Carriage of *Haemophilus influenzae* in Cape Town children

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Abstract **Little is known about the epidemiology of *Haemophilus influenzae* infections in South Africa. This study was designed to determine the prevalence, serotype distribution, antimicrobial susceptibility pattern and effect of age and hospitalisation on the carriage of *H. influenzae* in 322 Cape Town children.**

The overall and type b specific carriage rates in normal children (N = 107) were 45,8% and 4,7% respectively. The yield following nasopharyngeal culture was twice that following throat culture (P < 0,001). Children hospitalised with tuberculosis (N = 62) had significantly greater carriage rates, 66,1% and 37,1% respectively (P = 0,02). Institutionalised mentally handicapped children (N = 77) and children with tuberculosis attending an outpatient clinic (N = 76) had lower carriage rates (P < 0,02). Antimicrobial resistance was a major problem only in children hospitalised with tuberculosis (rifampicin 100%, penicillin 43,9%, erythromycin 85,4%, co-trimoxazole 82,9%). This universal resistance to rifampicin has not been reported previously. There was no difference in the mean age of children with positive or negative cultures, with the exception of those hospitalised with tuberculosis. In this group children infected with type b were much younger (mean 19,7 months) than those with other and non-typeable infections (32,1 months) and the non-infected (50,1 months) (P = 0,04). Duration of hospitalisation or outpatient therapy in the patients with tuberculosis did not influence carriage rates.

We conclude that carriage of *H. influenzae* in normal children is similar to that reported from other countries and that carriage, particularly of type b, in children hospitalised with tuberculosis was of significance and probably contributed to an outbreak of multi-resistant invasive *H. influenzae* disease in this group.

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Haemophilus influenzae is a major cause of severe bacterial disease (including meningitis, septicaemia, arthritis, epiglottitis and respiratory tract infections[1]) in infants and young children throughout the world. Although the pathogenesis of invasive disease is not entirely clear, one of the prerequisites is colonisation and infection of the nasopharynx.[2] The organism is a commensal of the upper respiratory tract and most children will have been infected by the age of 5 years. Surveys in open communities have shown that 25 - 80% of children are carriers of *H. influenzae*, with the encapsulated serotypes (i.e. types a - f) accounting for between 3% and 25% of all isolates.[1-4] Carriage of type b, which causes most cases of invasive disease, is generally below 5%. However, in closed communities, such as institutions, carriage of type b has been reported in as many as 70% of children.[5,6]

The epidemiology of *H. influenzae* is not well described in South Africa and there are no published data on nasopharyngeal carriage. The objectives of the study were to determine the prevalence of *H. influenzae* carriage in Cape Town children and the serotypes and antibiotic susceptibility of the isolates, and to ascertain whether there was a difference in the culture yield after nasopharyngeal or throat cultures and whether there was a relationship between age, duration of hospitalisation and carriage.

Methods

Four groups of children were studied: 107 normal children attending a well-baby clinic, 77 children in an institution for the mentally handicapped (the handicapped group), 62 children hospitalised with tuberculosis (TB inpatients) and 76 children with tuberculosis attending a community outpatient clinic (TB outpatients). All the children were from a similar poor socio-economic background. Children with tuberculosis were included in the study because during the months before the study there were a few cases of invasive disease in those hospitalised. Handicapped children were chosen as a control group for the hospitalised tuberculosis patients.

Nasopharyngeal and throat swabs were taken from all children. The nasopharyngeal swabs were obtained by passing a Dacron-tipped thin wire swab through the nostril and along the floor of the nasal cavity until it touched the posterior nasopharyngeal wall, where it was left for about 5 seconds and then removed. Throat swabs were taken by sweeping another swab across the oropharyngeal wall and over the tonsils. The swabs were then immediately plated onto Columbia agar (Oxoid, UK) and transported to the laboratory for processing. *Haemophilus* species were identified by colonial morphology, Gram stain, oxidase reaction, growth requirements for factors X and V and the satellite phenomenon. Beta-lactamase production was detected using the chromogenic cephalosporin method (Nitrocefin; Oxoid, UK). Capsular serotyping was performed by slide agglutination according to the manufacturer's recommendations (Wellcome Diagnostics, UK). The minimum inhibitory concentration (MIC) for antibiotics was performed using an agar dilution method as described by Cavanaugh *et al.*[7] Resistance was defined according to the following MIC values: penicillin $\geq$ 4 mg/l (the National Committee for Clinical Laboratory Standards recommendation for ampicillin), choramphenicol $\geq$ 8 mg/l, rifampicin $\geq$ 4 mg/l and trimethoprim-sulphamethoxazole $\geq$ 4/76 mg/l. Antibiotic resistance to tetracycline, gentamicin and cefotaxime was tested using disc sensitivity methods (Mast, UK).

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Statistical methods

Data were analysed by computer with the Epi-Info program, version 5. Continuous data were compared using the non-parametric Kruskall-Wallis test and categorical data using the χ^2-test.

Results

H. influenzae (a positive nasopharyngeal or throat swab) was isolated in 129 (40,0%) of the 322 children (Table I). In normal children the carriage rate was 45,8%. This was higher than the rate in the TB outpatients (P = 0,02) and the handicapped children (P = 0,003) but lower than that in the TB inpatients (P = 0,02). The rate in the latter was also higher than the rate in the TB outpatients (P < 0,001).

Serotyping of the *H. influenzae* isolated from these 129 children revealed that 85 (65,9%) were non-typeable, 30 (23,3%) were type b, 6 (4,7%) were other types (1 type a, 5 type e), and 8 (6,2%) were untypeable (i.e. organisms died before typing). Twenty-three of the 30 type b cultures (76,7%) were isolated in the TB inpatients. The type b specific carriage rate in this group was 37,1%. This was significantly higher than the type b carriage rates in the TB outpatients and the normal children (2,6% and 4,7% respectively). No type b isolates were found in the handicapped group.

Numbers of positive and negative cultures after nasopharyngeal and throat swabs are shown in Table II. The yield after nasopharyngeal culture was significantly higher (twofold) than that after throat swabs (P < 0,001). Sixty-six per cent of the children with a positive nasopharyngeal swab had a negative throat swab, while only 26% with positive throat swabs had negative nasopharyngeal swabs.

TABLE II.
Comparison of *H. influenzae* isolation after nasopharyngeal and throat swabs

	Throat swab		
	Pos.	Neg.	Total
Nasopharyngeal swab			
Pos.	40	77	117
Neg.	14	191	205
Total	54	268	322

P < 0,001.

Antimicrobial resistance was of significance only in the patients with tuberculosis (Table III). The TB inpatients and outpatients had rates of resistance to rifampicin of 100% and 42,9% respectively. The TB inpatients had a penicillin resistance rate of 43,9%, while the rate in the outpatients was no different to that in the normal children (4,8% and 4,1% respectively). Resistance rates of the type b and the non-b organisms cultured from the TB inpatients did not differ, except for penicillin, to which 15 of 23 (65,2%) type b and 3 of 18 (16,7%) non-b organisms were resistant (P = 0,005). No resistance to chloramphenicol, cefotaxime or tetracycline was documented in any group.

There was no difference between the mean ages of the children with positive *H. influenzae* cultures and those of the children with negative cultures (Table IV). However, in the TB inpatients the mean age differed according to the serotype; children with type b infections were much younger (19,7 ± 12,9 months) than those with non-typeable and non-b infections (32,1 ± 19,5 months) and those who had negative cultures (50,1 ± 52 months) (P = 0,04). There was also no relationship between the rate of positive cultures and increasing age (Table V).

TABLE I.
Carriage of *H. influenzae* in various groups of children

	All		Normal		Handicapped		TB inpatients		TB outpatients	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total	322		107		77		62		76	
Positive cultures	129	40,0	49	45,8	18	23,4	41	66,1	21	27,6
Type b	30	9,3	5	4,7	0		23	37,1	2	2,6
Other	6	1,9	1	0,9	0		1	1,6	4	5,3
Non-typeable	85	26,4	39	36,4	18	23,4	15	24,2	13	17,1
Untypeable*	8	2,5	4	3,7	0		2	3,2	2	2,6

* Organisms died before typing.

TABLE III.
Antimicrobial resistance profile of *H. influenzae*

	Normal		Handicapped		TB inpatients		TB outpatients	
	No.	%	No.	%	No.	%	No.	%
Positive cultures	49		18		41		21	
Resistant to								
Rifampicin	0		0		41	100	9	42,9
Penicillin/ampicillin	2	4,1	0		18	43,9	1	4,8
Erythromycin	2	4,1	6	33,3	35	85,4	1	4,8
Co-trimoxazole	0		0		34	82,9	0	
Gentamicin	0		0		23	56,1	0	

TABLE IV.
Age distribution (mo.) of children with positive and negative cultures of *H. influenzae*

	Positive culture		Negative culture		
	Mean ± SD	Median	Mean ± SD	Median	*P*
All children	38 ± 36	25	47 ± 44	33	0,08
Normal children	19 ± 18	15	16 ± 15	12	0,35
TB inpatients	30 ± 28	22	50 ± 52	24	0,63
TB outpatients	36 ± 22	32	32 ± 17	27	0,68
Handicapped	100 ± 33	96	92 ± 40	102	0,48

TABLE V.
Age distribution of children with positive *H. influenzae* cultures

	All			TB inpatients			Normal			TB outpatients			Handicapped		
Age (mo.)	No.	Pos.	%	No.	Pos.	%	No.	Pos.	%	No.	Pos.	%	No.	Pos.	%
< 12	78	34	44	18	10	56	52	22	42	6	2	33	2	0	0
13 - 24	70	30	43	14	11	79	28	13	46	28	6	21	0	0	0
25 - 36	45	22	49	11	10	91	16	8	50	13	4	31	5	0	0
37 - 48	39	12	31	5	4	80	6	3	50	20	4	20	8	1	12
49 - 60	14	6	43	1	1	100	4	2	50	2	1	50	7	2	29
61 - 72	16	9	56	4	2	50	0	0	0	5	3	60	7	4	58
> 72	60	16	27	9	3	33	1	1	100	2	1	50	48	11	23
Total	322	129	40,1	62	41	66,1	107	49	46	76	21	28	77	18	23

In the children who were hospitalised with tuberculosis, the duration of hospitalisation did not influence carriage rates. Duration of hospitalisation in children with type b carriage was 52,7 ± 55,5 days compared with 78,1 ± 42,9 days in children infected with other types and 78,3 ± 51,6 days in the non-infected children ($P = 0,09$). In addition, duration of therapy in the TB outpatients had no influence on carriage rate, 68 v. 71 days in the positive and negative carriers respectively ($P = 0,94$).

Discussion

This study found that the carriage rates of *H. influenzae* and specifically type b *H. influenzae* in normal children were 45,6% and 4,7% respectively. This is comparable with other surveys of normal children, which showed carriage rates of 25 - 80% and 2 - 15% respectively.[1-4] The overall rates in the institutionalised handicapped children (23,4%) and in the children attending a tuberculosis clinic (27,6%) were about half that of the normal chidren, and this may be due to the fact that the handicapped children and those attending the tuberculosis clinic were significantly older. Children hospitalised with tuberculosis had an overall rate (66,1%) and a type b rate (37,1%) significantly higher than rates for normal children. This may have been the result of nosocomial infection in children who have been hospitalised for long periods and who may have had some degree of immunosuppression as a result of the tuberculosis infection. Type b infections may have been absent in the handicapped children because they were significantly older than the others (mean age 96 months).

There has been a debate as to which method of specimen collection is the most efficient for the isolation of *H. influenzae*. Some studies have found no difference in the culture yield when comparing nasal with throat swabs,[8] while others have found throat swabs to be superior.[9] We found that nasopharyngeal swabs gave a significantly higher rate of positive cultures.

Antibiotic resistance was not of any importance in the normal children. The rates for penicillin and erythromycin (both 4,1%) were much lower than those reported in most other studies from both developed and developing countries.[10-14] However, antibiotic-resistant strains were extremely prevalent in the children hospitalised with tuberculosis. The universal resistance to rifampicin in these children has not been reported previously. This is extremely distressing, because rifampicin is the antibiotic of choice for chemoprophylaxis to prevent secondary cases of invasive *Haemophilus* infection[15] and has been used to eradicate multiple drug-resistant isolates.[16] Nosocomial cases of invasive type b disease had occurred sporadically among the children hospitalised with tuberculosis both before and after the study was completed. The prevention of disease in this context therefore poses a dilemma. Use of ciprofloxacin[17] as a chemoprophylactic agent is being considered (keeping in mind the possibility of side-effects such as arthropathy[18]), as is implementation of a policy of immunisation[19] with one of the conjugate vaccines in an attempt to control nosocomial outbreaks in this group of children.

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