

Epidemiology of *Haemophilus influenzae* infections in Malaysian hospitals

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Abstract

Haemophilus influenzae is a leading cause of meningitis in children and frequently associated with otitis media, sinusitis, pneumonia and septicaemia. This study in 7 centres in Malaysia showed that *H. influenzae* infections was seen in all age groups with the peak incidence occurring in children less than 10 years of age. Invasive diseases occurred in 92.9% of this age group of which 54.3% were infants. All *H. influenzae* recovered from meningitis occurred in infants between 4-11 months old. Out of 354 *H. influenzae* strains, 78 (22.0%) belonged to serotype *b* of which 12 (15.4%) were strains isolated from invasive infections. Resistance to ampicillin and chloramphenicol was noted in 13.5% and 2.6% of the isolates respectively. In view of high morbidity and mortality related to the disease, together with emergence of resistance strains, the available capsular polysaccharide protein-conjugate vaccine may have a useful role in our population.

Key Words: *Haemophilus influenzae* *b*, meningitis, childhood infection

Introduction

Haemophilus influenzae, although a common commensal of the upper respiratory tract of healthy individuals, is an important human pathogen. Capsulated strains are a frequent cause of meningitis and other invasive infections in children (Dyas & George, 1986). The most important group is encapsulated serotype *b*, which is associated with serious invasive diseases such as meningitis, epiglottitis, arthritis and cellulitis, mainly in children (Turk, 1984). Non-encapsulated strains found in the respiratory tracts of up to 75% of healthy adults are associated with localised infections in both children and adults, such as otitis media, sinusitis and conjunctivitis (Murphy & Apicella, 1987; Wald, 1989). These strains can also cause meningitis, pneumonia, and septicemia (Everett *et al.*, 1977; Spagnuolo *et al.*, 1982) and be the cause of exacerbation of cystic fibrosis or chronic bronchitis (Govan & Glass, 1990).

The diseases caused by *H. influenzae* serotype *b* (Hib) are life threatening. Severe long-term morbidity is almost always caused by Hib meningitis, although epiglottitis occasionally results in hypoxic brain injury and septic arthritis can result in deformity. Severe handicap occurs in 3 - 5% of survivors of Hib meningitis (Hanna & Wild, 1991; McInlyre, 1992; Murphy & Apicella, 1987) and hearing loss severe enough to require hearing aids occurs in another 3 - 5%.

Increasing emergence of *H. influenzae* strains resistant to ampicillin and chloramphenicol has complicated the management of these infections and has prompted the need for preventing such invasive infections by the use of available vaccines.

As epidemiological differences of the infections have

been associated with genetic and socioeconomic factors, a surveillance to determine the incidence of *H. influenzae* causing invasive infections in our hospitalised patients would indicate whether there is a need for vaccination of the population.

This study was carried out to determine the prevalence of *H. influenzae* infection in the Malaysian population, distribution of the diseases among age groups, the distribution of *H. influenzae* serotype *b* and their antibiotic susceptibility pattern.

Materials and Methods

Bacterial strains

Clinical isolates, accompanied by special forms containing relevant data, were obtained from 7 centres representing 5 geographical regions of Malaysia. These strains were isolates from clinical samples including blood, sputum, body fluid, pus, and others, received by the microbiology laboratories for isolation of pathogens, during the study period from October 1994 to September 1995.

The hospitals involved were Kota Bharu Hospital, Kelantan, Kuala Lumpur Hospital, Wilayah Persekutuan, Pulau Pinang Hospital, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Sultanah Aminah Hospital, Johor, University Hospital Kuala Lumpur and Bacteriological Laboratory, Institute for Medical Research (IMR). The identity of the strains were reconfirmed using standard methods and their antibiotic susceptibility pattern were repeated using the Kirby Bauer method.

Typing of bacterial strains

Haemophilus influenzae was identified by colonial mor-

phology on chocolate agar, gram's staining appearance, X and V factor dependence and satellitism with *Staphylococcus aureus*. The isolates were serotyped by slide agglutination with *H. influenzae* monovalent type *a* through *f* polyclonal rabbit antisera. Biotyping was determined based on the ability of the isolates to produce indole, urease, and/or ornithine decarboxylase as described by Kilian (1976).

Antibiotic susceptibility pattern

Antibiotic susceptibility pattern of the isolates were determined by modified Kirby-Bauer disc diffusion method recommended by National Committee for Clinical Laboratory Standards (NCCLS, 1993) against ampicillin, chloramphenicol, ceforaxime, ceftriaxone and cotrimoxazole. Minimum inhibition concentrations of antibiotic to which the strains were resistant were determined using E rest system from Biodisk Sweden. β -lactamase activity was tested using the chromogenic cephalosporin substrate, nitrocefin (Oxoid).

Results

From October 1994 to September 1995, 508 (0.33%) *H. influenzae* strains were isolated from 153,285 clinical specimens processed in the 7 centres, of which only 459 were still viable on arrival at the IMR. The strains were isolated from patients of all ages with the peak isolation rate (19.7%) occurring in children aged 10 years and below (Table 1). These children were admitted to the hospitals for various types of infection. Among the diagnosis given, pneumonia and meningitis were the two most common, being 34.3% and 24.3% respectively (Table 2). The percentage is slightly higher among the children aged 5 years and below which was 29.3% for pneumonia and 27.6% for meningitis. Most organisms (78.9%) were isolated from sputum while 5.5% were isolated from invasive infection, of which 3.1% were from blood and 2.4% were from

Table 1. *H. influenzae* isolated from various age groups

Age group (years)	<i>H. influenzae</i>
0 - 10	92 (18.1%)
11 - 20	42 (8.3%)
21 - 30	44 (8.6%)
31 - 40	66 (13.0%)
41 - 50	38 (7.5%)
51 - 60	66 (13.0%)
61 - 70	65 (12.8%)
71 - 80	40 (7.9%)
81 - 90	15 (2.9%)
Unknown	40 (7.9%)
Total	508

Table 2. *Haemophilus influenzae* diseases among children less than 10 years old

Diseases	No of cases	%
Pneumonia	24	34.3
Meningitis	17	24.3
Fever	9	12.9
Bronchitis	8	11.4
Upper Respiratory Tract Infections	5	7.1
Eye infections	4	5.7
Encephalitis	2	2.9
Otitis media	1	1.4
Total	70	100

Table 3. *H. influenzae* isolated from 7 centers (October 1994 - September 1995)

Source	<i>H. influenzae</i>
Sputum	401 (78.9%)
Blood	16 (3.1%)
Cerebrospinal fluid	12 (2.4%)
Nasal /Throat swab	39 (7.7%)
Tracheal nasopharynx aspirate	20 (3.9%)
Eye swab	7 (1.4%)
Sinus washing	3 (0.6%)
Others	10 (2.0%)
Total	508

cerebrospinal fluid (CSF) (Table 3). Of all the invasive infections, 92.9% were from children aged ≤ 10 year old. Fifty (54.3%) out of the 92 *H. influenzae* isolated from the children ≤ 10 years were from infants aged 1-12 months, of which 23 isolates were from invasive infections (Table 4). All the meningitis cases occurred in children aged between 4 and 11 months.

Among the younger age group the disease occurred slightly more in male, the male:female ratio being 1.4:1 and for invasive infection 1.2:1. Among the older age group ≥ 50 years, more *H. influenzae* strains were isolated from male patients, giving the male:female ratio of 2.6:1. Among this group of patients several underlying diseases were noted. Chronic obstructive airway diseases, pulmonary tuberculosis, followed by asthma, were among the commonest underlying diseases (Table 5).

Out of 459 strains, only 187(40.7%) were capsulated *H. influenzae* which belonged to either one or more of the serotypes, 167(36.4%) were untypable or non-encapsulated strains and 105(22.9%) strains could not be typed due to autoagglutination. Among the capsulated strains 78(41.7%) belonged to serotype *b* (Table 6) of which 12(15.4%) were from invasive infection. Serotyping of the 14 strains isolated from blood showed

Table 4. Source of *H. influenzae* from children aged 0-10 years

Age (year)	Blood	Cerebrospinal fluid	Sputum	Tracheal aspirate	Others	Total
0-1	11	12	7	7	13	50
>1-2	1	-	4	3	4	12
>2-3	-	-	-	2	4	6
>3-4	1	-	3	1	1	6
>4-5	-	-	3	-	-	3
>5-6	-	-	1	-	-	1
>6-7	-	-	4	-	-	4
>7-8	-	-	3	1	2	6
>8-9	1	-	1	-	-	2
>9-10	-	-	2	-	-	2
Total	14	12	28	14	24	92

Table 5. Underlying diseases noted to be associated with *Haemophilus influenzae* pneumonia

Underlying diseases	No. of cases	%
Pulmonary Tuberculosis	45	26.9
Chronic Obstructive Airway Disease	41	24.6
Bronchiectasis	6	3.6
Malignancy	10	6.0
Congestive Cardiac Failure	11	6.6
Bronchitis	11	6.6
Lung cancer	13	7.8
Asthma	30	17.9
Total	167	100

Table 6. *H. influenzae* serotype isolated from 7 centres (September 1994- October 1995)

Serotype	Invasive infection	Other infection	Total
a	5	55	66
b	12	66	78
c	-	4	4
d	1	2	3
e	-	2	2
f	1	10	11
Mix	-	29	29
Untypable	4	163	167
Autoagglutination	2	103	105
Total	25	434	459

rhat 7 were serotype *b*, 2 serotype *a*, one serotype *f* and 4 were untypable. Nine CSF isolates were serotyped, of which 5 were serotype *b*, 3 serotype *a* and one serotype *d*. The commonest biotypes were II and III. Invasive infection was more likely to be associated with biotype I (54.2%). β -lactamase was produced by 77 (16.8%) of 459 *H. influenzae* isolates. Resistance to ampicillin and chloramphenicol was noted in 13.5% and 2.6% of the isolates respectively (Table 7). Among the ampicillin resistant *H. influenzae* the MIC of ampicillin ranged from 8-256 μ g/ml and for chloramphenicol resistant strains, the MIC of chloramphenicol was from 4-12 μ g/ml.

Discussion

In 1995, 50,584 significant bacterial pathogens comprising of 99 different bacteria or group species (unpublished data) were isolated from 15 hospitals. *H. influenzae* was listed among the 25 most common organisms isolated, being 0.69% of the total. As observed in this study, the majority were isolated from sputum. The percentage of *H. influenzae* isolated from invasive infection was 4.23%.

H. influenzae is a leading cause of meningitis in children (Landgraf & Vieira, 1993). Meningitis accounted

Table 7. Antibiotic susceptibility patterns of *H. influenzae* isolates

Antibiotic	Susceptibility pattern (%)		
	S	I	R
Ampicillin	84.1	2.4	13.5
Chloramphenicol	97.0	0.4	2.6
Cefotaxime	97.0	0.0	3.0
Ceftriaxone	98.0	0.0	2.0
Cotrimoxazole	78.4	0.8	20.8

for 41-56% of various serious infection caused by *H. influenzae* in children (Alphen *et al.*, 1992). Based on the number of isolations and the total admission of 321,301 patients to the 7 centres during the study period, the incidence rate of *H. influenzae* infection in these centres was 150/100,000. In this study the number of pneumonia and meningitis cases due to *H. influenzae* infections is almost equal and account for more than half of all *H. influenzae* diseases in children. A local study by Choo *et al.* (1990) and Year *et al.* (personal communication) showed that 35 - 50% of organisms isolated from CSF of children with meningitis were due to *H. influenzae*. In Japan, 31% of culture positive purulent meningitis in childhood were due to *H. influenzae* type *b* (Taneda *et al.*, 1991) and it is the single most common organism causing meningitis in children older than 3 months. In Singapore (Low *et al.*, 1984) *H. influenzae* was the cause of 25% childhood bacterial meningitis above the neonatal age group.

In this study 12 out of 23(52.2%) typable strains causing invasive infection were Hib. The incidence of Hib meningitis is highest in infants with a peak at 6-10 months. In our study, meningitis due to *H. influenzae* occurred in a younger age group, ie between 3-11 months. In developed countries, the mortality from Hib meningitis was 20% in the 1950s, which decreased to 5% in the 1970s (Carter *et al.*, 1992). This was attributed to the improvement in treatment and the percentage has stayed at that level since then. In less developed countries the mortality from Hib meningitis is 20 to 50% (Bijlmer *et al.*, 1990). We noted that 5 of the 9 strains from CSF and 7 of the 14 strains from blood were Hib, which represent 52.2% of strains isolated from invasive infection. Serotypes other than serotype *b* were also isolated from cases of meningitis and septicemia. We also noted 4 untypable strains which represented the non-encapsulated strains and were associated with invasive infection. Epiglottitis and pneumonia are also potentially fatal, with a mortality rate of 2 to 5% (Gilbert *et al.*, 1990; Trollfors *et al.*, 1990). Acute lower respiratory tract infection is probably an important cause of mortality from Hib diseases in developing countries and may be more important than meningitis (Greenwood, 1992).

The high incidence of Hib meningitis in infants could be related to the disappearance of protective maternal antibody by 2 months of age and heavy colonization in infants < 6 month old particularly in developing countries as observed in Papua New Guinea (Shann *et al.*, 1984). Sell *et al.* (1973) noted that all children in their study were found to be carriers on one or more occasions from birth to 4 years old. The emergence of strains of *H. influenzae* resistant to ampicillin and other antibiotics has also become a major concern for future management of the disease. In this study we detected 13.5% ampicillin resistant strains compared to 1.9% in 1990 (NSAR data). In other studies this varied from 1-57%

(Campos *et al.*, 1986; Gunn *et al.*, 1974; Howard *et al.*, 1978; Powell & William, 1987). The *H. influenzae* type *b* (Hib) capsular polysaccharide-protein conjugate vaccines can protect children in the first year of life. In Finland the conjugate vaccine reduced the annual incidence rate from 52/100,000 in 1985 to 8.5/100,000 in 1989 and 0 in 1991 as observed by Eskola *et al.* (1990, 1991, 1992).

In adults, *H. influenzae* is frequently associated with pneumonia accounting for 5-10% of cases (Musher *et al.*, 1983; Pugh, 1985). This is particularly so in the elderly population with underlying disease, old pulmonary tuberculosis and asthma. This is also observed in this study and previously healthy people may also get the infection.

H. influenzae infections occur worldwide, affecting all ages with infants being the most susceptible age group. Invasive diseases particularly meningitis tend to occur in infants due to their lack of natural immunity. We found that more than 50% of invasive infections in this age group were due to *H. influenzae* type *b*. In view of the high rate of morbidity and mortality as well as neurological deficit among the survivors, in addition to the increase of resistance towards the principle antibiotics, protection from infection by vaccination is advisable as the efficacy of the vaccine has been proven in many parts of the world.

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