

Biotypes and Serotypes of *Haemophilus influenzae* from Patients with Meningitis in the City of São Paulo, Brazil

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A total of 1,094 *Haemophilus influenzae* isolates from cerebrospinal fluid were examined by biochemical and serological means. Most of them belonged to biotype I (70.9%) and to serotype b (99.4%). The relationship of biotypes I and II to the ages of the patients was shown to be significant ($P < 0.001$).

Haemophilus influenzae is one of the leading causes of bacterial meningitis, together with *Neisseria meningitidis* and *Streptococcus pneumoniae*. In some countries it has been the predominating agent of bacterial meningitis, as it is in the United States (24), Chile (8), Papua New Guinea (6), and Sweden (19) and as it was in Finland (21) prior to the large-scale *H. influenzae* type b vaccination period from 1986 to 1989, which resulted in an 84% decline of meningitis by this agent in children less than 5 years of age (5). Most of the capsulated *H. influenzae* strains are serotype b, with a percentage varying from 80% (23) to about 99% (3). Although many authors refer to serotype b and non-serotype b *H. influenzae* strains from meningitis in their reports (17, 24), all of Pittman's other capsular serotypes have been observed in *H. influenzae* isolates from cerebrospinal fluid (CSF). *H. influenzae* has been subdivided into eight taxonomic biotypes on the basis of differences in biochemical reactions (12). Biotype I has been shown to account for most of the *H. influenzae* meningitis strains in studies from Denmark and Norway (13), western Europe (22), and the United States (1), but in Papua New Guinea (6) it represents only 29% of the

biotyped strains isolated from CSF, in contrast to biotype II, which accounts for 62%.

Little information concerning the situation of *H. influenzae* meningitis in Brazil is available in the literature. Most of the studies, such as one developed in São Paulo, Brazil, from 1960 through 1977 (15), refer mainly to epidemiologic aspects. In another epidemiologic study reported in 1981, this bacterium was the third most common etiologic agent of meningitis in Rio de Janeiro, Brazil (16). In Salvador, northeastern Brazil, it was the second most frequently isolated meningeal pathogen from 1973 through 1982. However, at the end of that period, there was an increase in the incidence of this bacterium, so it emerged as the most common cause of bacterial meningitis in children under 15 years of age (4). In São Paulo, prior to 1975, it was cited as the third most common cause of bacterial meningitis when 15,067 CSF samples were analyzed (2).

In the Instituto Adolfo Lutz, the national reference center for meningitis, 50,622 CSF samples were examined from 1977 through 1991. As a public health laboratory, it conducts most of the CSF examinations in the city of São Paulo. This

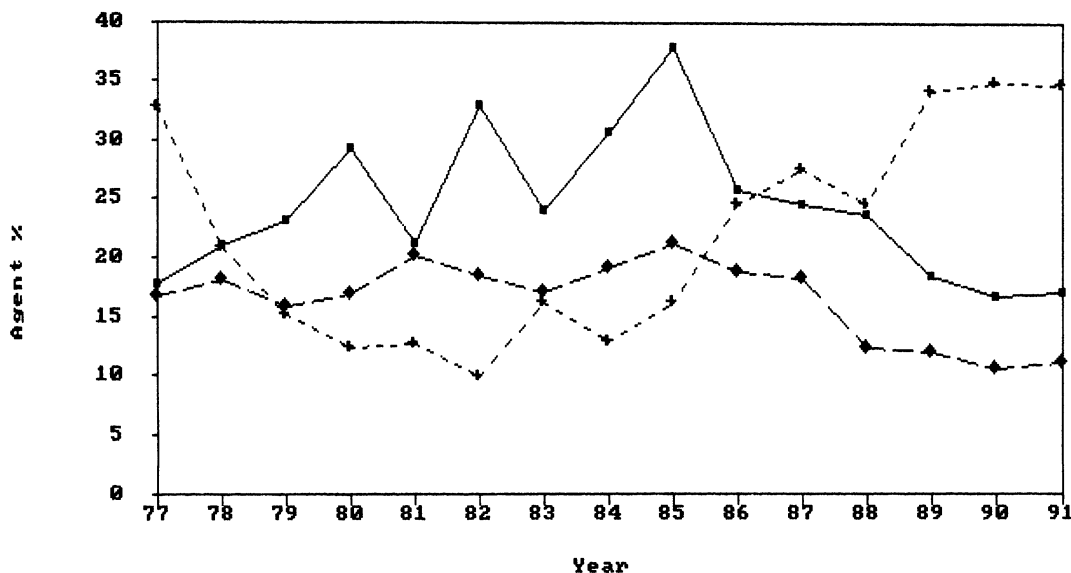


FIG. 1. Three leading bacterial meningitis agents identified in the Bacteriology Section, Instituto Adolfo Lutz, São Paulo, Brazil, from 1977 to 1991. Symbols: ■, *H. influenzae*; +, *N. meningitidis*; ◆, *S. pneumoniae*.

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TABLE 1. Frequency distribution of biotypes and serotypes of 1,015 *Haemophilus influenzae* strains from CSF and relationship to ages of patients

Age (yr) of patient	Biotype								Serotype						Total no. serotypeable	No. nonserotypeable	Total no. (%) of strains
	I	II	III	IV	V	VI	VII	VIII	a	b	c	d	e	f			
0-1	521	180	4	3	3	0	0	0	2	706	0	0	0	0	708	3	711 (70.0)
2-3	159	78	1	0	0	0	0	0	1	235	0	0	0	0	236	2	238 (23.4)
4-5	14	8	0	0	0	0	0	0	0	22	0	0	0	0	22	0	22 (2.2)
6-10	11	3	2	0	0	0	0	0	1	14	0	0	0	0	15	1	16 (1.6)
11-15	3	6	0	0	1	0	0	0	1	9	0	0	0	0	10	0	10 (1.0)
16-20	1	2	0	1	0	0	0	0	0	1	1	0	0	0	2	2	4 (0.4)
≥21	5	7	0	2	0	0	0	0	0	10	0	0	0	0	10	4	14 (1.4)

study showed that *H. influenzae* was the most frequently detected isolate by culture or by counterimmunoelectrophoresis from 1978 to 1986. In the year 1977 and over the last 5 years, 1987 to 1991, the relative proportion of *H. influenzae* cases decreased (Fig. 1). From these CSF samples, 1,094 isolates of *Haemophilus* species from patients whose ages varied from 23 days to 56 years were identified. Identification of isolates was done by colony and cellular morphology and the demonstration of growth requirements for V and X factors. The V requirement was tested by observing the satellite phenomenon on 5 to 10% (vol/vol) sheep blood agar (11) inoculated with a bacterial suspension in 0.85% saline to match the turbidity of a 0.5 McFarland density standard and further diluted to 1:100 with 0.85% saline. Two or three drops of this inoculum was inoculated on the blood agar surface, spread with a bent glass rod, and, after drying, cross-streaked with a strain of *Staphylococcus aureus*. The X factor dependence was determined by testing the ability to convert δ -aminolevulinic acid to porphyrins (12). Strains were further characterized by biochemical reactions by using the scheme described by Kilian (10, 12). Serotyping of the capsulated strains was done according to the method of Pittman (18) by slide agglutination with type-specific antisera a through f.

The 1,094 *Haemophilus* isolates were identified as *H. influenzae* (10). All isolates fermented glucose without gas and all but two fermented xylose (one each of biotypes I and II), whereas none of them fermented sucrose, lactose, or mannitol. All isolates conformed with one to five of the eight *H. influenzae* biotypes. Table 1 shows the distribution of biotypes and serotypes and the relationship to the ages of the patients. The ages of 79 (7.1%) of the patients were unknown. From these patients, 77 isolates were serotype b, one was serotype a, and one was nonserotypeable. Concerning the biotypes of these isolates, 61 (77.2%) were biotype I, 17 (21.5%) were biotype II, and one (1.3%) was biotype IV.

The majority of strains subjected to capsular polysaccharide typing belonged to serotype b (99.4%), which is consistent with what many authors have observed in different countries. Serotype a was isolated in only 0.5% of the cases, a proportion quite inferior to what was observed in the United States among the White Mountain Apaches (22.2%) (14); in Fajara, the Gambia (20.0%) (23); and in Papua New Guinea (12.8%) (6). Serotype c occurred once in a patient who was 17 years old. Nonserotypeable strains occurred in almost all age groups, as was reported also by Greene (7). However, Trollfors et al. found nonserotypeable isolates from invasive infections only in patients over 30 years old (20). Most of the nonserotypeable isolates of this study (75.0%) belonged to biotype II, III, or IV, a finding similar to that described by Kamme (9).

Most of the biotypes were biotype I (70.9%) but many were biotype II (27.5%), a finding quite similar to that observed in Finland (25.0%) (3) and in South Africa (31.0%) (3) but far less than that seen in Papua New Guinea (62.0%) (6) and in the United States among the White Mountain Apaches (55.6%) (14). Some 0.6% of the isolates belonged to biotypes III and IV, and 0.4% belonged to biotype V. It was noticeable that biotype I was predominant in patients under 10 years of age, whereas in patients over this age biotype II was more frequent. The difference in incidence of these two biotypes according to age groups was statistically analyzed by the chi-square test, and it was shown to be significant ($P < 0.001$).

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