THE JOURNAL OF INFECTIOUS DISEASES • VOL. 160, NO. 2 • AUGUST 1989
 0022-1899/89/6002-0008$01.00

Bacteriologic Epidemiology of *Hemophilus influenzae* Type b Strains Causing Invasive Infections in Finland

A. K. Takala, L. van Alphen, J. M. Musser, L. Geelen, R. K. Selander, J. Eskola, and P. H. Mäkelä

From the National Public Health Institute, Helsinki, Finland; the Department of Medical Microbiology, University of Amsterdam, The Netherlands; and the Department of Biology, Mueller Laboratory, Pennsylvania State University, University Park

Consecutive *Hemophilus influenzae* type b (Hib) isolates (333 total) from children with invasive disease in Finland in 1985–1986 were analyzed. All belonged to the common genetic clusters described in the USA and Europe. However, detailed typing demonstrated some characteristics unique to Hib strains in Finland. Of the isolates, 86% belonged to one of four distinct patterns according to the combination of outer membrane protein subtype, biotype, and lipopolysaccharide serotype: 1-I-1 (35%), 1-II-1 (25%), 1-II-9 (8%), and 1c-I-1 (18%). Pattern 1-II-9 has not been previously reported; it was most commonly found in the most densely populated area of Finland and among children cared for outside the home. Multilocus enzyme electrophoresis revealed that 87% of isolates with the pattern 1c-I-1 belonged to the electrophoretic type 21.8, which is seldom recovered from patients with invasive Hib disease in other countries.

More than 95% of serious *Hemophilus influenzae* infections in childhood are caused by serotype b organisms [1]. Incidence and age distribution of invasive *H. influenzae* type b (Hib) disease vary between countries [2, 3]. The highest rates have been reported among children <5 y of age, up to 500 per 100,000, among Alaskan Eskimos, Indians, and black children in the USA and aboriginal Australians [4–7], while most parts of USA and Europe have incidences comparable to that among children <5 y of age in Finland: 52 per 100,000 [3]. These differences may to a large part be due to genetic [8, 9] or socioeconomic factors.

The type b polysaccharide capsule is the major virulence determinant of Hib bacteria [10]. Anticapsular antibodies have been shown to prevent invasive disease [11]. Other surface-exposed determinants, e.g., outer membrane proteins (OMP) and lipopolysaccharides (LPS), may contribute to the virulence of Hib strains [12–18]. Differences in OMP, LPS, and biotype are used for subtyping Hib strains [19–22]. In addition multilocus enzyme electrophoresis has been recently introduced to study the epidemiology of invasive Hib disease [23]. Analysis of a large number of Hib strains recovered from different parts of the world has revealed that the distribution of Hib strains is strongly clonal [24].

A nationwide efficacy trial with a second-generation Hib capsular polysaccharide–diphtheria toxoid conjugate vaccine is being conducted in Finland [25]. We analyzed the OMP subtype, biotype, and LPS serotype of 333 consecutive Hib isolates and the multilocus enzyme genotype of 151 Hib isolates from children with invasive Hib disease in Finland to determine the bacteriologic epidemiology of Hib disease in Finland before the vaccination program.

Received for publication 21 December 1988 and in revised form 27 March 1989.

This work was supported by Connaught Lab Inc. as a part of the Hib vaccination project and by grant AI-24332 from the National Institute of Allergy and Infectious Diseases to R. K. S.

The authors thank Agaath Arends, Hannele Mattila, Eija Kela, Panu Rekola, Pirjo-Riitta Rönnberg, Paula Solukko, C. M. Sommers, and L. M. Tremblay for technical assistance and all contacts in bacteriologic laboratories in Finland who provided Hib strains in 1985–1986.

Please address requests for reprints to Dr. Aino Kaarina Takala, National Public Health Institute, SF-00300 Helsinki, Finland.

Patients and Methods

Patients. Hib was isolated between February 1985 and December 1986 from blood, cerebrospinal fluid, or other normally sterile body sites of 333 consecutive patients (0–15 y old) with invasive Hib disease. Each patient was represented by a single isolate: 46% had meningitis, 29% had epiglottitis, and 25% had other forms of invasive disease [3].

An intensified surveillance system was organized to cover all culture-proven invasive infections in children nationwide. The details of the surveillance system and the epidemiology of invasive Hib disease among Finnish children have been described in detail [3].

Hib isolates. *H. influenzae* was isolated and identified as Hib in the 25 collaborative laboratories by standard methods and then sent to the National Public Health Institute, where identification was confirmed and isolates stored in skim milk at −70°C. Isolates were mailed for further analysis to the Department of Medical Microbiology, University of Amsterdam, and the Department of Biology, University of Rochester, New York, in Stuart transport medium (Transpocult; Orion Diagnostica, Espoo, Finland).

Biotyping, OMP subtyping, and LPS serotyping. Biotyping (BT), OMP subtyping, and LPS serotyping were performed at the Department of Medical Microbiology, University of Amsterdam, as previously described [19, 22].

Multilocus enzyme electrophoresis. Multilocus enzyme electrophoresis was performed at the Department of Biology, University of Rochester, New York, with a representative set of 151 Hib isolates obtained between August 1985 and September 1986 as previously described [24, 26].

Statistical methods. The χ^2 test was used to compare the distribution of isolates with different OMP-BT-LPS patterns among the five University Central Hospital districts in Finland (figure 1). Two-tailed Poisson distribution was used to test for significance of differences between the incidence rates. The form of day care of patients in the Helsinki University Hospital district (HUCH) was evaluated using a questionnaire. Parents were asked to indicate whether day care was at home or outside the home (supervised care of two or more unrelated children for >4 h/w) 4 w before hospitalization of the child. The results were evaluated in relation to the OMP-BT-LPS pattern using Fisher's exact test.

Results

The predominant OMP subtype among all 333 isolates was subtype 1 (75%); about half of these were of biotype I (51%) and half biotype II (48%). Most isolates of OMP subtype 1 were of LPS serotype 1 (80%); 14% were of LPS serotype 9. The next most common OMP subtype was 1c (20%). These isolates were remarkably homogeneous: 91% were of biotype I and 96 were of LPS serotype 1 (table 1).

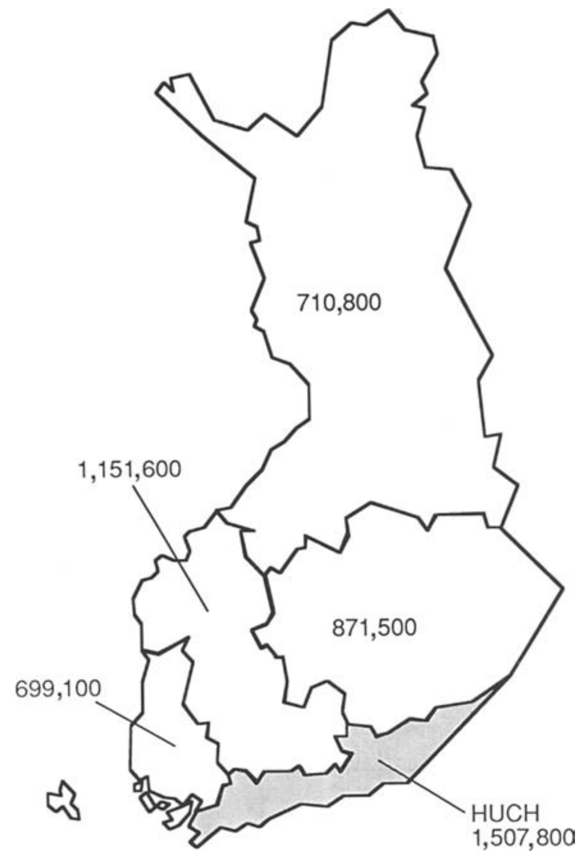

Figure 1. The five University Central Hospital districts in Finland with the number of inhabitants in each district. HUCH = Helsinki University Central Hospital district.

OMP-BT-LPS patterns. We identified four distinct OMP-BT-LPS patterns that made up 86% of all isolates: 1-I-1 (117 isolates), 1-II-1 (82), 1-II-9 (28), and 1c-I-1 (59). Isolates of the first three patterns were equally often recovered from patients with different disease entities. Further, there was no difference between patterns in age distribution or gender ratio of the patients. However, as reported previously [18], isolates of pattern 1c-I-1 caused proportionately more meningitis and affected mainly younger children.

When comparing the geographic distribution of the various OMP-BT-LPS patterns in the five districts, we identified differences within the country and within the study period (figure 2). The distribution of OMP-BT-LPS patterns in 1986 in the HUCH district was different from that in the rest of the country ($P < .001$). This dissimilarity was due to a cluster of 15 isolates of pattern 1-II-9 in the HUCH district,

Table 1. Outer membrane protein (OMP) subtypes, biotypes, and lipopolysaccharide (LPS) serotypes of 333 consecutive *Hemophilus influenzae* type b isolates from Finnish children with invasive disease in 1985–1986.

OMP subtype, no. (%) of isolates	Biotype				LPS serotype				
	I	II	III	IV	1	3	9	10	NT
1,* 251 (75)	129	120	1	1	201	3	34	1	12
1c, 67 (20)	61	6	—	—	64	—	1	1	1
Other,† 15 (5)	10	5	—	—	13	—	1	1	—

NOTE. NT = nontypable.

* Corresponds to OMP 3L in the scheme of Barenkamp et al. [20].

† NT (6 isolates), 2 (3), 1a (1), and 1b (1) according to the scheme of van Alphen et al. [19] and 2L (2), 1H (1), and 14L (1) according to the scheme of Barenkamp et al. [20].

where the incidence of Hib disease caused by isolates of this pattern was 5.06 per 100,000 compared with 0.69 per 100,000 in the rest of the country ($P <$ .01). Further, within the HUCH district, patients with Hib of this pattern were more frequently cared for outside the home (10/12) than were children infected with Hib with other patterns (33/58). This difference was, however, not statistically significant (P = .08).

Multilocus enzyme electrophoresis. Among the 151 isolates examined there were 19 distinct multilocus enzyme genotypes, or electrophoretic types (ETs) (table 2). Most were assigned to ET 12.8 (40%), 21.8 (24%), or 12.5 (23%). These ETs are closely related: the difference between ETs 12.5 and 12.8 is in the mobility of one of the 17 metabolic enzymes measured (carbamylate kinase) and ET 21.8 differs from 12.8 in the mobility of one enzyme (glucose-6-

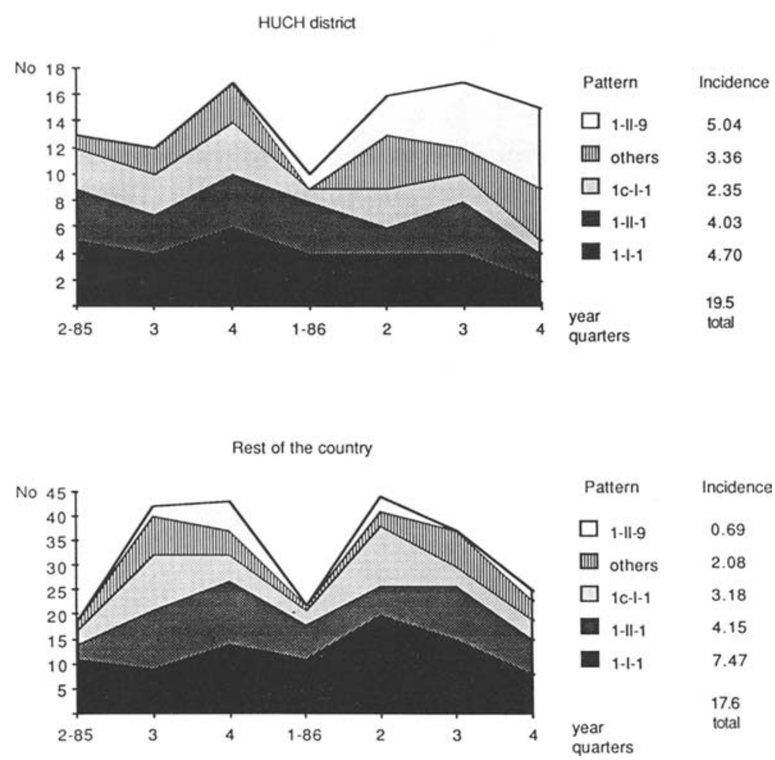

Figure 2. Incidence (cases per 100,000/y) of infection by *Hemophilus influenzae* type b of various outer membrane protein subtype, biotype, and lipopolysaccharide serotype patterns in children 0–15 y old during 1986. HUCH = Helsinki University Central Hospital district.

Table 2. Electrophoretic types (ETs) of 151 isolates of *Hemophilus influenzae* type b from Finnish children with invasive disease in 1985–1986 in relation to outer membrane protein (OMP) subtype, biotype, and lipopolysaccharide (LPS) serotype.

ET, no. (%) of isolates	OMP subtype			Biotype			LPS serotype			
	1	1c	other	I	II	IV	1	9	10	NT
1.9, 3 (2)	–	2	1	2	1	–	2	–	–	1
12.5, 34 (23)	30	2	2	32	2	–	31	1	–	2
12.7, 2 (1)	2	–	–	2	–	–	1	1	–	–
12.8, 60 (40)	56	2	2	8	51	1	44	12	–	4
12.9, 3 (2)	3	–	–	3	–	–	3	–	–	–
21.8, 36 (24)	1	34	1	32	4	–	36	–	–	–
71, 2 (1)	2	–	–	2	–	–	2	–	–	–
81, 2 (1)	2	–	–	2	–	–	2	–	–	–
Other,* 9 (6)	7	1	1	6	3	–	7	–	1	1
Total, 151	103	41	7	89	61	1	128	14	1	8

NOTE. Genetic clusters, according to Musser et al. [24], were A1 for ET 1.9 and A2 for ETs 12.5, 12.7, 12.8, 12.9, 21.8, 71, and 81. NT = nontypable.

* Single isolates of ET 8 (A1); ETs 12.0, 67, 82, 87, 93, 110, unclassified (A2); and ET 141 (B1).

phosphatase dehydrogenase). All the ETs found among the 151 isolates belonged to the common genetic clusters of Hib (A1, A2, and B1) found in the USA and Europe [24, 27].

Within most ETs represented by multiple isolates there was little diversity in OMP subtype or BT. For example, 88% and 93% of isolates assigned to ET 12.5 or ET 12.8, respectively, were of OMP subtype 1; similarly, 94% of ET 21.8 were of OMP subtype 1c. Most isolates of each ET had a single common LPS serotype; the exception was ET 12.8, in which 73% of isolates were of LPS serotype 1 and 20% were of LPS serotype 9 (table 2). Conversely, the ET varied among isolates of the two common OMP subtypes. Our analysis identified 14 distinct ETs among isolates of OMP subtype 1 and 5 ETs among isolates of OMP subtype 1c.

When the ET of isolates of the four OMP-BT-LPS patterns was analyzed, there was generally little diversity: 94% of isolates of pattern 1-II-1 and 92% of pattern 1-II-9 were of ET 12.8 and 86% of pattern 1c-I-1 were of ET 21.8. However, among isolates of the most common pattern, 1-I-1, 56% (26) were of ET 12.5, while 11 ETs were found among the remaining 20 isolates.

Discussion

Among the 333 isolates studied we found distinct OMP-BT-LPS patterns. The two main patterns, 1-I-1 and 1-II-1, are the most common patterns found in Europe [28]. Hib of pattern 1c-I-1 is rarely found but accounted for as much as 20% of isolates in our study.

Pattern 1-II-9, which accounted for 8% of isolates, has not previously been reported. Hib of LPS serotype 9 has been found in Iceland, but these were uniformly of OMP subtype 2 [28]. Hib of OMP subtype 2 is seldom found in Finland; among the present 333 isolates, only 3 were of OMP subtype 2 and none of these were of LPS serotype 9. Although variation in LPS structure during laboratory subculture and passage through infant rats has been reported [16, 29], passage in vitro or in vivo has not revealed variation in LPS serotype 9 (L. v. A., unpublished data).

Isolates of pattern 1-II-9 were found in a cluster in the HUCH district, with a rapid increase during 1986. Temporal changes of the OMP subtype of Hib have been reported earlier, but these extended over a long time [30]. Recently, a temporal shift in the dominant clones causing invasive Hib disease in one area in the USA has been reported [31].

The HUCH district is the most densely populated of Finland (figure 1). Further, within the HUCH district, children infected by Hib of pattern 1-II-9 tended more often to be those cared for outside the home than were children infected by Hib of other patterns. This suggests that Hib of pattern 1-II-9 spreads more effectively in dense populations and within groups with close human contact. The fact that low numbers of isolates of this pattern were present in the rest of the country in 1985 but did not spread (figure 2), and that once entering the HUCH district in 1986 Hib of this pattern was able to increase rapidly, further supports this hypothesis.

Multilocus enzyme electrophoresis of 151 isolates revealed that most (87%) belonged to three clones — ET 12.5, 12.8, and 21.8. The three are similar in overall chromosomal character: Only one enzyme differs between ETs 12.5 and 12.8 and between ETs 12.8 and 21.8 and there is a difference in mobility of two enzymes between ETs 12.5 and 21.8. Similarity among isolates assigned to ET 12.5 and ET 12.8 is also demonstrated by OMP subtyping; 92% of isolates belonged to OMP subtype 1.

The strong association of multilocus enzyme genotype and OMP subtype and biotype has been reported [23, 24] and seems to be characteristic of encapsulated *H. influenzae* [24]. However, the associations between ETs and LPS serotypes or OMP-BT-LPS combinations are new. The LPS serotype varied within certain ETs: Isolates of ET 12.8 could be divided in two groups according to their LPS serotype. There was little variation in the ETs of isolates of the OMP-BT-LPS patterns 1-II-1, 1-II-9, and 1c-I-1, but the ETs within pattern 1-I-1 varied considerably. This pattern has been the most common among Hib in Finland and the rest of Europe for the past two decades [19].

The combination of results of OMP subtyping, biotyping, and LPS serotyping (pattern 1c-I-1) was able to mark the clone ET 21.8 with distinct virulence properties [18]. The novel OMP-BT-LPS pattern 1-II-9 was, however, not distinguished by multilocus enzyme electrophoresis.

We described the bacteriologic epidemiology of Hib before large-scale immunization with a Hib conjugate vaccine. Hib isolates were recovered from children with invasive Hib disease and collected via an intensified surveillance to provide nationwide data on Hib disease and subtypes of Hib strains in Finland. This study revealed certain novel characteristics of Hib epidemiology in the country. The most notable of these may be the relatively common recovery of isolates of a unique OMP-BT-LPS pattern, 1-II-9, which increased rapidly in one region of the country, and the frequency of strains of the otherwise rare pattern 1c-I-1, which corresponds to ET 21.8. In a recent survey of 1975 Hib isolates recovered from 30 countries on 5 continents, isolates of ET 21.8 accounted for <2% of invasive isolates (J. M. M., unpublished data). However, all multilocus enzyme genotypes found in Finland belonged to the common genetic clusters frequently found in Europe and the USA.

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