

HAEMOPHILUS INFLUENZAE TYPE B STRAINS ISOLATED IN KUALA LUMPUR

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Summary

Eighty four strains of *Haemophilus influenzae* type b were isolated from 70 patients at the University Hospital, Kuala Lumpur, during a period of 6 years from September 1971 to August 1977. Forty one strains were recovered in pure cultures from 28 children between 3 months and 5 years of age. Twenty strains were isolated from CSF, 14 from blood, 3 from pleural fluid, 2 from joint aspirate and 1 each from the pus of a subdural abscess and a hand abscess. The remaining strains were recovered from mixed cultures, mainly from respiratory tract specimens of children and adults. Laboratory methods for the isolation, identification, serotyping and antibiotic susceptibility testing of these organisms are described. All the type b strains were susceptible to ampicillin and chloramphenicol. None of them produced β -lactamase.

Haemophilus influenzae is the aetiologic agent for a number of serious clinical infections, mainly in children. This small Gram-negative pleomorphic bacillus is a normal inhabitant of the upper respiratory tract. Most strains are non-capsulated. Six serotypes of encapsulated *H influenzae* are recognised and only type b is commonly responsible for severe clinical disease.¹ Pyogenic meningitis and acute epiglottitis are the most severe and common diseases caused by *H influenzae*. Otitis media, pneumonia, suppurative arthritis, pyogenic abscesses and cellulitis are also encountered but less frequently. The various disease patterns all appear to be local manifestations of Haemophilus septicaemia and more than one local manifestation may be present in a patient at the same time.^{2,3} *H influenzae* type b has been reported as the commonest cause of bacterial meningitis in children in North America.^{4,5} In Britain it is the second commonest cause of meningitis next to meningococcal infection.⁶ It has also been reported as the commonest cause of meningitis in Malaysian children.⁷

This is a report on the incidence, clinical significance and antibiotic susceptibilities of *H influenzae* type b strains isolated at the University Hospital, Kuala Lumpur between September 1971 and August 1977.

MATERIALS AND METHODS

Clinical specimens were cultured on ox blood agar and chocolate agar plates and incubated

overnight at 36°C in a candle jar. Strains of *H influenzae* were identified on the basis of: a) microscopic appearance in the Gram stain, b) colonial morphology on chocolate agar and lack of haemolysis on blood agar, and c) requirements for both X and V factors, using nutrient agar (Oxoid) and commercial disc containing the factors.

During the period of 6 years, 1810 strains of *H influenzae* were isolated. Strains recovered from clinical material other than sputum specimens and nasopharyngeal swabs were supplied by Drs K H Chai and S D Puthuchery.

Identification of type b strains

All freshly isolated strains of *H influenzae* were grown on Levinthal agar plates for 18 hours at 36°C. Capsulated strains showing bright iridescence, when viewed with obliquely transmitted light, were typed by the slide agglutination procedure using type specific antisera (Hyland Laboratories, California). Eighty four strains were identified as *H influenzae* type b. The strains were preserved for further tests by freezing Levinthal broth cultures at -70°C. Both Levinthal agar and broth were prepared according to the method described by Turk and May.³

Susceptibility Testing

Minimal inhibitory concentrations (MICs) were determined by an agar dilution method.⁸ The antibiotics tested were ampicillin, cephalo-

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ridine, chloramphenicol, erythromycin, penicillin, streptomycin and tetracycline. Serial two-fold dilutions of the antibiotics were made in sterile distilled water and the appropriate concentrations were added to chocolate agar held at 50°C in a water bath. Plates were poured immediately and used for testing the same day. Seventy isolates were tested, one from each patient. The test organisms were grown overnight in Levinthal broth and diluted to give a final inoculum of 10³ colony forming units. Using a phage multiple inoculator, each antibiotic plate and a control (without antibiotic) were spot inoculated with 25 strains. The MIC was considered the lowest concentration of the antibiotic inhibiting visible growth of *H influenzae* after overnight incubation at 36°C.

All the strains were tested for beta-lactamase production by the rapid iodometric procedure of Catlin.⁹ Two beta-lactamase producing strains of *H influenzae* type b, isolated in England and supplied by Dr D C Turk,

Radcliffe Infirmary, Oxford, were used as positive controls.

Patients

The 84 strains of *H influenzae* type b were isolated from 70 patients. Data provided on the bacteriology request forms and inpatients' case notes were analysed for age, sex, ethnic group and clinical diagnosis.

RESULTS

The clinical sources of the *H influenzae* type b strains are presented in Table 1. These strains represented 4.6% of all isolates of *H influenzae* in this hospital during the period of study. Forty one strains were recovered as pure cultures in 28 children and were of aetiological importance. Two isolates from different specimens were obtained from each of 12 patients and three isolates, again from different specimens, were obtained from one child. Forty three strains were recovered in mixed cultures from children and adults, mainly from the respiratory tract. One strain was recovered to-

TABLE I
CLINICAL SOURCES OF *H INFLUENZAE* TYPE B
STRAINS ISOLATED BETWEEN SEPTEMBER 1971 AND
AUGUST 1977

Source	Number of strains*
PURE CULTURES	41
CSF	20
Blood	14
Pleural fluid	3
Joint aspirate	2
Pus (subdural abscess)	1
Pus (hand abscess)	1
MIXED CULTURES	43
Blood	1
Ear discharge	1
Tracheal aspirate	3
Nasopharyngeal swabs	8
Sputum	30
Total	84

Total number of patients = 70

*Single strains were recovered from 57 patients
Multiple strains were recovered from 13 patients
from blood and CSF (10)
Blood and pleural fluid (2)
Blood, CSF and pleural fluid (1)

gether with *Streptococcus pneumoniae* from the blood culture of a child whose pleural fluid yielded pure growth of *H influenzae* type b. The strain recovered from the purulent ear discharge of a child with otitis media was isolated as the predominant organism on two occasions and was considered clinically significant. The other organism isolated from the ear discharge was a diphtheroid bacillus. Among the other strains recovered in mixed cultures were 10 strains from the respiratory tract of patients with pneumonia, including a child aged 10 months. Unfortunately, blood cultures had not been done and these strains could not be clearly established as aetiologic agents of the pneumonia. All the remaining patients had some form of acute respiratory symptoms prior to the recovery of *H influenzae* type b strains from their respiratory tract secretions. Five of the nasopharyngeal strains were recovered from older siblings of two of the children with meningitis.

Twenty eight strains (33%), all from sputum specimens were isolated from patients between 13 and 70 years of age. The other 56 strains were recovered from children below 5 years of age. Forty nine (70%) of the patients were males.

Table 2 present some features associated with severe *Haemophilus* infections in young children in this study. There were 21 cases of meningitis with 2 cases having pneumonia as well. Only 12 (57%) of these meningitis cases had bacteriologically confirmed septicaemia. In 1 case the organism was recovered from blood

but not from cerebrospinal fluid. All 5 cases with pneumonia had proven septicaemia and 3 also had empyema. However, no organism was recovered from blood cultures of the 2 patients with septic arthritis of the hip. Twenty (71%) of the patients were between 6 and 11 months of age. The youngest was 3 months old and the oldest over 4 years old. More males were affected than females. There were only 3 Chinese and one Caucasian while the rest were Malays and Indians.

The cumulative percentages of strains inhibited by increasing two-fold concentrations of the 7 antibiotics are shown in Table 3. All strains were inhibited by 0.5 mg/l of ampicillin, chloramphenicol, penicillin and erythromycin, indicating that these strains were very sensitive to all 4 antibiotics. The strains were also sensitive to streptomycin (MICs of 0.5 to 2.0 mg/l). With tetracycline, 66 strains (94%) were inhibited by 0.5 mg/l or less, while the other 4 strains had MICs of 4 to 16 mg/l and were considered resistant. These tetracycline resistant strains were recovered from the respiratory tract secretions of 3 adults and one child. Cephaloridine was less active against *H influenzae* with MICs ranging from 0.5 to 16 mg/l and a median inhibitory concentration of 4 mg/l. None of the strains produced beta-lactamase.

DISCUSSION

The spectrum of severe *Haemophilus* infections presented here is similar to those reported from other countries.^{2,3} However there was no case

TABLE III
CUMULATIVE PERCENTAGE OF 70 *HAEMOPHILUS INFLUENZAE*
TYPE B STRAINS INHIBITED BY ANTIBIOTICS

Antibiotic	MIC (mg/l)							
	0.12	0.25	0.5	1.0	2.0	4.0	8.0	16.0
Ampicillin	41	99	100					
Chloramphenicol	13	97	100					
Penicillin	10	96	100					
Erythromycin	4	49	100					
Streptomycin	3	10	30	81	100			
Tetracycline	9	89	94	94	94	97	99	100
Cephaloridine			3	20	39	76	97	100

TABLE II
SOME FEATURES ASSOCIATED WITH SEVERE HAEMOPHILUS INFECTIONS

Clinical Illness	No. Cases	Age (months)				Sex		Ethnic Group			
		0-5	6-11	12-23	24-60	M	F	Malay	Indian	Chinese	Others
Meningitis	19	2	12	3	2	9	10	7	9	2	1
Meningitis and pneumonia	2	-	1	1	-	2	-	2	-	-	-
Pneumonia	3	1	1	1	-	3	-	2	-	1	-
Septic arthritis (hip)	2	-	2	-	-	-	2	1	1	-	-
Subdural abscess	1	-	-	1	-	1	-	-	1	-	-
Abscess (hand)	1	-	1	-	-	-	1	-	1	-	-
Total	28	3	17	6	2	15	13	12	12	3	1

of acute epiglottitis. This potentially fatal infection is not uncommon in other countries and is often unrecognized unless there is local and personal awareness of its existence.¹⁰ Blood cultures are necessary for the laboratory diagnosis of this condition.

Antibiotic susceptibility testing of *H influenzae* requires special media and careful control of test conditions.^{11,12} The single disc diffusion method may yield unreliable results unless carefully standardized. The type of medium used and the size of the inoculum particularly affects the results obtained. Disc potencies used were usually 10 µg for ampicillin and 30 µg for chloramphenicol. Some authors, however, recommend the use of 2 µg disc of ampicillin in preference to a larger disc which may give spurious results.¹³ All disc tests should be confirmed by MIC methods. The agar dilution method was most useful for quantitative susceptibility testing of *H influenzae* when the inoculum size was kept small.^{11,12}

The range of MICs obtained in this study are, in general, similar to those reported in the literature.^{8,14,15,16} All fully sensitive strains of *H influenzae* type b had MICs of 1 mg/l or less for ampicillin and chloramphenicol. These concentrations are well within attainable blood levels after standard doses of the drugs.¹⁴ Ampicillin is the best single drug for initial treatment of bacterial meningitis because of its broad efficacy and relative nontoxicity. However, strains of *H influenzae* type b resistant to ampicillin have been recognised recently in many parts of North America and Europe.¹⁷ The ampicillin resistant strains had MICs of 4 to 128 mg/l. They were all sensitive to chloramphenicol, the alternative drug of choice. Many physicians, however, are reluctant to use chloramphenicol because of its potential haematologic toxicity. To date, only 2 chloramphenicol resistant strains of *H influenzae* type b have been reported in the literature.^{18,19} Both strains were fully sensitive to ampicillin. These 2 strains had MICs of 50 and 32 mg/l, respectively, for chloramphenicol.

The incidence of ampicillin resistant strains in the USA and in Britain has been reported to range from 1.5 to 11% depending on the geographical location.¹³ Most of these strains have been shown to produce beta-lactamase which destroys penicillin, ampicillin and cephalosporins.^{20,21} This beta-lactamase enzyme has

been characterized and found to resemble enzymes present in other species of Gram-negative bacilli. Its production has been shown to be R-factor mediated and to be transferred within the genus and to other susceptible genera.²² Thus, although ampicillin resistant type b strains were not encountered in this hospital in the period covered by this study, the possibility that they may occur in the near future cannot be ignored.

It has been recommended that the choice of antibiotic therapy in systemic *Haemophilus* infections be predicted on local experience with ampicillin resistance and upon whether facilities are available to detect resistant strains.¹⁷ A rapid beta-lactamase test can be performed at the time of primary isolation on chocolate agar.⁹ This allows for early selection of appropriate therapy with ampicillin or chloramphenicol. When the isolates prove to be ampicillin sensitive, many physicians prefer to use this safer drug. Ampicillin combined with chloramphenicol has been used for initial therapy of suspected *Haemophilus* meningitis in areas where ampicillin resistant strains are endemic. Recently, it was reported that a combination of ampicillin and chloramphenicol therapy in *Haemophilus* meningitis may increase the risk for long term sequelae.²³ Therefore, when both antibiotics are used in initial therapy, one drug should be discontinued as soon as the antibiotic sensitivity of the strain is known, provided the strain is fully sensitive to one or both antibiotics.

Even with sensitive organisms, treatment failures of *Haemophilus* infections have been reported.¹⁸ It is important that patient response be closely monitored regardless of the chemotherapeutic agent used. Continued surveillance of *H influenzae* type b strains and their susceptibility to antibiotics should be carried out wherever and whenever possible.

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