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Acquisition and invasiveness of different serotypes of *Streptococcus pneumoniae* in young children

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SUMMARY

Rates of acquisition and mean duration of nasal carriage of different serotypes of *Streptococcus pneumoniae* have been estimated by fitting a stochastic model to longitudinal carriage data in children from Papua New Guinea. Immunogenicity and two indices of relative invasiveness were determined for each serotype. Immunogenic serotypes were less frequently acquired and were carried for shorter periods, but no relationship between immunogenicity and invasiveness was apparent using either index of invasiveness. Frequent invasion was associated with a high acquisition rate and high frequency and prolonged duration of carriage. Carriage studies can provide a broad indication of which serotypes cause invasive disease but not the proportion of disease due to individual serotypes; some serotypes which cause invasive disease (e.g. serotype 46) are not found even in extensive carriage studies. The antibiotic resistance of carriage organisms, however, does approximate the resistance patterns of invasive organisms and thus may be used to monitor changing patterns of antimicrobial susceptibility in the community.

INTRODUCTION

Pneumonia due to *Streptococcus pneumoniae* remains a major cause of hospitalization and death among children in developing countries [1]. A total of 83 different serotypes of *S. pneumoniae* are recognized, but only one quarter commonly cause disease [2]. It is important to determine the frequency of different pneumococcal serotypes causing disease in any given population in order, firstly, to optimize the design of candidate vaccines and, secondly, to ensure appropriate antimicrobial therapy, since antimicrobial susceptibility may be serotype-dependent [3].

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Blood culture is an insensitive method of determining the aetiology of pneumonia and sensitive pneumococcal antigen detection methods have yet to be developed. Therefore it would be easier to characterize the pneumococci circulating in a community by typing pneumococci carried in the upper respiratory tract (URT). However, surveys of community carriage on their own may not tell us which serotypes are important pathogens since pneumococcal serotypes differ in virulence [2] and the serotype distribution in the URT does not necessarily reflect the distribution of serotypes causing invasive disease [3, 4]. If measures of invasiveness (i.e. the ability of an organism to reach and grow in normally sterile tissues) were available for the different serotypes it may be possible to use community carriage data to determine which serotypes are important pathogens in a given community.

A previous study in Papua New Guinea (PNG) [4] measured invasiveness by comparing the prevalence of URT carriage of a serotype in the community with the frequency of isolation from normally sterile tissue of children hospitalized with pneumonia. This measure is not necessarily the most appropriate index of invasiveness if, as reported elsewhere, the incidence of invasive disease is associated with the acquisition of a new serotype, rather than with long-term carriage [5]. Whether this association is generally true has not been determined. Assuming equal probabilities of invasion associated with any one acquisition event, the estimate of invasiveness obtained by comparing the frequency of isolation in normally sterile tissue with the frequency of carriage in the URT [4] will be lower for a serotype which colonizes the upper respiratory tract for extended periods than for a serotype which is acquired equally frequently but persists in the upper respiratory tract for a shorter period. As an alternative index of invasiveness, we propose the ratio of the frequency of a specific serotype in a series of blood cultures collected from children with pneumonia to the corresponding acquisition rate in the URT in the community.

Acquisition rates can be estimated only from longitudinal data for children at high risk of carriage. A longitudinal community-based study took place between 1985 and 1987 in a rural area of the Eastern Highlands Province, PNG [3, 6]. Thirty-nine different serotypes of *S. pneumoniae* were isolated at widely different frequencies [3]. This variation in carriage prevalence may reflect variation between serotypes in either the acquisition rate, or in the tendency of the serotype to persist in the URT, or both. By comparing the pneumococcal serotypes isolated from paired samples collected from the same host in the longitudinal study, we have been able to examine the relationship between the probability of acquisition of a given serotype and the duration of the interval between samples. Acquisition rates could then be estimated by fitting the reversible catalytic model of Muench [7]. This statistical model not only provides estimates of acquisition rates but also estimates of the mean rates of elimination of serotypes from the nose, and hence of the average duration of nasal carriage. Using these estimates we were then able to compare duration of carriage of specific groups of carriage isolates with that expected, given the particular serotype composition of the group. For instance, those serotypes which are most frequently carried are on average more frequently resistant to penicillin [3]. This might be because resistance is associated with persistence or because of the chance distribution of resistant strains across

serotypes. By comparing the numbers of resistant strains persisting with the numbers expected to persist given their distribution of serotypes, it was possible to test whether the duration of carriage is significantly longer for penicillin-resistant strains than non-resistant strains of the same serotype.

The host immune system is potentially one important determinant of carriage, but the role, if any, of the immune system in the control of nasal carriage is not well understood. We therefore investigated indirectly the hypothesis that humoral immune responses influence carriage of pneumococci in the nose by examining rates of acquisition and persistence in relation to serotype-specific antibody responses. As a measure of the immunogenicity of a serotype we used published data [8] on fold increases in antibody level following vaccination with a standard dose of polysaccharide antigen.

Data from PNG have suggested that the more immunogenic serotypes, for example 2, 3, 5 and 7, are also the more invasive serotypes compared to those serotypes which are poorly immunogenic, such as 6, 14, 19 and 23 [4, 9]. Here we investigate this hypothesis by examining the correlation of proposed indices of invasiveness with the immunogenicities of the corresponding serotypes.

METHODS

Two sets of data collected in the Eastern Highlands Province of PNG were used to estimate acquisition and elimination rates as well as duration of carriage of pneumococci from the URT and to obtain an index of invasiveness of different pneumococcal serotypes:

(*a*) Pneumococci isolated from nasal swabs collected from a cohort of 158 children attending village clinics which were held monthly in the Asaro Valley (near Goroka town) over a period of 18 months between 1985 and 1987 [3, 6]. Nasal swabs were also collected from the same children during episodes of acute lower respiratory tract infections.

(*b*) Pneumococcal isolates from blood of children admitted to Goroka Base Hospital with pneumonia between 1981 and 1987 [4, 10].

Standard bacteriological techniques used to isolate and identify bacteria have been described in detail previously [3, 4, 10]. These studies provided data for pneumococcal serotypes and serogroups but did not differentiate antigenically related types within groups (e.g. serogroup 6 consists of types 6A and 6B and serogroup 19 consists of types 19F, 19A, 19B and 19C).

Indices of immunogenicity

Fold increases in serotype-specific antibody titre in children following vaccination were used as measures of immunogenicity. Values were taken from a study of 249 Australian children aged 6–54 months who were injected with a vaccine containing 50 μg of each of 14 pneumococcal polysaccharides [8]. Sera were obtained at the time of injection and 4 weeks afterwards and pneumococcal antibody levels measured by radioimmunoassay. Seven of these 14 serotypes have been studied in the PNG highlands [11] and showed patterns of fold increases following vaccination similar to those recorded by Douglas and colleagues [8].

Table 1. *Distribution of length of interval between paired nasal swab specimens*

Interval length (days)	Frequency	Percent
1–13	43	6·2
14–27	82	11·7
28–41	331	47·4
42–55	109	15·6
56–69	72	10·3
70+	61	8·7
Total	698	

Statistical methods

Estimation of acquisition and loss rates of pneumococci. The specimens collected from each child in the community study were ranked in temporal order of collection and grouped into pairs such that each specimen defined the beginning or end of a single interval. Where an odd number of specimens had been collected from a child, the last specimen was omitted from the analyses.

The 698 intervals thus defined (Table 1) were treated as independent units in subsequent analyses. To estimate acquisition and loss rates, analyses proceeded by fitting stochastic models to the proportions of specimens which were shown to contain specific bacterial serotypes at the ends of the intervals [7]. A separate analysis was carried out for each serotype (Table 2). Acquisition was defined as the colonisation of the upper respiratory tract by a serotype not present at the beginning of the interval. Loss of a serotype was said to occur when a previously carried serotype was no longer present in the upper respiratory tract. Further details on the fitting of models for the estimation of acquisition (λ) and loss (μ) rates are given in the Appendix. Illustrative results for serotype 6 are given in Table 3 and Fig. 1.

The initial models make the assumption that the laboratory methods are 100% sensitive. Four bacterial colonies/plate were subcultured and serotyped. These were chosen to represent different morphologies where morphological diversity was evident [3]. False negatives would therefore necessarily occur if more than four serotypes were actually present. It is likely that false negatives for specific serotypes also occurred on other occasions. This complication was taken into account by the development of a more complex equation as the basis for the models. However, for no serotype was there a statistically significant improvement of the fit of the model when the sensitivity of bacteriological assay was estimated rather than fixed at 100%. The results presented here therefore consider only models where this sensitivity was assumed to be 100%.

Estimation of relative invasiveness of different serotypes. Two measures of relative invasiveness were calculated for each serotype:

(i) Relative invasiveness was measured by empirical odds ratio as in a previous study in Goroka [4]. The total frequencies of isolates of each pneumococcal serotype from blood in previous studies in Goroka [4, 10] are tabulated in Table 4. Data for nasal swabs refer to the dataset of Montgomery and co-workers [3].

The odds ratio is the ratio:

$$\omega = ad/bc,$$

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Table 2. *Estimates of acquisition and loss rates by pneumococcal serotype*

Sero-type	Initial (%) +ve*	Final (%) +ve*	Loss rate (per day)	S.E.	Acquisition rate (per day)	S.E.
1	0·000	0·143	—	—	—	—
2	0·143	0·143	0·032	0·019	0·00007	0·00005
3	1·576	1·289	0·054	0·007	0·00070	0·00027
4	2·006	1·719	0·046	0·004	0·00071	0·00025
5	0·143	0·573	—	—	—	—
6	25·931	26·504	0·016	0·002	0·00572	0·00074
7	1·289	2·149	0·031	0·004	0·00078	0·00023
8	1·003	0·287	0·043	0·007	0·00008	0·00006
9	3·009	3·438	0·041	0·005	0·00153	0·00035
10	3·582	3·295	0·038	0·003	0·00112	0·00031
11	1·576	0·573	—	—	—	—
12	1·146	0·573	0·089	0·008	0·00043	0·00025
13	3·152	3·009	0·033	0·003	0·00110	0·00041
14	7·307	8·596	0·023	0·005	0·00231	0·00092
15	5·301	5·301	0·031	0·003	0·00179	0·00038
16	1·862	1·146	0·034	0·002	0·00029	0·00014
17	0·573	1·289	—	—	—	—
18	1·146	1·719	0·027	0·004	0·00060	0·00022
19	22·063	23·496	0·018	0·002	0·00591	0·00075
20	0·716	0·716	0·150	0·113	0·00111	0·00097
21	2·722	3·725	0·050	0·006	0·00186	0·00046
22	2·436	2·149	0·029	0·002	0·00062	0·00020
23	13·897	14.327	0·024	0·002	0·00427	0·00060
24	0·573	0·716	—	—	—	—
25	0·287	0·143	—	—	—	—
28	0·287	0·287	—	—	—	—
29	2·006	1·003	0·059	0·006	0·00052	0·00023
31	1·003	1·003	0·079	0·013	0·00078	0·00034
33	5·444	4·298	0·067	0·012	0·00316	0·00080
34	3·438	2·722	0·036	0·003	0·00086	0·00026
35	3·582	4·441	0·017	0·002	0·00101	0·00021
36	0·143	0·000	—	—	—	—
38	1·433	1·576	—	—	—	—
39	0·287	0·287	—	—	—	—
42	1·003	0·860	—	—	—	—
43	0·143	0·000	—	—	—	—
45	0·860	0·287	—	—	—	—
46	—	—	—	—	—	—
47	0·143	0·000	—	—	—	—
Non-serotypable	10·029	12·034	0·063	0·010	0·00872	0·00172

Note. Results are not presented for those serotypes with limited data for which the model could not be fitted.

* Proportion of 698 paired nose swabs in which individual serotypes were isolated (initially or finally).

where a, is the number of invasive isolates of a specific serotype; b, is the number of invasive isolates of other serotypes; c, is the number of isolates of the specific serotype from nasal swabs; d, is the number of isolates of other serotypes from nasal swabs.

The empirical odds ratios were calculated by analogy with the empirical logistic transform [12] as the ordinary odds ratio with 0·5 added to each frequency [4].

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Table 3. *Percentage of final specimens from which serotype 6 was recovered*

Interval duration (days)	Initially negative		Initially positive		Overall	
	n	Final % positive	*n*	Final % positive	*n*	Final % positive
1–13	28	17·9	15	80·0	43	39·5
14–27	62	9·7	20	45·0	82	18·0
28–41	245	13·9	86	74·4	331	29·6
42–55	82	11·0	27	40·7	109	18·3
56–69	52	19·2	20	55·0	72	29·2
70+	48	18·8	13	38·5	61	23·0
Total intervals studied	517	14·1	181	61·9	698	26·5

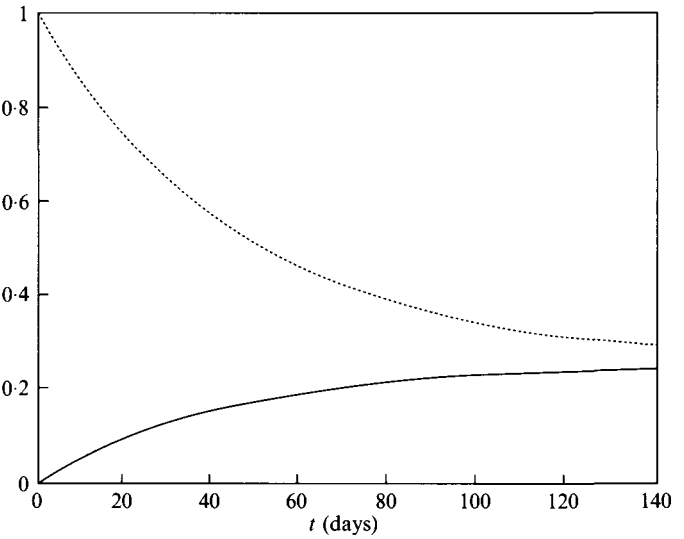

Fig. 1. Fitted acquisition curve (——) and loss curve (------) for the commonest serotype (type 6), i.e. the probability of carriage among individuals initially not carrying, $p(t, 0)$ (——) or carrying, $p(t, 1)$ (------), this serotype, plotted against interval (t) in days.

(ii) A second index of invasiveness is the serotype-specific ratio of the incidence of invasive disease to the rate of acquisition. Since we do not know the incidence of invasive disease, we can only calculate this ratio relative to some standard. Serotype 19 (the most commonly acquired serotype, Table 4) was chosen as the standard. Thus the invasion : acquisition ratio for individual serotypes in Table 4 is:

$$\frac{\text{Number invasive/total isolates}}{\text{acquisition rate}} \quad \text{for individual serotype,}$$

divided by

$$\frac{\text{Number invasive/total isolates}}{\text{acquisition rate}} \quad \text{for serotype 19.}$$

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Table 4. *Estimates of immunogenicity and invasiveness of pneumococcal serotypes*

Sero-type	Number of isolates*		Immuno-genicity†	Carriage		Estimates of invasiveness	
	Invasive	Carriage		Acquisition rate (per day)	Duration‡ (days)	Empirical odds ratio§	Invasion: acquisition ratio‖
1	1	1	1·3	—	—	35·4	—
2	1	2	5·5	0·0001	31·3	21·2	5·90
3	0	23	5·6	0·0007	18·5	0·7	0·00
4	0	27	2·9	0·0007	21·7	0·6	0·00
5	2	5	—	—	—	16·4	—
6	3	385	1·7	0·0057	62·5	0·3	0·31
7	8	24	5·1	0·0008	32·3	13·9	5·90
8	0	9	5·9	0·0001	23·3	1·8	0·00
9	3	46	5·2	0·0015	24·4	2·7	1·18
10	2	50	—	0·0011	26·3	1·7	1·07
11	0	15	—	—	—	1·1	—
12	0	12	1·7	0·0004	11·2	1·4	0·00
13	1	45	—	0·0011	30·3	1·1	0·54
14	7	118	1·4	0·0023	43·5	2·4	1·80
15	1	81	—	0·0018	32·3	0·6	0·33
16	3	21	—	0·0003	29·4	5·9	5·90
17	0	13	—	—	—	1·3	—
18	0	22	4·7	0·0006	37·0	0·8	0·00
19	10	337	1·6	0·0059	55·6	1·1	1·00
20	0	12	—	0·0011	6·7	1·4	0·00
21	0	45	—	0·0019	20·0	0·4	0·00
22	0	35	—	0·0006	34·5	0·5	0·00
23	6	205	1·8	0·0043	41·7	1·1	0·82
24	0	11	—	—	—	1·5	—
25	1	3	2·9	—	—	15·2	—
28	1	3	—	—	—	15·2	—
29	0	22	—	0·0005	16·9	0·8	0·00
31	1	14	—	0·0008	12·7	3·6	0·74
33	1	70	—	0·0032	14·9	0·7	0·18
34	1	44	—	0·0009	27·8	1·2	0·66
35	0	57	—	0·0010	58·8	0·3	0·00
36	0	1	—	—	—	11·6	—
38	0	21	—	—	—	0·8	—
39	0	4	—	—	—	3·9	—
42	0	14	—	—	—	1·2	—
43	0	1	—	—	—	11·6	—
45	0	7	—	—	—	2·3	—
46	3	0	—	—	—	257·2	—
47	0	1	—	—	—	11·6	—
Non-serotypable	0	159	—	0·0087	15·9	0·1	0·00
Total	56	1965					

* References [3, 4, 10].
† Fold increase following vaccination [8].
‡ Duration = loss rate^{-1}.
§ Reference [4].
‖ Ratio calculated relative to the ratio for serotype 19 (see statistical methods).

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Spearman rank correlations were computed between the serotype-specific measures of immunogenicity, invasiveness and carriage.

RESULTS

The results of the first 18 months of the hospital study are included in the paper of Barker and co-workers [4]. Montgomery and co-workers [3] have reported results on URT carriage in the community-based study.

Table 1 shows the distribution of lengths of intervals between paired nasal swab specimens included in the present analyses. The time interval between paired nasal swab specimens was 28–41 days for almost half of the specimen pairs. Acquisition and loss rates estimated from the two-parameter nonlinear model are tabulated in Table 2. The highest acquisition rate was observed for serotype 19 (0·0059/day) while the lowest acquisition rate was observed for serotype 2 (0·00007/day). The highest and lowest loss rates were observed for serotypes 20 (0·15/day) and 6 (0·016/day), respectively. The data were too sparse for the catalytic model to be fitted for many of the rarer serotypes, which presumably have the lowest acquisition rates. Fig. 1 illustrates the fitted acquisition and loss curves for the commonest serotype (type 6). A summary of the actual data from which the curves are estimated is given in Table 3. The fit of the model for this serotype is quite good but there is substantial scatter about the fitted curves.

In order to evaluate the fit of the models, the observed proportions of final specimens which were positive were compared with the numbers expected, given the actual distribution of durations of intervals. A good fit was observed.

If we assume that the probability of loss does not depend upon the length of time for which the child has been infected (i.e. that the immune response acquired through carriage does not affect carriage) then the mean distribution of carriage of a serotype follows an exponential distribution with mean μ^{-1} days and variance μ^{-2} days. Estimates made in this way indicate that serotypes 6, 19 and 35 were carried for prolonged periods (62·5, 55·6 and 58·8 days, respectively) while the mean duration of carriage was short for serotypes 20, 12, 31 and 33 (6·7, 11·2, 12·7 and 14·9 days, respectively) (Table 4).

Comparison of observed with expected positivity rates of subsets of the final specimens was used to explore whether the duration of carriage depended on the antimicrobial susceptibility of the bacterium to penicillin. The carriage rates at the end of the intervals were very similar to those expected, irrespective of the susceptibility to penicillin of the initial isolate (data not shown).

Measures of immunogenicity and invasiveness of individual pneumococcal serotypes are tabulated in Table 4. The total invasive isolates refer to 56 *S. pneumoniae* isolated from blood [4, 10]. Table 5 gives rank correlations between serotype-specific fold increases in antibody titre after vaccination (immunogenicity) and the various measures of nasal colonization and invasiveness of individual serotypes. Several of these measures are correlated with one another as a necessary consequence of the way in which they are calculated. These correlations are italicised in the table. This does not apply to any of the correlations between fold increases in antibody titre and the measures derived from the PNG studies. Immunogenicity, as measured in this way, was significantly

Table 5. *Correlations between different characteristics of pneumococcal serotypes*

	Frequency invasive isolates	Frequency carriage isolates	Immuno-genicity	Acqui-sition rate	Mean duration carriage	Empiri-cal odds ratio	Invasion: acquisition ratio
Frequency invasive isolates	*1·0* *(41)*						
Frequency carriage isolates	0·34* (41)	*1·0* *(41)*					
Immuno-genicity	−0·41 (14)	−0·28 (14)	*1·0* *(14)*				
Acqui-sition rate	0·41* (25)	*0·89* *(25)*	−0·65* (12)	*1·0* *(25)*			
Mean duration carriage	0·50* (25)	*0·50* *(25)*	−0·56 (12)	*0·21* *(25)*	*1·0* *(25)*		
Empirical odds ratio	*0·28* *(41)*	*−0·77* *(41)*	−0·01 (14)	*−0·41* *(25)*	*−0·10* *(25)*	*1·0* *(41)*	
Invasion: acqui-sition ratio	*0·90* *(25)*	*0·16* *(25)*	−0·13 (12)	*0·13* *(25)*	0·36 (25)	*0·70* *(25)*	*1·0* *(25)*

(i) Figures in parentheses indicate the number of serotypes included in the calculation.
(ii) Italicized correlations are those where there is a structural relationship between the variables (i.e. the same raw data contribute to estimates of both parameters).
(iii) Immunogenicity is determined by the fold increase in antibody titre to the specific serotypes in children as measured by Douglas and colleagues [8].
(iv) Measures of invasiveness are as tabulated in Table 4.
(v) * indicates $P < 0{\cdot}05$; Probability values are not indicated where there is a structural relationship between the variables.

negatively correlated with the serotype-specific acquisition rate; i.e. immunogenic serotypes were less frequently *acquired* in the upper respiratory tract than those serotypes which are poorly immunogenic. There was also a substantial, but non-significant, negative correlation ($r = -0{\cdot}56$) between immunogenicity and the mean duration of carriage of different serotypes, suggesting that the more immunogenic serotypes were carried for shorter periods than poorly immunogenic serotypes. However, the risk of invasion as determined by either the empirical odds ratio or the invasion: acquisition ratio was not related to the immunogenicity of a serotype.

There were also significant correlations between the frequency of invasive isolates (but not their inherent 'invasiveness') and nasal carriage of corresponding serotypes, as measured by the frequency of carriage ($r = 0{\cdot}34$), by the acquisition rate ($r = 0{\cdot}41$) or by the mean duration of carriage ($r = 0{\cdot}50$).

DISCUSSION

It has been possible to estimate acquisition and loss rates (and hence duration of carriage) for different pneumococcal serotypes in the URT by fitting stochastic models to longitudinal data and then to characterize serotypes according to these

rates and risk of invasiveness (using the pneumococcal serotype distribution in blood cultures collected from children with pneumonia).

Pneumococcal serotypes isolated from the URT vary widely in their acquisition rates and in the length of time for which they generally persist. Some serotypes are acquired frequently and are carried for long periods (types 6, 14, 19, 23), others are acquired infrequently and eliminated from the URT quickly (e.g. types 3, 12, 29, 31), others are infrequently acquired but carried for quite a long time (e.g. types 2, 7, 16, 18, 22) while some (e.g. type 33) are frequently acquired and eliminated quickly (Table 4). The mean duration of carriage (Table 4) differs by a factor of 10 between the least persistent serotype for which we can estimate a loss rate (serotype 20) and serotype 6, which is both frequently acquired and the most persistent. The duration of carriage is not affected by the antimicrobial susceptibility of the organism to penicillin.

Different measures of carriage are correlated with the frequency of invasive isolates of corresponding serotypes. Thus carriage studies can give a broad picture of serotypes causing invasive disease, particularly if examined in conjunction with the indices of invasiveness determined here. Nevertheless, data from carriage studies should be treated with caution since extensive community carriage surveys may not detect some serotypes (e.g. type 46) which are important causes of invasive disease. With the data available in PNG, it would be possible to determine whether the distribution of pneumococcal serotypes causing invasive disease is broadly similar throughout the country, but it would not be possible to extrapolate from one country to another since patterns of carriage and invasive disease may be markedly different. Community surveys can be used effectively to monitor the emergence of strains with diminished antimicrobial susceptibility [10].

Correlation analysis supports the view that invasion is correlated with carriage but not that it is more closely related to acquisition than to persistence, as has been found in North American children [5]. However, differences between the two studies should be noted: firstly, in the study of Gray and colleagues [5], acquisition and persistence were examined with respect to risk of infection, most of which was mild disease (otitis media), while our study examined acquisition and persistence in relation to risk of bacteraemia. Secondly, carriage rates in Papua New Guinean children are much higher than among North American children [3, 5]. It may be that in situations where serotypes 6, 19 and 23 are carried less frequently than in PNG, acquisition is associated with increased risk of invasion by these serotypes, but where children have constant very heavy colonization of the URT with these serotypes, this very heavy inoculum of bacteria in the URT will result in an increased occurrence of invasive disease. Similarly, in PNG, acquisition or persistence as a risk factor for invasive disease is likely to be serotype-dependent, i.e. for serotypes which are infrequently carried acquisition may be associated with an increased occurrence of invasive disease, while the sheer load of bacteria in the URT of those serotypes carried for prolonged periods may be associated with increased risk of invasive disease. Precipitating events such as viral infections may be important factors in determining attacks of bacterial pneumonia, but the bacteria must be resident or acquired during this critical phase to cause bacterial lower respiratory disease.

Both composition and quantity of capsular polysaccharide are thought to play a role in the virulence of *S. pneumoniae* [2]. For a given capsular type, strains which produce the largest amounts of polysaccharide are the most virulent [13]. Adhesiveness of the bacterium is also likely to play a role in determining virulence and risk of invasion [14].

In the present study we were unable to confirm the hypothesis that immunogenic serotypes are also those with an increased risk of invasion. However, fold increases in antibody level following vaccination represent only a crude measure of immunogenicity and do not allow for the fact that the pre-vaccination antibody titre for some serotypes is high (e.g. type 23) [8] in which case the fold increase after vaccination is likely to be low. In practice different serotypes will differ quantitatively in the antigenic challenge which they present and, in addition, antibodies to different serotypes will have different abilities to opsonize the target bacteria. It is also likely that cell-mediated immune responses play a role in the prevention of invasive disease [14]. The strength of serotype-specific cell-mediated responses need not be related to the corresponding humoral immunogenicity.

Previous studies have shown that nasal carriage of some pneumococcal serotypes (types 6, 19 and 23) does not elicit a humoral immune response [5, 15], while carriage or illness associated with the more immunogenic serotypes may afford protection against future illness and result in a serotype-specific antibody response [16]. In PNG, W. Pomat and colleagues (Epidemiology and Infection, in press) found serotype-specific humoral antibody levels to be independent of whether the serotype was carried in the nose. Yet our results show that immunogenic serotypes are less frequently acquired. This negative correlation between immunogenicity and acquisition therefore provides indirect evidence that a mucosal immune response to the capsular polysaccharide occurring in the upper respiratory tract plays a role in regulating the relative abundance of different serotypes. If this is so, it also suggests that such a mucosal response recognizes the same antigenic determinants as does the systemic immune system (which was the basis for determining immunogenicity).

The possible role played by interbacterial antagonism in determining carriage must also be considered. Antagonism between *Haemophilus influenzae* or *S. pneumoniae* and autochthonous microflora has been demonstrated but no antagonism could be demonstrated between strains of *H. influenzae* and *S. pneumoniae* [10]. In reverse, viridans streptococci and other local bacteria have been shown to inhibit growth of some potential pathogens, though the study in question did not investigate inhibition of growth of *S. pneumoniae* and *H. influenzae* [17].

The question remains as to why the more highly immunogenic serotypes ('adult' serotypes, based on their pattern of disease production in North American populations) produce so much disease in Papua New Guinean children but not in American children [9, 18]. The PNG situation is similar to that found in Africa [19]. Papua New Guinean children may have deficiencies in bactericidal capacity based on specific or nonspecific deficiencies in cell-mediated immunity [20] or on the load of new infections transmitted from adults living in the same households [21]. Such serotypes though highly immunogenic may also have more efficient

mechanisms for evading the destructive immune mechanisms of the host once they have invaded. Indeed, immunogenic serotypes which lack such defences are likely to have been eliminated by natural selection and thus would not appear in our analyses.

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APPENDIX

Estimation of acquisition and loss rates

Each interval was classified according to whether the particular serotype was isolated from the specimen marking the beginning of the interval. It follows from simple probability calculus that the probability that the same serotype will be isolated from the specimen taken at the end of the interval depends on the parameters of the first order differential equation:

$$dp/dt = (1-p)\lambda - p\mu, \tag{1}$$

where λ, is the acquisition rate; μ, is the loss rate; $p(t, a)$, is the probability that an individual carries the serotype at the end of an interval of duration t given initial status a; a, is an indicator taking the value 1 if carriage was detected initially and 0 otherwise.

This equation corresponds to the Reversible Catalytic model of Muench [7] and has the solution:

$$p(t) = \frac{\lambda}{\lambda+\mu} + c\exp(-(\lambda+\mu)t), \tag{2}$$

where c is a constant depending on the initial conditions. If we consider only individuals who were initially known to be infected:

$$p(t,1) = \frac{\lambda}{\lambda+\mu} + \frac{\mu}{\lambda+\mu}\exp(-(\lambda+\mu)t). \tag{3}$$

If we consider individuals who initially tested negative, the solution is:

$$p(t,0) = \frac{\lambda}{\lambda+\mu}(1-\exp(-(\lambda+\mu)t)). \tag{4}$$

Where the length of the interval is large, both equations (3) and (4) tend asymptotically towards the limit:

$$p(t) = \frac{\lambda}{\lambda+\mu}, \tag{5}$$

i.e. the expected ratio of positives to negatives is $\lambda:\mu$. If the process is stationary, then the initial ratio has the same expectation.

In order to estimate the parameters λ and μ, the probability of carriage at the end of the interval was assumed to be binomially distributed with probability $p(t, a)$. Parameters λ and μ were then estimated by maximum likelihood separately for each serotype using the SAS nonlinear regression procedure [22].

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