

Nasopharyngeal carriage of *S. pneumoniae* among young children in rural Nepal

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Summary

OBJECTIVES To provide epidemiologic data on *Streptococcus pneumoniae* (*Spn*) carriage in Nepal. **METHODS** Prospective, population-based study among children in Sarlahi, Nepal to estimate carriage prevalence, identify risk factors, and determine antibiotic susceptibility patterns and serotype distribution. Between December 2003 and July 2004, NP specimens were collected from 604 children aged 1–36 months with acute lower respiratory infection (ALRI) and 604 healthy, age- and season-matched controls.

RESULTS Of the 1100 specimens analysed, carriage prevalence was approximately 80% in both groups. In the multivariate analyses, significant risk factors for *Spn* carriage in controls were Muslim religion [adjusted odds ratio (AOR): 2.93] and no latrine in the household (AOR: 2.41). Those treated for a recent illness had lower carriage rates (AOR: 0.37). Results were similar for ALRI cases with the addition of age ≥ 12 months (AOR: 1.68), and symptomatic infection (AOR: 3.78) as risk factors. The antibiotics and proportions of isolates resistant to them were as follows: penicillin 4.5%, cotrimoxazole 89.2%, chloramphenicol 1.4%, erythromycin 1.5% and tetracycline 22.7%. The most prevalent serogroups/types were 6, 19, 23, 15, 9 and 10.

CONCLUSIONS Young children in rural Nepal experience high rates of *Spn* carriage. Most isolates were resistant to cotrimoxazole. Current conjugate *Spn* vaccines may substantially reduce the risk of a severe pneumonia and other *Spn* infections.

keywords *S. pneumoniae*, nasopharyngeal carriage, Nepal, epidemiology, children

Introduction

Streptococcus pneumoniae (*Spn*) is a leading cause of life-threatening pneumonia in young children globally (Mulholland 2007). It is estimated that *Spn* accounts for 30–50% of the two million pneumonia deaths that occur annually in children <5 years of age (Wardlaw *et al.* 2006; Rudan *et al.* 2008). The effectiveness of empiric therapy for *Spn* infections is being threatened by the spread of strains resistant to first-line antibiotics (Lee *et al.* 2001; Jacobs 2003). In poor settings, second-line antibiotics are often unavailable, which may increase treatment failures and facilitate disease transmission.

Nasopharyngeal (NP) colonisation is the necessary first step in the pathogenesis of *Spn*-associated invasive respiratory tract infections (Garcia-Rodriguez & Fresnadillo Martinez 2002; Bogaert *et al.* 2004). Results from observational studies have shown rapid acquisition of *Spn* in

early infancy among populations with a high burden of infections (Huebner *et al.* 1998; Coles *et al.* 2001). *Spn* conjugate vaccines are highly efficacious in the prevention of invasive disease in young children caused by vaccine serotypes (Klugman 2001; Greenwood *et al.* 2007) and in lowering disease transmission by reducing NP carriage of these strains, which, in turn, may stem the spread of antibiotic-resistant strains (Klugman 2001; Kayhty *et al.* 2006). A licensed seven-valent *Spn* conjugate vaccine is in routine use in the USA and Europe. There are also new 10- and 13-valent vaccine formulations under evaluation for use in developing countries. Several issues have delayed the introduction of conjugate vaccines in low-income countries: the vaccines are expensive, the *Spn* burden of disease is ill-defined and the distribution of pathogenic serotypes is known to vary geographically, raising concerns about whether these vaccines provide sufficient coverage of local strains (Levine *et al.* 2006; Pletz *et al.* 2008).

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In Nepal, as in many low-income countries, there are insufficient epidemiologic data on *Spn* infections. Surveillance of NP *Spn* isolates from healthy children is frequently used to provide information on antibiotic susceptibility and serotypes circulating in the community responsible for *Spn* infections, and is key for the development of rational therapeutic guidelines for *Spn* disease and assessing vaccine coverage. There is evidence that acute infection may facilitate NP carriage while recent antibiotic therapy may reduce carriage of susceptible strains (Arason *et al.* 1996; Syrjanen *et al.* 2001). It is possible that these factors may also alter serotype and drug resistance profiles.

We studied the epidemiology of *Spn* carriage in children aged 1–36 months living in rural southern Nepal with acute lower respiratory infection (ALRI) and in healthy, age- and season-matched controls.

Materials and methods

Study population

The *Spn* carriage study was nested within a community-based, cluster-randomised, double-blind, placebo-controlled, 2 × 2 factorial micronutrient prophylaxis trial conducted in rural Nepal. The aim of the Nepal Nutrition Intervention Program, Sarlahi – 4 (NNIPS4) trial was to evaluate the effect of daily supplementation with zinc and iron/folate prophylaxis on mortality and infectious morbidity in 41 276 children aged 1–36 months. The trial was conducted between October 2001 and January 2006 in Sarlahi, a rural district in southern Nepal. In November 2003, the iron/folate and iron/folate with zinc arms of the trial were halted on the recommendation of the study's data safety monitoring board because of a lack of evidence for any treatment effect. Clusters that had been randomised to iron/folate arms were subsequently re-randomised to zinc or placebo. The trial results showed no difference in ALRI incidence or in ALRI mortality rates between zinc and placebo groups (Tielsch *et al.* 2006, 2007).

NNIPS4 enrolment and study procedures

All children aged 1–35 months who resided in the study area were eligible for enrolment. There were no exclusion criteria. Once enrolled, interviewers collected information on household characteristics and recorded children's mid-upper arm circumference before they received their first supplement. Study workers visited households 2 days per week to give children their assigned tablets. Caregivers were responsible for administering the tablets to children between staff visits. The workers visited the households of participants in the morbidity substudy on a weekly basis to

record data on signs and symptoms of illness. All enrolled children were followed until death, migration out of the study area or discharge at 36 months of age.

Carriage study enrolment

Enrolment in the carriage study was open to all children participating in the NNIPS4 morbidity substudy. Between December 2003 and July 2004, 604 acute lower respiratory infection (ALRI) cases and 604 healthy, age- and season-matched controls were enrolled in the carriage study.

Identification and ascertainment of ALRI cases

A case was defined by the presence of the following three criteria: (1) cough, (2) fever or shaking chills and (3) fast breathing, difficulty breathing or chest retractions occurring concurrently on one or more of the prior seven days (i.e. the recall period). Interviewers informed NP specimen collectors of presumptive ALRI cases identified during their weekly household morbidity assessment. Collectors returned to the household within 24 h of notification to enrol the case. Parents of children who fulfilled the ALRI case definition were provided with information about the carriage study. If enrolled, a collector recorded the presence of ALRI symptoms, noted whether the child had been treated for any illness <7 days before their visit, and then collected an NP sample from the child. Children with symptomatic illness at the time of specimen collection were referred to the nearest health post for treatment. The end of an ALRI episode was defined when all three ALRI criteria were absent for at least seven consecutive days. Once a child was enrolled as a case, at least 4 weeks had to elapse between ALRI episodes to be eligible for enrolment as a new case.

Selection of controls

Children who had not met the ALRI case definition in the past 4 weeks were eligible to be controls. For each case, a control was randomly selected from a list of potential controls identified from a list of substudy participants from the same cluster. The controls were individually matched on age (± 3 months), and month of enrolment. In the event that an appropriate age- and season-matched control could not be identified within the same cluster, a suitable matched control was selected from the cluster nearest to the case. Specimen collectors attempted to enrol controls within 7 days of enrolling the cases with whom they were matched. Once enrolled, collectors examined the controls for any ALRI symptoms and recorded their history of recent treatments before collecting NP specimens. Controls

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found to meet the case definition during their examination were enrolled as new cases.

Ethical review

The NNIPS4 trial and *Spn* carriage study protocols received approval by the Nepal Health Research Council and The Committee for Human Research of the Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA. Children were included in the studies on the basis of oral informed consent from parents or guardians. Verbal consent was considered appropriate this community because of the high prevalence of illiteracy.

Data collection

Nasopharyngeal specimens were collected by trained field workers following a set protocol. A flexible calcium alginate-tipped swab (Fisher Scientific, Pittsburgh, USA) was inserted into the posterior nasopharynx for a minimum of 5 s before removal. The swab tips were then inserted in a plastic vial containing skim milk–tryptone–glucose–glycerol medium (STGG) (O'Brien *et al.* 2001), and transported on ice to the field office within 6 h. Specimens were stored at -20°C at the field office and then transported on ice to the microbiology laboratory at the Institute of Medicine in Kathmandu on a weekly basis.

In the laboratory, all NP specimens were frozen at -80°C until they were processed. Swabs were inoculated onto tryptic soy agar plates (Becton Dickinson, Cockeysville, MD, USA) containing 5% sheep blood and 5 $\mu\text{g}/\text{ml}$ gentamicin (Nathan Pirumal, Bombay, India). Inoculated plates were incubated at 37°C in 5% CO_2 for 18–24 h. Colonies exhibiting classic *Spn* morphology were confirmed by optochin (Taxo) inhibition or bile solubility testing. All culture-positive specimens were then sub-cultured and frozen in STGG at -20°C until they were serogrouped/typed. *Spn* isolates were serogrouped/typed using PneumoTest kits (Staten Serum Institut, Copenhagen, Denmark). The kit antisera reacts with a total of 23 SGTs: serotypes 1, 2, 3, 4, 5, 8, 14 and 20 and serogroups 6, 7, 9, 11, 12, 15, 17, 18, 19, 22, 23 and 33. All non-groupable/typable isolates were submitted to the WHO Pneumococcal Reference Laboratory at Staten Serum Institut in Denmark for identification. SGTs were further categorised based on their inclusion in three current *Spn* conjugate formulations: the licensed seven-valent vaccine (Pneumovax, Wyeth Vaccines, Pearl River, NY, USA) as well as a 10-valent (10vPnC, GlaxoSmithKline, Rixensart, Belgium) and a 13-valent (13vPnC, Wyeth Vaccines, Pearl River, NY, USA) undergoing evaluation for use in developing countries. Pneumovax includes serotypes 4, 6B, 9V, 14,

18C, 19F and 23 F, 10vPnC includes all of the previous seven along with serotypes 1, 5 and 7F and 13vPnC includes all 10 serotypes with the addition of serotypes 3, 6A and 19A.

The Bauer and Kirby disk diffusion method (Bauer *et al.* 1966) was used to screen isolates for susceptibility to first-line antibiotics commonly used for the treatment of infections in low-income countries: penicillin, cotrimoxazole, tetracycline, erythromycin and chloramphenicol. Isolates were classified as susceptible, intermediately resistant or resistant to the antibiotics based on the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards 2002). Minimum inhibitory concentrations (MIC) were determined using E-test (AB Biodisk, Solna, Sweden) to confirm penicillin resistance in *Spn* isolates that screened resistant to oxacillin. Isolates found to be non-susceptible to three or more antibiotics were classified as 'multi-drug resistant'.

Statistical analysis

Data were analysed with STATA 9 software (Stata Corporation, College Station, TX, USA). McNemar's tests for paired contingency data and paired *t*-tests for continuous data were used to evaluate differences in baseline characteristics between ALRI cases and matched controls. Bivariate associations between potential risk factors and NP colonisation were evaluated using logistic regression models. Variables statistically significant at the level of $P \leq 0.10$ in the bivariate analyses were included in multivariate logistic regression models. Odds ratios and 95% confidence intervals were used to measure the association between potential risk factors and NP colonisation. A $P \leq 0.05$ was considered statistically significant in multivariate analyses. A final, more parsimonious model was fit with covariates that were significant in the initial multivariate model.

Results

Enrolment

A total of 604 ALRI cases and 604 healthy, age- and season-matched controls were identified and enrolled during the study. There were no refusals. In the parent trial, rates of death, migration and refusal to continue receiving the assigned supplements among the 41 276 children were 1.6%, 8% and 6%, respectively. Thus, it is unlikely that these outcomes had an impact the results of this cross-sectional study. The mean ($\pm\text{SD}$) number of days that elapsed between enrolling a case and a matched

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control was 1.1 (± 2.5) days. Among 23 of 550 pairs (4.2%) the duration between enrolling the case and control was >7 days. Similarly, the mean (\pm SD) age difference between cases and controls 0.9 (± 1.0) months. However, in 16 of the 550 pairs (2.9%) the age differences were >3 months, and in three of the 550 pairs (0.5%) the age difference was >3.7 months. The errors in age matching are likely to be related to errors in the initial reporting of the dates of birth that were later corrected. The mean age of the controls was 18 days younger than that of the entire morbidity subsample (11.8 [± 6.2] *vs.* 12.4 [± 6.3] months, $P = 0.043$) at the time of entry into the morbidity substudy. Of the 604 cases, 54 (8.9%) had experienced their last ALRI symptoms >7 days before NP specimen collection and were omitted from the analysis along with their matched controls. Among the 1100 children included in the analysis, 8% (44/550) of those children initially enrolled as controls were eventually enrolled as cases during the study.

Demographic characteristics

Half of the 1100 children were male and 75% were >12 months of age at the time of enrolment (Table 1). Three-quarters of the participants had at least one sibling <5 years of age. Their families were primarily Hindu and members of marginalised communities. Approximately 80% of the mothers of study participants had had no

formal education. While the prevalence of maternal smoking was low, it was significantly greater among cases than controls. The majority of families of the study participants were of low socioeconomic status; <20% lived in homes with electricity and <11% had a latrine in their household.

Prevalence of *Spn* NP carriage

Spn was isolated in 79.4% (873/1100) of the study children. The proportion of carriers was significantly greater among healthy controls than among ALRI cases (82.0% *vs.* 76.7%, $P = 0.031$). Among children aged ≥ 12 months, the prevalence of *S. pneumoniae* carriage was similar between ALRI cases and healthy controls (80.0% *vs.* 83.0%, $P = 0.267$). However, among infants, the prevalence of carriage was significantly lower among the cases than the controls (68.0% *vs.* 79.2%, $P = 0.028$).

Risk factors for NP carriage of *S. pneumoniae*

Among controls, seven factors were significantly associated with pneumococcal carriage in the bivariate analyses (Table 2). The risk of colonisation among Muslim children was three times greater than among Hindus. Madhesi children had a twofold greater risk of carriage as compared to Pahadis. Similarly, children of 'low' Hindu castes had

Characteristic	Healthy controls		ALRI cases	
	No./total no.	%	No./total no.	%
Male sex	268/550	48.7	275/550	50.0
Child's age <12 months	149/550	27.1	150/550	27.2
Ethnic group				
Pahadi	127/550	23.1	130/550	23.7
Madhesi	423/550	76.9	419/550	76.3
Religion				
Hindu	490/550	89.1	491/550	89.3
Muslim	60/550	10.9	59/550	10.7
Hindu caste				
Brahmin/Chhetri	60/490	12.2	72/491	14.7
Vaiysha/Shudra	430/490	87.8	419/491	85.3
One or more siblings < age 5 years	395/526	75.1	416/521	79.8
Mother with <1 year of education	439/548	80.1	448/546	82.0
Mother smokes cigarettes/bedis**	51/500	10.2	75/482	15.6
Electricity in household	108/550	19.6	94/550	17.1
Low socioeconomic index	227/550	41.3	217/550	39.5
Family owns land	366/549	66.7	385/550	70.0
No latrine in household	491/550	89.3	503/547	92.0
Child received zinc supplements	292/550	53.1	281/550	51.1

Table 1 Characteristics of children with ALRI and healthy matched controls enrolled in the *S. pneumoniae* carriage study, Sarlahi, Nepal ($n = 1100$)*

*Characteristics were compared using McNemar's test for paired data.

** $P \leq 0.05$.

C. L. Coles *et al.* ***Streptococcus pneumoniae* carriage in young children in rural Nepal****Table 2** Bivariate and multivariate analysis of risk factors for *S. pneumoniae* carriage among healthy controls ($n = 550$) and ALRI cases ($n = 550$), Sarlahi, Nepal ($n = 550$)

Risk factor	<i>n</i> /total <i>n</i>	(%)	Bivariate model		Final multivariate model		
			Crude OR	95% CI	Adjusted OR	95% CI	
Healthy controls							
Male	214/268	79.9	0.88	0.49–1.16			
Age ≥ 12 months	333/401	83.0	1.28	0.80–2.07			
One or more siblings <5-years old	326/395	82.5	1.06	0.63–1.77			
Madeshi ethnic group	356/423	84.2	1.71	1.10–2.89			
Muslim	56/60	93.3	3.37	1.19–9.51	2.93	1.03–8.39	
Vaiysha/Shudra (Hindu caste)	353/430	82.1	1.96	1.07–4.00			
Low socioeconomic status	184/220	83.6	1.21	0.77–1.89			
Family does not own land	159/183	86.9	1.68	1.02–2.77			
Latrine distant from household	411/491	83.7	2.44	1.34–4.43	2.41	1.31–3.44	
Mother <1 year of formal education	369/440	83.9	1.82	1.10–3.00			
Treated for illness 1–7 days before NP Sampling	47/71	66.2	0.36	0.21–0.63	0.37	0.21–0.64	
ALRI cases							
Male	207/275	75.3	0.85	0.57–1.26			
Age ≥ 12 months	320/400	80.00	1.88	1.23–2.90	1.63	1.04–2.53	
Siblings <5 years of age	320/416	76.9	1.09	0.67–1.81			
Madeshi	332/419	79.2	1.69	1.09–2.64			
Muslim	51/59	86.4	2.06	0.95–4.47	2.43	1.10–5.38	
Vaiysha/Shudra (Hindu caste)	327/419	78.0	2.26	1.33–3.82			
Mother smokes cigarettes/bedis	61/75	81.3	1.29	0.69–2.41			
Low socioeconomic status	176/209	84.2	2.06	1.33–3.20			
Family does not own land	130/165	78.8	1.18	0.76–1.83			
Latrine distant from household	392/503	77.9	2.02	1.05–3.86	2.15	1.08–4.28	
Mother has <1 year of formal education	351/448	78.4	1.18	0.98–2.59			
Treated for illness 1–7 days before NP sampling	252/353	71.4	0.40	0.25–0.63	0.40	0.25–0.65	
Symptomatic ALRI at NP sampling*	112/124	90.3	3.49	1.86–6.57	3.78	1.97–7.24	

Data were analysed using logistic regression.

*ALRI: Cough + fever + (fast breathing, difficulty breathing or retractions).

double the risk of carriage compared to those of 'high' Hindu castes. In addition, controls whose mothers had <1 year of formal education had nearly twice the risk of carriage as controls whose mothers had received more schooling. Children of families who did not own any land or did not have a latrine on their property were also at greater risk of *Spn* colonisation. In contrast, having received any treatment for an illness 1–7 days before NP sampling was associated with a >50% reduction in the odds of NP colonisation. A number of the same factors were found to be significantly associated with carriage among ALRI cases. These included Muslim religion, Madeshi ethnicity, 'low' Hindu caste, lack of maternal education, the absence of a latrine on household premises and treatment of an illness 1–7 days before NP sampling. In addition, ALRI cases who were ≥ 12 months of age were had significantly higher risk of carriage as compared to infants. Moreover, symptomatic ALRI cases had nearly a fourfold greater risk of carriage compared to asymptomatic cases. In the final multivariate analyses, Muslim religion,

absence of a toilet and recent treatment for an illness, remained significantly associated with *Spn* carriage in controls and ALRI cases. Age ≥ 12 months and symptomatic illness remained risk factors among cases.

Susceptibility of *Spn* isolates by antibiotic

Antibiotic susceptibility profiles to penicillin, cotrimoxazole, erythromycin, tetracycline and chloramphenicol were available for the 869 of the 873 (99.5%) *Spn* isolates. Of this number, 136 (15.7%) were resistant to oxacillin. However, only 4.5% of the 869 isolates had MICs > 0.064 $\mu\text{g/ml}$ to penicillin, all of which were intermediately resistant. In contrast, the proportion of isolates resistant to cotrimoxazole was 89.2% (775/869). Approximately, 22.4% (196/869) of the isolates were resistant to tetracycline. Less than 1.6% of the isolates were resistant to erythromycin and chloramphenicol. The proportion of resistant isolates to each of the five antibiotics did not differ by case status, symptomatic illness or recent treatment.

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Approximately 14% (123/873) of the isolates were not viable after thawing. Of the 750 remaining isolates, the most common SGTs among healthy controls who had not been treated for a recent illness were, order of prevalence 6, 19, 23, 15, 9, 10, 14, 17, 33, 3, 8 and 35 (Table 3). This pattern did not differ significantly by case or treatment status. Antibiotic susceptibility data was available for all but four of the 750 serotyped isolates. SGTs included in the 13vPnc vaccine accounted for approximately 62% of the resistant isolates. Over 70% of all isolates in the 13 SGTs were resistant to cotrimoxazole (Table 4). In contrast, the prevalence of tetracycline resistance exceeded 30% for only two SGTs – 14 and 19. Other than SGT's 14 and 1 the

prevalence of serotypes resistant to oxacillin was low. The prevalence of chloramphenicol and erythromycin resistance ranged from 0% to 8% for each for each of the 13 SGTs. The susceptibility profile for the 13vPnc serotypes was not associated with case status, symptomatic infection or recent treatment.

Distribution of serogroups/types included in current *Spn* conjugate vaccine formulations

We estimated the level of coverage provided by each of the three *Spn* conjugate vaccine formulations without information on the serotypes within serogroups. Our estimates assumed that there was 100% cross-protection between serotypes within serogroups, which is likely to

Table 3 Most commonly isolated *S. pneumoniae* serogroups/types in colonised children stratified by recent treatment status and case status in order of overall prevalence, Sarlahi, Nepal*

Serogroup/type	Not treated for recent illness				Treated for recent illness			
	Healthy controls		ALRI cases		Healthy controls		ALRI cases	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
6	79	22.8	31	20.3	10	23.8	29	13.9
19	41	11.9	19	12.4	3	7.1	31	14.8
23	36	10.4	17	11.1	4	9.5	14	6.7
15	26	7.5	16	10.5	3	7.1	22	10.5
9	18	5.2	4	2.6	3	7.1	17	8.1
10	15	4.3	7	4.6	2	4.8	11	5.3
14	12	3.5	3	2.0	2	4.8	9	4.3
17	10	2.9	4	2.6	0	0	11	5.3
33	12	3.5	5	3.3	0	0	7	3.4
3	11	3.2	3	2.0	1	2.4	6	2.9
8	7	2.0	7	4.6	1	2.4	5	2.4
35	0	0	8	5.2	2	4.8	4	1.9
Other	79	22.8	29	19.0	11	26.2	43	20.1
Total	346	100.0	153	100.0	42	100.0	209	100.0

*Includes all serogrouped/typed isolates.

Table 4 Profile of non-susceptible serogroups/types included in the 13-valent conjugate vaccine (13vPnc) by antibiotic*†‡

Serogroup/ type	1 (n = 9)	3 (n = 21)	4 (n = 13)	5 (n = 5)	6 (n = 149)	7 (n = 7)	9 (n = 42)	14 (n = 25)	18 (n = 17)	19 (n = 93)	23 (n = 71)
Antibiotic*	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
COT	9 (100)	19 (91)	13 (100)	5 (100)	134 (90)	5 (71)	32 (76)	23 (92)	14 (82)	90 (97)	65 (92)
TET	1 (10)	2 (10)	3 (23)	1 (20)	11 (7)	0 (0)	10 (24)	14 (56)	4 (23)	40 (43)	14 (20)
OXA	3 (33)	2 (10)	2 (15)	0 (0)	16 (4)	0 (0)	6 (14)	15 (60)	1 (6)	10 (11)	17 (24)
CHL	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	1 (1)
ERY	0 (0)	1 (5)	0 (0)	0 (0)	1 (0)	0 (0)	1 (2)	2 (8)	0 (0)	1 (1)	2 (3)

*COT, cotrimoxazole; TET, tetracycline; OXA, oxacillin; CHL, chloramphenicol; ERY, erythromycin.

†13vPnc serotypes: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23C.

approximate to the upper limits of coverage. Among healthy controls, the SGTs in the Prevnar, 10vPnc and 13vPnc vaccines accounted for 58.0%, 60.1% and 63% of the isolates, respectively. The coverage was similar among ALRI cases, with the Prevnar, 10vPnc and 13vPnc vaccines accounting for 51.7%, 55.5% and 58.0% of the isolates, respectively.

Discussion

Our results show a high prevalence of *Spn* NP carriage among young children in rural southern Nepal. The prevalence is comparable to rates observed in south India (Coles *et al.* 2001), and the Gambia (Hill *et al.* 2008) and approaches the 100% prevalence reported among children older than age three months in New Guinea (Montgomery *et al.* 1990).

Among cases and controls, Muslim religion and lack of a latrine in the household were associated with increased risk of *Spn* carriage in the multivariate analyses. Muslims are a poor and underprivileged minority group in Sarlahi. In addition, age ≥ 12 months was identified as risk factor carriage among cases but not controls. Poverty, membership in a disadvantaged community and age have consistently been reported as risk factors of carriage (Ghaffar *et al.* 1999; Greenwood 1999; Garcia-Rodriguez & Fresnadillo Martinez 2002). The potential for the modification of these factors is small, as absolute differences in demographic and socioeconomic status in this population are minor.

Children who received treatment for any illness in the 10 days before NP specimen had a significantly lower risk of carriage. Treatment comprised all remedies provided by traditional healers, 'village doctors', drug shops and health posts. It was not possible to determine antibiotic use with any accuracy given the high levels of illiteracy in this population. Although we did not collect information on why treatment was sought or what treatment was received, anecdotal evidence suggests that use of antibiotics for paediatric illness is common in Sarlahi. Antibiotic therapy has been shown to reduce NP carriage of susceptible *Spn* strains, yet treatment may result in replacement carriage with antibiotic-resistant pathogenic strains (Arason *et al.* 1996; Varon *et al.* 2000).

Among cases, evidence of symptomatic infection at the time of NP specimen collection was also associated with increased risk of carriage. This finding may suggest the greatest risk for pathogen transmission is during the initial stage of infection. Thus, prompt treatment of ALRI symptoms is likely to reduce disease transmission of *Spn* disease. Evidence suggests an association between increased pneumococcal carriage and symptomatic acute

otitis media though this relationship may be modified by prior antibiotic therapy (Faden *et al.* 1991; Syrjanen *et al.* 2005; Konno *et al.* 2006).

There was a high frequency of antibiotic-resistant *Spn* carriage in Nepalese children. Drug resistance profiles were not modified by case status, symptomatic infection or treatment status. Most isolates were resistant to cotrimoxazole, which is inexpensive and readily available as an over-the-counter medication. Moreover, the Nepalese government is scaling up national efforts to control paediatric pneumonia through community-based programs in which field health workers administer cotrimoxazole to children diagnosed with ALRI (Dawson *et al.* 2008). Evidence from Pakistan, which has a similar pattern of cotrimoxazole resistance, shows that its use in the treatment of non-severe pneumonia was associated with a clinical treatment failure rate of 18–21% (Hazir *et al.* 2006; Noorani *et al.* 2006). Thus the use of cotrimoxazole may have a detrimental effect on ALRI control in Nepal in the long term. While the spread of penicillin-resistant *Spn* is major concern in high- and low-income countries (Amsden 2004), only a small proportion of the isolates screened were resistant to penicillin. The pattern of antibiotic resistance to cotrimoxazole and other first-line drugs is consistent with published data from three Indian studies of carried and invasive isolates (Coles *et al.* 2002; Jain *et al.* 2005; Goyal *et al.* 2007).

There were no significant differences in SGT distribution between ALRI cases and healthy controls nor was the distribution affected by symptomatic infection or treatment for recent illness. The serotype distribution is similar to that reported in a study of south Indian infants (Coles *et al.* 2001). The licensed seven-valent *Spn* vaccine (Prevnar) may protect against 58% of SGTs identified in our study population, assuming 100% cross protection between serotypes within serogroups. It is estimated that the 10vPnc and 13vPnc, vaccines would extend coverage of the identified SGTs by an additional 2% and 3%, respectively.

Estimates of vaccine coverage are subject to bias. NP bacterial carriage is a dynamic process, and the duration of carriage with the same serotype can vary considerably (Gray *et al.* 1980). It is possible that we missed capturing SGTs that colonise for shorter periods. In addition, while individuals may be colonised with up to four serotypes at any one time, our laboratory techniques restricted us to identifying the predominant strains. A further limitation of this study is that we did not type any of the serogrouped isolates. There are also no published SGT data on invasive *Spn* isolates from hospitalised paediatric cases in Nepal with which to compare our results. However, our estimates of vaccine coverage are consistent with the results from two studies of invasive isolates collected from children

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<5 years hospitalised for severe *Spn* disease in India and other developing countries (Anonymous 1999; Hausdorff *et al.* 2007).

In summary, our findings indicate that young children in rural Nepal experience a high prevalence of *Spn* carriage. Carriage was increased in children with symptomatic ALRI infection but reduced in children who had received treatment for a recent illness. Risk factors identified for *Spn* are largely not amenable to modification. Most of the *Spn* isolates were resistant to cotrimoxazole, which has important implications for Nepal's community-based ALRI control program. Monitoring of local antibiotic susceptibility patterns will optimise effectiveness of ALRI case management. The introduction of *Spn* conjugate vaccines in Nepal is likely to decrease childhood morbidity and mortality and circulation of resistant strains associated with *Spn* infection assuming that carriage of vaccine serotypes is replaced by non-vaccine strains of low virulence.

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