## **Title**

Upper airways colonisation of *Streptococcus pneumoniae* in adults aged 60 years and older: a systematic review of prevalence and individual participant data meta-analysis of risk factors

## **Running title**

Nasopharyngeal colonisation with *Streptococcus pneumoniae* in adults aged 60 years and older: Prevalence and risk factors

## **Authors**

Emma L. Smith MBChB1 \*, India Wheeler MBChB1 \*, Hugh Adler PhD1, Daniela M. Ferreira PhD1, Raquel Sá-Leão PhD2 Osman Abdullahi PhD3, Ifedayo Adetifa PhD 4,5,6, Sylvia Becker-Dreps MD7, Susanna Esposito MD8, Helmia Farida PhD9, Rama Kandasamy DPhil10,11, Grant A. Mackenzie PhD12,13,14,15, J. Pekka Nuorti PhD16,17, Susan Nzenze PhD18,19, Shabir A. Madhi PhD18,19, Omar Ortega PhD20,21, Anna Roca PhD12, Dodi Safari PhD22, Frieder Schaumburg23, Effua Usuf PhD12, Elisabeth A.M. Sanders MD24, Lindsay R. Grant PhD25, Laura L. Hammitt MD25, Katherine L. O’Brien25, Prabhu Gounder MD26, Dana J.T. Bruden MD26, Michelle C. Stanton PhD27, Jamie Rylance PhD1.

\* these two authors contributed equally

## **Corresponding author**

Dr Jamie Rylance, Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK, L3 5QA. Telephone: +447904242353. Email: Jamie.Rylance@lstmed.ac.uk

## **Affiliations**

1 Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK.

2 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal.

3 Department of Public Health, School of Health and Human Sciences, Pwani University, Kilifi, Kenya.

4 Epidemiology and Demography Department, KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya.

5 Department of Infectious Diseases Epidemiology, London School of Hygiene and Tropical Medicine, Keppel Street, WC1E 7HT, London, United Kingdom.

6 Department of Paediatrics and Child Health, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria.

7 Departments of Family Medicine and Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA.

8 Pediatric Clinic, Department of Surgical and Biomedical Sciences, Università degli Studi di Perugia, Perugia, Italy.

9 Faculty of Medicine, Diponegoro University, Semarang, Indonesia.

10 Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Oxford, OX3 7LE, United Kingdom.

11 NIHR Oxford Biomedical Research Centre, Oxford, OX3 7LE, United Kingdom.

12 Medical Research Council The Gambia Unit at LSHTM, Banjul, The Gambia.

13 Faculty of Infectious and Tropical Diseases, The London School of Hygiene & Tropical Medicine, UK.

14 Infection and Immunity Theme, Murdoch Children’s Research Institute, Melbourne, Australia.

15 Department of Paediatrics, University of Melbourne, Melbourne, Australia.

16 Health Sciences Unit, Faculty of Social Sciences, Tampere University, Finland.

17 Department of Health Security, National Institute for Health and Welfare (THL), Helsinki, Finland

18 Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand, Johannesburg, South Africa.

19 Department of Science and Technology/National Research Foundation: Vaccine Preventable Diseases, University of the Witwatersrand, Johannesburg, South Africa.

20 Gastrointestinal Physiology Laboratory, Department of Surgery, Hospital de Mataró, Universitat Autónoma de Barcelona, Mataró, Spain.

21 Centro de Investigación Biomédica en Red de enfermedades hepáticas y digestivas (CIBERehd), Instituto de Salud Carlos III, Barcelona, Spain.

22 Eijkman Institute for Molecular Biology, Jl. Diponegoro no. 69 Jakarta
Indonesia.

23 Institute of Medical Microbiology, University Hospital Muenster, Muenster, Germany.

24 Department of Pediatric Immunology and Infectious Diseases, Wilhelmina Children's Hospital, University Medical Center Utrecht, The Netherlands.

25 Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

26 Arctic Investigations Program, Division of Preparedness and Emerging Infections, Center for Disease Control and Prevention, Anchorage, Alaska.

27 Lancaster Medical School, Lancaster University, UK.

## **Word count**

Abstract: 244

Full text: 2,775

**List of figures and tables**

Figure 1: PRISMA flowchart for systematic review

Figure 2: Forest plot of proportion of pneumococcal colonisation by publication, grouped by country income category

Figure 3: Summary data panels of risk factors for colonisation at individual and study level

Table 1: Multiple imputation generalised linear mixed model of risk factors for pneumococcal carriage using participant level data

**Supplementary tables**

Supplementary Table 1: Strengthening The Reporting Of Observational Studies in Epidemiology (STROBE) Checklist for all included studies

Supplementary Table 2: Details of contact with authors of all eligible journal articles

Supplementary Table 3: Summary of all included studies in the systematic review and meta-analysis

Supplementary Table 4: Univariate model of risk factors for pneumococcal carriage using participant level data

# **Abstract**

**Background:** Colonisation with *Streptococcus pneumoniae* can lead to invasive pneumococcal disease and pneumonia. Pneumococcal acquisition and prevalence of colonisation are high in children. In older adults, a population susceptible to pneumococcal disease, colonisation prevalence is reported to be lower, but studies are heterogeneous.

**Methods:** This is a systematic review and meta-analysis of prevalence of, and risk factors for, pneumococcal colonisation in adults ≥ 60 years of age (PROSPERO #42016036891). We identified peer-reviewed studies reporting the prevalence of *S. pneumoniae* colonisation using MEDLINE and EMBASE (until April 2016), excluding studies of acute disease. Participant-level data on risk factors were sought from each study.

**Findings**: Of 2202 studies screened, 29 were analysable: 18 provided participant-level data (representing 6290 participants). Prevalence of detected pneumococcal colonisation was 0-39% by conventional culture methods and 3-23% by molecular methods. In a multivariate analysis, colonisation was higher in persons from nursing facilities compared with the community (odds ratio (OR) 2·30, 95% CI 1·26-4·21 and OR 7·72, 95% CI 1·15-51·85 respectively), in those who were currently smoking (OR 1·69, 95% CI 1·12-2·53) or those who had regular contact with children (OR 1·93, 95%CI 1·27-2·93). Persons living in urban areas had significantly lower carriage prevalence (OR 0·43, 95%CI 0·27-0·70).

**Interpretation:** Overall prevalence of pneumococcal colonisation in older adults was higher than expected but varied by risk factors. Future studies should further explore risk factors for colonisation, to highlight targets for focussed intervention such as pneumococcal vaccination of high-risk groups.

**Funding:** No funding was required.

**Key words:**

Pneumococcal, colonisation, adults, risk factors

**Research in context:**

**Evidence before this study:**

Nasopharyngeal colonisation with Streptococcus pneumoniae is a pre-requisite for pneumonia and invasive pneumococcal disease. Carriage prevalence in children can be as high as 90%, and geographic location is a strong determinant of rates. In older people, carriage spot-prevalence and risk factors are less clear. Defining these has implications for understanding transmission, for example in nursing home and outbreak settings, and to identify modifiable factors or at-risk groups.

**Added value of this study:**

This systematic analysis includes extensive participant level data in adults over 60 years of age to define prevalence and risk factors of upper airway pneumococcal colonisation. We incorporate 18 studies and more than 6000 patients from contrasting geographical and residential settings. Adults over the age of 60 had point prevalence between 0 and 39% using classical microbiology and 3 to 23% using bacterial DNA detection methods. Individuals who have contact with children, smoke and those who reside in a nursing home had a higher prevalence of carriage.

**Implications of all the available evidence:**

This evidence is a distillation of previously fragmented data which increase our understanding of population colonisation and risk factors for acquisition. The findings confirm that rates are lower than in children, but identify a significant proportion of individuals which could be protected by developing vaccines which reduce carriage. Using such tools, targeted intervention might reduce the considerable burden of pneumococcal disease in older adults.

# **Introduction**

*Streptococcus pneumoniae* represents a leading cause of morbidity and mortality, resulting from pneumonia, and invasive pneumococcal disease (IPD, encompassing bacteraemia and meningitis). Pneumococcal disease is most common at the extremes of age;1–3 those over 65 years old are particularly susceptible, in part from the effects of immunosenescence and frequent co-morbidities.4The reported overall incidence of IPD in Europe was 6.2 cases per 100,000 population in 2017.5 Higher rates occur in low income countries, although data are fewer*.*6–7

*S. pneumoniae* is a frequent transient colonizer of the human upper respiratory tract (naso- and oropharynx). In a minority of cases this can lead to invasive disease but more usually, asymptomatic colonisation generates specific immune responses, and protects against future re-colonisation by the same serotype.8–12

Pneumococci spread by droplet transmission of nasopharyngeal bacteria. Young children with high density colonisation are generally considered to be the reservoir for pneumococcal transmission in the population13 but adult-to-adult transmission has also been documented, for example in nursing home populations, particularly in outbreak settings.14–15 An understanding of population level colonisation and dynamics is important in order to monitor transmission and help to reduce the burden of invasive disease.

Rates of detection of *S.pneumoniae* in the nasopharynx of children are reported to be as high as 90%.16–18 In ageing adults, rates from highly disparate cohort studies are widely variable. This variation may be driven by study-level factors, including geographical location, site of sampling (nasopharyngeal vs oropharyngeal) and detection methods (e.g., classical microbiological culture in the case of low-density colonisation in adults may be less sensitive than molecular methods). A recent study highlighted a decrease in colonisation density at older ages, particularly >50 years old, and showed improved detection of pneumococcal colonisation when molecular methods were used.19 Putative determinants of colonisation include overcrowding, smoking, increasing age, institutionalization, recent respiratory tract infection and contact/cohabitation with young children*.*1,20–23

The burden of pneumococcal disease has been successfully reduced by the introduction of pneumococcal conjugate vaccination regimens into infant immunisation programmes.24 Since the introduction of the seven-valent pneumococcal conjugate vaccine (PCV) in 2000, there has been a general decline in vaccine serotype IPD in children and all other age groups, indicating an indirect effect. This has, however, been accompanied by a concurrent increase in non-vaccine serotype carriage in vaccinated children and adults, resulting in relatively unchanged overall carriage prevalence*.*11,17,25–30

An understanding of population level colonisation and the influence of risk factors is important to assess the risk of acquisition and colonisation and subsequent disease in both the individual and the community. Furthermore, it allows identification of potentially modifiable risk factors to reduce the burden in populations with the highest incidence of IPD (i.e. older adults).

The objective of the systematic review and meta-analysis was to define the prevalence and risk factors for upper airway pneumococcal colonisation in adults over 60 years of age using participant level data from previously published reports.

# **Methods**

We searched for articles from MEDLINE and EMBASE databases in April 2016 for English language manuscripts published since 1946 using the following search terms: (“pneumococcus” [ti] OR “Streptococcus pneumoniae” [MESH]) AND (“carriage” [ti] OR “colonis\* [ti]). Additional articles were identified through discussion with experts with knowledge of other sources. Inclusion and exclusion criteria are given in Box 1.

Two independent reviewers screened the resulting titles and abstracts, reaching agreement through consensus to include studies reporting rates of nasopharyngeal and/or oropharyngeal colonisation of *S. pneumoniae* by detection through classical culture and/or PCR. We searched any adult study, excluding studies which: had no participants over the age of 60 years as documented in the manuscript or by subsequent confirmation with the corresponding author; measured colonisation in participants with acute lower respiratory tract infection or hospitalized participants; lacked peer review or abstract only available; used inappropriate bacterial sampling techniques; lacked clear methodology on population or sampling method; abstract unavailable as English translation. Duplicate articles were removed. Full text articles of the relevant titles/abstracts were evaluated independently by ELS and IW. The review was registered with PROSPERO (#42016036891).

## **Assessment of study quality**

Study quality data were extracted using the STrengthening the Reporting of Observational studies (STROBE) criteria for epidemiological studies (see Supplementary table 1).

## **Summary data**

Data were extracted from original manuscripts into a spreadsheet, disaggregated by age. Where sufficient contextual information was available, we inferred at the study-level pre-defined variables, which were hypothesised or previously documented risk factors for colonisation.

For each included article, we invited the corresponding author to contribute de-identified individual participant level data including potential risk factors for colonisation, and metadata relating to the study. These risk factors were pre-defined by assessing previous research in pneumococcal disease and other respiratory illness. All participants within their respective studies gave their informed written consent to participate in the original study, and each study was approved by their respective ethics committee.

## **Data extraction**

We extracted information on study country and setting, dates that the study was conducted as well as information regarding pneumococcal vaccination programmes ongoing when the study was conducted. We recorded the number of participants over the age of 60 years as well as the recorded prevalence of *S. pneumoniae* colonisation in that age group, where possible. Where available, we extracted data of the prevalence of carriage across pre-defined risk factors into a spreadsheet'. De-identified participant level data (PLD) were cleaned and imported to R v3.4.3 for analysis.

## **Data analysis**

We calculated exact binomial confidence intervals around the estimate of proportion colonised for each study. For meta-analysis of all available studies, we generated a generalized linear mixed-effects model of logit transformed proportion using study as a random effect.

For meta-analysis of participant level data, a joint modelling approach with multiple imputation (R package jomo)31 was fitted to the colonisation data, to account for ‘missing’ data as a result of differences in the risk factors being collected by each individual study. Models fitted to the data assumed an initial fixed common variance matrix across all individual studies. We used the Markov Chain Monte Carlo (MCMC) algorithm (jomo1ran function) with a burn in of 500,000 and a thinning factor of 1000. Two-hundred imputed datasets were simulated, and a generalised linear mixed model was fitted to each of the simulated datasets, including all imputed risk factors. Imputed estimates of the log-odds associated with each risk factor were combined according to Rubin’s rules.32

# **Results**

We screened 2202 studies with 2134 records excluded at this stage (as detailed in Figure 1). Sixty-eight full text articles were assessed for eligibility of which, 23 studies were excluded due to: (1) no data on elderly participants (n=13); (2) study article unavailable in English (n=5); study contained only data on IPD serotype, not on colonisation (n=2); study included participants with acute respiratory illness (n=2) and; alternative method of detecting carriage used (n=1).

Data were sought from 45 studies, and successfully extracted from 29. Seventeen studies had data disaggregated by age in the manuscript and had relevant data on carriage in participants over 60 years old. We received PLD from 18 studies. In the remaining 27 studies PLD was not obtained due to: authors not contactable (n=8), authors contacted but no data on adults >60yrs (n=4), authors contacted but could not give PLD for various reasons (n=15). Three further studies could not give PLD but were able to give age-specific prevalence that had not been detailed in the original manuscript.

## **Summary-level data**

Studies represented varied geographies: Europe (n=13), Africa (n=7), the Americas (n=6), South-East Asia (n=2) and the Western Pacific (n=1). Studies were predominantly from high income countries (n=21), with low income countries presented by two cohorts. Study quality analysis is given in Supplementary Table 1.

Prevalence of pneumococcal carriage in included studies was highly variable, ranging from 0%-38·8% in those studies using standard classical culture techniques of naso- and/or oropharyngeal swabs to detect *S. pneumoniae* carriage (Figure 2). For classical microbiological detection, the pooled estimate of colonisation was 9% (95% confidence interval (CI) 6-14%) representing data from 7823 participants. Using molecular diagnostics like PCR for detection, colonisation prevalence was 2·7%-22·7% (Figure 2), with pooled estimate of 9% (95% CI 5-24%), representing 1224 individuals. One study compared classical microbiology and PCR detection methods in the same participant group and found significantly higher prevalence when using PCR (7·6%, 95% CI 5·2-10·9 vs 22·7%, 95% CI 18·2-27·2).33

Known and putative risk factors for pneumococcal carriage at the study level are shown in Figure 3, and in the Supplementary Table 4.

## **Participant level data**

Participant level data from 6290 participants were available within 18 studies. Studies that used microbiological culture as the method of detection of *S. pneumoniae* carriage were included in a multivariate analysis (5680 participants). Due to many missing values (more than 70% of data), the variables “passive smoking”, “lives with children”, “number sleeping in household”, and “alcohol use” were excluded. The “season” variable was excluded due to inconsistencies in recording the timing of sampling.

In the univariate model, the variables “accommodation”, “setting” and “contact with children” were found to be significantly associated with colonisation (Supplementary Table 4). Those residing in a skilled nursing facility compared to living in the community, those living in a rural area compared to an urban area and those who had regular contact with young children (aged<6 years) were found to have increased rates of pneumococcal colonisation.

The results from the generalised linear mixed model applied to our dataset with imputed values are outlined in Table 1. For all variables used in this analysis, data were obtained in at least 65% of participants. This model showed a significant increase in the prevalence of pneumococcal colonisation in those living in assisted living and more so those in a nursing facility, compared with those living in community (OR 2·30, 95% CI 1·26-4·21 and OR 7·72, 95% CI 1·15-51·85 respectively). Participants from urban areas had significantly lower prevalence of pneumococcal colonisation than did those living in rural settings (OR 0·43, 95%CI 0·27-0·70). Current active smoking and regular (at least weekly) contact with children aged ≤6 years was also associated with significantly higher prevalence of pneumococcal colonisation (OR 1·69, 95% CI 1·12-2·53 and OR 1·93, 95%CI 1·2-2·93 respectively).

Male participants had significantly lower pneumococcal colonisation than females (OR 0·76, 95% CI 0·59-0·98). Participants’ age was not significantly associated with colonisation.

**Discussion**

Our systematic review and meta-analysis incorporate 29 studies and more than 7000 participants from a variety of geographical and residential settings. Adults aged 60 years and older had point prevalence rates of *S. pneumoniae* colonisation between 0% and 38.8% using classical microbiology, and 2.7% and 22·7% using bacterial DNA detection methods. Pooled estimates for both detection techniques were similar (9%), with the three largest studies reporting amongst the lowest rates, notably from European populations.1,28,34 While substantially lower than rates in paediatric populations, these findings challenge the convention that adults aged over 60 years are only infrequent carriers.

Previous studies have found the average duration of colonisation in young adults is 2-3 weeks. 35,36 In the absence of such studies in the elderly population we assume duration of colonisation will be similar.

If 9% of older adults are colonised at any one time, with a median duration of around three weeks, this may have implications for the reservoir of circulating pneumococcus at the community level.  For example, recent vaccine impact studies in the UK have identified a high proportion of invasive disease caused by a few key vaccine-type (e.g. 3, 19A) and non-vaccine type (8, 10A, 15A) pneumococci.37 Some of these serotypes (3, 8, 19A) were infrequent/absent in childhood colonisation surveys carried out during the same period,38 but have been identified in adult colonisation,39 which might explain their persistent circulation.

The most important risk factors were smoking, contact with children and residence in a nursing home compared to living in the community. Living in an urban setting and being male was associated with lower prevalence of carriage. Our findings are consistent with previous studies. Cigarette smoking is a well-documented risk factor for colonisation both directly in the elderly population1 and also exposure to passive smoke in children.40 Cigarette smoking has also been shown to be a strong independent risk factor for invasive pneumococcal disease in the immunocompetent non-elderly adult population.41 Potential mechanistic explanations include the effect of cigarette smoke on the oral microbiome42–44 with a higher proportion of disease causing organisms and fewer bacteria with interfering capabilities in smokers compared with non-smokers.45 Smoking also increases host susceptibility to respiratory viruses through the potential mechanisms of: impaired mucocilary flow and increased permeability of the respiratory epithelium.46

Contact with young children, as an independent risk factor for adult colonisation, has been previously documented, and confirms children being the most important reservoir for circulating strains.41,47,48 Adult-to-adult pneumococcal transmission has also been documented within nursing homes and other institutions, evidenced by cluster analysis of disease outbreaks within these settings.12,15,49,50 Our meta-analysis identified nursing home residence as a risk factor, although total participant numbers were small in this subgroup. Findings suggest that this, in combination with reports of nursing home pneumococcal outbreaks, could support the designating of residents as higher-risk and therefore worthy of PCV and pneumococcal polysaccharide vaccine (PPV) vaccination to protect against severe pneumococcal disease. A recent study confirmed that PCV causes a transient reduction in vaccine serotype carriage in older adults51, and protection against vaccine serotype pneumonia, further supporting a potential benefit of vaccination of higher-risk groups.

The data demonstrate stark intra-cohort differences in prevalence across different geographic settings, ranging from no documented carriage in a Spanish community setting52 to 40% carriage in a community survey in Gabon.53 Living in a tropical climate was not identified as an individual risk factor, however the observed higher carriage rates in the tropics may reflect higher population or living densities.54,55 Nonetheless these individual factors may relate only to associations with other aspects of lower economic status. Indeed, in keeping with paediatric data, the increased risk of colonisation associated with residing in a rural setting is partly explained by the economic conditions in these settings, as seen in Aboriginal communities in Australia21,56 and in Sub-Saharan Africa.57,58

Our results derive from rigorous systematic and approved methodology, with strenuous effort to obtain participant-level data. However, the results should be interpreted in light of several considerations. As described, our data was derived from vast geographical settings and as such it is difficult to draw comparisons between such starkly contrasting populations. We used both participant-level data (from 18 studies), and meta-data derived from the study setting and inclusion/exclusion criteria. Not all studies followed the WHO recommendation for measuring nasopharyngeal colonisation.59 Our analysis therefore combines nasopharyngeal and oropharyngeal colonisation into a single categorical variable, in order to maximise statistical power. The combination of oro- and nasopharyngeal sampling may have influenced results, incurring a bias towards lower values compared to using nasopharyngeal sampling alone.

Risk factors are necessarily restricted to those for which data were collected (or inferable); some, including alcohol consumption, number of household members and participants’ cohabitation with children had to be excluded due to insufficient completion rates. We were also unable to codify data on seasonality. In order to analyse the remaining variables, we used imputation. Imputation may introduce bias, and although this overall dataset does represent a very large proportion of published data, publication or reporting bias may contribute significantly. There may also be other potential sources of bias in the data as a result of recruitment or sampling methods, which might favour those with certain risk factors for carriage giving a falsely elevated or decreased carriage risk

We have been unable to fully ascertain the association of colonisation with some important risk factors, particularly immunisation. Data on individual elderly participant’s pneumococcal vaccination history (with PPV) was included in the meta-analysis and was found to not be a significant factor in pneumococcal colonisation. National paediatric pneumococcal conjugate vaccine policy was not codified into a study variable. This was due to studies being conducted over a number of years, during vaccination policy change, as well as a number of studies in which vaccination policy was not outlined within the journal article. Results from adult populations in high income countries demonstrate reduced vaccine-serotype carriage where paediatric immunisation has been programmatically introduced.29,30,60,61 However, in some cohorts from low income countries, the effect on even paediatric circulating serotypes has been suboptimal, demanding better vaccination coverage and time to eradicate PCV serotype circulation.62 It may therefore be useful for a longitudinal study to be conducted investigating colonisation in the elderly in the context of childhood vaccination programmes.

We also could not capture potentially significant differences in living environment (for example crowding and paediatric prevalence rates), due to lack of granularity. Data for colonisation densities were unavailable. We recommend that a consistent approach to data capture and reporting is taken in studies; we have suggested a core set of variables which should be considered in the design of such future research (see Box 1). Standardising sampling methods as described by the World Health Organisation59, or clarifying the site and diagnostic method within the study protocol is also a priority to enable accurate comparisons to be made between population groups.

Paradoxically, older adults have high incidence rates and case-fatality of pneumococcal disease, despite low observed carriage rates. Given a limited and transient reduction in colonisation after PCV1351 in older adults, we need to understand this relationship and the factors which drive progression from colonisation to severe illness. When performing epidemiological and laboratory-based research, this review identifies the core measures which should be consistently reported.

# **Acknowledgments**

We would like to thank all of the study participants for their contribution and all study teams involved in the original research. This paper has used data provided by the KEMRI Wellcome Trust Research programme, Nestle Research, Centre of Disease Control, and the Medical Research Council (The Gambia) in accordance with the consent provided by participants and approved by the various Ethics Review Committees.

We would like to thank Professor Elizabeth Miller, Stefan Flasche, Albert Jan Van Hoek and Public Health England for collecting and sharing their data. We would also like to thank Professor Andrew Pollard, Associate Professor Pere Clave and Dr Olga Slawinski for their expertise and contribution to the manuscript.

# **Declaration of interests**

# SBD reports research grants from Pfizer during the conduct of the study and personal fees from Pfizer outside of the submitted work.

# PG reports an investigator-initiated grant by Wyeth Pharmaceuticals (now Pfizer) on a pneumococcal colonisation study. The funding agency provided funding support only - they did not provide any input into the study design.

SE reports grants and personal fees from GSK, personal fees from Merck, grants from Abbott, grants and personal fees from Sanofi Aventis, grants and personal fees from Vifor, grants from DMG, outside the submitted work.

GM reports grants from Bill & Melinda Gates Foundation, grants from GAVI the Vaccine Alliance, grants from Pfizer Ltd, during the conduct of a pneumococcal colonisation study.

EAMS reports grants from Wyeth, grants from Pfizer, during the conduct a pneumococcal study

LLH reports grants from Pfizer, GSK, and Merck, outside the submitted work.

LRG reports grants from Pfizer, GSK, and Merck, outside the submitted work and honorarium from Pfizer and Merck.

# **References**

1. Almeida ST, Nunes S, Santos Paulo AC, et al. Low prevalence of pneumococcal carriage and high serotype and genotype diversity among adults over 60 years of age living in Portugal. *PLoS One*. 2014;**9(3)**:1–10.

2. Faden H, Duffy L, Wasielewski R, Wolf J, Krystofik D, Tung Y. Relationship between nasopharyngeal colonization and the development of otitis media in children. Tonawanda/Williamsville Pediatrics. *J Infect Dis*. 1997;**175(6)**:1440–1445.

3. Leach AJ, Stubbs E, Hare K, Beissbarth J, Morris PS. Comparison of nasal swabs with nose blowing for community-based pneumococcal surveillance of healthy children. *J Clin Microbiol*. 2008;**46(6)**:2081–2082.

4. Krone CL, van de Groep K, Trzcinski K, Sanders EAM, Bogaert D. Immunosenescence and pneumococcal disease: an imbalance in host-pathogen interactions. *Lancet Respir Med*. 2014;**2(2)**:141–153.

5. European Centre for Disease Prevention andControl. Annual Epidemiological Report 2016 – Invasive pneumococcal disease. https://ecdc.europa.eu/en/publications-data/invasive-pneumococcal-disease-annual-epidemiological-report-2017. Date accessed 21 January 2019.

6. Meiring S, Cohen C, Quan V, et al. HIV Infection and the Epidemiology of Invasive Pneumococcal Disease (IPD) in South African Adults and Older Children Prior to the Introduction of a Pneumococcal Conjugate Vaccine (PCV). *PLoS One*. 2016;**11(2)**:e0149104.

7. Iroh Tam P-Y, Thielen BK, Obaro SK, et al. Childhood pneumococcal disease in Africa - A systematic review and meta-analysis of incidence, serotype distribution, and antimicrobial susceptibility. *Vaccine*. 2017;**35(15)**:1817–1827.

8. Adler H, Ferreira DM, Gordon SB, Rylance J. Pneumococcal capsular polysaccharide immunity in the elderly. *Clin Vaccine Immunol*. 2017;**24(6)**:e00004–17.

9. Ansaldi F, De Florentiis D, Canepa P, et al. Carriage of Streptoccoccus pneumoniae in healthy adults aged 60 y or over in a population with very high and long-lasting pneumococcal conjugate vaccine coverage in children: Rationale and perspectives for PCV13 implementation. *Hum Vaccines Immunother*. 2013;**9(3)**:614–620.

10. Bogaert D, De Groot R, Hermans PWM. Streptococcus pneumoniae colonisation: the key to pneumococcal disease. *Lancet Infect Dis*. 2004;**4(3)**:144–154.

11. Simell B, Auranen K, Kayhty H, Goldblatt D, Dagan R, O’Brien KL. The fundamental link between pneumococcal carriage and disease. *Expert Rev Vaccines*. 2012;**11(7)**:841–855.

12. Zivich PN, Grabenstein JD, Becker-Dreps SI, Weber DJ. Streptococcus pneumoniae outbreaks and implications for transmission and control: a systematic review. *Pneumonia (Nathan Qld)*. 2018;**10**:11.

13. Hill PC, Townend J, Antonio M, et al. Transmission of Streptococcus pneumoniae in rural Gambian villages: a longitudinal study. *Clin Infect Dis*. 2010;**50(11)**:1468–1476.

14. Flamaing J, Peetermans WE, Vandeven J, Verhaegen J. Pneumococcal colonisation in older persons in a nonoutbreak setting. *J Am Geriatr Soc*. 2010;**58(2)**:396­–398.

15. Nuorti JP, Butler JC, Crutcher JM, et al. An Outbreak Of Multidrug-Resistant Pneumococcal Pneumonia And Bacteremia Among Unvaccinated Nursing Home Residents. *N Engl J Med*. 1998;**338(26)**:1861–1868.

16. Sa-Leao R, Nunes S, Brito-Avo A, et al. High rates of transmission of and colonization by Streptococcus pneumoniae and Haemophilus influenzae within a day care center revealed in a longitudinal study. *J Clin Microbiol*. 2008;**46(1)**:225–234.

17. Garcia-Rodriguez JA, Fresnadillo Martinez MJ. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *J Antimicrob Chemother*. 2002;**50 Suppl S**:59–73.

18. Usuf E, Bojang A, Hill PC, Bottomley C, Greenwood B, Roca A. Nasopharyngeal colonization of Gambian infants by Staphylococcus aureus and Streptococcus pneumoniae before the introduction of pneumococcal conjugate vaccines. *New microbes new Infect*. 2015;**10**:13–18.

19. Sutcliffe CG, Grant LR, Cloessner E, et al. Impact of Laboratory Methods, Colonization Density and Age on Detection of Streptococcus Pneumoniae in the Nasopharynx. *Am J Epidemiol*. 2019. doi:10.1093/aje/kwz191

20. Mosser JF, Grant LR, Millar E V, et al. Nasopharyngeal carriage and transmission of Streptococcus pneumoniae in American Indian households after a decade of pneumococcal conjugate vaccine use. *PLoS One*. 2014;**9(1)**:3–10.

21. Mackenzie GA, Leach AJ, Carapetis JR, Fisher J, Morris PS. Epidemiology of nasopharyngeal carriage of respiratory bacterial pathogens in children and adults: cross-sectional surveys in a population with high rates of pneumococcal disease. *BMC Infect Dis*. 2010;**10(1)**:304.

22. Farida H, Severin JA, Gasem MH, et al. Nasopharyngeal carriage of Streptococcus pneumonia in pneumonia-prone age groups in Semarang, Java Island, Indonesia. *PLoS One*. 2014;**9(1)**:16–18.

23. Adetifa IMO, Antonio M, Okoromah CAN, et al. Pre-vaccination nasopharyngeal pneumococcal carriage in a Nigerian population: Epidemiology and population biology. *PLoS One*. 2012;**7(1)**:e30548.

24. Hammitt LL, Bruden DL, Butler JC, et al. Indirect Effect of Conjugate Vaccine on Adult Carriage of *Streptococcus pneumoniae:* An Explanation of Trends in Invasive Pneumococcal Disease. *J Infect Dis*. 2006;**193(11)**:1487–1494.

25. Esposito S, Mari D, Bergamaschini L, et al. Pneumococcal colonization in older adults. *Immun Ageing*. 2016;**13(1)**. doi: 10.1186/s12979-016-0057-0

26. Coughtrie AL, Whittaker RN, Begum N, et al. Evaluation of swabbing methods for estimating the prevalence of bacterial carriage in the upper respiratory tract: A cross sectional study. *BMJ Open*. 2014;**4(10)**. doi:10.1136/bmjopen-2014-005341

27. Brugger SD, Frey P, Aebi S, Hinds J, Mühlemann K. Multiple Colonization with S. pneumoniae before and after Introduction of the Seven-Valent Conjugated Pneumococcal Polysaccharide Vaccine. *PLoS One*. 2010;**5(7)**:1–8.

28. 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)**:e335.

29. Collins DA, Hoskins A, Bowman J, et al. High nasopharyngeal carriage of non-vaccine serotypes in western australian aboriginal people following 10 years of pneumococcal conjugate vaccination. *PLoS One*. 2013;**8(12)**:4–12.

30. Flasche S, van Hoek AJ, Sheasby E, et al. Effect of pneumococcal conjugate vaccination on serotype-specific carriage and invasive disease in England: A cross-sectional study. *PLoS Med*. 2011;**8(4)**:14.

31. Quartagno M, Carpenter J. jomo: Multilevel Joint Modelling Multiple Imputation. November 2016. http://datacompass.lshtm.ac.uk/397/. Date accessed September 10 2018.

32. Campion WM, Rubin DB. Multiple Imputation for Nonresponse in Surveys. *J Mark Res*. 2006;**26(4)**:485.

33. van Deursen AMM, van den Bergh MR, Sanders EAM, et al. Carriage of Streptococcus pneumoniae in asymptomatic, community-dwelling elderly in the Netherlands. *Vaccine*. 2016;**34(1)**:4–6.

34. Palmu AA, Kaijalainen T, Saukkoriipi A, Leinonen M, Kilpi TM. Nasopharyngeal carriage of Streptococcus pneumoniae and pneumococcal urine antigen test in healthy elderly subjects. *Scand J Infect Dis*. 2012;**44(6)**:433–438.

35. Gritzfeld JF, Crèmers AJH, Ferwerda G, Ferreira DM, Kadioglu A, Hermans PWM, and Gordon SB. Density and duration of experimental human pneumococcal carriage. *Clinical Microbiology and Infection.* 2014; **20(12):**1145-1151.

36. Rylance J, de Steenhuijsen Piters WAA, Mina MJ, Bogaert D, French N, Ferreira DM. Two Randomized Trials of the Effect of Live Attenuated Influenza Vaccine on Pneumococcal Colonization. *Am J Respir Crit Care Med.* 2019; **199(9)**: 1160-1162.

37. Ladhani SN, Collins S, Djennad A, Sheppard CL, Borrow R, Fry NK, et al. Rapid increase in non-vaccine serotypes causing invasive pneumococcal disease in England and Wales, 2000-17: a prospective national observational cohort study. *The Lancet Infectious Diseases.* 2018; **18**: 441-451.

38. Southern J, Andrews N, Sandu P, Sheppard CL, Waight PA, Fry NK, et al. Pneumococcal carriage in children and their household contacts six years after introduction of the 13-valent pneumococcal conjugate vaccine in England. *PLOS One.* 2018; **13(5):** e0195799.

39. Adler H, Nikolaou E, Gould K, Hinds J, Collins AM, Connor V, et al. Pneumococcal Colonization in Healthy Adult Research Participants in the Conjugate Vaccine Era, United Kingdom, 2010-2017. *The Journal of Infectious Diseases.* 2019; **219(12):** 1989-1993.

40. Brook I, Gober AE. Recovery of potential pathogens in the nasopharynx of healthy and otitis media-prone children and their smoking and nonsmoking parents. *Ann Otol Rhinol Laryngol*. 2008;**117(10)**:727­–730.

41. Nuorti JP, Butler JC, Farley MM, et al. CIGARETTE SMOKING AND INVASIVE PNEUMOCOCCAL DISEASE. *N Engl J Med*. 2000;**342(10)**:681–689.

42. Morris A, Beck JM, Schloss PD, et al. Comparison of the respiratory microbiome in healthy nonsmokers and smokers. *Am J Respir Crit Care Med*. 2013;**187(10)**:1067–1075.

43. Munck C, Helby J, Westergaard CG, Porsbjerg C, Backer V, Hansen LH. Smoking Cessation and the Microbiome in Induced Sputum Samples from Cigarette Smoking Asthma Patients. *PLoS One*. 2016;**11(7)**:e0158622.

44. Charlson ES, Chen J, Custers-Allen R, et al. Disordered Microbial Communities in the Upper Respiratory Tract of Cigarette Smokers. *PLoS One*. 2010;**5(12)**:1–10.

45. Brook I, Gober AE. Recovery of potential pathogens and interfering bacteria in the nasopharynx of smokers and nonsmokers. *Chest*. 2005;**127(6)**:2072–2075.

46. Sherman CB. The health consequences of cigarette smoking: Pulmonary Diseases. *Med Clin North Am*. 1992;**76(2)**:355–375.

47. Regev‐Yochay G, Raz M, Dagan R, et al. Nasopharyngeal Carriage of *Streptococcus pneumoniae* by Adults and Children in Community and Family Settings. *Clin Infect Dis*. 2004;**38(5)**:632–639.

48. Usuf E, Badji H, Bojang A, et al. Pneumococcal carriage in rural Gambia prior to the introduction of pneumococcal conjugate vaccine: A population-based survey. *Trop Med Int Heal*. 2015;**20(7)**:871–879.

49. Thomas HL, Gajraj R, Slack MPE, et al. An explosive outbreak of Streptococcus pneumoniae serotype-8 infection in a highly vaccinated residential care home, England, summer 2012. *Epidemiol Infect*. 2015;**143(9)**:1957–1963.

50. Olver WJ, Cavanagh J, Quinn M, Diggle M, Edwards GFS. Investigation and control of a cluster of penicillin non-susceptible Streptococcus pneumoniae infections in a care home. *J Hosp Infect*. 2008;**70(1)**:80–83.

51. van Deursen AMM, van Houten MA, Webber C, et al. The Impact of the 13-Valent Pneumococcal Conjugate Vaccine on Pneumococcal Carriage in the Community Acquired Pneumonia Immunization Trial in Adults (CAPiTA) Study. *Clin Infect Dis*. 2018;**67(1)**:42–49.

52. Ortega O, Sakwinska O, Combremont S, et al. High prevalence of colonization of oral cavity by respiratory pathogens in frail older patients with oropharyngeal dysphagia. *Neurogastroenterol Motil*. 2015;**27(12)**:1804–1816.

53. Schaumburg F, Alabi A, Von Eiff C, et al. Streptococcus pneumoniae colonization in remote african pygmies. *Trans R Soc Trop Med Hyg*. 2013;**107(2)**:105–109.

54. Huang SS, Finkelstein JA, Rifas-Shiman SL, Kleinman K, Platt R. Community-level predictors of pneumococcal carriage and resistance in young children. *Am J Epidemiol*. 2004;**159(7)**:645–654.

55. Adegbola RA, DeAntonio R, Hill PC, et al. Carriage of Streptococcus pneumoniae and Other Respiratory Bacterial Pathogens in Low and Lower-Middle Income Countries: A Systematic Review and Meta-Analysis. *PLoS One*. 2014;**9(8)**:1–17.

56. Mackenzie G, Carapetis J, Leach AJ, Hare K, Morris P. Indirect effects of childhood pneumococcal vaccination on pneumococcal carriage among adults and older children in Australian Aboriginal communities. *Vaccine*. 2007;**25(13)**:2428–2433.

57. Hammitt LL, Akech DO, Morpeth SC, et al. Population effect of 10-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of Streptococcus pneumoniae and non-typeable Haemophilus influenzae in Kilifi, Kenya: Findings from cross-sectional carriage studies. *Lancet Glob Heal*. 2014;**2(7)**:e397–e405.

58. Nzenze SA, Shiri T, Nunes MC, et al. Temporal changes in pneumococcal colonization in a rural african community with high HIV prevalence following routine infant pneumococcal immunization. *Pediatr Infect Dis J*. 2013;**32(11)**:1270–1278.

59. 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.

60. Scott JR, Millar E V., Lipsitch M, et al. Impact of more than a decade of pneumococcal conjugate vaccine use on carriage and invasive potential in native American communities. *J Infect Dis*. 2012;**205(2)**:280–288.

61. Van Hoek AJ, 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*. 2014;**32(34)**:4349–4355.

62. Kamng’ona AW, Hinds J, Bar-Zeev N, et al. High multiple carriage and emergence of Streptococcus pneumoniae vaccine serotype variants in Malawian children. *BMC Infect Dis*. 2015;**15**:234.

63. Roca A, Hill PC, Townend J, et al. Effects of community-wide vaccination with PCV-7 on pneumococcal nasopharyngeal carriage in the Gambia: A cluster-randomized trial. *PLoS Med*. 2011;**8(10)**. doi:10.1371/journal.pmed.1001107

64. Becker-Dreps S, Kistler CE, Ward K, et al. Pneumococcal carriage and vaccine coverage in retirement community residents. *J Am Geriatr Soc*. 2015;**63(10)**:2094–2098.

65. Reisman J, Rudolph K, Bruden D, Hurlburt D, Bruce MG, Hennessy T. Risk factors for pneumococcal colonization of the nasopharynx in alaska native adults and children. *J Pediatric Infect Dis Soc*. 2014;**3(2)**:104–111.

66. Grant LR, Hammitt LL, O’Brien SE, et al. Impact of the 13-Valent Pneumococcal Conjugate Vaccine on Pneumococcal Carriage Among American Indians. *Pediatr Infect Dis J*. 2016;**35(8)**:907–914.

67. Abdullahi O, Nyiro J, Lewa P, Slack M, Scott JAG. The descriptive epidemiology of Streptococcus pneumoniae and Haemophilus influenzae nasopharyngeal carriage in children and adults in Kilifi District, Kenya. *Pediatr Infect Dis J*. 2008;**27(1)**:59–64.

68. Millar EV, Watt JP, Bronsdon MA, et al. Indirect Effect of 7‐Valent Pneumococcal Conjugate Vaccine on Pneumococcal Colonization among Unvaccinated Household Members. *Clin Infect Dis*. 2008;**47(8)**:989–996.

69. Dodi S, Harimurti K, Khoeri MM, et al. Staphylococcus Aureus and Streptococcus Pneumoniae Prevalence Am. *Southeast Asian J Trop Med Public Health*. 2015;**46(3)**:465–471.

## **Box 1**

|  |
| --- |
| Data elements to include in studies of colonisation rates for *Streptococcus pneumoniae* in older adults\* |
| **Individual*** Age
* Sex
* Smoking and passive smoking
* Accommodation type (community, institutional facility)
* Accommodation density (# per house and per bedroom)
* Contact with children (<5 years) in the home
* Contact with children (<5 years) at work
* Exposure to household air pollution (by specific domestic fuel types)
* Antibiotic use (in the preceding 1 month)
* Hospital admission (in the preceding 3m)
* Influenza vaccine in the preceding year
* Pneumococcal vaccine (type and date)
* Vaccination programme in children, time since introduction and coverage
* Chronic respiratory illness (COPD, asthma)
* Diabetes

**Study*** Sample timing (relative to climate and ‘flu season)
* Urban or rural setting
* Country pneumococcal vaccination policy
 |

\* Based on the appearance in multiple existing studies, or plausible or measurable effect on colonisation to allow future meta-analysis.

**Table 1: Multiple imputation generalised linear mixed model of risk factors for pneumococcal carriage using participant**

**level data**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Risk factor** |  | **Missing data (%)** | **Odds ratio** | **95% confidence interval**  |
| Age (years) |  | 0 | 0·99 | 0·98-1·01 |
| Sex | Female | 0·05 |  |  |
| Male |  | 0·76 | 0·59-0·98 |
| Accommodation type | Community | 0 |  |  |
| Assisted living |  | 2·30 | 1·26-4·21 |
| Nursing facility |  | 7·72 | 1·15-51·85 |
| Geographical location  | Rural | 0·07 |  |  |
| Sub-urban |  | 0·42 | 0·17-1·02 |
| Urban |  | 0·43 | 0·27-0·70 |
| Obstructive lung disease | No | 27·3 |  |  |
| Yes |  | 1·65 | 0·97-2·81 |
| Asthma | No | 32·4 |  |  |
| Yes |  | 0·71 | 0·27-1·86 |
| Pneumococcal vaccination\* | No | 0·09 |  |  |
| Yes |  | 1·25 | 0·49-3·23 |
| Smoker | No | 2·9 |  |  |
| Yes |  | 1·69 | 1·12-2·53 |
| Regular contact with children † | No  | 10·1 |  |  |
| Yes |  | 1·93 | 1·27-2·93 |
| Respiratory illness ‡ | No | 9·1 |  |  |
| Yes |  | 1·28 | 0·93-1·74 |
| Received antibiotics in the past 3 months  | No | 4·1 |  |  |
| Yes |  | 0·71 | 0·38-1·32 |
| Climate | Temperate | 0 |  |  |
| Sub-tropical |  | 1·40 | 0·39-5·07 |
| Tropical |  | 2·90 | 0·86-9·76 |

\*Participant has ever received pneumococcal vaccination (pneumococcal polysaccharide vaccine or pneumococcal conjugate vaccine)

† Regular contact was defined by at least weekly contact with a child/children under the age of 6 years

‡Any viral or bacterial respiratory illness within the past 2 weeks

**Figure 1:** PRISMA flowchart describing the identification and inclusion of data sources

****

**Figure 2:** Forest plot of pneumococcal colonisation rates by publication, grouped by country income category. ‘n’ indicates total participants contributing data. ‘data’ indicates participant level data (PLD), or agglomerated result from manuscript which includes adults of ages 60+ or 65+ (Study>60 and Study>65 respectively. ‘site’ indicates nasopharyngeal or oropharyngeal sampling (NP and OP respectively). Proportions colonised are given with exact binomial 95% confidence intervals for each study. Summary using a random effects logistic model with study as the random effect.

****

**Figure 3:** Each panel describes the colonisation rates for a given variable. Studies are indicated by dots which size describes the number of participants contributing data (see key). Lines connect data from the same study where these are available (where dots exist with no line, all participants who were colonised from that study fell into the given category). Graphs indicate studies with participant level data (grey circles) and those with only summary data (red triangles).

****