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Nasopharyngeal colonization of infants in southern India with *Streptococcus pneumoniae*

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SUMMARY

To investigate the dynamics of nasopharyngeal colonization with *Streptococcus pneumoniae*, and to determine the prevalent serogroups/types (SGT) and their antimicrobial susceptibility, we studied 100 infants attending our well-baby clinic. Nasopharyngeal swab specimens were obtained at 6, 10, 14, 18 and 22 weeks and at 9 and 18 months of age and submitted for culture, serotyping and antimicrobial susceptibility testing of *S. pneumoniae*. Colonization with pneumococcus was seen on at least one occasion in 81 infants. The median age of acquisition was 11 weeks and the median duration of carriage was 1·3 months. The common SGTs identified were 6, 19, 14 and 15. SGT 1, which was a common invasive isolate in children in our hospital during this period, was not isolated from these children. Sequential colonization by 2, 3 or 4 SGTs was observed in 18, 5 and 2 children, respectively. Resistance to penicillin, chloramphenicol, cotrimoxazole and erythromycin was observed in 0, 13 (6%) 11 (5%) and 5 (3%) isolates, respectively. There was a significant difference in susceptibility to cotrimoxazole between colonizing and invasive isolates (5% vs. 40%, $P < 0{\cdot}0001$).

INTRODUCTION

Invasive disease due to *Streptococcus pneumoniae* and *Haemophilus influenzae* are among the commonest causes of death among children under 5 years of age in developing countries [1]. Nasopharyngeal colonization with these organisms is the initial event leading in some instances to invasive disease [2, 3]. Higher rates of upper respiratory tract colonization and an early age of acquisition of these bacteria may, in part, account for higher rates of disease in developing countries. Early age of colonization with a multiplicity of bacterial types is postulated as a cause of persistent carriage leading to Eustachian tube damage and recurrent otitis media in Australia aboriginal infants [4]. However, there is limited data on nasopharyngeal colonization with *S. pneumoniae* from the developing countries to confirm whether this observation is valid. We are unaware of data published from India, where nearly 25% of all the world's babies are born (25 million births per year). This study was undertaken to describe the dynamics of nasopharyngeal colonization of infants by pneumococci and to determine the prevalence of different serogroups/types (SGT) of colonizing pneumococci.

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MATERIALS AND METHODS

This prospective observational study was conducted among infants attending the Well Baby Clinic of the Christian Medical College and Hospital, Vellore, India. Infants who were born in, and residing in three

geographically contiguous areas, namely the Vellore and Tiruvannamalai districts of Tamilnadu State and Chittoor district of Andhra Pradesh were included in the study, after obtaining consent from the parent or guardian. They were recruited into the study during their first immunization visit at 6 weeks of age when demographic data were collected for each of the study infants and their length and weight recorded.

Nasopharyngeal swabs were obtained at the time of recruitment and at every subsequent immunization visit (i.e. 10, 14, 18 and 22 weeks and at 9 and 18 months) with calcium alginate swabs on a flexible aluminium wire (Hard Wood Products, Guilford, ME, USA) using the technique recommended by the World Health Organization [5].

The swabs were transported immediately to the microbiology laboratory where they were inoculated on to culture plates containing trypticase soy agar (Baltimore Biologicals, MA, USA) with 5% sheep blood (TSBA) and 5 μg of gentamicin sulphate per ml of the medium. Plates were incubated at 37 °C overnight in a CO_2 extinction jar and then examined for the presence of α-haemolytic colonies. Such colonies were screened by Gram's stain. If the morphological appearance was suggestive of *S. pneumoniae*, then the identity was confirmed by susceptibility to optochin and bile solubility [6]. Isolates of *S. pneumoniae* were serogrouped/typed by a co-agglutination test using pneumococcal antiserum supplied by the Statens Serum Institute, Copenhagen [7]. A single colony was picked from the primary plate and subcultured. A sweep from the subculture on TSBA was used for serogrouping/typing.

Antimicrobial susceptibility testing was done using Kirby Bauer's disk diffusion method on plates containing Mueller–Hinton agar (Baltimore Biologicals, MA, USA) with 5% sheep blood [8]. The following antibiotics (concentration per disk) were used for susceptibility testing: penicillin (10 U), oxacillin (1 μg), erythromycin (15 μg) chloramphenicol (30 μg) (Becton–Dickinson, Cockeysville, MD, USA) and cotrimoxazole (25 μg) (Span Diagnostics Ltd., Sachin, India). The 1 μg oxacillin disc was used to screen for penicillin susceptibility [9]. Estimation of minimum inhibitory concentration (MIC) of penicillin using the agar dilution technique was carried out when screening with an oxacillin disk suggested resistance to penicillin. Acquisition was defined as the recovery of any pneumococcal SGT for the first time, or by the recovery of a SGT not previously detected, in a given child. The date of acquisition was taken as the midpoint between the first positive culture and the previous negative culture. The duration of carriage was calculated from the date of acquisition to the midpoint between the last consecutive positive culture and the subsequent negative culture, if only one culture was positive, duration of colonization was considered to be 1 month.

Student's t test was used to compare means and the χ^2 test or Fisher's test to compare proportions. Odds ratios and 95% confidence limits were determined using EPi-Info 6.0 software.

RESULTS

One hundred healthy infants, 52 female and 48 male, were recruited to the study from July 1994 to October 1995. The median age at first visit was 8 weeks and the median age at the last visit was 34·4 weeks. They were observed for a median duration of 28 weeks (range: 4–44 weeks). Fifty-five infants completed all six scheduled visits for primary immunization (i.e. 6, 10, 14, 20 and 22 weeks and 9 months) and 79 infants completed four or more visits. In addition, 29 children made one additional visit to the clinic after 9 months of age. The median age for this visit was 18 months (range 9·5–20·5 months).

A total of 506 nasopharyngeal cultures was obtained from these 100 infants during the study period. Of these, 202 (40%) were positive for *S. pneumoniae*.

Nasopharyngeal colonization with *S. pneumoniae* was noted at least once during the period of observation in 81 (81%) of the 100 infants studied. Of the 81 colonized infants, 58% were colonized with pneumococcus before the age of 18 weeks (Table 1). The median age of acquisition of *S. pneumoniae* was 11 weeks (range 5–42 weeks). Only six children first acquired pneumococci after the age of 30 weeks.

The median duration of carriage of *S. pneumoniae*, irrespective of the SGT, was 1·3 months (range 0·9–15·9 months). In over 75% of colonized infants, the duration of carriage was less than 4 months, in five (7%) of the 81 infants the duration of colonization was 7 months or more.

The media age of acquisition of a second SGT of *S. pneumoniae* was 5·6 months (range 3·4–12 months) and the media duration of carriage of the second SGT was 1·15 months (range 0·9–6·2 months).

Serogroup/type analysis

Thirty *S. pneumoniae* SGTs were isolated. Of these, the four most common types were 6, 19, 14 and 15.

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Table 1. *Age at first colonization*

Age (weeks)	No. (%) colonized	Cumulative %
< 6	13 (13)	13
7–10	18 (18)	31
11–14	17 (17)	48
15–18	10 (10)	58
19–22	7 (7)	65
23–26	10 (10)	75
⩾ 7	6 (6)	81

Table 2. *Common SGTs of pneumococcus isolated from nasopharyngeal culture*

SGT	No. of isolates	No. of children colonized
6	31	18
19	24	15
14	14	8
15	14	8
23	12	10
11	10	7
35	10	8
9	8	6
13	7	2
39	6	3
4	4	1
33	4	2
8	3	2
16	3	3
Others	23	16
Not typed	29	24
Total	202	81

These SGTs formed 48% of all isolates for which SGT data were available (Table 2). Two separate SGTs were isolated from 5 specimens (32 and 23 in 1, 29 and 35 in two, and 35 and 42 in 2) and 3 SGTs of pneumococci (types 29, 35 and 42) in each of 3 other specimens. SGTs 29, 35 and 42 are known to cross-react during typing. Hence, the identification of these SGTs need not necessarily indicate colonization with different SGTs. In 29 isolates SGT data were not available, either because the isolate was not available for typing or because the isolate was not typable.

In 12 children the same SGT of pneumococcus was isolated from the nasopharynx after it was not detected on one or more visits. The SGT isolated in these cases included types 19 (in 4 children), 6 (in 3 children) and 10, 33, 15, 11 and 41 (in 1 child each). In 25 children, 2 or more SGTs were isolated during the period of follow up. In 18 children colonization with 1 SGT was followed by the acquisition of a second SGT, 5 children were sequentially colonized with 3 different SGTs and 2 with 4 different SGTs.

Table 3. *Age of acquisition and duration of colonization of selected pneumococcal SGTs*

SGT	Median (range) age at colonization (months)	Median (range) duration of colonization (months)
Any	2·90 (1·3–10·5)	1·30 (0·9–15·9)
19	2·90 (1·3–9·3)	1·20 (0·9–4·1)
6	3·50 (1·4–10·2)	1·10 (0·9–6·4)
15	3·70 (1·5–12·6)	1·15 (1·0–12·1)
14	4·60 (3·4–5·8)	2·40 (0·9–5·5)

The age of acquisition and duration of carriage of the common SGTs of *S. pneumoniae* are shown in Table 3. SGT 14 was acquired at a later age when compared with the other types. Also, the duration of the colonization with SGT 14 was more than twice that of the other SGTs. However, the longer gap between the last three visits (22 weeks and 9 months, and 9 and 18 months, respectively) compared with the earlier visits (at 4-weekly intervals) may account for the longer duration of carriage, since duration was defined as the midpoint of the last positive culture and the subsequent culture.

The frequencies of isolation of the various SGTs from the nasopharynx were compared with those obtained from blood or CSF of children aged less than 5 years in Vellore during a multicentre study in India in 1993–7 called the Invasive Bacterial Infection Surveillance (IBIS) study [unpublished] (Table 4). Odds ratios were calculated to determine the relative invasiveness of the different SGTs [10]. Pneumococcal SGTs 1 and 5 were significantly more often invasive than carried. SGTs 1, 5, 7 and 12 were not isolated from the nasopharynx though they were isolated from children with invasive disease. SGT 23 showed a low invasive potential (OR 0·00, 95% CL 0·00, 0·81, $P = 0·01$).

Seasonal variation

The rate of isolation of *S. pneumoniae* by calendar month is shown in Figure 1. The percentage of positive cultures was lowest in July and highest in December and January. July to November are the wettest months of the year, whereas December to February are the coldest; the temperature seldom goes below 15 °C even in the cold season.

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Table 4. *Comparison of frequency of common serogroups/types of pneumococcus from blood or CSF from children under 5 years from Vellore (1993–7), and from nasopharynx*

SGT	Invasive isolates (%) ($n = 67$)	Nasopharyngeal isolates (%) ($n = 109$)	Odds ratio (95% CI)	*P*-value*
1	7 (10)	0 (0)	∞†	< 0·001
4	3 (4)	1 (1)	5·06 (0·46, 129)	0·15
5	4 (6)	0 (0)	∞	0·02
6	18 (27)	18 (16·5)	1·86 (0·83, 4·15)	0·10
7	1 (2)	0 (0)	∞	0·38
12	2 (3)	0 (0)	∞	0·14
14	6 (9)	8 (7)	1·24 (0·36, 4·19)	0·15
15	2 (3)	8 (7)	0·39 (0·06, 2·07)	0·32
18	2 (3)	2 (2)	1·62 (0·16, 16·57)	0·63
19	5 (7)	15 (14)	0·50 (0·15, 1·59)	0·20
23	0 (0)	10 (9)	0·00 (0·00, 0·81)	·01

* Fisher's exact or χ^2 test.
† ∞, undefined.

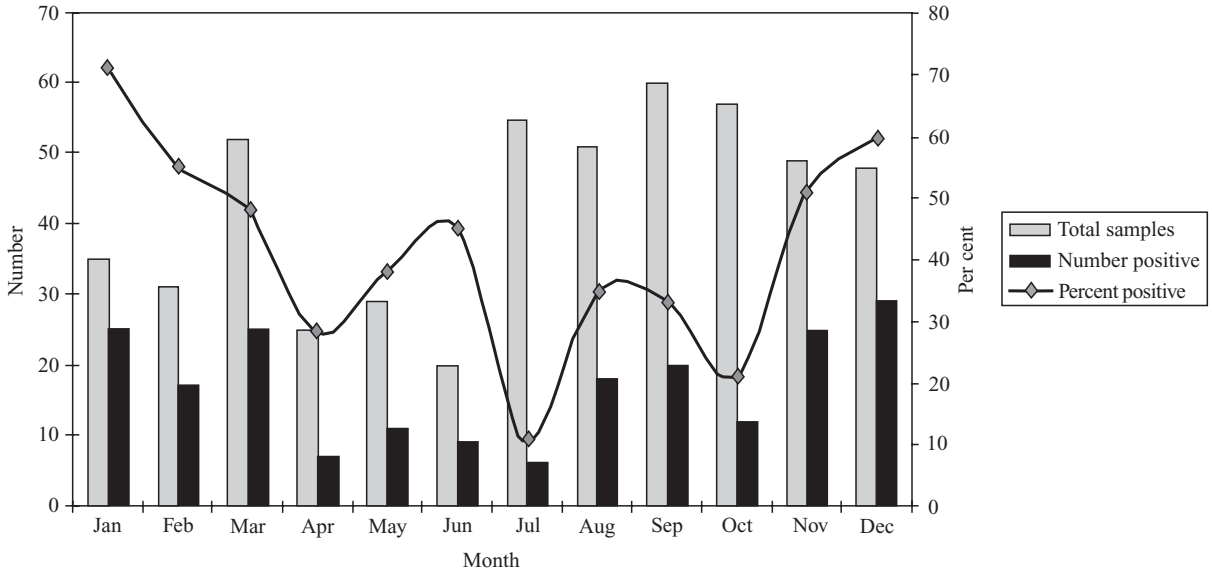

Fig. 1. Month-wise distribution of specimens collected and percent positive for pneumococcus.

Antimicrobial susceptibility

All the *S. pneumoniae* isolates were susceptible to penicillin. Resistance to chloramphenicol, cotrimoxazole and erythromycin were observed in 13 (6·4%), 11 (5·4%) and 5 (2·5%) of the isolates, respectively. Resistance to chloramphenicol, cotrimoxazole and erythromycin was seen in 4 (6%), 27 (40%) and 1 (1·5%), respectively, of 67 invasive isolates of pneumococci from children under 5 years of age during the IBIS study [unpublished]. A significantly higher number of invasive isolates showed resistance to cotrimoxazole when compared with the nasopharyngeal isolates ($P < 0·0001$). Two of the invasive isolates showed intermediate susceptibility to penicillin.

DISCUSSION

This study documents that the majority of children in southern India are colonized with *S. pneumoniae* during infancy; 81% of children were colonized on one or more occasions. The age of acquisition of these bacteria is later than in Australian aboriginal infants [4] or infants from Papua New Guinea [3], but earlier than in children in North America [11] or Europe [12]. The subjects in this study mainly belonged to urban,

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middle-income families and the results from this study may not reflect colonization in other population groups. Further studies in other population groups are required to substantiate our data.

The commonest SGTs of pneumococcus isolated in this study were 6, 19, 15, 14 and 23 in decreasing order of frequency. These five SGTs comprised 55% of pneumococcal isolates for which data were available. These are similar data to those from the United States where types 6, 19, 23, 15 and 14 comprise close to two-thirds of all colonizing types [11, 13] and Papua New Guinea where these SGTs formed 79% of all the isolates [3]. SGTs 6 and 19, which are commonly isolated from the nasopharynx were also the common invasive SGTs. However, SGT 1 was not isolated from the nasopharynx in any of the infants but was the second most frequent invasive isolate. Similarly, SGTs 5, 7 and 12 were also never isolated from the throat but produced invasive disease in children. The high invasive potential of *S. pneumoniae* type 12 in children has been described previously [14]. On the other hand, SGT 23, which frequently colonized infants, was not a common invasive isolate. Data from North America [15], Finland [16] and Papua New Guinea [10] indicate that SGT 14 has high invasive potential. We found that this SGT was isolated with equal frequency from the nasopharynx and from potentially sterile body sites. This suggests that the strains of pneumococcus type 14 in our region are less invasive than those described in western countries. Genotyping of pneumococcal isolates in Finland showed that all the tested SGT 14 isolates appeared to belong to a single clone. It is possible that genotype variation may account for the difference in the invasive potential of this specific SGT in different regions.

The acquisition and duration of carriage varied among the different SGTs. Among the common SGTs, type 19 was acquired the earliest and type 14 the latest. The duration of carriage of type 14 was twice as long as that of the other SGTs, though the increased interval between the last two visits may have influenced these results.

The transmission of pneumococci has been linked to upper respiratory tract infection. Simultaneous transmission of rhinovirus and pneumococcus has been reported [17]. It is also known that upper respiratory infections are more common during the winter months. So it was postulated that pneumococcal carriage in the nasopharynx would be increased in winter. Gray and colleagues found that there were quarterly peaks, in the months of March, June, September and December with a higher peak in the months of December, January and February [11]. On the other hand, in Papua New Guinea, there was no apparent seasonal trend [3]. In our study we found that *S. pneumoniae* was isolated from 71% of the nasopharyngeal cultures in the month of December, which is one of the colder months in this region, whereas only 10% the cultures taken in the month of July, the onset of the wet season, yielded pneumococcus. These data suggest that the increase in the proportion of the colonized infants during the cooler months of the year may be associated with viral respiratory infections, which are common during this period in southern India [18].

Penicillin resistance among pneumococci is a disturbing trend, but has not been reported in India. In this study no penicillin resistant strains were detected. It has been suggested that antimicrobial susceptibility patterns of *S. pneumoniae* isolated from the nasopharynx may be used for surveillance of antimicrobial resistance. Studies from Pakistan [19] and Egypt [20] have shown that antimicrobial susceptibility of nasopharyngeal isolates of pneumococci and *H. influenzae* from clinic patients were similar to those of invasive isolates. However, our study of healthy children has documented a substantial difference in resistance to cotrimoxazole between nasopharyngeal and invasive isolates. Further studies comparing the antimicrobial susceptibility patterns of invasive and nasopharyngeal isolates of pneumococci are required to determine the suitability of surveillance for antimicrobial susceptibility using nasopharyngeal isolates.

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