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Pneumococcal carriage amongst Australian aborigines in Alice Springs, Northern Territory

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SUMMARY

In Alice Springs and its vicinity, a single nasal swab was collected from 282 Australian aborigines in May 1981 to determine nasal carriage rates of pneumococci. Each swab was inoculated on blood agar and on gentamicin blood agar. The carriage rates were 89 % in children, 39 % in adolescents and 34 % in adults. In all, 27 serotypes of pneumococci were met with and 15 (4 %) of subjects yielded two or more serotypes. In children, types 23, 19, 6, 22 and 6 were predominant (in that order), whereas type 3 was commonest in older subjects. Approximately 25 % children and 5 % adults yielded drug-insensitive pneumococci. Resistance to benzylpenicillin, tetracycline and co-trimoxazole was met with, resistant pneumococci showed five resistance patterns and belonged to nine serotypes, predominantly types 19 and 23. All isolates were sensitive to chloramphenicol, erythromycin, lincomycin and rifampicin. The carriage rate of drug-insensitive pneumococci was 100-fold higher amongst children sampled than in non-aboriginal children in Australia.

INTRODUCTION

Australian aboriginal children often suffer from chronic lung disease, especially bronchiectasis, probably as a consequence of repeated attacks of respiratory infection, including pneumonia. The prevalence of chronic otitis media is also high: thus Kamien (1975) showed that 60 % of aboriginal children in Bourke, in western New South Wales, showed opaque, perforated, retracted or scarred tympanic membranes, and detected some degree of deafness in at least one ear in 35 % of aboriginal children, compared with 4 % of Caucasian children in the same region. Careful microbiological studies to establish the aetiology of these infections are lacking.

Encapsulated, potentially pathogenic pneumococci are often carried in the human upper respiratory tract. Surveys amongst healthy subjects have shown that the carrier rate is higher amongst children, especially young children, than it is amongst adults and that, at least in young children, nasal carriage is commoner than is throat carriage (Masters *et al.* 1958). Occasionally, two or more (as many

as six) serotypes are demonstrable in the same individual, especially if repeated sampling is done (Gray, Converse & Dillon, 1980). Carriage of the same serotype may persist for weeks or months (Loda *et al.* 1975). Although high carrier rates have been found in tropical regions, it is likely that the carrier rate in a community is more closely related to living conditions and to standards of hygiene than it is to climate (or to race).

Little is known of the carriage rates of potentially pathogenic bacteria, including pneumococci, amongst aborigines. Although some of the first isolates of penicillin-insensitive pneumococci were from aborigines, few studies to ascertain the prevalence of drug-resistant strains have been done. The licensing of pneumococcal vaccine in Australia in 1981 and the imminence of a controlled study of its efficacy in preventing infections gave us an opportunity to conduct a pilot study of pneumococcal carriage amongst aborigines.

MATERIALS AND METHODS

During a 5-day period, 18–22 May 1981, we collected nasal swabs from aborigines living either in Alice Springs or in aboriginal settlements within 50 km of the town, including Amoonguna and Jay Creek. Although most of them lived in brick houses and had access to running water, the houses had been poorly maintained. Flies were often seen on the children's faces, feeding on nasal and ocular secretions. Alice Springs has a hot, dry climate with a rainfall of 246 mm per annum; however, at the time of our visit the weather was mild (minimum 3 °C, maximum 24 °C). A single cotton-wool swab was used to sample both anterior nares, the swab was then inserted into Stuart's Transport medium (TM), which was held at environmental temperature (usually about 15 °C) until plating was carried out, usually about 3–5 h after collection (also see below). Each swab was plated on horse blood agar (BA) and on horse blood agar containing gentamicin 5 μm per ml (GBA); the media were incubated in a jar containing a tube of water and added carbon dioxide. Pneumococci were preserved temporarily in semi-solid blood agar and then transported to Adelaide, where serotyping and antimicrobial susceptibility tests were done.

In order to ascertain how many subjects carried two or more serotypes of pneumococci, 25 of the original nasal swabs were replated and clones from four separate colonies serotyped. The survival of pneumococci in TM was studied by replating the original swab (stored in TM) from 30 subjects after its refrigeration from 3 to 14 days.

Pneumococci were typed by the capsular reaction using sera from the State Serum Institute, Copenhagen. Antimicrobial susceptibility tests were done on BA 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. The inoculum was a 4 h culture in serum broth diluted approximately 1/50 in buffered saline, pH 7·3, this usually yielded semiconfluent growth. The plates were incubated overnight in a jar with added Carbogen (5 % carbon dioxide in oxygen). With selected strains of pneumococci, which had shown various degrees of susceptibility to co-trimoxazole (Table 5), disk diffusion tests were done on BA and Isosensitest BA, using individual discs of co-trimoxazole (25 μg).

Table 1. *Pneumococcal nasal carriage in Australian aborigines in Alice Springs*

	Subjects sampled		Subjects yielding pneumococci		
Category	No.	(%)	No.	(Isolates)	(%)
Children (< 14 years)	174	(62%)	155	(169)	(89%)
Adolescents (14–20 years)	23	(8%)	9	(10)	(39%)
Adults (⩾ 21 years)	85	(30%)	29	(30)	(34%)
Total	282	(100%)	193	(209)	(68%)

Susceptibility to co-trimoxazole, penicillin and tetracycline was tested quantitatively by plate titration. For tests with co-trimoxazole we used Bactrim for Infusion (Roche), which contained 16 mg trimethoprim and 80 mg sulphamethoxazole per ml (ratio of trimethoprim to sulphonamide 1:5); this also contained propylene glycol 40%, which did not prove inhibitory to pneumococci in the final concentration used. Dilutions of co-trimoxazole were incorporated in Isosensitest agar (Oxoid). Pure samples of penicillin G and tetracycline powders were used to prepare sterile solutions and the drugs were incorporated in meat-extract agar. To all these media horse blood 5% (v/v) was added. To prepare inocula, the pneumococci were grown in serum broth for 4h and diluted 1/50 in phosphate-buffered saline, pH 7·3. To inoculate the drug-containing plates a replica plating device was used (Annear, Norcott & Ruhen, 1974) which delivered an inoculum of 10^3 viable units in a volume of 1 μl. Tests were incubated overnight in Carbogen for 16 h.

RESULTS

Nasal swabs were collected from 282 individuals of whom 193 yielded smooth (typable) pneumococci; some subjects yielded more than one strain of pneumococcus (see below) and the total yield was 209 isolates (Tables 1 and 2). Although there was often a marked growth of a pneumococcus, it was rarely the predominant bacterium on aerobic culture on blood agar. The carriage rate was 89% for children, 39% for adolescents and 34% for adults (Table 1). The yield of pneumococci was higher from GBA than from BA and GBA frequently yielded a pneumococcus in pure culture. GBA inhibited, completely, the swarming of proteus. Pneumococci survived well on swabs in TM, with no fall-off in recovery apparent for 10 days. This finding was utilized by re-culturing many of the swabs, which yielded additional isolates.

In all, 27 serotypes of pneumococci were isolated: types 2–23, 29, 33, 34, 35, 39 (Table 2). In children types 23, 19, 6, 22 and 16 were predominant and together these five types constituted 61% of the total, whereas type 3 was commonest in adults (Table 2). Fifteen (5%) subjects (of whom 13 were children) yielded two or more serotypes of pneumococci; 13 yielded two serotypes and 2 yielded three serotypes (3, 6, 16; 3, 13, 21). In the sample of 25 subjects where additional serotypes were deliberately sought, three (12%) yielded an additional type. In all, 12 (4%) subjects yielded a pneumococcus belonging to an epidemic serotype; type 2 (two subjects), 4 (two), 5 (three), 7 (four) and 12 (one); type 1 was not encountered.

Table 2. *Pneumococcal nasal carriage in Australian aborigines in Alice Springs: serotypes of pneumococci in order of prevalence*

	Children (< 14 years: $n = 174$)				Adolescents and adults (⩾ 14 years: $n = 108$)			
Rank	Serotype	No.	%	Cumulative %	Serotype	No.	%	Cumulative %
First	23	32	18·8	18·8	3	5	12·5	12·5
Second	19	28	16·6	35·4	21	4	10·0	22·5
Third	6	22	12·9	48·5	7	3	7·5	30·0
Fourth	22	11	6·5	55·0	22	3	7·5	37·5
Fifth	16	10	5·9	60·9	23	3	7·5	45·0
Sixth	21	8	4·7	65·7	29	3	7·5	52·5
Seventh	9	7	4·1	69·8	13	2	5·0	57·5
Eighth	11	6	3·5	73·4	15	2	5·0	62·5
Ninth	13	6	3·5	76·9	16	2	5·0	67·5
Tenth	15	6	3·5	80·5	18	2	5·0	72·5
Other types	*	33	19·5	19·5	*	11	27·5	27·5
Total		169	100·0	100·0		40	100·0	100·0

* Other serotypes encountered were: children 2–5, 7, 8, 12, 14, 17, 20, 33–35, 39; adolescents and adults 2, 4–6, 10, 13–17, 19, 34.

Table 3. *Carriage rate of drug-insensitive pneumococci amongst Australian aborigines in Alice Springs*

		No. of subjects yielding drug-resistant pneumococci						Carriage rate of resistant strains	
Category	No.	P	T	Co	CoP	PT	Total	All subjects*	Carriers†
Children‡	174	27 (28)§	4	4 (5)	6	5	46 (48)	26%	29%
Adolescents	23	1	1	0	0	0	2	9%	22%
Adults	85	3	2	0	0	0	5	5%	14%

P, penicillin; T, tetracycline; Co, co-trimoxazole.

* Percentage of total subjects in each age group who yielded drug-resistant strains.

† Percentage of pneumococcal carriers in each age group who yielded drug-resistant strains.

‡ One child yielded a type-7 pneumococcus resistant to tetracycline and a type-11 pneumococcus resistant to co-trimoxazole. Another yielded type 14 and type 23 pneumococci, both penicillin-insensitive.

§ Figures in parentheses are number of isolates.

Drug-insensitive pneumococci were isolated from 26% of the children, 9% adolescents and 5% adults; 55 (26%) of the 209 pneumococci isolated were drug-insensitive. Resistance to penicillin G, tetracycline and co-trimoxazole was met with and some strains showed dual resistance (Table 3). All isolates were sensitive to chloramphenicol, erythromycin, lincomycin and rifampicin. The commonest form of resistance was insusceptibility to penicillin G alone, with minimal inhibitory concentration (MIC) values ranging from 0·1 to 1·0 μg penicillin/ml. These penicillin-insensitive (P) strains belonged to five serotypes of which types 19 and 23 were predominant (Table 4). All 12 strains of pneumococci resistant to tetracycline showed a similar degree of resistance with a MIC value

Table 4. *Pneumococci from Australian aborigines: results of quantitative susceptibility tests with penicillin G*

MIC*	No. of isolates	Serotypes (no. of isolates)				
⩽ 0·02	151	See text and Table 2				
0·05	15					
0·1	27			16 (4)	19 (2)	23 (19)
0·2	8	9 (5)	14 (2)		19	
0·5	2				19 (2)	
1·0	6		14		19 (5)	
Total	209					

* Minimal inhibitory concentration as μg penicillin G/ml.

Table 5. *Results of susceptibility tests with co-trimoxazole*

(A) Pneumococci sensitive to co-trimoxazole on disk diffusion testing (see text)

			Results of disk tests (see text)			
			BA†		IBA†	
MIC*	No. (%)	No.	Mean	Range	Mean	Range
⩽ 1	21 (28)	10	19·6	18·2–21·2	25·0	22·4–26·9
2	42 (55)	10	19·1	17·6–20·6	24·3	21·5–27·5
5	10 (13)	10	12·6	9·1–16·6	16·2	11·9–20·7
10	3 (4)	2	0‡	0	8·2	8·2
Total	76 (100)					

(B) Pneumococci resistant to co-trimoxazole on disk diffusion testing

		Results of disk tests			Serotypes (no. of isolates)		
MIC*	No.	No.	BA (mean)	IBA (mean)			
20	6	6	0	< 10	6	19 (4)	23
50	3	3	0	0	11	19 (2)	
100	2	2	0	0	11 (2)		
Total	11						

* Minimal inhibitory concentration (μg co-trimoxazole/ml).
† Diameter of inhibition zone (mm).
‡ No inhibition zone (disk diameter 7 mm).

of 50 μg tetracycline/ml, these belonged to types 3, 6, 7 (4 isolates), 14 (2) and 19 (4). When tested quantitatively, 76 consecutive isolates of pneumococci sensitive to co-trimoxazole on disk diffusion testing showed MIC values ranging from 1 to 10 μg co-trimoxazole per ml (Table 5) with a median value of 2 μg co-trimoxazole per ml. For pneumococci resistant to co-trimoxazole, degrees of resistance varied, with MIC values from 20 to 100 μg per ml (Table 5).

When co-trimoxazole susceptible strains were tested by disk diffusion on Isosensitest BA (IBA) and BA in parallel, using individual disks of co-trimoxazole (25 μg), wider zones of inhibition were evident on IBA (Table 5). For strains with

MIC values $\geqslant 10\ \mu g$ co-trimoxazole/ml, inhibition zones were small or non-detectable. When tested by a similar technique, tetracycline-resistant strains showed growth to the margin of a 10 μg tetracycline disk (data not shown). Four strains of pneumococci tested in parallel as controls (types 1–4) showed these MIC values: penicillin G $\leqslant 0{\cdot}02\ \mu g$/ml, tetracycline $\leqslant 0{\cdot}5\ \mu g$/ml, co-trimoxazole 2 μg/ml. For the most resistant strains the resistance ratios were penicillin 50, tetracycline 100 and co-trimoxazole 50.

DISCUSSION

The carrier rate found in a survey will depend upon the 'true' (intrinsic) rate and upon the sampling and cultural techniques employed. Although BA is usually used for primary culture, several methods have been used to increase the yield of pneumococci. For example, by using an enrichment medium in addition to plating on BA, Masters and his colleagues (1958) were able to increase the yield by about 10 %. Other workers have used mouse inoculation for this purpose. Gentamicin blood agar has been introduced as a selective medium for pneumococci (Converse & Dillon, 1977). By using GBA in addition to BA for primary culture the isolation rate in the present survey was increased by 20 % (data not shown). We found that it was necessary to use BA as well as GBA because some strains of pneumococci grew either poorly (as atypically small colonies) or not at all on GBA.

In the present study the nasal carriage rate of pneumococci in children was 89 % and in adults was 34 % (Table 1). Studies in Europe and North America have shown carriage rates of about 50 % in young children, with lower rates in children over 8 years of age and in adults (Masters *et al.* 1958); Loda *et al.* 1975; Gray, Converse & Dillon, 1980). Recent studies in young caucasian children in Adelaide have shown a carriage rate of about 35 % (Hansman & Douglas, unpublished work). The lower rate in Caucasian than in aboriginal children may be related to the former's more favourable living conditions. Young aboriginal children often have a 'running' nose and respiratory secretions containing pneumococci (and other potential pathogens) could be transferred by hand contact or by the agency of insects, especially flies.

Infectious diseases are a major contributor to the high morbidity and mortality rates amongst aborigines in Australia. This applies especially to children. In the Northern Territory (NT) the mortality rate amongst 'full-blooded' aboriginal children under 2 years of age was 172 per 1000 live births in 1965–7, compared with 28 per 1000 for Australia as a whole in 1958–60, and in the second year of life the mortality rate was 20-fold higher in aboriginal children. After the neonatal period the leading causes of death in aboriginal children are 'gastro-enteritis' and 'pneumonia' (Moodie, 1969); and, as mentioned above, the prevalence of chronic otitis media is also exceptionally high. Although the necessary aetiological studies have not been done yet, it is probable that pneumococci are a major cause of pneumonia and otitis media in the NT. In this context, what is the significance of the exceptionally high carriage rate of pneumococci amongst aboriginal children in Alice Springs? In Australian children (as in children elsewhere) the serotypes chiefly causing systemic infections (bacteraemia and meningitis) are 4, 6, 14, 18, 19 and 23 and for otitis media types 3, 14 and 19 (Hansman, 1983). Whilst the

studies yielding this information were done with caucasian children, there is no reason to believe that the important serotypes are different in aboriginal children. Although several of these types, namely 6, 19 and 23, were commonly carried by the aboriginal children, the other important paediatric types 4, 14 and 18 were not. Moreover, types 16 and 22, which were often carried by the children, uncommonly cause disease (Hansmann, 1983). These findings suggest that a high carriage rate of pneumococci is not the only factor responsible for the apparently high prevalence of pneumococcal disease in aboriginal children. In the aboriginal children the types most commonly carried, 23, 19 and 6 (in that order) are those most often carried by children in Europe and North America and similar findings have been made in Adelaide (Hansman, unpublished).

Antibiotic-resistant pneumococci were encountered first in Australia. One of the earliest reports described a penicillin-insensitive strain from an aboriginal child in Ernabella, a remote area in northern South Australia (Hansman *et al.* 1971). However, almost no systematic studies have been done to ascertain the prevalence of drug-resistant strains in aborigines.

In Australia, Europe and North America the prevalence of penicillin-insensitive pneumococci is 3% or less (Ahronheim, Reich & Marks, 1979), whereas in Papua New Guinea (PNG) rates of 15% amongst isolates from carriers are common and a study in Port Moresby showed a prevalence of 30% amongst isolates from patients with severe pneumococcal disease (Gratten, Naraqi & Hansman, 1980). The high prevalence of penicillin-insensitive strains in Alice Springs, 21% of isolates from children, approximates to that observed in PNG. Although fewer individuals carried pneumococci resistant to other drugs, overall 26% of children and 5% of adults carried pneumococci resistant to one or more of three drugs: co-trimoxazole, penicillin, tetracycline. These rates are much higer than those encountered amongst caucasian children in Australia. For example, in Adelaide the carriage rate of drug-insensitive pneumococci amongst children is about 0·2%. The rate amongst aboriginal children in Alice Springs was some 100-fold greater. Most of the drug-insensitive pneumococci isolated belonged to types 19 and 23 (Tables 4 and 5). These types are commonly carried by healthy children and also commonly cause disease. In Australia, Europe and PNG drug resistance has been encountered in types 19 and 23 and in South Africa a multiply-resistant type 19 pneumococcus has caused bacteraemia in black children (Hansman *et al.* 1974; Cybulska *et al.* 1970; Jacobs *et al.* 1978).

The high prevalence of drug-resistant pneumococci, especially penicillin-insensitive pneumococci, amongst aboriginal children is unexplained at present. However, it may be related to their high carriage rate of pneumococci and to frequent courses of antimicrobial drugs, especially penicillins, for suspected respiratory infections. The high carriage rate of drug-insensitive pneumococci points to the possibility of a future increase in the proportion of pneumococcal infections that are caused by drug-resistant strains.

In 1977 a 14-valent polysaccharide pneumococcal vaccine (Merck) was licensed for use in the United States. This provided about 80% coverage for patients with pneumococcal disease in Australia (Hansman, 1983), while the 23-valent vaccine (Merck) that was licensed in the United States in 1983 would provide about 90% coverage. However, the latter vaccine is not available in Australia, where the

currently licensed vaccine is a 17-valent vaccine comprising types 1, 2, 3, 4, 6A, 7F, 8, 9N, 11A, 12F, 14, 15F, 17F, 18C, 19F, 23F and 25 (Smith Kline) with about 85% coverage. These data apply to pneumococcal serotypes causing bacteraemic and meningeal infections amongst caucasians in Australia. It is desirable for studies to be carried out amongst Australian aborigines to ascertain whether or not a similar type distribution occurs.

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