**Pneumococcal colonization in healthy adult research participants in the conjugate vaccine era, United Kingdom, 2010—2017**

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**Summary**

We detected pneumococcal colonization in 52/795 subjects using nasal wash culture. The majority were non-vaccine serotypes, but we identified persistent circulation of serotypes 3, 19A and 19F and the emergence of antimicrobial resistance in the latter years of the study.

**Abstract**

Pneumococcal colonization is rarely studied in adults, except as part of family surveys. We report the outcomes of colonization screening in healthy adults (non-smokers without major comorbidities or contact with children under five years) who had volunteered to take part in clinical research. Using nasal wash culture, we detected colonization in 6.5% (52/795) of volunteers. Serotype 3 was the commonest serotype (10/52). The majority of the remainder (35/52) were non-vaccine serotypes, but we also identified persistent circulation of serotypes 19A and 19F. Resistance to at least one of six antibiotics tested was found in 8/52 isolates.

**Background**

Colonization with *Streptococcus pneumoniae* is a key precursor to invasive pneumococcal disease (IPD). Colonization is more common in children than in adults, and contact with children aged <5 years is the main risk factor for adult colonization [1]. Pneumococcal conjugate vaccines (PCV) prevent pediatric pneumococcal disease, and induce herd protection against vaccine-serotypes through reductions in colonization in vaccinated children, and indirectly, unvaccinated adults [2].

In the United Kingdom (UK), PCV13 (covering serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F, 19A, and 23F) was introduced to the national immunization program in April 2010, replacing PCV7. Longitudinal studies of IPD in the UK have demonstrated serotype replacement following PCV13 introduction [3]. Serotype replacement has also been demonstrated in colonization studies of children and their families: in 2015/16, 51.1% of children <5 years were colonized with non-vaccine type (NVT) pneumococci, versus 1% with PCV13 serotypes [4]. Parental colonization rates were low, with only 2.8% of parents testing positive in 2015/16.

Antimicrobial resistance rates in pneumococcal colonization and disease vary markedly between different European countries [5, 6]; resistance is relatively uncommon in the UK, with <10% of IPD isolates non-susceptible to penicillin [5].

When comparing the serotypes found in IPD and those found in childhood colonization, there is some discordance—for example, serotype 8 has emerged as the commonest disease-causing serotype [3], whereas no colonization with serotype 8 was identified in the most recent childhood survey [4]. In addition, IPD studies have identified incomplete herd protection in adults against some PCV13 serotypes, particularly the highly-virulent 3 and 19A, even though childhood colonization with these serotypes was exceedingly rare. This led the researchers to conclude that many disease-causing serotypes have high case-carrier ratios, or perhaps only colonize for short durations [4]. It also suggests that serotype replacement in childhood colonization is an imperfect surrogate for replacement in invasive disease

Adults without regular contact with children may be less susceptible to the ecological effects of childhood PCV programs, and thus could represent a reservoir of vaccine-type pneumococci [7]. However, studies of such populations are few, typified by low sampling yield and reliance on molecular detection methods which limits their ability to identify serotypes and assess antimicrobial susceptibility [8].

Volunteers for the experimental human pneumococcal colonization (EHPC) research program in the Liverpool School of Tropical Medicine undergo screening for pneumococcal colonization prior to participation. In this paper we use these volunteers as a surrogate for the general healthy adult population to report the rates of colonization, serotype distributions and antimicrobial susceptibility profiles in healthy adults over the first seven years of screening.

**Methods**

The rationale, methodology and inclusion/exclusion criteria for EHPC studies have been previously reported [9]. In brief, the studies are open to healthy adults aged ≥18 years, excluding those with important risk factors for pneumococcal disease, colonization or transmission, including: cigarette smoking; close contact with children aged <5 years; healthcare work or caring responsibilities; steroid therapy and respiratory or immunosuppressive comorbidities. Recent antibiotic therapy (within two weeks) and prior pneumococcal vaccination are also exclusion criteria. The majority of study recruitment events are held in local universities.

All volunteers are screened for community-acquired pneumococcal colonization by nasal wash at their first visit. We reviewed all screening nasal washes obtained between October 2010 and March 2017. A summary of the original studies is provided in the Supplementary Appendix. All studies were approved by the local National Health Service Research Ethics Committee, and all participants provided written informed consent.

Nasal washes were performed as previously described [9]: 5mL of 0.9% sodium chloride solution was introduced using a syringe and held for a few seconds in the nose before being expelled in to a sterile container. The participants were advised to occlude their pharynx during the procedure (e.g. by pressing their tongue against their hard palate), which was repeated twice in each nostril, thus using 20mL saline in total. Nasal wash samples were transported to the laboratory within one hour of collection, where they were plated on gentamicin/blood agar and incubated overnight at 37°C with 5% CO2. Community-acquired pneumococcal colonization was defined as the identification of *S. pneumoniae* using standard microbiological techniques, with serogroup identified by latex agglutination test. Where required, the serotype was confirmed using the Senti-SP v1.6 molecular serotyping microarray (BUGS Bioscience) as previously described [10]. We tested all isolates for susceptibility to penicillin, clarithromycin, doxycycline, levofloxacin, trimethoprim-sulfamethoxazole and vancomycin using disc diffusion and Etest (bioMérieux, Basingstoke, UK), following the recommendations and clinical breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, version 7.1, [11], see Supplementary Appendix). Zones of inhibition were measured by two independent reviewers, and all resistant isolates were tested a second time for confirmation. We used meningitis breakpoints when interpreting penicillin minimum inhibitory concentrations.

For purposes of analysis, we assumed that maximal vaccine coverage of under-5s with the primary series of PCV13 was achieved five years after its introduction [3]. We compared the proportion colonized, the proportion colonized with PCV13 serotypes and the proportion of isolates displaying antibiotic resistant phenotypes before and after April 1, 2015 (early and late periods) by χ2 or Fisher’s exact test where appropriate, using SPSS version 24 (IBM Inc., NY, USA).

**Results**

795 healthy volunteers met the inclusion criteria and underwent nasal wash screening (see TABLE). The median age was 21 years (IQR 20—23 years) and 452 (57%) were female. Pneumococcal colonization was detected in 52 participants (6.5%, 95% CI 5.0—8.5%).

We identified PCV13 serotypes in 17/52 (32.7%); see FIGURE and TABLE S3 (Supplementary Appendix). Serotype 3 was the commonest isolate (10/52 isolates); the next most common VTs were 19A and 19F (3 isolates each). The most common NVTs were 23B (five isolates), 8, 11A, 35F and 37 (four isolates each). Colonization rates were not significantly different between the early and late periods (24/368 [6.5%] and 28/427 [6.6%] respectively, p = 0.98). Among colonized participants, 8/24 (33.3%) were carrying PCV13 serotypes before 01 April 2015 versus 9/28 (32.1%, p = 0.93) afterwards.

We identified resistance to at least one antibiotic in 8/52 isolates (15.4%, 95% CI 8.0—27.5%) (see FIGURE S1 and TABLE S4 in the Supplementary Appendix). The majority of resistant isolates (6/8) were NVTs. The highest rate of resistance was against penicillin (5/8 isolates; all susceptible to amoxicillin and ceftriaxone), followed by clarithromycin and doxycycline (4/8 each). We did not detect any levofloxacin or vancomycin resistance. We identified resistance to three antibiotic classes in two isolates, and to two classes in a further four isolates. All resistant isolates were detected after 01 April 2015 (8/28 [28.6%] compared with 0/24 before this cut-off, p = 0.005).

**Discussion**

We found a higher rate of colonization than has previously been reported in the general European adult population [1, 12]. This finding is notable given that the exclusion criteria for EHPC studies resulted in all participants lacking major risk factors for pneumococcal acquisition [9]. When our results are compared with a recent colonization study of UK children <5 years and their parents, we identified more frequent colonization and a greater range of serotypes than were found in the parents, but less colonization and fewer serotypes than were found in the children [4].

Although our data collection spanned the first seven years of PCV13 implementation, the relative distribution of serotypes did not change over this time. Our colonization patterns were dominated by NVTs, reflecting current trends in IPD and colonization in the UK [3, 4]. Serotype 8 is currently the commonest cause of IPD in the UK and was the joint third-most frequently-detected serotype in our study, even though it was not detected at all in the most recent UK pediatric colonization survey [3, 4]. A cross-sectional study in 2010—2011 identified PCV13 serotypes (specifically 3, 19F and 19A) in 5/36 colonized UK adults [6]; we identified ongoing circulation of these same serotypes throughout the PCV13 era. This could explain why serotypes 3 and 19A remain common in IPD even though they are rarely identified in pediatric colonization [3, 4]. This reservoir in young adults could have the potential to re-colonize the pediatric population after the UK reduces the childhood PCV13 schedule from three to two doses [13]. Conversely, the failure of the current pediatric vaccine schedule to generate herd protection against these serotypes could support a call for vaccination of at-risk adults, although the 23-valent pneumococcal polysaccharide vaccine is already employed for direct protection of this population in the UK.

While our study outperformed the pediatric colonization survey in detecting certain serotypes with invasive potential (e.g. 3, 8, 19A), the pediatric survey frequently identified carriage with other invasive serotypes (e.g. 15B/C, 10A) which were poorly represented in our cohort [4]. Therefore, it seems that colonization surveys should optimally include a wider range of subjects, rather than being restricted to households with children. This would maximize their potential to quantify serotype replacement and complement IPD surveillance in guiding future vaccine formulation recommendations.

Colonization studies have typically focused on children and their close contacts to maximize the yield from screening. We are aware of one other study that deliberately recruited a control cohort of adults who lacked close contact with children [8]. The authors detected colonization in 10% (29/298) Dutch parents versus 2% (5/323) controls using culture-based methods. Molecular testing increased the yield to 7% in controls (21/323), similar to our findings using culture-based methods in UK adults.

Antimicrobial resistance in pneumococci is a growing global health concern [14]. We detected the emergence of antimicrobial resistance (AMR) during the later period of this study, with no resistant isolates identified during the early period. However, a cross-sectional study including data from UK primary care practices in 2010 did identify cases of resistant pneumococcal colonization in adults, suggesting that the absence of resistance in the early years of our data collection may be due to either chance or specific local factors [1]. The resistance profiles of different pneumococcal serotypes in adult colonization have not been previously reported in the UK. Although our numbers are small, it is concerning that the majority of resistant isolates displayed resistance to >1 class of antibiotic. Clustering of resistance in NVTs suggests that the childhood PCV13 program is unlikely to reduce this reservoir of AMR in the community.

The main constraint of the study is the limited demographic and medical history data available. However, the strict inclusion criteria mandated by EHPC studies should have resulted in a homogeneous study population, defined by the absence of significant risk factors for pneumococcal colonization. Another limitation is that our sampling was all performed in a single city. Culture-based colonization determination, allowing accurate serotyping and resistance measurement, is a strength of our study—molecular testing of oropharyngeal samples may have increased our yield, but would not allow phenotypic confirmation of antimicrobial resistance [8]. Our use of nasal wash rather than nasal or nasopharyngeal swab may have contributed to the high pneumococcal yield obtained in this study [15]: a study employing nasal swabs in >3000 UK adults only identified colonization in 1.8% [1].

In summary, we describe pneumococcal colonization in a population of adults not normally targeted by colonization studies, including the persistence of some PCV13 serotypes, epidemiologically-important NVTs, and emerging antimicrobial resistance. Inclusion of a wider sample of adults in colonization studies would complement ongoing surveillance of invasive disease isolates to inform national pneumococcal vaccination policies.

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**Conflicts of interest**

Adam Roberts reports lecture fees from Merck Sharp & Dohme, outside the submitted work; Jason Hinds reports grants from Pfizer, Sanofi Pasteur and GSK, outside the submitted work, and being co-founder, board member and shareholder of BUGS Bioscience, a not-for-profit spin-out company of St George's University of London, but receives no personal income from this activity; Katherine Gould reports being sub-contracted to BUGS Bioscience, but receives no personal income from this activity; all other authors report no disclosures.

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Figure: Serotype distribution during the two time periods

This bar chart shows the numbers of subjects colonized with vaccine serotypes (left) and non-vaccine serotypes (right), before (white bars) and after (gray bars) the five-year anniversary of the introduction of the 13-valent pneumococcal vaccine (PCV13) in the UK.