Persistent circulation of vaccine serotypes and serotype replacement after five years of UK infant immunisation with PCV13.

Rama Kandasamy DPhil FRACP[1,2], Merryn Voysey MBiostat[1,2,3], Sarah Collins MPH[4], Guy Berbers PhD[5], Hannah Robinson MSc[1,2], Irene Noel MSc[1,2], Harri Hughes MSc[1,2], Susan Ndimah MSc[1,2], Katherine Gould PhD[6,7], Norman Fry PhD[4], Carmen Sheppard PhD[4], Shamez Ladhani PhD MRCPCH[4], Matthew D. Snape MD FRACP[1,2], Jason Hinds PhD[6,7], and Andrew J. Pollard PhD FRCPCH[1,2]

Affiliations:

[1]Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Oxford, OX3 7LE, United Kingdom.

[2]NIHR Oxford Biomedical Research Centre, Oxford, OX3 7LE, United Kingdom.

[3]Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, UK.

[4]Public Health England, 61 Colindale Avenue, London NW9 5EQ, United Kingdom.

[5]Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, the Netherlands.

[6]Institute for Infection and Immunity, St. George's, University of London, London, United Kingdom.

[7]BUGS Bioscience, London Bioscience Innovation Centre, London, United Kingdom.

Correspondence to: Rama Kandasamy. Oxford Vaccine Group. CCVTM, Churchill Hospital, OX3 7LE, United Kingdom. Tel: +44 (0) 1865 611 400 Fax: +44 (0) 1865 611 400

Email: rama.kandasamy@paediatrics.ox.ac.uk

Keywords: Pneumococcus, children, adults, carriage, sero-prevalence, invasive, disease

Main point: There is emergence of non-PCV13 serotype pneumococcal disease and evidence for a differential effect of PCV13 among vaccinated UK children, most notably for pneumococcal serotypes 3 and 19A carriage and disease.

ABSTRACT

Background

Following programmatic introduction of the 13-valent pneumococcal conjugate vaccine (PCV13), there is residual carriage and disease due to PCV13 covered serotypes.

Methods

988 PCV13-immunised children aged 13-48 months were enrolled between February 2014 and August 2015 (late-PCV13), and had nasopharyngeal pneumococcal carriage compared with 567 PCV7-immunised children enrolled into a study between November 2010 and September 2011 (early-PCV13). Nasopharyngeal pneumococci were molecular-serotyped by microarray. Invasive pneumococcal disease (IPD) cases were identified through enhanced national surveillance. Blood collected from the late-PCV13 cohort was assessed for levels of serotype-specific serum IgG by multiplex immunoassay.

Results

Compared with PCV7-immunised children, carriage among PCV13-immunised children was significantly lower for serotypes 19A (OR=0.08, 95% CI 0.02-0.25), 6C (OR=0.11, 95% CI 0.03-0.32) and 7F (8 vs 0 cases).

IPD incidence in children <5 years was significantly lower for serotypes 1 (IRR=0.03, 95% CI 0-0.19) and 7F (IRR=0.13, 95% CI 0.05-0.36) but not 19A (IRR=0.6, 95% CI 0.3-1.12) or serotype 3 (IRR=2.3, 95% CI 0.86-6.15) in the late-PCV13 period than in the early-PCV13 period. The most significant rises in IPD incidence were for serotypes 8, 12F, and 24F.

Children from the late-PCV13 period, who had serum analysed, and were not carrying a PCV13 serotype, had high levels of antibody presumed to be due to natural exposure, to serotypes 3 (24/204, 11.76%) and 19A (14/204, 6.86%).

Conclusions

PCV13 has reduced serotype 19A carriage among vaccinated children however, disease is not fully controlled. We also found, no impact of PCV13 on serotype 3 carriage or disease, and emergence of non-PCV13 serotype disease.

INTRODUCTION

Before introduction of the 7-valent pneumococcal conjugate vaccine (PCV7), *Streptococcus pneumoniae* was responsible for over 8000 cases of invasive pneumococcal disease (IPD) per year across all ages in England and Wales.[1] PCV7 administered at 2, 4, and 12-13 months, coupled with a catch-up programme for children aged up to 24 months, was introduced into the UK infant schedule in September 2006.[2] A reduction in carriage and IPD due to serotypes included in PCV7 was initially observed in the vaccine-eligible cohort, followed by a decline in older, unvaccinated children and adults (herd protection).[1,3,4] At the same time, there was an increase in non-PCV7 type IPD (serotype replacement) across all age groups, particularly serotypes 1, 7F, and 19A.[3] In April 2010 the 13-valent PCV (PCV13), covering the additional serotypes 1, 3, 5, 6A, 7F, and 19A, replaced PCV7 in the UK childhood immunisation schedule without any catch-up.[5]

PCV13 introduction in the UK led to a reduction in overall PCV13-type disease and carriage, with just over 4,000 IPD cases reported across all ages in England and Wales between July 2013 and June 2014.[6,7] A similar effect was also seen in carriage, with one study reporting a reduction in children <5 years of age carrying one of the additional PCV13 serotypes from 9.9% prior to PCV13 introduction to 0.4% after PCV13 introduction in the UK.[7] At the serotype level, the effect is less consistent, exemplified by a trial comparing carriage in children randomised to receive either PCV7 or PCV13, which described no detectable effect of PCV13 on serotype 3 carriage in Israeli children prior to community-wide PCV13 roll-out, and another report showing no significant reduction of serotype 3 IPD in UK children <5 years of age, 4 years after PCV13 introduction.[6,8,9] For serotype 19A, there was an increase in IPD incidence following PCV7 introduction and a subsequent decline after PCV13 introduction across all age groups during the first four years of PCV13 use in England and Wales, but since then serotype 19A IPD incidence has remained static, especially in adults and the elderly.[6,10] In children, this serotype is also the most common cause of vaccine failure.[11]

The rapid increase in serotype replacement IPD following PCV7 introduction, in particular, serotypes 7F and 22F, which were infrequently identified among asymptomatic carriers (high attack-rate) raises

the question whether a similar phenomenon would occur post-PCV13.[1,3] To date however, there has only been a statistically significant rise in serotype 24F IPD among English and Welsh children <5 years reported since PCV13 introduction.[6]

The purpose of this study is to examine the effect of PCV13 on pneumococcal carriage and disease in UK children and adults, through the examination of changes in pneumococcal serotypes carried and causing IPD after the first five years of PCV13 use in the UK childhood immunisation programme.

METHODS

Study design

A cross-sectional observational study to establish the point-prevalence of pneumococcal nasopharyngeal carriage in children 13-48 months of age and their parents/legal guardians from the Thames Valley region of the UK was conducted between February 2014 and August 2015 (late-PCV13 era). Following informed consent children were included in the study if they were in good health and had received three doses of PCV13, at 2, 4, and 12 months of age, as per the recommended UK infant immunisation schedule. Children who had not received a complete course of PCV13, had taken antibiotics in the preceding 30 days, were febrile, had a respiratory illness, or had a health condition that may have influenced the study were excluded. Enrolled parents were in good health and resided in the same household as their participating child. Parents who were febrile, had a respiratory illness, or had a health condition that may influence the study were excluded. Demographic information was recorded before participants underwent sampling. Data collected in this present study were compared with data from a similar study of children vaccinated with three doses of PCV7 conducted in the same region from November 2010 to September 2011 (early-PCV13 era).[12] All clinical investigation was conducted according to the principles expressed in the Declaration of Helsinki. Written ethics approval for the study was obtained from the Oxford Research and Ethics Committee (ID: 13/SC/0578).

Sample size

Sample size was calculated to allow detection of a 50% reduction in serotype 19A pneumococcal carriage when comparing carriage amongst PCV7 with PCV13-immunised children. We used a prevalence of 4.7% for serotype 19A in PCV7-immunised children and determined that 600 participants would be required to produce a two-sided 95% confidence interval with a width equal to 3.6% (i.e. 95% CI = 3.1% to 6.7%). A sample size of 600 would provide 90% power to detect an absolute reduction in prevalence of serotype 19A of 2.4% (i.e. from 4.7% to 2.3%) at 5% level of significance. In order to compare children recruited in this study with those in the previous study for

prevalence of 19A we aimed to recruit 600 children aged 21-48 months. We also recruited an extra 400 children aged 13-48 months to provide a cohort for future matching across the whole age-range for subsequent cross-sectional studies and to provide a point estimate of carriage among children.

Outcomes

The primary outcome was the presence of serotype 19A pneumococci on children's swabs. Secondary outcomes included, presence of pneumococcal serotypes on children's swabs; presence of pneumococcal serotypes on parent's/legal guardian's swabs; molecular serotype of nasopharyngeal carriage isolates from children and parents/legal guardians; serotype-specific odds of carriage compared to IPD amongst children; and serotype-specific pneumococcal antibody levels in children.

Nasopharyngeal swab analysis

Swabs were collected and processed according to WHO guidelines.[13] A single flexible aluminum shaft with rayon tip (MWE, Wiltshire, UK) was passed through the anterior nares as far as the posterior pharynx, rotated 360 degrees before removal and placed into a tube of 0.5-1ml of skim-milk-tryptone-glucose-glycerin transport medium. Swabs were plated on Columbia-blood agar and incubated overnight before morphologically distinct alpha-haemolytic colonies were selected and sub-cultured overnight in the presence of optochin. Pneumococcal isolates underwent DNA extraction followed by molecular serotyping using the Senti-SPv1.5 microarray (BUGS Bioscience, UK). Secondary phenotyping by Quellung reaction (Serum Statens Institute, Denmark) was performed on variant genotypes.[14]

Blood collection and measurement of serum IgG

Blood was collected from children using finger-prick. A finger was lanced and blood collected in a micro-tube (multivette 600, Sarstedt, Leister, UK). Pneumococcal serotype-specific serum IgG against PCV13 serotypes were measured by multiplex immunoassay (Bio-Rad Laboratories, Hercules, CA, USA) with Luminex technology by the National Institute for Public Health and the Environment, The Netherlands.[15] Sera analysed were those from individuals who were carrying a PCV13

serotype (serotype 3, n=7; serotype 19A, n=8; and serotype 19F, n=1) and a random selection of NVT carriers (n=98), and non-carriers (n=107).

IPD surveillance

IPD data were obtained from the active surveillance of cases conducted across England and Wales by Public Health England.[6] Data were extracted from this database for the time periods between November 2010 - September 2011 and February 2014 - August 2015.

Statistical analysis

Details of the statistical analysis are described in the supplement. All statistical analyses were performed using R version 3.3.1.[17] P-values <0.05 were considered significant for comparisons of demographic data. For serotype-specific comparisons of carriage and IPD, a more conservative threshold of $p<0.01$ was considered significant due to the multiplicity of comparisons.

RESULTS

Between February 2014 and August 2015, following screening and informed consent, 1005 children and 204 parents were enrolled into the study as the late-PCV13 cohort (Figure 1). There were 17 children who withdrew resulting in 988 swabs for analysis. Blood samples from 220 children were tested for pneumococcal antibodies. From the parent cohort 4 participants withdrew resulting in 200 swabs for analysis. During the carriage sample collection period there were 408 cases of IPD in children under 5 years of age and 2697 cases in 18-65 year olds across England and Wales. The study of the early-PCV13 cohort, enrolled participants and collected IPD data from November 2010 to September 2011.[18]

Nasopharyngeal carriage in the late-PCV13 era

481/988 (48.7%, 95% CI 45.5-51.9%) children and 21/200 (10.5%, 95% CI 6.6-15.6%) adults were carrying at least one pneumococcal serotype (Table 1). One swab from a child had two serotypes (17F and NT) detected following culture, whilst five samples from children had more than one serotype detected by microarray and were classified as mixed (Figure 2). Carriers and non-carriers were similar in age, household makeup, and prevalence of smoking.

In children, non-PCV13 serotypes (NVTs) represented the majority of carriage isolates (463/482, 96.1%), followed by the serotypes in PCV13 but not PCV7 (18/482, 3.7%), and PCV7 only serotypes (1/482, 0.2%) (Figure 2). Serotypes 19A and 3, which are serotypes included in PCV13 but not PCV7, were identified on 9/482 (1.9%) occasions each. Serotype 19F (included in PCV7 and PCV13) was identified once. Serotype 15B was the most prevalent serotype, identified on 59/482 (12.2%) occasions. Serotype 3 carriers were significantly older (mean age 3.1 years) than NVT carriers (mean age 2.5 years) (p=0.0446) whilst there was no significant difference in age between serotype 19A (mean age 2.1 years) and NVT carriers (p=0.087) (Supplemental Figure 1).

The effect on transmission and hence, herd protection from carriage was examined via the parent cohort, within which there were no detected PCV13 serotypes from the 21 pneumococcus positive swabs (Figure 2). The most prevalent serotypes identified were 21, 23B, and 38.

Comparison of serotypes identified in carriage with IPD

During the study period, in children under 5 years of age, the most common serotypes causing IPD were 12F (49/408, 12%), 24F (30/408, 7.4%), and 23B (28/408, 6.9%). Eleven serotypes (5.4% of isolates), were detected in IPD but not in carriage of which serotype 27 was most common (5/408, 1.2%). PCV13 serotypes accounted for 11.8% (48/408) of IPD cases. The odds of each serotype being detected in IPD samples compared with carriage samples, was higher in children for serotypes 8 and 12F, which are not included in PCV13 (Supplementary Table 1).

Effect of PCV13 on carriage and IPD

To study the effect of PCV13 on pneumococcal carriage and IPD, comparisons were made for 24-48 month old children enrolled in a prior study of pneumococcal carriage in the Thames Valley region in the initial year of PCV13 introduction (N=567) and age matched children in the current study (N=642). Carriage was detected in 268 (47.2%) of early-PCV13 children compared with 305 (47.5%) late-PCV13 children.[12] Significant reductions in late-PCV13 carriage were seen for the PCV13 serotypes 7F, and 19A (Table 2). Serotype 6C also became less common. A trend towards a reduction in serotype 3 carriage among the late-PCV13 cohort was also noted. There was a significant increase in carriage of serotypes 15A and 15B. A trend towards an increase in serotype 9N and 24F carriage among the late-PCV13 cohort was also noted. The PCV13 serotypes 1, 6B, 19F, and 23F were detected in small numbers in the early-PCV13 cohort but were not detected in the late-PCV13 cohort.

408 IPD cases occurred in children < 5 years over the 18-month study period during the late-PCV13 era, compared with 98 cases in total for the 10-month observation period in the early-PCV13 period. In children <5 years of age, there was a lower incidence of IPD in the late-PCV13 period for serotype 1, and 7F, but no change in rates for serotypes 3, 5, 19A, or 19F (Table 3). In contrast there were

significantly higher rates of serotype 8, 9N, 10A, 12F, 15B/C, 23B, and 24F IPD in the late-PCV13 compared with the early-PCV13 cohort. Of these, the most significant rises in IPD were for serotypes 12F ($p<0.0001$), 24F ($p<0.0001$), and 8 ($p=0.0002$), with serotype 12F accounting for the greatest number of cases (49 cases, incidence 9.74 per 10^6 per year). A trend towards an increase in rates for serotypes 15A, 22F, 33f, 35B, and 38 was noted among the late-PCV13 when compared with the early-PCV13 cohort.

In adults aged 18-64 years, there was a significant decrease in IPD incidence for PCV7 serotypes 6B, 9V, and 23F from late-PCV13 compared with adults in the early-PCV13 period. There was also a significant decrease in IPD incidence for the additional PCV13 covered serotypes 1, 3, 6A, 7F, and 19A; for vaccine related serotype 6C; and for three other non-vaccine serotypes (11A, 22F, and 38). A trend towards a decrease in rates for serotype 14, 16F, and 23A was also noted. Increases were observed for serotypes 8, 9N, 10A, and 12F, with serotype 8 accounting for the greatest number of cases (544 cases, incidence 11.13 per 10^6 per year). A trend towards an increase in rates for serotype 34 was noted.

Comparison of carriage to IgG levels in children

The seven children carrying serotype 3 who had serum assayed, had higher geometric mean IgG concentrations against serotype 3 than carriers of NVTs (GMR=5.98, 95% CI 1.86-19.16, p=0.003) or non-carriers (GMR=6.17, 95% CI 1.96-19.47, p=0.0022) (Supplementary Table 2).

The eight children carrying serotype 19A who had serum assayed, had higher IgG concentrations against serotype 19A and 19F than carriers of NVT (GMR=6.89, 95% CI 2.62-18.13, p=0.0001, and GMR=3.76, 95% CI 1.51-9.31, p=0.0048 for 19A and 19F respectively). Similar results were seen when comparing with non-carriers (Supplementary Table 3).

Raised antibody levels due to natural boosting occurred most frequently for serotypes 3 and 19A, with 24/204 (11.76%) and 14/204 (6.86%) of children having high antibody levels for these serotypes

(Supplemental Figures 2, 3, and 4). Of these, 4/24 (16.7%) and 2/14 (14.3%) were also carrying serotype 3 or 19A/F respectively. 23 participants had high antibody levels for more than one PCV13 serotype with the maximum number being four. The mean age of children with naturally-boosted antibody levels was significantly higher than the average age of children sampled (3 vs 2.1 years, $p<0.0001$).

DISCUSSION

Five years after PCV13 introduction, serotypes 3 and 19A continue to circulate in carriage and in disease, despite some evidence of a reduction in carriage in children for both serotypes between the early and late PCV13 periods. Ongoing analysis of IPD data through national surveillance will provide greater power to clarify the direction and magnitude of any changes.[19] To explore the current exposure of children to these serotypes, we examined children's antibody levels against PCV13 serotypes and identified participants with higher than normal antibody levels for their age, which would be consistent with prior colonisation by circulating strains. These measurements were highest for pneumococcal serotype 3 followed by serotype 19A, providing further evidence that these serotypes are still transmitted amongst children and may be a factor explaining why serotype 3 and 19A IPD continues to occur in the post-PCV13 era. Further studies examining whether the PCV13 effect on carriage and disease could be further optimised, for example, by improving antibody concentrations and persistence with higher vaccine antigen doses and/or alternative schedules, are needed.

Early-PCV13 compared with late-PCV13 carriage and IPD

Serotype replacement, due to expansion of NVTs, appears to be responsible for overall carriage prevalence being unchanged between the two cohorts presented, and is consistent with findings in other settings.[20] One interesting exception was the significant reduction in serotype 6C carriage in children, which may be due to cross-protective antibody generated towards closely-related vaccine types 6A and 6B.[21]

Comparing PCV13 with PCV7-immunised children, there was an overall reduction in proportion of vaccine serotypes carried. At a serotype-specific level, reductions in serotype 3 and 19A were seen, although only the latter was statistically significant. This finding is consistent with a trial in Israel which demonstrated a significant reduction in serotype 19A but not serotype 3 carriage in children receiving PCV13 compared with those who received PCV7.[8]

There were few cases of serotype 3 IPD in children at the time of PCV13 introduction in the UK so it is difficult to demonstrate a decrease post-PCV13 from this low baseline. Recent data demonstrates serotype 3 IPD amongst children to be fluctuating, a pattern that may partly be due to natural disease outbreak cycles.[6,19] In adults and older adults however, serotype 3 is currently one of the most prevalent causes of IPD.[19]

In contrast to the overall reductions in IPD due to PCV13 serotypes, the significant increase in incidence of IPD in children caused by serotypes 8, 12F, and 24F is concerning, with these serotypes accounting for a quarter of IPD in children < 5 years, and around 40% of adult cases in the late-PCV13 period. These serotypes have high attack-rates indicating that these strains colonise briefly, circulate rapidly, and possess features conferring high virulence. Increases in IPD due to serotypes 8, 10A, 12F, 15A, and 24F among 5-64 year-olds have previously been reported. However, only an increase in serotype 24F was statistically significant in children <5 years in England and Wales during 2013/14.[6] Priority should be given to the inclusion of serotypes 8, 12F, and 24F in higher-valent PCVs as vaccination against these serotypes could have the greatest impact on IPD incidence, without substantially affecting distribution of less virulent serotypes found in carriage.

Detection of natural boosting events using serum antibody levels

The high number of natural boosting events elicited by serotypes 3 and 19A reflects continued circulation of these serotypes. Detection of serotype 3 carriage in healthy vaccinated children who are older than other children in the study and have significantly lower serum IgG levels against other vaccine serotypes than NVT carriers, support the conclusion that age-related decay in antibody levels has a role in conferring susceptibility to carriage of serotype 3. The same pattern of findings is not as apparent for serotype 19A carriers with a number of carriers detected within the first year of boosting. This brings into question whether vaccine stimulated antibody generated to serotype 19A capsule polysaccharide needs to have higher concentrations and/or improved avidity to achieve the same effect on carriage as seen for other vaccine serotypes.

Strengths and limitations of the study

Strengths of the study include the use of microarray technology to accurately detect serotypes, the sample size, and the time-point and timescale of collection. The microarray is able to discriminate closely related *Streptococcus spp.* which are often detected as NTs by Quellung. Seasonality has been shown to effect the prevalence of carriage, however the sample collection in this study spanned across all seasons reducing the likelihood of confounding due to this factor. It should be noted however, that the early-PCV13 period spanned 11 months compared with the late-PCV13 period of 19 months.

Limitations of the study are, only nasopharyngeal samples being collected from adults, and differences between the carriage and IPD populations. In this present study a single nasopharyngeal swab was collected from adults, when it is currently recommended that adults ideally have both a nasopharyngeal and oropharyngeal sample collected for detection of pneumococcus, although if only one sample is to be collected it should be a nasopharyngeal swab.[22] Notably these recommendations are based on very limited size studies which show a mixed pattern of results when comparing nasopharyngeal to oropharyngeal samples for pneumococcal detection. When making comparisons across numerous serotypes it is important to consider the possibility that some observations may be due to chance alone. Additionally, the reason why some observations may be apparent in the carriage data set, and not reflected in the IPD data set is that the carriage population is a subset of the national population. Thus there may be variations in one population that are not captured in the other. Further, children in the carriage study were selected to be of good health and fully immunised, whilst cases of IPD may be under-immunised or have inter-current illness.

Conclusions

Serotypes 3 and 19A continue to circulate amongst UK children, five years after PCV13 introduction. The absence of PCV13 serotypes carried by adults and the reduction in IPD incidence due to serotypes 1, 3, 6A, 6B, 7F, 9V, 19A, and 23F however, is consistent with PCV13 use amongst children providing herd protection for the unvaccinated population. We have also shown that there are high numbers of natural boosting events for serotypes 3 and 19A in children and that this is correlated

with increasing age. This observation is most likely due to differential antibody decay to vaccine serotypes. If this is the case, schedules which have better antibody persistence against serotype 3 and 19A after the booster dose at 1 year of age could further reduce circulation of these serotypes.[23] This study also highlights that it is possible some serotype 19A circulation is due to antibody decay prior to boosting. Further studies which directly assess natural exposure in the 6-12 month age range, prior to boosting at 12 months, are needed.

Serotype replacement in IPD has been realised in England and Wales with significant increases due to the high virulence serotypes 8, 12F, and 24F seen in children. Strategies to develop vaccines against these serotypes either by inclusion in increased valency PCVs or pan-pneumococcus vaccines should be a priority.

Notes

Acknowledgements. The authors would like to thank Emma Plested for her assistance in participant recruitment.

Potential conflicts of interest. AJP has previously conducted vaccine studies on behalf of Oxford University that were sponsored by manufacturers of pneumococcal vaccines but no longer does so. The University of Oxford has received unrestricted educational grants from pneumococcal vaccine manufacturers in 2016. This study was funded by an investigator-initiated grant to Oxford University. AJP is chair of the Department of Health's Joint Committee on Vaccination and Immunisation but the views expressed herein do not represent necessarily those of DH or JCVI. He is also a member of WHO's SAGE. RK received the Robert Austrian Award in Pneumococcal Vaccinology, which was supported by Pfizer, at the 10th International Symposium on Pneumococci and Pneumococcal Diseases 2016. MDS is an investigator on studies conducted on behalf of the University of Oxford that are sponsored and/or funded by vaccine manufacturers including Pfizer, Glaxosmithkline, Janssen, Novavax and Medimmune. Prior to 2017 MDS spoke at industry sponsored symposium and attended advisory boards; payment for these activities was made to the University of Oxford and

MDS received no personal financial benefit from these activities. JH receives no personal financial benefit from these activities.

Financial support. This work was supported by an Investigator initiated Grant from Pfizer Ltd and the NIHR Oxford Biomedical Research Centre.

REFERENCES

1. Miller E, Andrews NJ, Waight PA, Slack MP, George RC. Herd immunity and serotype replacement 4 years after seven-valent pneumococcal conjugate vaccination in England and Wales: an observational cohort study. Lancet Infect Dis [Internet]. **2011** [cited 2016 Jun 7]; 11(10):760–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21621466
2. Planned changes to the routine Childhood Immunisation Programme [Internet]. 2006 [cited 2016 May 28]. Available from: http://webarchive.nationalarchives.gov.uk/20130107105354/http://www.dh.gov.uk/en/Publicationsandstatistics/Lettersandcirculars/Dearcolleagueletters/DH_4128120
3. Flasche S, Hoek AJ Van, Sheasby E, et al. Effect of pneumococcal conjugate vaccination on serotype-specific carriage and invasive disease in England: a cross-sectional study. PLoS Med [Internet]. **2011**; 8(4):e1001017. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21483718
4. Andrews N, Waight PA, Borrow R, et al. Using the indirect cohort design to estimate the effectiveness of the seven valent pneumococcal conjugate vaccine in England and Wales. PLoS One [Internet]. **2011** [cited 2016 Jun 7]; 6(12):e28435. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22164292
5. JCVI meetings 2010 : Department of Health - Joint Committee on Vaccination and Immunisation (JCVI) [Internet]. [cited 2018 Aug 17]. Available from: http://webarchive.nationalarchives.gov.uk/20120907095400/http://www.dh.gov.uk/ab/JCVI/DH_107556#_5
6. Waight PA, Andrews NJ, Ladhani SN, Sheppard CL, Slack MPE, Miller E. Effect of the 13-valent pneumococcal conjugate vaccine on invasive pneumococcal disease in England and Wales 4 years after its introduction: an observational cohort study. Lancet Infect Dis [Internet]. **2015** [cited 2016 Feb 19]; 15(5):535–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25801458
7. Hoek AJ van, Sheppard CL, Andrews NJ, et al. Pneumococcal carriage in children and adults two years after introduction of the thirteen valent pneumococcal conjugate vaccine in England.

Vaccine [Internet]. **2014**; 32(34):4349–4355. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24657717

8. Dagan R, Patterson S, Juergens C, et al. Comparative immunogenicity and efficacy of 13-valent and 7-valent pneumococcal conjugate vaccines in reducing nasopharyngeal colonization: a randomized double-blind trial. Clin Infect Dis [Internet]. **2013** [cited 2015 Dec 14]; 57(7):952–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23804191
9. Demczuk WHB, Martin I, Griffith A, et al. Serotype distribution of invasive Streptococcus pneumoniae in Canada after the introduction of the 13-valent pneumococcal conjugate vaccine, 2010-2012. Can J Microbiol [Internet]. **2013** [cited 2016 Jul 5]; 59(12):778–88. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24313450
10. Fry N, Kapatai G, Sheppard C, et al. The fall and rise of serotype 19A in invasive pneumococcal disease: application of whole genome sequencing to investigate the recent rise in England and Wales. 10th The Int Symp Pneumococci Pneumococcal Dis [Internet]. 2016. Available from: https://ep70.eventpilot.us/web/page.php?page=Inthtml&project=ISPPD16&id=abstract_199615
11. Oligbu G, Collins S, Andrews N, et al. Characteristics and Serotype Distribution of Childhood Cases of Invasive Pneumococcal Disease Following Pneumococcal Conjugate Vaccination in England and Wales, 2006–2014. Clin Infect Dis [Internet]. Oxford University Press; **2017** [cited 2018 Mar 27]; 65(7):1191–1198. Available from: http://academic.oup.com/cid/article/65/7/1191/4077000
12. Hamaluba M, Kandasamy R, Ndimah S, et al. A cross-sectional observational study of pneumococcal carriage in children, their parents, and older adults following the introduction of the 7-valent pneumococcal conjugate vaccine. Med [Internet]. **2015**; 94(1):e335. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25569650
13. Satzke C, Turner P, Virolainen-Julkunen A, et al. Standard method for detecting upper respiratory carriage of Streptococcus pneumoniae: updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. Vaccine [Internet]. **2013**;

32(1):165–179. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24331112

14. Streptococcus pneumoniae: textbook in Serotyping, Virulence Factors and Enzyme-linked Immunosorbent Assay (ELISA) for Measuring Pneumococcal Antibodies. Statens Serum Institut; 2013.

15. Elberse KEM, Greeff SC de, Wattimena N, et al. Seroprevalence of IgG antibodies against 13 vaccine Streptococcus pneumoniae serotypes in the Netherlands. Vaccine [Internet]. **2011** [cited 2016 Mar 23]; 29(5):1029–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21129397

16. Office for National Statistics - Population Estimates for UK, England and Wales, Scotland and Northern Ireland [Internet]. 2016 [cited 2016 Aug 31]. Available from: https://www.ons.gov.uk/peoplepopulationandcommunity/populationandmigration/populationestimates/datasets/populationestimatesforukenglandandwalesscotlandandnorthernireland

17. R Core Team (2016). R: A language and environment for statistical computing. [Internet]. R Foundation for Statistical Computing, Vienna, Austria; Available from: https://www.r-project.org/

18. Hamaluba M, Kandasamy R, Ndimah S, et al. A cross-sectional observational study of pneumococcal carriage in children, their parents, and older adults following the introduction of the 7-valent pneumococcal conjugate vaccine. Med (United States). **2015**; 94(1).

19. Ladhani SN, Collins S, Djennad A, et al. Rapid increase in non-vaccine serotypes causing invasive pneumococcal disease in England and Wales, 2000-17: a prospective national observational cohort study. Lancet Infect Dis [Internet]. Elsevier; **2018** [cited 2018 Mar 27]; 18(4):441–451. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29395999

20. Lee GM, Kleinman K, Pelton SI, et al. Impact of 13-Valent Pneumococcal Conjugate Vaccination on Streptococcus pneumoniae Carriage in Young Children in Massachusetts. J Pediatric Infect Dis Soc [Internet]. **2014** [cited 2015 Nov 30]; 3(1):23–32. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3933044&tool=pmcentrez&rendertype=abstract

21. Grant LR, O'Brien SE, Burbidge P, et al. Comparative immunogenicity of 7 and 13-valent

pneumococcal conjugate vaccines and the development of functional antibodies to cross-reactive serotypes. PLoS One [Internet]. **2013**; 8(9):e74906. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24086394

22. Satzke C, Turner P, Virolainen-Julkunen A, et al. Standard method for detecting upper respiratory carriage of Streptococcus pneumoniae: Updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. Vaccine. **2013**; 32(1):165–179.

23. Goldblatt D, Southern J, Andrews NJ, et al. Pneumococcal conjugate vaccine 13 delivered as one primary and one booster dose (1 + 1) compared with two primary doses and a booster (2 + 1) in UK infants: a multicentre, parallel group randomised controlled trial. Lancet Infect Dis [Internet]. Elsevier; **2018** [cited 2018 Jul 5]; 18(2):171–179. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29174323

Figure Legends

Figure 1. Overview of study recruitment. UK Children who had received three doses of PCV13 along with a subset of their parents were recruited into the carriage study between February 2014 and August 2015. A subset of serum samples collected from children at the same study visit, were analysed for IgG specific for each of the PCV13 serotypes. IPD data from enhanced national surveillance was extracted from the database held by the Department of Public Health England for the corresponding time-period.

Figure 2. Serotype-specific prevalence of pneumococcal carriage amongst UK children who had received three doses of PCV13 and their parents between February 2014 and August 2015. 481/988 children (light green bars) and 21/200 parents (dark green bars) had at least one serotype of pneumococcus isolated from their respective nasopharyngeal swabs. VT=PCV13 serotypes. NVT=non-PCV13 serotypes.

Figure 1

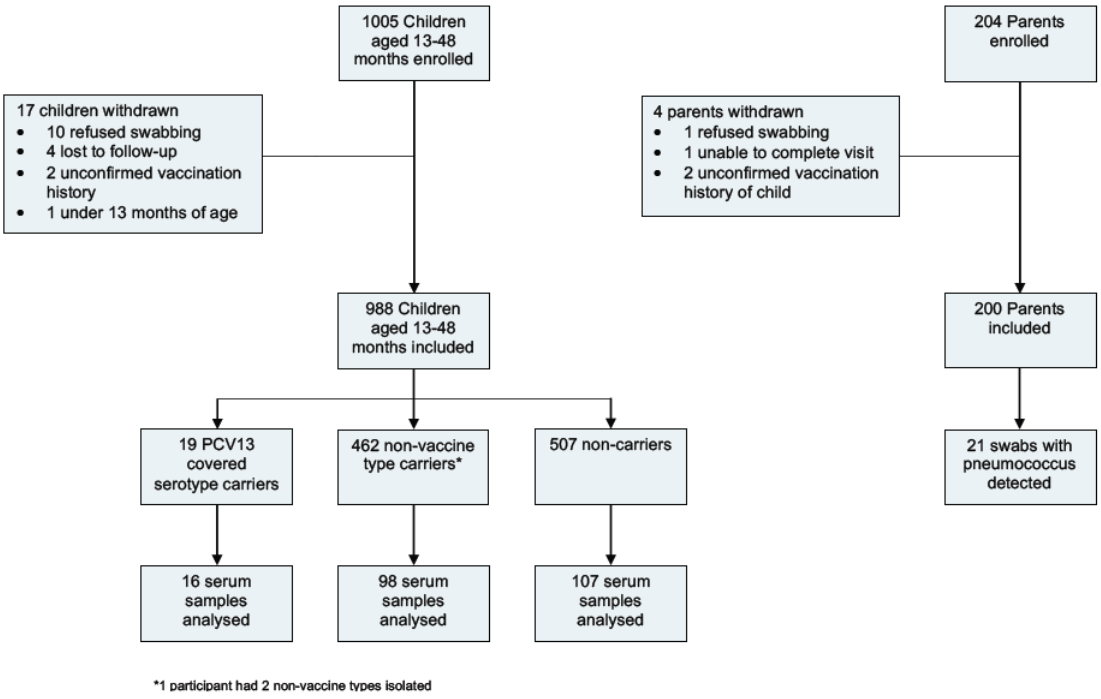

Figure 2

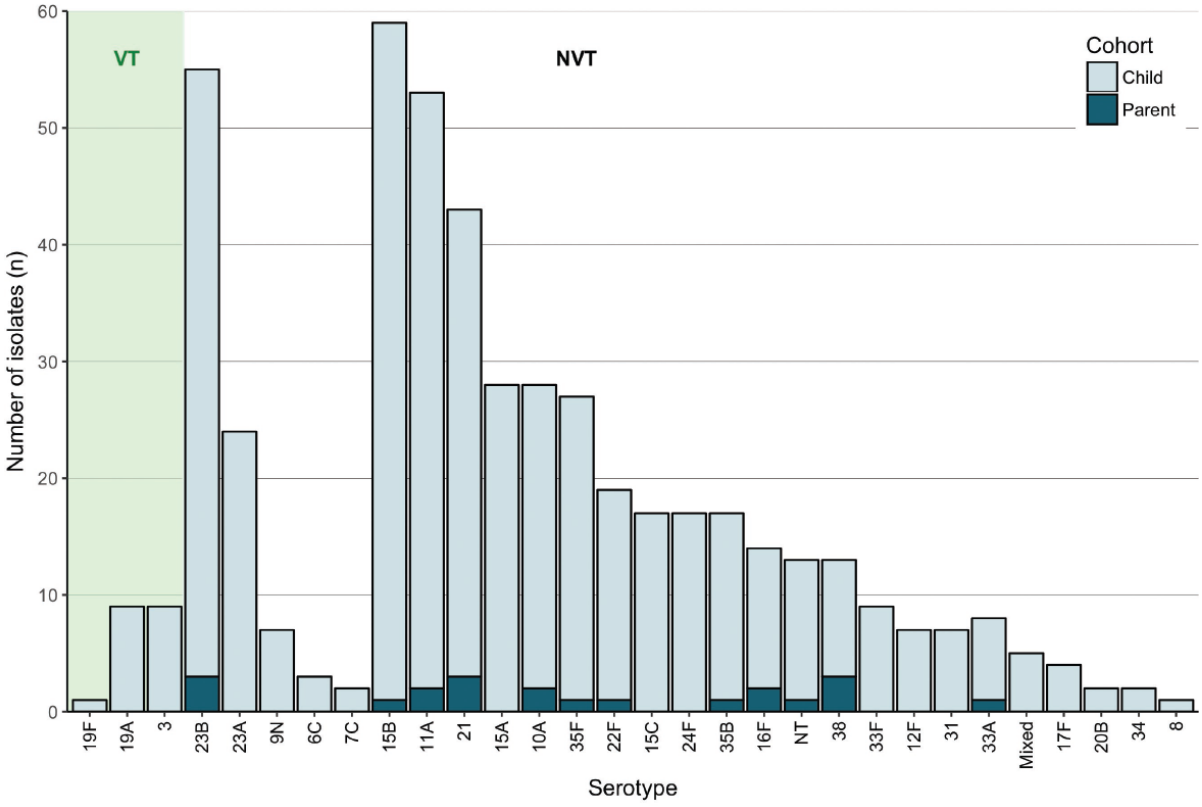

Table 1. Characteristics of the child and adult carriage cohorts.

Children		**All (N=988)**	**Carriers (N=481)**	**Non-carriers (N=507)**	**p***
Mean age, years (SD)		2.5 (0.9)	2.5 (0.9)	2.5 (0.9)	0.2007#
Males, N (%)		496 (50.2)	257 (53.3)	239 (47.1)	**0.0488**†
Ethnicity, N (%)	White	884 (89.5)	434 (90.2)	450 (88.8)	
	African Heritage /Afro-Caribbean	22 (2.2)	9 (1.9)	13 (2.6)	
	Asian	20 (2)	12 (2.5)	8 (1.6)	
	Other	62 (6.3)	26 (5.4)	36 (7.1)	
Household size	Mean (SD)	3.9 (0.9)	3.9 (0.9)	3.9 (0.9)	0.7547#
	Median [min, max]	4 [0, 8]	4 [2, 8]	4 [0, 7]	
Number of siblings	Mean (SD)	0.9 (0.8)	0.9 (0.9)	0.9 (0.8)	0.464§
	Median [min, max]	1 [0, 5]	1 [0, 5]	1 [0, 5]	
Children with a smoker in the house	n (%) [95% CI]	106 (10.7) [8.9-12.8]	45 (9.4) [6.9-12.3]	61 (12) [9.3-15.2]	0.155†
Daycare or preschool attendance per week	Mean hours (SD)	15.5(12.3)	16.2 (12.1)	14.9 (12.4)	0.0506§
	Median hours [min, max]	15 [0, 52]	15 [0, 50]	15 [0, 52]	
Adults		**All**	**Carriers**	**Non-carriers**	**p***

		(N=200)	(N=21)	(N=179)	
Mean age, years (SD)		36.8 (5)	35.8 (5)	36.9 (5)	0.3448[#]
Males, N (%)		20 (10)	1 (4.8)	19 (10.6)	0.7011[†]
Ethnicity, N (%)	White	189 (94.5)	21 (100)	168 (93.9)	
	African Heritage /Afro-Caribbean	1 (0.5)	0 (0)	1 (0.6)	
	Asian	4 (2)	0 (0)	4 (2.2)	
	Other	6 (3)	0 (0)	6 (3.4)	
Household size	Mean (SD)	3.8 (0.9)	3.8 (0.8)	3.8 (1)	0.828[§]
	Median [min, max]	4 [2, 7]	4 [3, 5]	4 [2, 7]	
Parents with a smoker in the house	n (%) [95% CI]	23 (11.5) [7.4-16.8]	2 (9.5) [1.2-30.4]	21 (11.7) [7.4-17.4]	1[†]
Parents who smoke	n (%) [95% CI]	11 (5.5) [2.8-9.6]	1 (4.8) [0.1-23.8]	10 (5.6) [2.7-10]	1[†]
Parents with a child who was a carrier	n (%) [95% CI]	105 (52.5) [45.3-59.6]	12 (57.1) [34-78.2]	93 (52) [44.4-59.5]	0.8180[†]

[*]Carriers versus non-carriers. [#]Welch Two Sample t-test. [†]Fisher's Exact two-sided test. [§]Mann-Whitney U test, two-sided. P values <0.05 are in bold font.

Table 2. Serotype-specific carriage in PCV7 compared with PCV13 vaccinated children aged 24-48 months from the Thames Valley.

Serotype	PCV7 vaccinated (early-PCV13) n (%) N=567	PCV13 vaccinated (late-PCV13) n (%) N=642	OR (95% CI) PCV7 vaccinated/ PCV13 vaccinated	p
Serotypes included in PCV7				
6B	2 (0.35)	-	-	0.2197*
19F	1 (0.18)	-	-	0.4690*
23F	1 (0.18)	-	-	0.4690*
Additional serotypes included in PCV13				
1	1 (0.18)	-	-	0.4690*
3	18 (3.17)	7 (1.09)	0.36 (0.14-0.83)	0.0224
7F	8 (2.99)	-	-	**0.0022***
19A	27 (4.76)	3 (0.47)	0.08 (0.02-0.25)	**<0.0001**
Non-vaccine types				
6C	24 (4.23)	3 (0.47)	0.11 (0.03-0.32)	**0.0004**
7C	-	1 (0.16)	-	1*
8	-	1 (0.16)	-	1*
9N	-	6 (1.97)	-	0.0324*

10A	10 (1.76)	21 (3.27)	1.93 (0.91-4.34)	0.0959
11A	24 (4.23)	37 (5.76)	1.28 (0.74-2.22)	0.3807
12F	2 (0.35)	3 (0.47)	1.45 (0.24-11.1)	0.684
15A	4 (0.71)	19 (2.96)	4.37 (1.61-15.24)	**0.0081**
15B	2 (0.35)	32 (4.98)	13.85 (4.12-86.17)	**0.0004**
15C	8 (1.41)	9 (1.4)	0.92 (0.33-2.54)	0.8714
16F	8 (1.41)	5 (0.78)	0.37 (0.09-1.26)	0.1264
17F	1 (0.18)	2 (0.31)	1.32 (0.09-31.05)	0.834
20B	-	1 (0.16)	-	1*
21	12 (2.12)	27 (4.21)	1.98 (1-4.15)	0.0565
22F	13 (2.29)	12 (1.87)	0.86 (0.38-1.91)	0.7029
23A	12 (2.12)	17 (2.65)	1.19 (0.55-2.63)	0.6543
23B	24 (4.23)	33 (5.14)	1.29 (0.75-2.25)	0.356
24F	1 (0.18)	12 (1.87)	10.87 (2.1-199.08)	0.0227
29	5 (1.87)	-	-	0.0219*
31	7 (1.23)	6 (0.93)	0.82 (0.26-2.5)	0.731
33A	-	2 (0.31)	-	0.5015*
33F	7 (1.23)	5 (0.78)	0.52 (0.14-1.76)	0.304
34	-	2 (0.31)	-	0.5015*

35B	11 (1.94)	11 (1.71)	0.94 (0.4-2.22)	0.8872
35F	15 (2.65)	11 (1.71)	0.55 (0.23-1.26)	0.1675
38	4 (0.71)	8 (1.25)	1.8 (0.55-6.86)	0.3451
NT	9 (1.59)	9 (1.4)	0.96 (0.37-2.48)	0.928

Odds ratios (OR) and the associated p values were adjusted for age and sex unless otherwise specified. P values <0.01 are in bold font. PCV7=seven-valent pneumococcal conjugate vaccine. PCV13=thirteen-valent pneumococcal conjugate vaccine. NT=non-typeable. *Fisher's Exact test. Time-period of collection from PCV7 vaccinated children; November 2010-September 2011. Time-period of collection from PCV13 vaccinated children; February 2014-August 2015.

Table 3. Serotype-specific incidence of IPD in children and adults in England and Wales, at the time of PCV13 introduction (early-PCV13) and 5 years following PCV13 use (late-PCV13).

	Children <5 years				Adults 18-64 years			
Serotype	**Cases (IR) early-PCV13*** N=98 cases in 10 months	**Cases (IR) late-PCV13*** N=408 cases in 18 months	**IRR (95% CI)† (Late-PCV13/ early-PCV13)**	**p**	**Cases (IR) early-PCV13*** N=1892 cases in 10 months	**Cases (IR) late-PCV13*** N=2697 cases in 18 months	**IRR (95% CI)† (Late-PCV13/ early-PCV13)**	**p**
PCV7 covered serotypes								
4	-	-	-	-	10 (0.31)	16 (0.33)	1.05 (0.48-2.28)	0.9075
6B	-	-	-	-	31 (0.97)	5 (0.1)	0.11 (0.04-0.26)	**<0.0001**
9V	-	-	-	-	22 (0.69)	4 (0.08)	0.12 (0.04-0.33)	**<0.0001**
14	-	-	-	-	17 (0.53)	11 (0.23)	0.42 (0.2-0.89)	0.0192
18C	-	-	-	-	11 (0.34)	10 (0.2)	0.6 (0.26-1.38)	0.2206
19F	1 (0.31)	8 (1.59)	5.11 (0.64-40.55)	0.0851	26 (0.81)	26 (0.53)	0.65 (0.38-1.12)	0.1168
23F	-	-	-	-	22 (0.69)	4 (0.08)	0.12 (0.04-0.33)	**<0.0001**
Additional serotypes covered by PCV13								
1	23 (7.15)	1 (0.2)	0.03 (0-0.19)	**<0.0001**	63 (1.97)	49 (1)	0.51 (0.35-0.73)	**0.0002**
3	5 (1.56)	18 (3.58)	2.3 (0.86-6.15)	0.0873	207 (6.47)	135 (2.76)	0.43 (0.35-0.53)	**<0.0001**
5	-	2 (0.4)	-	0.2582	2 (0.06)	2 (0.04)	0.65 (0.1-4.48)	0.6636
6A	-	-	-	-	44 (1.38)	5 (0.1)	0.07 (0.03-0.18)	**<0.0001**

7F	20 (6.22)	4 (0.8)	0.13 (0.05-0.36)	**<0.0001**	213 (6.66)	153 (3.13)	0.47 (0.38-0.58)	**<0.0001**
19A	16 (4.98)	15 (2.98)	0.6 (0.3-1.12)	0.1422	267 (8.35)	134 (2.74)	0.33 (0.27-0.4)	**<0.0001**
Non-vaccine types								
6C	-	5 (0.99)	-	0.0738	89 (2.78)	36 (0.74)	0.26 (0.18-0.39)	**<0.0001**
7C	-	-	-		-	3 (0.06)	-	0.1611
8	-	21 (4.18)	-	**0.0002**	130 (4.06)	544 (11.13)	2.74 (2.27-3.31)	**<0.0001**
9N	-	15 (2.98)	-	**0.0020**	40 (1.25)	144 (2.95)	2.36 (1.66-3.3)	**<0.0001**
10A	3 (0.93)	24 (4.77)	5.11 (1.55-16.9)	**0.0029**	19 (0.59)	75 (1.53)	2.58 (1.57-4.26)	**0.0001**
10F	-	-	-	-	3 (0.09)	3 (0.06)	0.65 (0.14-3.15)	0.5942
11A	1 (0.31)	8 (1.59)	5.11 (0.65-40.55)	0.0851	38 (1.19)	28 (0.57)	0.48 (0.3-0.78)	**0.0022**
12B	-	-	-	-	1 (0.03)	4 (0.08)	2.62 (0.3-23.05)	0.3676
12F	2 (0.62)	49 (9.74)	15.66 (3.82-64.27)	**<0.0001**	39 (1.22)	391 (8)	6.56 (4.73-9.11)	**<0.0001**
15A	3 (0.93)	20 (3.98)	4.26 (1.27-14.26)	0.0104	58 (1.81)	90 (1.84)	1.02 (0.73-1.41)	0.9253
15B/C[#]	4 (1.24)	27 (5.37)	4.31 (1.52-12.27)	**0.0028**	23 (0.72)	48 (0.98)	1.37 (0.84-2.23)	0.2114
16F	-	2 (0.4)	-	0.2582	36 (1.13)	34 (0.7)	0.62 (0.39-0.98)	0.0385
17F	2 (0.62)	3 (0.6)	0.96 (0.16-5.59)	0.9627	20 (0.63)	23 (0.47)	0.75 (0.42-1.36)	0.3430
20	-	3 (0.6)	-	0.1661	21 (0.66)	24 (0.49)	0.75 (0.42-1.33)	0.3213
21	1 (0.31)	7 (1.39)	4.47 (0.56-36.03)	0.1228	1 (0.03)	2 (0.04)	1.31 (0.12-	0.8233

							14.02)	
22F	6 (1.87)	24 (4.77)	2.56 (1.05-6.22)	0.0317	167 (5.22)	156 (3.19)	0.61 (0.49-0.76)	**<0.0001**
23A	-	2 (0.4)	-	0.2582	54 (1.69)	51 (1.04)	0.62 (0.42-0.9)	0.0112
23B	2 (0.62)	28 (5.57)	8.95 (2.14-37.44)	**0.0003**	28 (0.88)	50 (1.02)	1.17 (0.74-1.85)	0.5026
24F	-	30 (5.96)	-	**<0.0001**	21 (0.66)	49 (1)	1.53 (0.92-2.53)	0.0981
27	-	5 (0.99)	-	0.0738	1 (0.03)	-	-	0.1993
29	1 (0.31)	1 (0.2)	0.64 (0.04-9.72)	0.7452	1 (0.03)	-	-	0.1993
31	2 (0.62)	1 (0.2)	0.32 (0.03-3.33)	0.3140	20 (0.63)	19 (0.39)	0.62 (0.34-1.15)	0.1271
33A					-	2 (0.04)	-	0.2526
33F	4 (1.24)	20 (3.98)	3.2 (1.1-9.29)	0.0241	57 (1.78)	86 (1.76)	0.99 (0.71-1.37)	0.9407
34	-	-	-		3 (0.09)	-	-	0.0262
35A	-	1 (0.2)	-	0.4240	-	5 (0.1)	-	0.0704
35B	-	9 (1.79)	-	0.0165	23 (0.72)	24 (0.49)	0.68 (0.39-1.2)	0.1807
35C	-	-	-		2 (0.06)	-		0.0695
35F	1 (0.31)	8 (1.59)	5.11 (0.65-40.55)	0.0851	26 (0.81)	35 (0.72)	0.88 (0.53-1.45)	0.6193
37	-	-	-	-	1 (0.03)	3 (0.06)	1.96 (0.21-18.49)	0.5478
38	1 (0.31)	13 (2.58)	8.31 (1.09-63.19)	0.0143	24 (0.75)	14 (0.29)	0.38 (0.2-0.73)	**0.0023**

Serotypes 9L, 18A, 18F, and 35A were only found on one occasion in the pre-PCV13 period in children. Serotypes 7B, 11B, and 12A were only found on one occasion in the pre-PCV13 period in adults. Serotypes 9L, 10B, 11C, and 15F were only found on one occasion in the post-PCV13 period in adults. 8 isolates were classified as 16A/F in

pre-PCV13 adults. P values <0.01 are in bold font. PCV13=Thirteen-valent pneumococcal conjugate vaccine. [*]Incidence rates (IR) are quoted as cases per million population per year. IRR=incidence rate ratio. [#]The final serotype of some serotype 15B/C pneumococci could not be determined. [†]No IRR could be calculated as there were no detected cases in either the early-PCV13 time-period (November 2010-September 2011) or late-PCV13 time-period (February 2014-August 2015).