# A longitudinal household study of *Streptococcus pneumoniae* nasopharyngeal carriage in a UK setting

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## SUMMARY

A 10-month longitudinal household study of pre-school children and their families was undertaken with monthly visits collecting epidemiological data and nasopharyngeal swabs in Hertfordshire, England from 2001 to 2002. Pneumococcal culture was with standard methods. In total, 121 families (489 individuals) took part. Mean prevalence of carriage ranged from 52% for age groups 0–2 years, 45% for 3–4 years, 21% for 5–17 years and 8% for ⩾18 years. Carriage occurred more than once in 86% of children aged 0–2 years compared to 36% of those aged ⩾18 years. The most prevalent serotypes in the 0–2 years age group were 6B followed by 19F, 23F, 6A and 14. Young children were responsible for the majority of introductions of new serotypes into a household. Erythromycin resistance (alone or in combination) occurred in 10% of samples and penicillin non-susceptibility in 3·7%. Overall the recently licensed 7-valent conjugate vaccine (PCV) would protect against 64% of serotypes with no intra-serogroup cross protection and 82% with such protection. Nasopharyngeal carriage of *S. pneumoniae* is common in a UK setting in the pre-conjugate vaccine era. PCV would protect against a large proportion of carriage isolates. However, the impact of vaccination on non-vaccine serotypes will need to be monitored.

## INTRODUCTION

*Streptococcus pneumoniae* (Pneumococcus or Pnc) is a common component of the nasopharyngeal (NP) flora in healthy individuals, with more than 90 different serotypes identified. Infection with Pnc ranges from asymptomatic carriage through a variety of clinically mild, common mucosal infections (otitis media and sinusitis) to more serious, less common invasive presentations such as pneumonia, septicaemia and meningitis [1, 2]. Pnc carriage rates are generally far in excess of disease rates–with marked variation by age, serotype and geographical area [3–10].

A heptavalent Pnc conjugate (PCV) vaccine was licensed in the United States in 2000 for universal use

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in children aged 2–23 months and for children aged 24–59 months who are at increased risk for pneumococcal disease [11]. This vaccine has now been licensed in Europe including the United Kingdom, where it has been recommended since 2001 for children aged from 2 months to 5 years considered at high risk. Discussions are ongoing whether to include universal PCV vaccination within the childhood immunization schedules of many European countries.

In clinical trials, PCV has proved to be protective for invasive pneumococcal disease caused by vaccine serotypes [12] as well as reducing their carriage [3], and hence a possible herd immunity effect. The observed reduction in vaccine serotypes has also been associated with an increase in carriage of non-vaccine serotypes in vaccinated children in carriage studies [13] and in vaccine trials for PCV against otitis media [14]. This phenomenon of serotype replacement has also been explored by mathematical modelling [15–17]. These observations and models raised the question of whether such increases in non-vaccine serotypes may also occur for invasive disease after large-scale use of the vaccine in the population: an observation recently made in the United States [17].

To be able to predict the impact of mass introduction of PCV in the United Kingdom, baseline information on the epidemiology of Pnc (including carriage by multiple serotypes) in this setting is required. Few studies have been published in recent years on the descriptive epidemiology of pneumococcal carriage in the United Kingdom [8, 18], and none on the dynamics of infection in a household setting to enable these evaluations to be undertaken.

In this study, the prevalence of NP carriage in households with young children and their families was studied monthly over a 10-month period in one geographical area in Southern England. The description of the households and families, the epidemiology of pneumococcal carriage including serotype, antibiotic resistance patterns and coverage of the different formulations of the conjugate vaccine (7-, 9-, 11-valent) are presented in this paper. Detailed analysis of Pnc transmission within the household and from the community, with estimates of acquisition and clearance rates, are reported in Melegaro et al. [19]. In depth analysis of risk factors for Pnc carriage, serological aspects of carriage and the transmission dynamics of infection will be presented separately.

## MATERIAL AND METHODS

### Participants

The index children were aged from birth to 3 years old at study entry and were recruited by study nurses together with their entire households. The sampling frame was the primary health-care child registers of the four main general practices in Hertfordshire. The study started on 1 October 2001, followed by monthly home visits and was completed by the end of July 2002.

Individuals (and the entire family if the index child fulfilled an exclusion criteria) with the following conditions were excluded from the study: moderate to severe disability; cerebral palsy; syndromes and neurological disorders affecting swallowing; ear, nose and throat (ENT) disorders affecting the anatomy of the ear (i.e. malformed ears); confirmed or suspected immunodeficiency (congenital or acquired); immuno-suppressive therapy or enrolment in a pneumococcal vaccine trial.

The local and national ethics committees approved the study protocol. Written informed consent was obtained from all adult study participants and from a parent/guardian of all study children prior to enrolment.

### Study design

The study was a 10-month longitudinal prospective follow-up of a population-based sample of pre-school children and their households. A study nurse undertook monthly visits. At each visit, basic epidemiological data on each household member and the household were gathered together with NP swabs and other biological specimens including serum and urine.

Following recruitment of a family, a study nurse administered a pre-tested structured, detailed questionnaire at the initial visit to each family member. Both household and individual data were gathered on potential risk factors or confounders. Individual data included age, gender, education, day-care attendance, smoking, health status, contact history in and out the house, any medication including antibiotics. Household data included socio-demographic factors such as type of house, number of rooms and parental occupation. A shorter questionnaire was administered at each monthly visit to each family member to gather information on any changes in individual and household details.

NP swabs were obtained at the initial home visit from all family members, followed by monthly swabs

over the 10-month period. Sampling of absent members or members with illness was performed at a later date but within a 14-day period. Swabs were taken by the study nurse and transported to the Respiratory and Systemic Infection Laboratory (RSIL) at the Central Public Health Laboratory (CPHL), London within 24 h of collection.

Data were collected on a standard case report form (CRF), which were doubly entered onto a database housed at the Communicable Disease Surveillance Centre (CDSC). Laboratory data were entered on a laboratory form at CPHL and entered into the common database.

## Determination of sample size

Simulation was used to determine that a total of 150 index children with a stable prevalence of carriage and a mean duration of carriage of 1 month would be sufficient to estimate a 25% acquisition rate per month within the index children with a 95% confidence interval (CI) of 22–29%.

## Sample collection

The study nurse used a flexible wire shaft with a calcium alginate tip to obtain NP swabs. All NP samples were handled according to the Standard Operating Procedures of the WHO–Pnc trialists' network [20]. Briefly, the swab was plated immediately by the study nurse, first on non-selective Columbia horse blood agar then on a Streptococcus selective Columbia horse blood agar (Oxoid, Basingstoke, UK) for detection of Pnc. The tip was then placed in a medium containing skim milk, tryptone, glucose, and glycerol (STGG) [21] and the inoculated plates and STGG broths were kept cool until transfer to the laboratory.

## Laboratory procedures

On arrival at RSIL, the primary pool of inoculum on both plates was 'streaked-out' with a loop and an optochin disc applied to the primary streak of the non-selective plate. The STGG broth was subcultured onto Columbia horse blood agar and Streptococcus selective Columbia horse blood agar plates. All plates were incubated overnight with 5% $CO_2$ (18–24 h) and examined for $\alpha$-haemolytic colonies with morphology typical for Pnc. To identify Pnc, suitable colonies from all plates were tested for optochin sensitivity. The bile solubility test was used if the colony morphology was suggestive of Pnc but the organism was optochin resistant. Minimal inhibitory concentrations (MICs) of penicillin, erythromycin and cefotaxime were determined for all confirmed Pnc isolates by agar dilution as previously described [22]. Isolates were classified as susceptible, intermediate or resistant to the agents tested using the criteria of the British Society for Antimicrobial Chemotherapy [23].

Pneumococcal isolates from one or more (if morphologically distinct) colonies were serotyped by standard methods [24, 25] using sera from Statens Serum Institut (SSI), Denmark. After final identification, all Pnc isolates were stored in glycerol blood broth at $-80$ °C.

## Analysis

Descriptive data analyses were performed in Microsoft Excel. Confidence intervals on risk ratios were calculated using Taylor series in Epi-Info Version 6 (CDC, Atlanta, GA, USA). The number of times a new serotype was introduced in the family was derived for different age groups. A *new introduction* was defined as the situation in which one family member was found to be carrying a serotype that was not present in that family at the previous test point. *Ongoing* was defined as the type carried at the previous test point by either the same individual in the family or other family members and *concurrent* as someone else in the family carrying the same type at the same time. Serotype-specific odds ratios (ORs) were calculated with use of serotype 14 as the reference group as described by Bruggemann et al. [26].

## RESULTS

### Household

A total of 132 families gave informed consent (representing 534 persons including 151 index children). Household relationship to the index cases included 132 mothers (24·7%), 125 fathers (23·4%), 101 siblings (18·9%) and 25 others including three grandparents.

Household data was available on 129 families: they came from across the socio-economic and educational spectrum, with 23 (17·8%) living in local authority-owned rental accommodation and 103 (79·8%) in owner-occupied housing. Household size ranged from two to seven persons (mean and median four persons).
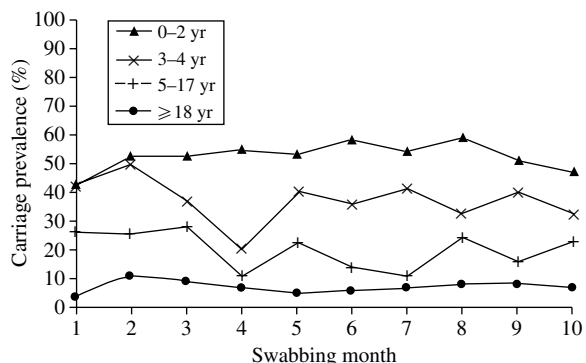
Fig. 1. Pnc carriage rates by age group of the study participants at the start of the study and by month of swabbing (October 2001 to July 2002).

Although 132 families were recruited, 11 withdrew before the first swab. Of the remaining 121 families, 106 remained until the end of the study (overall drop-out rate of 20%). Of the 26 families that failed to complete the entire study, three families immediately withdrew before completing the household questionnaire, four withdrew due to discomfort of NP swabs, three had social reasons (i.e. separation) that made it unreasonable to expect them to continue in the study and the remaining 16 withdrew either due to inconvenience related to time involved or because they left the country.

**Individual**

*Demographic and health*

In total, 121 families (with 138 index cases) were visited at least once by the study nurse (representing a total of 489 individuals).

*Prevalence of Pnc carriage*

NP swabbing was performed at least once in these 489 individuals. This represents 121 families out of the 129 that started the study, with eight families leaving the study during the first month and thus, never being swabbed. In total 3753 swabs were taken from these 489 individuals (77%) and 932/3752 (25%) were positive for Pnc carriage. Among those 121 families that started the study the acceptance rates gradually fell over the study period, although they remained at ~70% or above, throughout.

Mean prevalence of carriage by age group over the entire study period ranged from 52% for age groups 0–2 years, 45% for 3–4 years, 21% for 5–17 years to 8% for those aged ⩾18 years. There were no significant seasonal differences in the prevalence of carriage
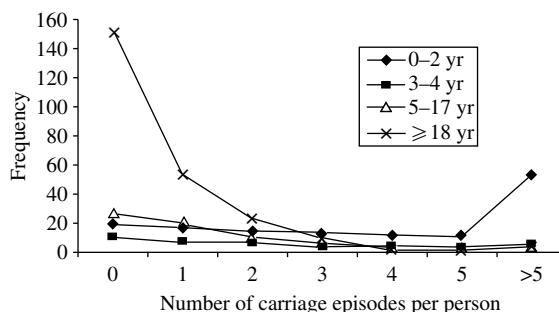


Fig. 2. Number of reported Pnc carriage episodes per person by age group during the 10-month study period (n = 488, information on age missing for one individual).

by age group over the 10-month period from October to July (Fig. 1) (*t* test trend > 0·05 for all age groups).

Among all study participants, 282/489 (58%) individuals were found to be Pnc carriers at least once throughout the study period. At least one episode of pneumococcal carriage was noted amongst 112 of the 121 families swabbed (93%) over the study period. When stratified by age the proportion carrying Pnc at least once ranged from 86% in the 0–2 years age group to 36% in those aged ⩾18 years (Fig. 2). Indeed, among the 0–2 years group, 39% were found to carry Pnc more than five times during the study follow-up time compared to 1% of those ⩾18 years (Fig. 2).

Simultaneous carriage of more than one serotype in an individual at a particular time-point was only detected on six (0·16%) occasions. Five of the individuals were ⩽5 years of age.

*Distribution of serogroups/types and vaccine coverage*

In total, 901/932 (97%) pneumococcal isolates were serotyped (the remainder being non-capsulate): 575 (98%) in age group 0–2 years, 93 (95%) in the 3–4 years group, 88 (92%) in the 5–17 years group and 144 (97%) in those aged ⩾18 years. In one circumstance the age was not recorded. Thirty-four distinct serotypes were found in the isolates (Fig. 3). The most prevalent five serotypes in children aged 0–2 years were 6B followed by 19F, 23F, 6A and 14. Among those persons ⩾3 years old, serotypes 6A followed by 23F, 14, 19F and 6B were again the most frequent, although isolates of 6B were much less frequently found compared to those aged 0–2 years.

Overall the 7-valent conjugate vaccine protected against 64% of the circulating serotypes when no cross protection between serotypes within serogroups was assumed (Table, Fig. 3). The potential coverage
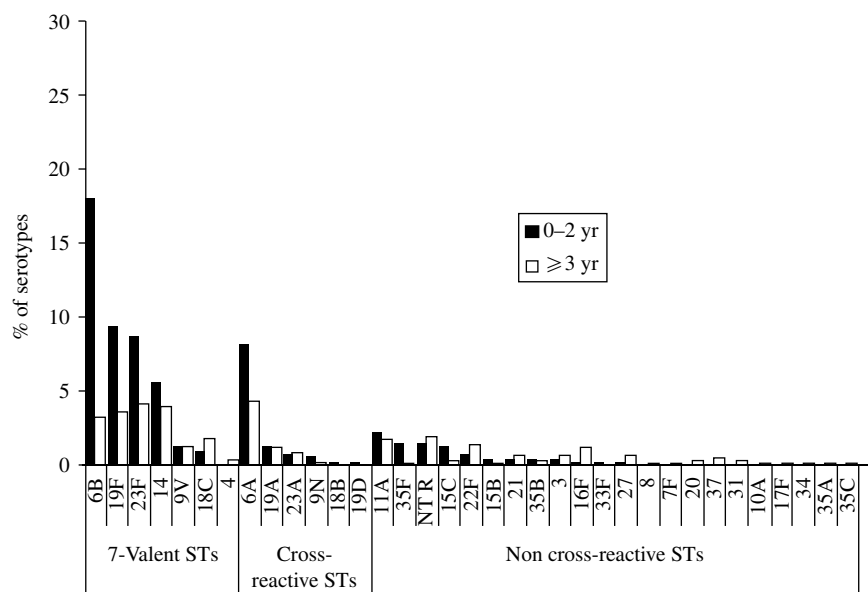
**Fig. 3.** Proportion of pneumococcal serotypes (STs) ($n=931$, information on age missing for one individual) isolated in age groups 0–2 and $\geqslant 3$ years by serotype and according to coverage with 7-valent conjugate vaccine.

was 82% when cross protection was assumed. Coverage for the 9-valent vaccine was identical to the 7-valent vaccine as serotypes 1 and 5, the additional types contained in the 9-valent were not observed in this study. An 11-valent vaccine would provide a small amount of additional coverage compared to the 7- or 9-valent groups.

By age group, potential vaccine coverage was highest in those <3 years of age at ∼70% if no cross protection within serogroups was assumed, rising to 88% assuming cross protection (Table). Coverage declined to 50–60% for the older age groups. Gaps in coverage were particularly due to the non-vaccine serogroups 11, 16 and 22.

### Antibiotic resistance

For the 932 isolates, the proportion with any erythromycin resistance (alone or in combination) was 10% and the proportion with any degree of penicillin resistance (alone or in combination) was 3·7%. Cefotaxime resistance was noted in only 0·3% of isolates. The most common serotypes showing resistance to at least one of the three antibiotics were serotypes 14 (45%, $n=92$), 19F (11%, $n=128$), 6B (14%, $n=214$), 6A (4%, $n=117$), 23F (4%, $n=117$) and 9V (17%, $n=23$). These serotypes represent 90% of all serotypes that are resistant to one or more of these three antibiotics. All of these serotypes (including cross-protection) are included in the 7-valent PCV.

Table. *Coverage of the 7-, 9- and 11-valent vaccines in different age groups*

| | Age group (years) | | | | |
|---|---|---|---|---|---|
| Coverage | 0–2 | 3–4 | 5–17 | $\geqslant 18$ | Total |
| No cross protection | | | | | |
| 7- and 9-valent | 70% | 52% | 53% | 55% | 64% |
| 11-valent | 71% | 53% | 53% | 60% | 65% |
| With cross protection | | | | | |
| 7- and 9-valent | 88% | 76% | 64% | 76% | 82% |
| 11-valent | 88% | 77% | 64% | 81% | 84% |

### Comparison with invasive isolates

Comparison of the distribution of carriage serotypes with the distribution of invasive Pnc isolates in England and Wales reported during 2000 demonstrated large differences in odds ratios between serotypes (Fig. 4). Serotypes 1 (OR 15·1), 4 (OR 6·3), 7F (OR 15·7) and 8 (OR 10·4) in particular were more likely to be invasive than carried compared to the reference serotype 14. The 7-valent Pnc conjugate vaccine does not cover three of these serotypes (1, 8 and 7F).

### Role of the household

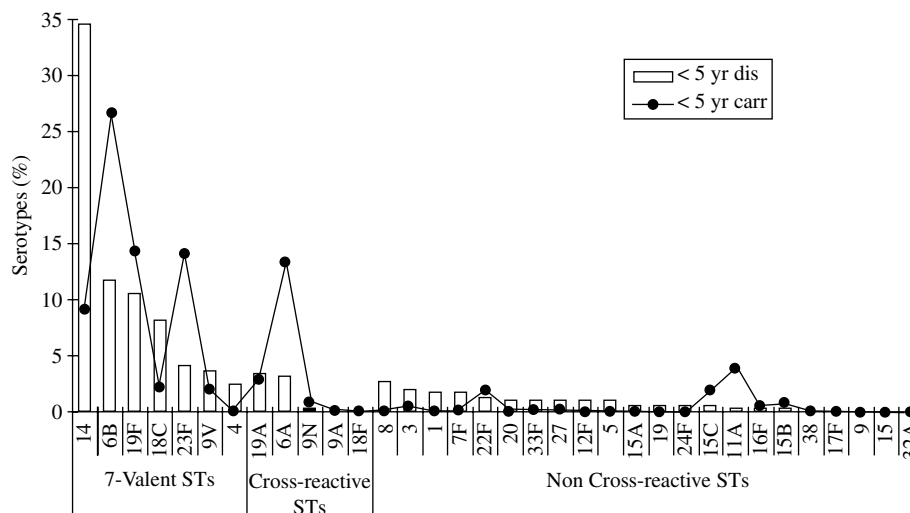The relative importance of intra- and extra-household exposure to Pnc infection by age group was evaluated

**Fig. 4.** Comparison of serotypes (STs) distribution in children <5 years of age in the Pnc carriage study (*n*=686) with Pnc invasive isolates reported in England and Wales (CDSC/RSIL 2000) (*n*=540).
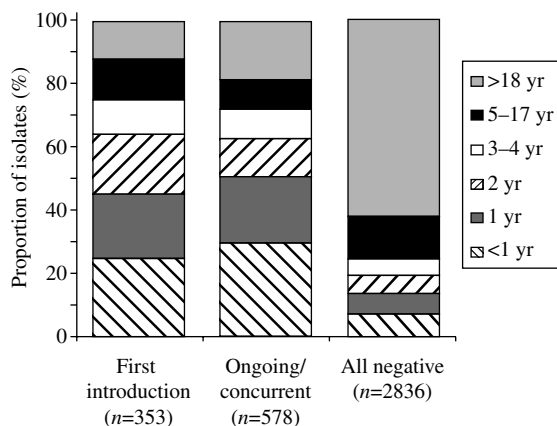


**Fig. 5.** Distribution of episodes by age group according to whether it was a new introduction, an ongoing or concurrent episode of carriage of a specific serotype or a negative sample.

by examining introductions of new serotypes into the household (Fig. 5). A marked age-specific role for first introductions was demonstrated: 25% of first introductions were due to individuals in their first year of life, 20% in the second and 19% during the third year. This then declined to an average of 5·5% per year for those aged 3–4 years and to 1% per year for ages 5–17 years.

The effect of within family exposure on pneumococcal carriage was also assessed by examining the number of changes in individual status over the observation intervals stratified by age group and background carriage in other family members. Individual pneumococcal carriage was found to be associated

with carriage in the other family members by age group with risk ratios of 1·42 (95% CI 1·26–1·61), 1·25 (95% CI 0·95–1·64) and 2·25 (95% CI 1·65–3·08) for age groups 0–2, 3–4 and ⩾5 years respectively.

## DISCUSSION

There are few longitudinal studies of Pnc carriage in the literature, and even fewer studies that have swabbed both children and adults in a closed setting such as a household [18, 27]. This study clearly shows that despite the invasive nature of swabbing studies, it is possible to achieve and maintain high levels of compliance over a sustained period of time, although as with other studies [27] obtaining samples from some groups such as adult males can be more difficult.

The study confirms that NP carriage of Pnc is common in the United Kingdom, with ∼60% of individuals and 93% of families carrying the organism at least once over a 10-month period. As has been reported previously [5, 18, 27], higher carriage rates are observed in children <5 years of age compared to older family members. Indeed, in this study approximately half of the children <3 years of age carried the organism for over half of the total study period, which is in agreement with a previous study conducted in Oxford [8]. This presumably reflects both longer duration of carriage in children, as has been previously reported [4, 10] together with more frequent acquisition compared to adults. Our results also suggest children are frequently the route of entry of the bacterium into the household, with a preponderance

of those identified to be carrying for the first time being young children. Although this finding could be confounded by children tending to carry Pnc for longer periods than adults, recent modelling work has confirmed the important role of children in introducing Pnc into households, despite longer duration of carriage [19].

The prevalence of carriers appeared constant throughout the study period, with no evidence of seasonal fluctuations. Results from previous studies have been mixed with some demonstrating an increase in carriage during the winter months [6, 28], whereas others have not [7]. The reasons for these differences are uncertain. However, since pneumococcal-associated disease (invasive disease, pneumonia and otitis media) all peak in the winter, it is important to understand whether such increases in disease are due to increased carriage (which this study suggests not) or other mechanisms, such as exposure to other respiratory pathogens [such as influenza or respiratory syncytial virus (RSV)], which may contribute to the pathogenic process.

The serotype distribution was broadly similar to that observed in two previous studies conducted in different geographical settings in England [8, 18]. No additional protection against carriage was provided by the 9-valent formulation of the vaccine, as the two additional serotypes that are present in the latter (1 and 5) were again not found in this carriage study. However, a comparison with the distribution of invasive isolates reported to the national surveillance system to those in the carriage study, found some serotypes were more likely to be invasive than carried. Several of these serotypes, e.g. serotype 1 are not covered by the 7-valent vaccine. These serotype-specific variations in 'invasiveness' have been noted by other groups both in the United Kingdom [26] and elsewhere [10]. However, this comparison should be undertaken with caution as it is well documented that serotype-specific duration of carriage varies – thus, serotype 1 for example, may be carried more widely, but for a very short period of time.

The rates of antimicrobial resistance observed in this carriage study compare to the rates observed for invasive isolates in 2000 for the United Kingdom. Observed rates were lower for both penicillin (3·7% for the carriage study vs. 7% for invasive disease) and erythromycin resistance (10% in the current study vs. 15% for invasive disease). The vast majority of serotypes demonstrating antibiotic resistance would be covered by a 7-valent PCV.

One of the concerns expressed about the introduction of Pnc conjugate vaccines into national immunization programmes has been replacement with non-vaccine serotypes. In the United States, a large reduction in invasive Pnc disease rates has been observed since the introduction of the infant immunization programme particularly in young children [29]. More recently, a significant increase in serotype replacement with non-vaccine serotypes has been published [17] In this study simultaneous carriage with more than one serotype was not detected commonly, suggesting that unmasking is not likely to be an important component of any potential serotype replacement. However, this still leaves the issue of replacement. This study together with long-term studies and ongoing surveillance will provide valuable data to better understand the transmission dynamics of Pnc and thus, the potential importance of replacement.

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