Nasopharyngeal carriage of resistant pneumococci in young South Indian infants

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SUMMARY

Streptococcus pneumoniae is the leading bacterial cause of life-threatening infections in infants. Although antibiotic resistance affects management of pneumococcal infections, few data on patterns of resistance are available for India. We examined nasopharyngeal carriage of antibiotic-resistant pneumococci in 464 South Indian infants between 2 and 6 months. Newly acquired serotypes were screened for susceptibility to cotrimoxazole, erythromycin and penicillin using disk diffusion. Cumulative prevalence of pneumococcal carriage rose from 53·9% at 2 months to 70·2% at 6 months. The prevalence of strains that were not susceptible to penicillin, cotrimoxazole and erythromycin was 3·4, 81·1 and 37·2%, respectively. Carriage of erythromycin non-susceptible strains declined significantly between ages 4 months and 6 months (44·1 *vs.* 10·7%). More than 87% of the isolates screened were non-susceptible to ⩾1 antibiotic. Serogroups/types that were most frequently non-susceptible to 1 or more antibiotics were 6, 9, 14, 19 and 23. Less than 1% of the isolates were multi-drug resistant. Widespread use of antibiotics in South India has resulted in *S. pneumoniae* becoming non-susceptible to some commonly used antibiotics. Monitoring trends in antibiotic susceptibility and making antibiotics available only through prescription from a health care worker may slow the spread of resistant pneumococci and improve management of pneumococcal infections in South India.

INTRODUCTION

Streptococcus pneumoniae is a leading cause of severe pneumonia among infants in developing countries [1, 2]. It is estimated that pneumococcal disease is responsible for more than 1 million deaths in children under 5 years of age each year. Approximately, 42%

of these deaths occur in infants less than 6 months of age [3]. In addition, *S. pneumoniae* is a common bacterial cause of otitis media and sepsis in children under 2 years of age and is a leading cause of meningitis [4]. The emergence and spread of antibiotic-resistant pneumococci threatens to make management of pneumococcal infections less effective and more expensive [5, 6]. In most countries, newer second-line antimicrobial agents are expensive and, therefore, unavailable to the majority of the population, causing many infections to become effectively untreatable.

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Current data on the susceptibility of pneumococcal isolates to antibiotics are necessary to minimize the impact of drug-resistance on case management. There are few published data available on community-level anti-microbial susceptibility patterns of pneumococci for India [7, 8]. Antibiotic therapy is empiric, and blood cultures are not routinely done and have little value in areas where antibiotic use prior to seeking medical care is common. Alternatively, nasopharyngeal isolates of healthy children may be used to gauge the pattern of pneumococcal antibiotic-resistance in local populations [9–12].

We studied the pattern of antibiotic-susceptibility of nasopharyngeal pneumococcal isolates from a cohort of South Indian infants in the first 6 months of life. Isolates were subjected to penicillin, cotrimoxazole and erythromycin, three agents commonly used for treating pneumococcal infections in developing countries.

SUBJECTS AND METHODS

Study population

The Infant Pneumococcal Acquisition/Carriage in Tamilnadu (InPACT) study was nested within an on-going, randomized, double-blinded, placebo-controlled study known as the Vitamin A Supplementation in Newborns (VASIN) trial. VASIN was a 3 year trial designed to evaluate the impact of vitamin A supplementation at birth on the morbidity, mortality and growth among 9000 newborns through the first 6 months of life. The trial was conducted in Natham and Karriyapatty, two rural areas near the South Indian city of Madurai. These areas were selected because they share characteristics including endemic vitamin A deficiency, high incidence of acute respiratory infections, and demographic similarities with many rural communities in South Asia. The methods and data collection procedures for the VASIN trial have been described previously [13].

InPACT enrolment

From 22 October 1998 to 30 June 1999, nasopharyngeal specimens were obtained from 464 infants living in Natham block. Infants born into the VASIN trial who survived to 2 months of age but were not older than 2·5 months of age, and who resided in one of eight selected supervisory areas in Natham block were eligible for enrolment in the InPACT study. The larger and denser population of Natham compared to Karriyapatty made it the optimal choice for the InPACT study. The eight supervisory areas with the highest birth rates in Natham were selected.

Ethical review

The VASIN and InPACT study protocols were approved by the Ethical Committee of the Aravind Center for Women, Children and Community Health, Lions Aravind Institute for Community Ophthalmology and by the Committee on Human Research of the Johns Hopkins University Bloomberg School of Public Health. The protocols were also approved by an ethical committee constituted by the government of Tamilnadu. Infants were included in each study based on oral informed consent from parents or guardians. Verbal consent was considered appropriate given the level of literacy in the community.

Data collection

Nasopharyngeal specimen collection

Three nasopharyngeal specimens were collected from each infant in the study, at 2, 4 and 6 months of age. Specimens were collected by five trained field workers following a set protocol. A small, flexible rayon-tipped swab (DIFCO CultureSwab Transport System with Amies Media) was inserted into the posterior nasopharynx for a minimum of 5 s or rotated 180 degrees before removal. Swabs containing specimens were placed in Amies transport medium and delivered to the microbiology laboratory at the Aravind Eye Hospital within 10 h of collection.

Laboratory procedures

The nasopharyngeal swabs were inoculated onto tryptic soy agar plates (Becton Dickinson) with 5 % sheep blood and 5 μg/ml gentamicin (Nathan Pirumal, Bombay, India) within 12 h of arrival at the hospital microbiology laboratory. When laboratory staff were not able to carry out inoculation within 12 h, the swabs were transferred from the Amies media to 0·5 ml of skim milk media and frozen at $-20\,^{\circ}$C for no longer than 2 days before processing. Inoculated plates were incubated at 37 °C in 5 % CO_2 for 18–24 h. Optochin (Taxo) inhibition and bile solubility (Himedia) tests were used to confirm colonies that showed classic pneumococcal morphology. Quality control was maintained by the use of American Type

Culture Collection (ATCC) 5603, a pneumococcal reference strain. Serogrouping/typing of penumococcal isolates resistant to one or more antibiotics was carried out using PNEUMOTEST kits (Staten Serum-institute, Copenhagen, Denmark) [14]. The antisera included in the kits reacts with serotypes 1, 2, 3, 4, 5, 8, 14, 20 and with serogroups 6, 7, 9, 11, 12, 15, 17, 18, 19, 22, 23 and 33, those included in the licensed 23-valent pneumococcal polysaccharide vaccine.
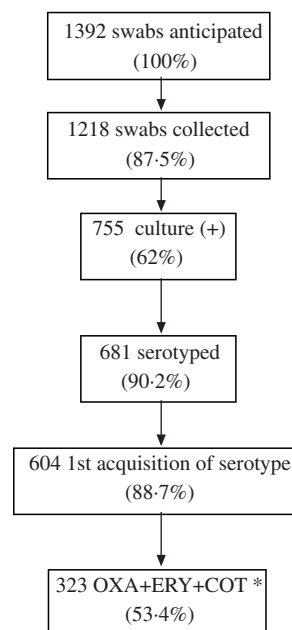
Specimens with an optochin inhibition zone larger than 13 mm were classified as 'culture-positive'. Those with a 9–13 mm inhibition zone were considered 'culture-indeterminate'. Culture-indeterminate specimens were verified with the bile solubility test. Infants were considered to be carrying pneumococci if they had a culture-positive or bile sensitivity test-confirmed specimen.

The Bauer and Kirby disk diffusion method was used to screen pneumococcal isolates for sensitivity to cotrimoxazole (trimethoprim/sulfamethoxazole, $1 \cdot 25/23 \cdot 75 \mu g$ disk), erythromycin ($15 \mu g$ disk) and penicillin (oxacillin, $1 \mu g$ disk). Isolates were classified as sensitive, intermediately resistant or resistant to cotrimoxazole and erythromycin according to the recommendations of the National Committee for Clinical Standards (NCCLS) [15]. Similarly, isolates were categorized as either sensitive or resistant to oxacillin based on the results of the screening test. However, no confirmatory testing was done for isolates that screened resistant to oxacillin. All susceptibility testing was carried out using Mueller–Hinton media with 5% lysed sheep blood. Isolates that were sensitive to any of the three antibiotics were categorized as 'susceptible'. Conversely, isolates not susceptible to any of the three antibiotics were categorized as 'non-susceptible'. Strains found to be non-susceptible to three antibiotics were classified as 'multi-drug resistant'.

Statistical analysis

Two-tailed χ^2 analysis or Fisher's exact test for contingency data were used, as appropriate, to assess differences in the frequency of non-susceptible colonization with newly acquired serotypes by antibiotic (Stata 6.0, Stata Corporation, College Station, Texas). Logistic regression was used to evaluate age as a risk factor for carriage of non-susceptible pneumococcal strains adjusted for the effect of season of acquisition.

No. of children enrolled: 464
No. of nasal swabs anticipated: $464 \times 3 = 1392$

1392 swabs anticipated (100%)
↓
1218 swabs collected (87·5%)
↓
755 culture (+) (62%)
↓
681 serotyped (90·2%)
↓
604 1st acquisition of serotype (88·7%)
↓
323 OXA+ERY+COT * (53·4%)

* OXA, Oxacillin; ERY, Erythromycin; COT, Cotrimoxazole

Fig. 1. Nasopharyngeal specimen collection.

RESULTS

Participant follow-up

For the 464 infants enrolled at age 2 months, follow-up rates at ages 4 months and 6 months were 87·5% (406/464) and 77·3% (359/464), respectively.

Specimen collection

Among the 464 infants, 1218 (87·5%) out of 1392 possible specimens were collected (Fig. 1). Of the number collected, 62% (755/1218) of the specimens were culture-positive for pneumococci. Isolates were often frozen until they could be serotyped; 8·8% (74/755) of the isolates were not viable after thawing and were not serotyped. Of the remaining 681 isolates, 605 (88·8%) represented newly acquired (incident) serotypes.

Susceptibility testing

Not all of the isolates could be tested against all three antibiotics at the same time for logistical reasons (shipping delays, inadequate storage space and lack of availability of antimicrobial disks). Of 604 new

acquisitions, 323 (53·4%) available isolates were tested against all three antibiotics. We compared the serotype/group (SGT) distribution of isolates resistant to one or more antibiotics and the proportion of resistant isolates by drug and age group between isolates tested against three drugs to those tested against one or two drugs and found that the differences were not statistically significant.

Demographic characteristics

Most of the families were Hindu and were from marginalized communities. About one-third of the infants weighed less than 2500 g at birth. All study infants were breast-fed and more than 80% received colostrum. Approximately 9% of the mothers reported experiencing night blindness during pregnancy, a classic symptom of vitamin A deficiency. The majority of infants (86·1%) had one or no sibling. The families of the infants were of low socioeconomic status and nearly 50% of the mothers had no formal education.

Prevalence of pneumococcal nasopharyngeal carriage

S. pneumoniae was isolated from 86·2% (400/464) of study infants during the first 6 months of life. Infants acquired pneumococci at an early age; 53·9% were colonized by age 2 months. The carriage prevalence at ages 4 and 6 months was 64·1 and 70·2%, respectively.

Susceptibility of pneumococcal isolates by antibiotic

Antibiotic susceptibility profiles to penicillin, cotrimoxazole and erythromycin were available for 323 (53·4%) of the isolates representing newly acquired strains. The prevalence of penicillin non-susceptibility was low in all age groups; 3·4% of the isolates were not susceptible to penicillin. The proportion of penicillin non-susceptible isolates did not vary significantly by age group. In contrast to penicillin, the prevalence of cotrimoxazole non-susceptible isolates was high; of the 81·1% of those that were non-susceptible, 64·7% were completely resistant. At 2 months of age, 83·8% of isolates were resistant. This proportion was similar in the 4 and 6 months age groups. Approximately 37·0% of the 323 isolates were not susceptible to erythromycin. The majority (105/120) were of intermediate resistance. The pattern of non-susceptibility to erythromycin at ages 2 and 4 months were comparable (53·0 vs. 44·1%), however, by age 6 months the prevalence dropped to 10·7%. The

odds of non-susceptible erythromycin carriage at age 2 months was approximately 4 times greater than among children aged 6 months (odds ratio 3·8 [1·9, 7·9]; $P < 0·001$).

Antibiotic susceptibility profile

Two hundred and seventy-eight (87·1%) of the 323 isolates were not susceptible to one or more antibiotics (Table 1). More than 51% were not susceptible to one drug and 33·7% were resistant to two drugs. The prevalence of susceptibility to multi-drugs was low, 0·9%. Most isolates (92·7%) at age 2 months were resistant to at least one antibiotic, while the proportion of isolates at ages 4 and 6 months resistant to one or more drugs was 85·7 and 77·6%, respectively. The prevalence of multi-drug resistance among the isolates was low, ranging from 1·5% at age 2 months to none at age 6 months.

Serogroup/type (SGT) distribution of isolates resistant to one or more antibiotics

Among the 278 isolates that were not susceptible to one or more antibiotics, the most prevalent SGTs were 6 (10·1%), 14 (8·3%), 15 (6·8%), 19 (7·6%) and 23 (15·8%) (Table 2). This pattern was similar across age groups. In total, 52 (18·7%) of isolates were from serotypes not included in the 23-valent pneumococcal polysaccharide vaccine.

DISCUSSION

Our results show a high prevalence of pneumococcal nasopharyngeal colonization among South Indian children in early infancy. More than one half of the infants were colonized by 2 months of age. By 6 months of age, 83% of the infants had been colonized at least once. The observed colonization rates are similar to those reported from India and other developing countries [8, 16–18].

There was a high frequency of resistant pneumococcal nasopharyngeal colonization in South Indian infants. The majority of the isolates tested were not susceptible to cotrimoxazole, the least expensive, orally administered antibiotic, which is readily available over the counter in India and recommended for use in the Integrated Management of Childhood Illness programme of the World Health Organization. The majority of these were completely resistant to cotrimoxazole. In contrast, about one-third of the

Table 1. *Antibiotic susceptibility patterns of newly acquired pneumococcal nasopharyngeal strains* ($n = 323$) *isolated from InPACT study infants*

Antibiotic resistance pattern*	Age 2 months		Age 4 months		Age 6 months		First 6 months	
	Freq	%	Freq	%	Freq	%	Freq	%
Resistance to 1 drug								
PEN	0	0	0	0	0	0	0	0
COT	49	36	32	38·1	69	67·0	150	46·4
ERY	12	8·8	3	3·6	1	1·0	16	5·0
Subtotal	61	44·8	35	41·7	70	68	166	51·4
Resistance to 2 drugs								
PEN+COT	5	3·7	3	3·6	0	0	8	2·5
PEN+ERY	0	0	0	0	0	0	0	0
COT+ERY	58	42·7	33	39·3	10	9·7	101	31·3
Subtotal	63	46·4	36	42·9	10	9·7	109	33·8
Resistance to 3 drugs								
PEN+COT+ERY	2	1·5	1	1·2	0	0	3	0·9
Susceptible	10	7·4	12	14·3	23	22·3	45	13·9
Total	136		84		103		323	

* PEN, penicillin; COT, cotrimoxazole; ERY, erythromycin.

isolates were not susceptible to erythromycin, the majority of which were of intermediate resistance. The rapid spread of penicillin-resistant pneumococci is a major health concern in the industrialized and developing world. Yet, we observed that less than 4% of the isolates tested screened resistant to penicillin. Our finding is consistent with recently published data from six Indian referral centres that showed the prevalence of penicillin-resistant isolates was 1·5% [7].

Nearly half the isolates were not susceptible to erythromycin during the first 4 months. However, this declined significantly by age 6 months. Most studies in developed and developing countries show that prevalence of resistant strains tend to increase with age, when there is increased exposure to and contact with carriers, mainly other children [6, 19]. The negative association between age and carriage of erythromycin-resistant strains remained after adjusting for the effect of season of strain acquisition. One possible explanation for the observation is that we under-reported the number of isolates that were not susceptible to erythromycin at age 6 months due to missing susceptibility data. However, when we tested this assumption by recoding all the missing erythromycin data to 'non-susceptible', the negative association between age and erythromycin-resistant carriage remained statistically significant.

The prevalence of strains resistant to at least one antibiotic was very high. Most of the strains obtained from the infants in this study were resistant to at least one antibiotic. In contrast, Jebaraj et al. reported little antibiotic resistance among nasopharyngeal pneumococci collected from an urban, middle class, paediatric cohort in northern Tamilnadu [8]. One possible explanation for our observation is antibiotic prophylaxis. There is anecdotal evidence to suggest that many young infants in our study area are given repeated courses of antibiotics as treatment for mild illness and also for prophylaxis. It is also possible that in our study population adult members of the household may be carriers of resistant strains and these strains are then passed to infants; this has been described for meningococci [20]. Neonatal antibiotic prophylaxis and familial pneumococcal carriage warrant investigation because these factors have implications for the prevention and treatment of pneumococcal infections.

SGTs 6, 14, 15, 19 and 23 were the most prevalent SGTs associated with strains resistant to one or more antibiotics in our cohort. These five SGTs comprised 57% of the resistant isolates tested. A 7-valent pneumococcal conjugate vaccine is licensed for use in the United States. Other conjugate vaccine formulations under investigation for use in developing countries contain 9–11 serotypes [21–23]. The licensed 7-valent vaccine contains serotypes 4, 6B, 9V, 14, 18C, 19F, 23F, which accounts for approximately 80% of serotypes associated with invasive pneumococcal infections in the United States [24]. The formulation of the 9-valent vaccines contains the

Table 2. *Serotype distribution of newly acquired nasopharyngeal pneumococcal strains (n = 278 isolates) resistant to ⩾ 1 antibiotics isolated from InPACT study infants*

Serogroup	Age 2 months		Age 4 months		Age 6 months		First 6 months	
	Freq	%	Freq	%	Freq	%	Freq	%
1	3	2·4	1	1·4	0	0·0	4	1·4
3	1	0·8	0	0·0	0	0·0	1	0·4
4	1	0·8	0	0·0	1	1·3	2	0·7
5	1	0·8	1	1·4	0	0·0	2	0·7
6	11	8·7	8	11·1	9	11·3	28	10·1
7	1	0·8	1	1·4	2	2·5	4	1·4
8	1	0·8	0	0·0	0	0·0	1	0·4
9	8	6·4	5	6·9	3	3·8	16	5·8
11	6	4·8	3	4·2	4	5·0	13	4·7
12	0	0·0	4	5·6	0	0	4	1·4
14	12	9·5	4	5·6	7	8·8	19	6·8
15	9	7·1	5	6·9	5	6·3	19	6·8
17	2	1·6	4	5·6	1	1·3	7	2·5
18	5	4·0	2	2·8	1	1·3	8	2·9
19	7	5·6	6	8·3	8	10·0	21	7·6
20	2	1·6	0	0·0	1	1·3	3	1·1
22	5	4·0	0	0·0	2	2·5	7	2·5
23	18	14·3	13	18·1	13	16·3	44	15·8
33	7	5·6	3	4·2	9	11·3	19	6·8
NVT*	26	20·6	12	16·7	14	17·5	52	18·7
Total	126		72		80		278	

* NVT, Vaccine serogroups/types not included in the 23-valent pneumococcal polysaccharide vaccine.

same 7 antigens with the addition of serotypes 1 and 5. The 11-valent vaccines include the same 9 antigens with the addition of serotypes 3 and 7V. We estimated that the 7-, 9- and 11-valent pneumococcal conjugate vaccine formulations would provide coverage against 53%, 55% and 56% of SGTs resistant to one or more drugs, respectively. This estimate assumes that the nasopharyngeal isolate SGTs are representative of those causing disease in this community and that there is cross-protection between serogroups included in the vaccines. In contrast, the 7-valent vaccine protects against 85% of the serotypes commonly associated with infection in the United States paediatric population [21].

One limitation of this study was our reliance on disk diffusion assays for determining susceptibility to penicillin. The use of oxacillin, while appropriate for screening, only allows categorization of isolates as 'sensitive' or presumptively resistant. Confirmatory testing, using microdilution or E-testing, is recommended because of the low specificity associated with use of oxacillin alone. Therefore our results are likely to overestimate the problem of penicillin resistance in this region. However, our results showed a low prevalence of penicillin resistance, one that is consistent with data from other studies conducted in the region [7].

We have detected a high prevalence of resistant *S. pneumoniae* strains isolated from the nasopharynx of young infants in South India. The majority of isolates were resistant to cotrimoxazole, the least expensive of the first-line therapies for pneumococcal disease and other bacterial infections. However, unlike most other regions of the world penicillin resistance is uncommon in South India. Most antibiotics are available over the counter in India. One option for limiting the impact of drug resistance in India is to make antibiotics available only by prescription. Moreover, our study indicates a need for on-going surveillance of antibiotic resistant pneumococcal nasopharyngeal strains to ensure that the treatment guidelines keep pace with local resistance patterns. In addition, our results suggest that pneumococcal conjugate vaccines may lower the prevalence of resistant pneumococcal strains in this community, if administered soon after birth.

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