NTHi accounted for the majority (n = 191) of cases. The 77 encapsulated isolates were defined as Hib (n = 29), Hif (n = 44), Hie (n = 1), or 'encapsulated non-type b' (n = 3). A statistically significant increase in invasive disease by NTHi (constant = 0.079, 95% CI 0.046–0.111, p ≤0.001) and Hif (constant = 0.023, 95% CI 0.003–0.043, p 0.025) was observed, whereas the incidence

TABLE 1. Total numbers of *Haemophilus influenzae* isolates and population base per study year

Year	All Hi (CSF)[a]	Secondary analysis					Total population base	Population base, secondary analysis[e]
		NTHi[b]	Hib[b]	Hif[b]	Non-b[c]	N/A[d]		
1997	25 (2)	4	2	1	1	1	2 407 000	1 102 000
1998	15 (3)	8	2	2	0	0	2 684 000	2 074 000
1999	16 (0)	3	0	1	0	1	2 433 000	1 222 000
2000	17 (2)	6	0	2	0	2	2 507 000	1 981 000
2001	20 (2)	3	0	0	0	1	2 720 000	801 000
2002	35 (5)	8	1	0	0	1	2 753 000	810 000
2003	31 (3)	6	1	0	0	1	2 774 000	1 352 000
2004	46 (4)	26	10	2	2	6	2 795 000	2 795 000
2005	35 (4)	20	2	3	0	1	2 815 000	2 274 000
2006	32 (4)	16	3	5	1	1	2 844 000	2 299 000
2007	49 (1)	35	4	8	0	2	2 874 000	2 874 000
2008	48 (5)	30	2	16	0	0	2 909 000	2 909 000
2009	41 (3)	26	2	4	0	0	2 935 000	2 164 000
Total	410 (38)	191	29	44	4	17		

[a]All invasive *H. influenzae* isolates during the study period and included in the primary analysis. Cerebrospinal fluid (CSF) isolates among the total number are given within parantheses.
[b]Numbers of isolates typed by PCR: NTHi, non-typeable *H. influenzae*; Hib, *H. influenzae* type b; Hif, *H. influenzae* type f.
[c]Comprising 'encapsulated non-type b' strains that were previously typed by a *capB* PCR using *bexA* as target. These strains had not been stored and were consequently not available for further analysis. The only *H. influenzae* type e isolate (from 2006) is also included here.
[d]N/A; not analysed. These isolates were not available for PCR serotyping, but were included in the secondary analysis as described in Materials and Methods.
[e]Population base including only years when >50% of strains were serotyped by PCR at each laboratory unit. This particular population out of the total population base was used in the secondary analysis.

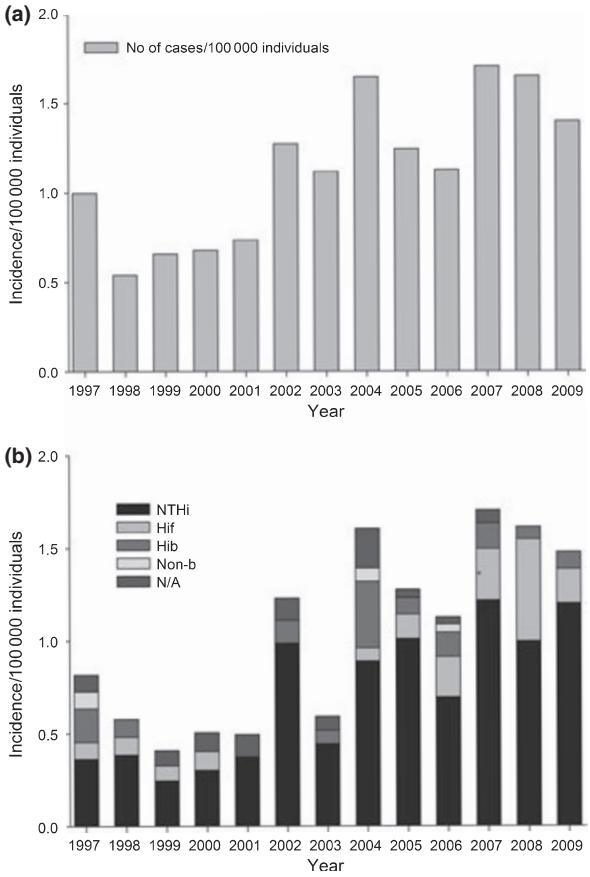

FIG. 1. The incidence of invasive *Haemophilus influenzae* disease increased significantly during the years 1997–2009. (a) Total incidence of invasive *H. influenzae* disease per 100 000 individuals and year from the primary analysis (regardless of serotype) (n = 410). The increase in incidence was statistically significant ($p \leq 0.001$). (b) Results from the secondary analysis, comprising 285 defined cases are shown. The increase was statistically significant (p 0.001 and p 0.025 for non-typeable *H. influenzae* (NTHi) and *H. influenzae* type f (Hif), respectively). Isolates (n = 17) that had not been saved or previously defined by PCR are indicated as not analysed (N/A). Regardless of the hypothetical outcome of the 17 non-analysed isolates, the increase of invasive NTHi disease remained statistically significant.

of Hib disease was unchanged during the study period. No *H. haemolyticus* isolate was identified in the material.

Increased incidence of invasive *H. influenzae* in individuals >60 years of age

The median ages of Hib, Hif and NTHi case patients were 38, 60 and 71 years, respectively. Only 11 of the 410 cases (3%) occurred in neonates (<28 days of age). NTHi was predominant in all age groups, including children <5 years of age. Based on an age-stratified population at risk (Fig. 2), it

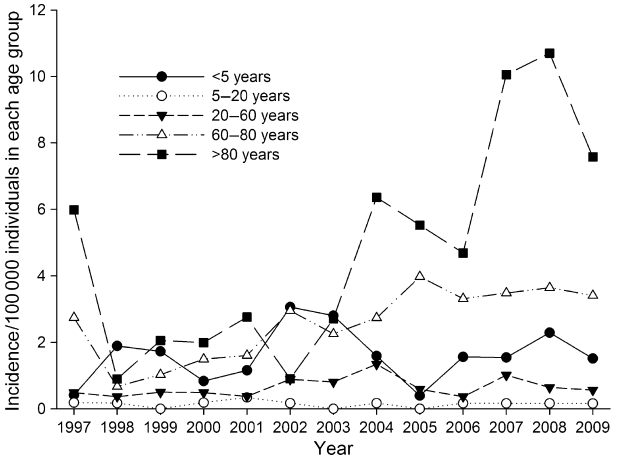

FIG. 2. The incidence per patient age group of invasive *Haemophilus influenzae* disease per 100 000 age-group-sorted individuals at risk is shown. The incidence increased significantly in the age groups 60–80 years and >80 years during the course of the study (p 0.002 and p 0.006, respectively). When the results from PCR serotyping were taken into account, the increase was mainly ascribed to non-typeable *H. influenzae* (NTHi), but in part also to *H. influenzae* type f (Hif).

was evident that the observed increase of invasive *H. influenzae* disease mainly occurred in individuals >60 years of age. The increase in both age groups 60–80 years (constant = 0.212, 95%CI 0.098–0.326, p 0.002) and >80 years (constant = 0.602, 95% CI 0.212–0.992, p 0.006) was statistically significant. No gender difference could be identified for any of the subspecies.

High proportion of sepsis severity in non-type b *H. influenzae* cases

When medical records were analysed in detail we found that the most common clinical presentation of invasive non-type b disease (in this context presence of bacteria in blood or CSF) was pneumonia (70%) but a wide variety of other presentations such as meningitis, epiglottitis, soft tissue infections and cholangitis was seen. As many as 48% of NTHi cases (n = 101) met the criteria of severe sepsis or septic shock, and 20% were admitted to intensive care units (Table 2). Interestingly, 62% of invasive NTHi cases occurred in individuals without evidence of immunocompromise, although one-third of these patients were >80 years of age. The case mortality (within 28 days) was 8%, whereas the 1-year mortality of invasive NTHi cases was 29%. Hif generally caused severe disease; 59% of Hif cases (n = 22) met the criteria of severe sepsis or septic shock, and 36% of cases were admitted to intensive care units. The majority of invasive Hif cases (68%) occurred in individuals without evidence of immunocompromise, whereas <10% of patients were

TABLE 2. Disease severity, need for intensive care treatment, mortality and data on state of immunocompromise from a total of 123 cases of invasive *Haemophilus* non-type b disease in Skåne county 1997–2009

	NTHi (*n* = 101)			Hif (*n* = 22)		
ACCP/SCCM category	**Sepsis**	**Severe sepsis**	**Septic shock**	**Sepsis**	**Severe sepsis**	**Septic shock**
Patients (total *n*)	53	41	7	9	10	3
Immunocompromise per sepsis category (*n*)[a]	20	15	3	5	1	1
Age >80 years per sepsis category (*n*)[b]	12	10	1	1	1	0
Need for intensive care (*n*)		20			8	
28-day mortality (*n*)		8			3	
1-year mortality (*n*)		29			5	
Immunocompromise[c] (total *n*)		38			7	
Acquired immunodeficiency (*n*)		25 (6 solid tumours and 19 blood cancers/myelomas)			5 (2 solid tumours and 3 blood cancers)	
Chronic disease (*n*)		9 (3 severe COPD, 3 dialysis patients, 2 dysregulated diabetes mellitus, and 1 heart disease)			1 (severe COPD)	
Iatrogenic condition (*n*)		3 (1 organ transplant and 2 chemotherapy)			1 (chemotherapy)	

ACCP/SCCM, American College of Chest Physicians/Society of Critical Care Medicine; COPD, chronic obstructive pulmonary disease; Hif, *H. influenzae* type f; NTHi, non-typeable *H. influenzae*.
[a]All ages of patients including individuals >80 years of age with immunocompromise.
[b]Patients >80 years of age with no sign of immunocompromise.
[c]In addition to acquired immunodeficiency, chronic disease and iatrogenic conditions, one patient with a primary immunodeficiency was identified in the material. This particular patient with Good's syndrome presented with an NTHi sepsis.

>80 years of age. Case mortality for Hif was 14% and 1-year mortality was 27%. Patients with invasive Hib infections (*n* = 13) were included as a control group and 77% of these patients met the criteria of severe sepsis/septic shock and 54% were admitted to an intensive care unit.

Increased clinical burden of invasive non-type b *H. influenzae*

To monitor the clinical burden of invasive *H. influenzae* non-type b disease, the demand for hospitalization days per year and individual at risk was studied (Fig. 3; filled circles). A statistically significant increase of hospital days/100 000 individuals at risk was identified (Constant = 1.31, 95% CI = 0.03–2.60, p 0.046). During the same time period, the average duration of hospitalization per case of any bacterial pneumonia or sepsis decreased in the same geographical area (Fig. 3; black and grey bars, respectively). Hence, despite shorter hospitalization periods for severe infections in general, the required days of hospitalization for *H. influenzae* non-type b invasive disease/100 000 individuals increased, indicating that the nominal increase in incidence was followed by an increase in clinical burden.

Discussion

The present study shows an unambiguous increase in the incidence of invasive disease caused by *H. influenzae* in southern Sweden from 1997 to 2009. The observed increase is explained by an increase in invasive disease caused by NTHi and Hif, mainly in individuals >60 years of age, so this does not imply that the vaccination campaign against Hib has been unsuccessful. The incidence of invasive Hib disease in Sweden remains stable at a very low level. However, the Hib cases that were studied in detail were generally severe, with a high

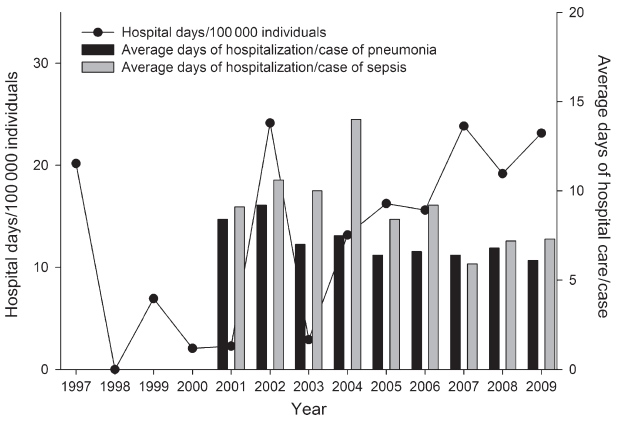

FIG. 3. The clinical burden from invasive *Haemophilus influenzae* non-type b disease is increasing. The line with filled circles shows total days of hospitalization of patients with invasive *H. influenzae* non-type b disease per 100 000 individuals at risk in the county of Skåne (left *y*-axis). The number from 2002 is high because of two cases with unusually long periods of hospitalization (>50 days). The increase in hospitalization days during the years 1997–2009 was significant (p 0.046). For comparison, the black and grey bars show the decreased average times of hospitalization/case due to pneumonia and sepsis in the city of Malmö, Skåne county 2001–2009 (right *y*-axis).

proportion of cases requiring intensive care treatment. The findings therefore support continued vaccination against and surveillance of Hib. Our study has a few limitations. We have no data from before 1997 because of the unavailability of isolates. However, there was still 13 years of observations, allowing for trends to be observed. Furthermore, not all recorded isolates could be retrieved and serotyped by PCR. This was addressed by the performance of a secondary analysis excluding years and laboratories where <50% of isolates could be retrieved.

In a recent European surveillance study [15], the total incidence of invasive *H. influenzae* disease was 0.4–0.5 cases per 100 000 individuals (2000–2006). The incidence varied between regions from 0.02 cases per 100 000 individuals in Italy to 1.0 case per 100 000 individuals in Norway. In our study, the incidence of invasive *H. influenzae* disease in Sweden almost reached 2.0 cases per 100 000 individuals, numbers supported by Swedish national surveillance data for 2007–2009 [7]. This suggests that Sweden has a current incidence of invasive *H. influenzae* disease that is three-fold higher than Europe in general, and 50- to 100-fold higher than in Italy. These conflicting data may be the result of actual, unexplained, differences in incidence, but could also merely reflect problems with regional variations in surveillance.

The present epidemiological observations are in line with studies from other parts of the world. The epidemiology of invasive *Haemophilus* disease in the post-Hib-vaccine era seems to be shifting from Hib in children towards non-type b strains in individuals >60 years of age [9,22]. Non-type b strains now dominate in all age groups, including children <5 years of age. We further identified that a major proportion of invasive non-type b cases met the criteria for severe disease. While Hib cases had the highest proportion of severe disease (77%), as many as 47% of invasive NTHi and 59% of invasive Hif cases met the criteria of severe sepsis or septic shock. Invasive disease by non-type b strains was not confined to immunocompromised patients or individuals of extreme age; findings that are in contrast to the widely held view that invasive disease by *H. influenzae* non-type b is mild and opportunistic in nature. Invasive non-type b cases with a severe clinical presentation often occurred in patients with no evidence of immunocompromise, suggesting a significant impact of bacterial virulence in these cases. However, fatal outcome was mainly observed in elderly or immunocompromised patients, and this small group of cases seemed truly opportunistic. Interestingly, the most common immuno compromising conditions that are associated with invasive *Haemophilus* disease were chronic lymphatic leukaemia and multiple myeloma, highlighting the role of humoral immunity in the control of *H. influenzae* disease [23,24]. Less efficient B-cell function is also a major part of immunosenescence [25], which is intriguing considering the high incidence of invasive *Haemophilus* disease in elderly individuals.

The increased incidence of invasive *H. influenzae* disease is probably explained by a combination of contributing factors. Though the Swedish incidence of multiple myeloma or chronic lymphatic leukaemia has not increased in recent years [26], prolonged survival may have increased disease prevalence. Another possible contributing factor is that the total number of blood cultures taken in Sweden increased during the study period. This may have led to the identification of more cases of pneumonia with bacteraemia. New guidelines in Swedish pneumonia care stressing blood culture as a quality indicator were introduced in 2007. However, the proportion of severe invasive cases did not decrease during the study period, indicating that modified clinical routines cannot fully explain the observed increase in incidence.

The introduction of the conjugated Hib vaccine has decreased Hib airway carriage in children [27], and arguably in adults. NTHi is a common colonizer of the human airway, with or without airway disease, but information on nasopharyngeal carriage rates of encapsulated *Haemophilus* other than type b in healthy children is scarce. Interestingly, *Streptococcus pneumoniae* and *H. influenzae* are niche competitors in the upper airway and are suggested to be negatively correlated [28]. Pneumococcal vaccines may, however, affect the future incidence of invasive *H. influenzae* disease because an increased burden of *H. influenzae* disease following pneumococcal vaccination has been suggested for other conditions, such as otitis media [29]. In fact, a heptavalent pneumococcal vaccine was introduced in the national vaccination programme for children in Sweden in 2008 or 2009 depending of geographical region, and most probably had no effect on the epidemiological results of the present study. Another issue is that a conjugated pneumococcal vaccine with *H. influenzae* protein D is now widely available, and the effect of this vaccine on the occurrence of invasive *H. influenzae* disease remains to be studied.

In conclusion, the present study demonstrates a statistically significant increase in the incidence of invasive *H. influenzae* disease in southern Sweden 1997–2009, explained by an increase in NTHi and Hif infections in individuals >60 years of age. Strikingly, many patients with invasive non-type-b disease presented no evidence of immunocompromise, and a surprisingly high proportion of cases were severe according to the ACCP/SCCM grading system. The results call for continued surveillance and active monitoring of invasive disease caused by *H. influenzae*.

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Transparency Declaration

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Figure S1. Map of Sweden including population density and geographical regions that are covered in the present study.

Table S1. Study definition of immunocompromise.

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