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Pneumococcal carriage amongst children in Adelaide, South Australia

By D. HANSMAN and SYLVIA MORRIS

Department of Microbiology, Adelaide Children's Hospital, North Adelaide, South Australia.

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SUMMARY

Amongst 1267 healthy children 6 months to 4·5 years of age in Adelaide, the pneumococcal carriage rate from a single nasal swab sampling was 29% in the period 1980–1. Of 269 children, sampled monthly on five occasions, 91% carried a pneumococcus on one or more occasions: 55% carried a single type, 33% carried two types, 2% carried three types and 1% carried four types; 18% carried a pneumococcus on either 4 or 5 occasions. The commonest types encountered were types 6, 19 and 23 in that order, and these three types constituted 57% of the total: other common types (> 5% of the total) were types 14, 15 and 11, and the six commonest types constituted 77% of the total. Of these, types 6, 14, 19 and 23 commonly cause systemic disease in children; on the other hand types 11 and 15 cause disease infrequently. The number of strains showing antimicrobial drug resistance was low: on quantitative testing 0·7% of 291 isolates examined showed relative resistance to benzylpenicillin and 0·7% were resistant to tetracycline; 10·9% of 230 isolates examined showed resistance to co-trimoxazole; dual or multiple drug resistance was not detected, and all isolates tested were susceptible to chloramphenicol, erythromycin, lincomycin and rifampicin.

INTRODUCTION

Since the 1960s, with the realization that pneumococcal disease had not disappeared with the advent of penicillin and other antimicrobial drugs, there has been a renewed interest in the pneumococcus and in pneumococcal infections. The appearance of drug-resistant pneumococci, especially pneumococci showing resistance to tetracycline or erythromycin, relative resistance to penicillin or mulitple drug resistance, was a factor which encouraged the re-development of pneumococcal polysaccharide vaccines and the re-investigation of the mechanisms whereby pneumococci colonize and invade the human host. There have been fresh studies also on the epidemiology of pneumococcal diseases, including the relationship of upper respiratory carriage to infection (Gray, Converse & Dillon, 1980). The conduct of clinical trials in Adelaide, in which the efficacy of a 14-valent pneumococcal vaccine in preventing respiratory infections in children was tested, gave us an opportunity to study pneumococcal carriage amongst the healthy children who were the subjects of the trial (Douglas *et al.* 1983). These

carriage studies form the substance of this report. The aims of this study were to ascertain the carriage rate, serotype distribution and antimicrobial drug susceptibility pattern of the pneumococci isolated.

MATERIALS AND METHODS

A total of 1267 healthy children living in the north-eastern suburbs of Adelaide, who were aged between 6 months and 4·5 years and whose parents agreed to their participation in this study, had one or more nasal swabs collected. The initial swab was collected on the same day as pneumococcal vaccine or placebo was administered, during the period August 1980 to April 1981. In addition, 327 of these children were to have nasal swabs collected monthly for five consecutive months, beginning 4–9 months after their entry into the study. Both anterior nares were swabbed with a single swab which was inoculated onto a blood agar plate and incubated overnight at 37 °C in an atmosphere containing added carbon dioxide. Next morning the plates were examined for colonies resembling pneumococci and suspect colonies were tested for susceptibility to optochin. Isolates showing optochin-susceptible colonies were identified as presumptive pneumococci and sent to the Microbiology Department, Adelaide Children's Hospital, where the isolates were tested as follow.

Pneumococci were serotyped by the capsular reaction using sera obtained from the State Serum Institute, Copenhagen. Antimicrobial susceptibility tests were done on blood agar using a Mastring (Mast Laboratories Limited) containing benzylpenicillin 0·6 μg (1 unit), tetracycline 10 μg, chloramphenicol 10 μg, erythromycin 5 μg, lincomycin 5 μg, rifampicin 2 μg, co-trimoxazole 25 μg and optochin 5 μg (Hansman *et al.* 1985). Susceptibility to co-trimoxazole, benzylpenicillin and tetracycline was tested quantitatively by plate titration (Hansman *et al.* 1985).

Of the initial 301 isloates in the study, 294 (98%) were typable. Of these 294 isolates 291 (99%) were tested by disk diffusion and also quantitatively for susceptibility to benzylpenicillin and to tetracycline, and 230 isolates (78%) were tested quantitatively for susceptibility to co-trimoxazole. Criteria for drug resistance were similar to those used in our previous studies. Pneumococci are normally inhibited by $\leqslant 0{\cdot}02$ μg benzylpenicillin/ml, $\leqslant 1{\cdot}0$ μg tetracycline/ml, $\leqslant 5{\cdot}0$ μg co-trimoxazole/ml. Pneumococci relatively resistant to penicillin show MIC values in the range 0·1–1·0 μg benzylpenicillin/ml and resistant to penicillin $\geqslant 2{\cdot}0$ μg benzylpenicillin/ml. Pneumococci relatively resistant to tetracycline show MIC values in the range 2–5 μg tetracycline/ml and resistant to tetracycline $\geqslant 10$ μg tetracycline/ml. Pneumococci relatively resistant to co-trimoxazole show an MIC value of 10 μg co-trimoxazole/ml and resistant to co-trimoxazole $\geqslant 20$ μg co-trimoxazole/ml.

RESULTS

Expressed as the proportion of children who were shown to be pneumococcal carriers on initial sampling, the carriage rate was 29% (368 of 1267 children). When derived similarly, the predominant serotypes of pneumococci were types 6,

Table 1. *Pneumococcal nasal carriage in Adelaide: serotypes of pneumococci in order of prevalence**

Rank	Serotype	No.	%	Cumulative %
First	6	83	22·6	22·6
Second	19	66	17·9	40·5
Third	23	58	15·8	56·3
Fourth	14	36	9·8	66·0
Fifth	15	22	6·0	72·0
Sixth	11	19	5·2	77·2
Seventh	9	16	4·4	81·5
Eighth	18	15	4·1	85·6
Ninth	21	7	1·9	87·5
Tenth	33	7	1·9	89·4
Other types	†	39	10·6	100·0
Total		368		100·0

* These figures only include typing results for pneumococci isolated on initial sampling (see results).

† Other types encountered were 3, 4, 10, 13, 16, 17, 20, 22, 24, 31, 34, 35, 37, 38.

Table 2. *Pneumococcal carriage Adelaide children.*

	Persistence of carriage (see text) Months*						
Category	1	2	3	4	5	Total	%
Carriage of same type	70	37	23	12	5	147	54·7
Carriage of two or more types							
2 types	0	26	36	15	12	89	33·1
3 types	0	0	4	2	0	6	2·2
4 types	0	0	0	1	1	2	0·7
Sub-total (all carriers)	70	63	63	30	18	244	90·7
	26·0%	23·4%	23·4%	11·2%	6·7%		
Non-carriage						25	9·3
Total						269	100·0

* This refers to the number of months, not necessarily consecutive, during which a pneumococcus was isolated on monthly nasal swabbing.

19 and 23 in that order, and these three types together constituted 56% of the total (Table 1). Also common (> 5% of the total) were types 14, 15 and 11, the five commonest and ten commonest types constituting 72% and 89% of the total, respectively. In all, 24 serotypes were encountered on initital sampling (Table 1). On second, third and subsequent samplings five additional serotypes were encountered (types 1, 5, 7, 8 and 28).

A complete set of five swabs was collected from 269 (82%) of the 327 children who were to have monthly nasal swabs collected for 5 months. As is shown in Table 2, 91% of these children carried a pneumococcus on one or more occasions. 'Persistent' carriers were defined as those who carried a pneumococcus on 4 or 5 occasions, irrespective of a change of serotype, and these constituted 18% of the total (Table 2). Seventeen (6%) of the persistent carriers had a single type and 31 (12%) carried two or more types during the period of the study. Carriage of more

Table 3. *Pneumococci from healthy children in Adelaide.*

Results of antimicrobial drug susceptibility tests by disk diffusion

Category	No.	%
Penicillin relatively resistant	2	0·7
Tetracycline relatively resistant	3	1·0
Tetracycline-resistant	2	0·7
Co-trimoxazole relatively resistant	2	0·7
Co-trimoxazole-resistant	17	5·8
Susceptible to all drugs tested	265	91·1
Total	291	100·0

Table 4. *Pneumococci from healthy children in Adelaide.*

Results of quantitative antimicrobial drug susceptibility tests*

Penicillin G			Tetracycline			Co-trimoxazole		
MIC	No.	%	MIC	No.	%	MIC	No.	%
< 0·01	70	24·1	< 0·2	114	39·2	< 1	9	3·9
0·02	211	72·5	0·5	172	59·1	2	153	66·6
0·05	8	2·7	1·0	2	0·7	5	31	13·5
0·1	0	0	2	1	0·3	10	12	5·2
0·2	2	0·7	5	0	0	20	11	4·8
—	—	—	10	1	0·3	50	10	4·4
—	—	—	20	1	0·3	100	4	1·7
Total	291	100·0		291	99·9		230	100·0

* MIC, minimal inhibitory concentration μg/ml. MIC values for controls: pneumococcus serotypes 1–4: penicillin $\leqslant$ 0·02, tetracycline $\leqslant$ 0·2–0·5, co-trimoxazole 2·0.

than three types was uncommon, and in no child was carriage of more than four types demonstrated.

The results of antimicrobial susceptibility tests are summarized in Table 3 (results of disk diffusion tests) and in Table 4 (results of plate titration tests). On disk-diffusion testing 0·7% of 291 isolates tested were relatively resistant to benzylpenicillin, 0·7% were resistant to tetracycline and 5·8% were resistant to co-trimoxazole. Pneumococci which showed relative resistance to penicillin (both isolates were type 6) had an MIC 0·2 μg benzylpenicillin/ml and tetracycline resistant pneumoccocci (types 3 and 9) MIC 10–20 μg tetracycline/ml. Of 230 isolates tested quantitatively 10·9% showed resistance to co-trimoxazole (types 6, 9, 14 and 23); these included some strains which had appeared susceptible on disk-diffusion testing; on the other hand most (11 of 13) strains which had appeared resistant on disk-diffusion testing gave MIC values in the range 20–100 μg co-trimoxazole/ml on quantitative testing, so confirming their resistance. Resistance was not detected to erythromycin or to the other drugs tested. No strain showed dual or multiple drug resistance.

DISCUSSION

In the present study of 1267 healthy Europoid (Caucasian) children aged 6 months to 4·5 years, the nasal carriage rate of pneumococci was 29% on a single

swabbing. This rate is similar to that encountered in young children in other westernized countries. For example, Hendley *et al.* (1975) encountered a rate of 38% amongst pre-school children in Virginia in 1972. Somewhat higher rates were found by Loda *et al.* (1975) amongst children in North Carolina 1970–4: for example, carriage rates were 47% in children aged 1 year and 52% in children aged 4 years; these children attended a day-care centre which may have accounted for the higher rates. An earlier study in the United Kingdom by Masters *et al.* (1958) showed carriage rates of about 50% amongst children in Paddington (London) in the 1950s.

What factors affect carriage rates of pneumococci? Possible factors are the age of the subject, the season of the year, socio-economic factors and climate; of these age is important. The detailed studies by Gray, Converse & Dillon (1980) in Alabama, who followed pneumococcal carriage in children from birth to 2 years of age, showed that the age at which infants first acquired a pneumococcus ranged from 4 days to 18 months, with a mean age of acquisition of 6 months. North American studied have shown a peak carriage rate at about 2 years of age. Amongst children studies by Loda *et al.* in North Carolina carriage rates declined from about 50% in children aged 2 to 30% in 7-year-old children and to between 12% and 25% in children aged 8–10 years.

Other factors which undoubtedly affect carriage are living conditions, which include standards of housing and especially the degree of crowding. Exposure to other young children may be particularly important. Whilst the children in the present study came from families of socio-economic classes 4 and 5, most lived in well-maintained, detached houses and the children were well nourished and cared for. This may account for a lower carriage rate (29%) than was found in some other studies, for example in Paddington in the 1950s, where a carriage rate of 50% was encountered amongst families who lived in more crowded conditions.

There is some evidence that seasonal factors affect carriage rates. For example in Gray's study (Gray, Converse & Dillon, 1980) acquisition and carriage rates tended to be higher during the winter. As yet we do not know whether the seasonal temperature is important *per se* or whether the longer time spent indoors in winter is the factor responsible. Does climate affect carriage rate? High carriage rates (exceeding 50% in children) have been encountered in Papua New Guinea, which has a hot and humid (tropical) climate throughout the year (Hansman, 1972). A high rate (89% in children) has been met with amongst Australian aborigines in Central Australia with its hot temperate climate and low humidity (Hansman *et al.* 1985). While further information is required on this aspect of carriage, including data on the carriage rates of children living in cold climates, it is probable that carriage rates are more closely related to living conditions, especially the degree of crowding, than to climate *per se*.

Of the pneumococcal serotypes encountered in the present study, types 6, 14, 19 and 23 were especially common. These types often cause disease in children, including children of the age group studied. Thus in Australia systemic pneumococcal infections in children (bacteraemia, bacteraemic pneumonia and meningitis) are caused principally by types 14, 18, 6, 19 and 23 (in that order) and cases of otitis media are caused principally by types 19, 3, 6 and 23 (Hansman, 1983). Neither type 18, which is an important cause of systemic disease in children

(especially meningitis) nor type 3, which is a leading cause of otitis media, were commonly carried. On the other hand, types 11 and 15, which rarely cause disease, were fairly common amongst the carriers. Thus, whilst there was some concordance between common disease types and carrier types, there were several notable exceptions. The types which were commonly carried by Adelaide children are also commonly carried amongst children in North America: thus Loda *et al.* (1975) found that types 6, 23 and 19 predominated amongst young children in North Carolina. Although a much higher carriage rate was found amongst aboriginal children in Alice Springs, the predominant serotypes were similar (types 6, 19 and 23); however, type 14 was commoner in Adelaide and type 22 in Alice Springs.

In the present study almost one-fifth of the children were shown to be 'persistent' carriers of pneumococci (Table 2). However, most were either transient or intermittent carriers. About one-tenth of children were consistently non-carriers. We do not know whether non-carriage resulted because of the lack of the child's exposure to carriers or because of an individual's resistance to the carrier state. For other aspects of carriage the reader is referred to the study by Gray and his colleagues who demonstrated, *inter alia*, the important finding that there was a 15% risk of pneumococcal disease developing during the first month of the carrier state.

The number of strains showing antimicrobial drug resistance was low in the present study: strains which were relatively resistant to penicillin or resistant to tetracycline comprised $< 1\%$. However, 11% of pneumococci tested were resistant to co-trimoxazole. Resistance to chloramphenicol or erythromycin was not encountered. Similar findings have been encountered in pneumococci isolated from children and adults with pneumococcal infections in Australia (Hansman, unpublished data). On the other hand, the 1981 study of pneumococci isolated from Australian aborigines in Alice Springs, Northern Territory, showed a high prevalence of drug resistance: about 25% of pneumococci isolated from children were resistant to one or more antimicrobial drugs (Hansman *et al.* 1985).

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