**University of Southampton**

Faculty of Medicine

Clinical and Experimental Sciences

# The Epidemiology of *Moraxella catarrhalis*

By

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Thesis for the Degree of MPhil/PhD Infection Inflammation & Immunity

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## Abstract

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*Moraxella catarrhalis* is a pathogen of increasing importance and is recognised as one of the most common causes of respiratory tract infection. In particular, it is known for its role in causing otitis media (OM) in children and exacerbation of chronic obstructive pulmonary disease (COPD) in adults. Increasing interest in the development of vaccines against *M. catarrhalis* necessitates a better understanding of carriage and disease epidemiology; to inform both vaccine development and implementation strategies. To that end a community wide carriage study (the Solent SMART Study) was designed and undertaken to investigate the epidemiology of *M. catarrhalis*, including risk factors for carriage.

As the acquisition of new strains of *M. catarrhalis* is a known risk factor for exacerbation of COPD, a better understanding of the carriage of *M. catarrhalis* in those with COPD is of benefit. Care/nursing home residents are a cohort with no prior data related to *M. catarrhalis*. As a cohort who often suffer from COPD and frequent RTI, there is certainly a need to investigate *M. catarrhalis* carriage in this vulnerable group. Carriage of *M. catarrhalis* was therefore investigated in these cohorts of interest.

In total 1701 participants were recruited, from which 228 isolates of *M. catarrhalis* were obtained. In total 8% (CI: 6.7-9.4%) of community-based participants, 19% (CI: 11.0-29.4%) of care/nursing home residents and 4.7% (CI: 1.6-10.7%) of those with COPD carried *M. catarrhalis*. Carriage site, age, microbial co-carriage, up-to-date vaccination status, recent/concurrent cold and recent use of antibiotics were all significantly associated with the carriage of *M. catarrhalis*.

Antimicrobial resistance was investigated amongst the *M. catarrhalis* isolated from this study and isolates from disease, providing data which could inform public health strategies. Both carriage and disease isolates showed low levels of resistance, with the most resistance seen for ciprofloxacin. Lastly the prevalence of carriage and AMR over time was investigated using supplementary data from another study. A significant increase in carriage was observed over recent years, however prevalence of AMR remained low with no significant change.

This study is important as it provides an insight into the epidemiology of *M. catarrhalis*, as well as factors that perhaps impact the disease potential of this common pathogen. It also addresses the paucity of data in certain ages and cohorts and helps provide clarity where existing research is inconsistent.

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# Research Thesis: Declaration of Authorship

Print name: DENISE MORRIS

Title of thesis: The Epidemiology of *Moraxella catarrhalis*.

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. None of this work has been published before submission.

Signature: ..... Date: .....

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I dedicate this thesis to those lost along the way.

# List of Abbreviations

AfeA - Chelated iron ATP-binding cassette (ABC) transporter substrate binding protein (SBP)

AI – Auto inducer

AMR - Antimicrobial resistance

AOM - Acute Otitis Media

BACH - Columbia agar with chocolated horse blood and bacitracin

CBA - Columbia blood agar with horse blood

CEACAM - carcinoembryonic antigen related cellular adhesion molecule

CHOC - Columbia blood agar with chocolated horse blood

CI - Confidence Intervals

CLSI - Clinical Laboratory Standards Institute

CNA - Columbia blood agar with colistin and nalidixic acid

CopB - Catarrhalis outer membrane protein B

COPD - Chronic Obstructive Pulmonary Disease

CRN - Clinical Research Network

CSOM - Chronic Suppurative Otitis Media

CysP - Sulphate-binding protein CysP

DHFR – Dihydrofolate reductase

ECM - Extracellular matrix

ERGO - Ethics and Research Governance Online

EUCAST - European Committee on Antimicrobial Susceptibility Testing

FET - Fischer's exact tests

GCP – Good Clinical Practice

HCP Health care professional

HiB - Haemophilus influenzae B

HRA- Health Research Authority

HumA - Hemin Utilization Protein

Ig - Immunoglobulin

IRAS - Integrated Research Application System

LbpA - Lactoferrin binding protein A

LbpB - Lactoferrin binding protein B

LOS A, B and C - Lipooligosaccharide A, B and C

LRT - Lower Respiratory Tract

LRTI - Lower Respiratory Tract Infection

M35 - Outer membrane porin M35

MASP - Mannose-binding lectin-Associated Serine Protease

MBL - mannose-binding lectin

McaP - M. catarrhalis adherence protein

MclS - M. catarrhalis a cardiolipin synthase

McmA - M. catarrhalis metallopeptidase-like Adhesin

MenB/MenC - Meningitis B/C

MhaB1 - Moraxella haemagglutinin-like protein B1

MhaB2 - Moraxella haemagglutinin-like protein B2

MhaC -Moraxella haemagglutinin-like protein C

MID/ Hag - M. catarrhalis immunoglobulin D binding outer membrane protein

ModM - *M. catarrhalis* type III restriction-modification system methyl-transferase

Msp22 - Moraxella surface protein 22

Msp75 - Moraxella surface protein 75

Msp78 - Moraxella surface protein 78

NICE - National Institute for Health and Clinical Excellence

NIHR - National Institute for Health Research

NP - Nasopharynx/Nasopharyngeal

OlpA - Opa like protein A

OM - Otitis Media

OME - Otitis Media with Effusion

OMPCD - Outer membrane protein CD

OMPE - Outer membrane protein E

OMPG1a - Outer membrane protein G1a

OMPG1b - Outer membrane proteinG1b

ONS – Office of National Statistics

OP - Oropharynx/Oropharyngeal

OppA - Oligopeptide permease protein A

OR - Odds Ratio

PAMP - Pathogen Associated Molecular Pattern

PCV - Pneumococcal conjugate vaccine

PHE - Public Health England

PilA - M. catarrhalis type IV pilin A

PilQ - M. catarrhalis type IV pilus biogenesis secretin

PilT - M. catarrhalis type IV pilus retraction NTPase

PIS - Participant information sheet

PPI - Patient and Public Involvement

PPR - Pattern Recognition Receptor

PPV - Pneumococcal polysaccharide vaccine

QS - Quorum signalling

R&D - Research and development

REC - Research Ethics Committee

RT - Respiratory Tract

RTI - Respiratory Tract Infection

Sbp2 - Substrate Binding Protein 2

SH – second hand

SHS - second hand smoke

SOP – Standard Operating Procedure

SR - Seroresistant

S-S – Self-swab/self-swabbing

SS - Serosensitive

STGG - skim milk, tryptone, glucose, and glycerin storage medium

TbpA - Transferrin Binding Protein A

TbpB - Transferrin Binding Protein B

TLR -Toll-like Receptor

UHS – University Hospital Southampton

UoS - University of Southampton

URT - Upper Respiratory Tract

URTI - Upper Respiratory Tract Infection

UspA1 - Ubiquitous surface protein A1

UspA2 - Ubiquitous surface protein A2

UspA2H - Ubiquitous surface protein A2H

VT - Vaccine type

X2 - chi squared

β-lactamase - Beta lactamase

# Chapter 1 Introduction

## 1.1 *Moraxella catarrhalis*

*Moraxella catarrhalis* is a Gram negative, piliated, auto-agglutinating, non-encapsulated pathobiont (Public Health England, 2015b). First described in 1896, it was originally named *Mikrokokkus catarrhalis* in German (Frosch, 1896) and later *Micrococcus catarrhalis* in English (Dunn and Gordon, 1905). In 1963, differences in nitrate and nitrite reduction and tributyrin hydrolysis led to *Micrococcus catarrhalis* being split into two distinct *Neisseria* species; *Neisseria catarrhalis* and *Neisseria cinerea* (Berger, 1963). In 1970, *Neisseria catarrhalis* was determined phylogenetically distinct due to its lack of chromosomal homology with *Neisseria*, thus was renamed *Branhamella* catarrhalis (Catlin, 1970). In 1984, *B. catarrhalis* was reclassified as *Moraxella catarrhalis* based on the genetic relatedness of its 16S rRNA sequence to *Moraxella* (Enright *et al.*, 1994; Pettersson *et al.*, 1998). The *Moraxella* genus belongs to the family Moraxellaceae. There are 22 *Moraxella* species including *M. catarrhalis*, all are non-motile and aerobic. *M. catarrhalis* are kidney shaped diplococci with a diameter of 0.5-1.5μm (Public Health England, 2015b).

A common commensal of the upper respiratory tract (URT) (Frosch, 1896), *M. catarrhalis* was once considered non-pathogenic. However, towards the end of the 1970's and throughout the 1980's, its pathogenic potential was demonstrated through isolation from cases of disease (McNeely, Kitchens and Kluge, 1976; Johnson, Drew and Roberts, 1981; McLeod *et al.*, 1983; Feder and Garibaldi, 1984; Hager *et al.*, 1987; Catlin, 1990). *M. catarrhalis* is now recognised as one of the most common causes of respiratory tract infection (RTI) (Murphy, Bakaletz and Smeesters, 2009; Bosch *et al.*, 2013), particularly exacerbations in chronic obstructive pulmonary disease (COPD) in adults (Wilkinson *et al.*, 2017) and otitis media (OM) in children (Sillanpää *et al.*, 2016).

*M. catarrhalis* has two distinct lineages which evolved independently, whilst divergent strains with lower homology have also been identified. One lineage expanded alongside hominid expansion ~5 million years ago (Wirth *et al.*, 2007; Earl *et al.*, 2016), is complement-resistant and adheres to epithelial cells, thus is known as the seroresistant (SR) subpopulation. It shows extensive homologous recombination and greater mutation at housekeeping genes than lineage two (Wirth *et al.*, 2007). This second lineage is less pathogenic and known as the serosensitive (SS) subpopulation. It is more clonal in structure, is up to 70 million years old, adheres less efficiently to the epithelium and is commonly complement sensitive (Bootsma *et*

*al.*, 2000a; Schaller *et al.*, 2006; Wirth *et al.*, 2007). The SR lineage comprises of 16S type 1 isolates, whilst 16S type 2 and 3 isolates fall into the SS lineage (Bootsma *et al.*, 2000a; Earl *et al.*, 2016). Whilst disease burden is greater from strains belonging to the SR lineage, all 16S types can cause disease (Verhaegh *et al.*, 2008; Earl *et al.*, 2016). Despite their separate evolution, distinct core genomes and differing genome size (~1.89Mb for the SR lineage, ~1.93Mb for the SS), both lineages show regular horizontal gene transfer and conserved genes for the majority of known virulence factors (Earl *et al.*, 2016).

## 1.2 Carriage of *M. catarrhalis*

Bacterial carriage is a dynamic balance of environmental, host and bacterial interaction, made more complex by the presence of and interaction with other microbes. Each of these factors impacts the duration of bacterial carriage as well as the development (or lack thereof) of infection and disease. Carriage is defined as the asymptomatic colonisation by bacteria and can be transient or longer term; disease occurs when cells and tissues are damaged as a result of bacterial infection causing signs or symptoms of illness (Weiss-Salz, 2010; Bosch *et al.*, 2013; de Steenhuijsen Piters, Sanders and Bogaert, 2015; Man, de Steenhuijsen Piters and Bogaert, 2017). *M. catarrhalis* is commonly carried in the human nasopharynx (NP) and oropharynx (OP) and is accepted as one of the most prevalent bacteria of the human URT (Pettigrew *et al.*, 2008; Dunne *et al.*, 2012). It has also been isolated from the conjunctiva and genital tract, although these are not common sites of carriage (Blackwell, Young and Bain, 1978; Wilhelmus, Peacock and Coster, 1980). Whilst *M. catarrhalis* can be transiently carried by animals, it is only pathogenic to humans (Murphy and Parameswaran, 2009; Abrahamian and Goldstein, 2011) and certain primates such as cynomolgus macaques (VandeWoude and Luzarraga, 1991). Long-term colonisation of *M. catarrhalis* has not been achieved in non-human primates and vertebrates (Murphy, 1996; Perez and Murphy, 2017).

### 1.2.1 Age

As is the case for many respiratory pathobionts, the carriage prevalence of *M. catarrhalis* is associated with age, with higher carriage shown in young children and the elderly. Differences in carriage prevalence in children versus adults are thought to be due to increased immunity in adults through increased levels of immunoglobulin G (IgG) (Ejlertsen *et al.*, 1994b; Verduin *et al.*, 2002; Bernhard, Spaniol and Aebi, 2012). However high levels of maternally acquired IgG, means children aged <1 month tend to exhibit low carriage of *M. catarrhalis*. Such passive immunity is limited and from 3 months of age carriage of *M. catarrhalis* increases due to a decrease in maternal IgG (Ejlertsen *et al.*, 1994b). Development of antibody responses to *M. catarrhalis*, particularly IgG3, correlate with decreased

colonisation; IgG3 appears around the age of four prompting the reduction of carriage prevalence seen from the age of five years. IgG3 promotes complement-dependent killing, and the development of a systematic IgG response specific to *M. catarrhalis*, as observed in adults and older children (Chen *et al.*, 1999; Verduin *et al.*, 2002; Bernhard, Spaniol and Aebi, 2012). Increased carriage in the elderly is associated with a reduction of immunoglobulin A (IgA) and M (IgM) (Buckley and Dorsey, 1970; Simell *et al.*, 2008). Lower Ig levels further explain why young children and the elderly are commonly colonised with *M. catarrhalis* longer than other age groups (Perez and Murphy, 2019).

A definitive prevalence of *M*. *catarrhalis* carriage is unclear, particularly in children, due to the disparity in findings between studies. Carriage prevalences of 27% (Faden, Harabuchi and Hong, 1994), 36% (Brorson and Malmvall, 1981), 42.7% (Dunne *et al.*, 2018) and 46% (Lundgren and Ingvarsson, 1986; Van Hare *et al.*, 1987) have been reported in healthy 0-2 year olds. Furthermore, data suggests 66% of children are colonised at least once in the first year of life and 78% by the second year of life. Young children frequently lose and gain new strains of *M. catarrhalis* (Faden, Harabuchi and Hong, 1994). Additional research reports carriage prevalences of 10.7% (Coughtrie *et al.*, 2014) - 54% (Vaneechoutte *et al.*, 1990) in children aged 4 and under and 86% in children aged 2-4 year (Thors *et al.*, 2016b). Carriage reduces in older children; prevalences of 17% in 6 and 7 years olds (Lundgren and Ingvarsson, 1986), 13% in 6-9 year olds (Brorson and Malmvall, 1981), 49% in 3-12 year old (Ejlertsen *et al.*, 1994a), 67% in 3-6 year old pre-schoolers (Jourdain *et al.*, 2011), 7% in 4-15 year olds (Ejlertsen *et al.*, 1994b) and 4.5% of 6-17 year olds (Coughtrie *et al.*, 2014) have previously been reported.

Such differences in carriage prevalence may be explained by differences in sampling technique, method of bacterial identification, sampling period (year or season), the age distribution of those sampled and/or geographical location. For example, in one study culture techniques showed a prevalence of 18.5%, whilst molecular techniques showed a 45% prevalence from the same samples (Coughtrie *et al.*, 2018). Additional factors may include differences in the social mixing patterns of participants (e.g., whether pre-school aged children attend nursery versus a more isolated household setting).

Adults more consistently exhibit a lower carriage prevalence (~3% on average) (Jousimies-Somer, Savolainen and Ylikoski, 1989; Ejlertsen, 1991; Verduin *et al.*, 2002; Coughtrie *et al.*, 2014) until the age of 60 when carriage increases (Vaneechoutte *et al.*, 1990); however an increase isn't seen in all studies (Coughtrie *et al.*, 2014). Adults frequently lose and acquire

different strains of *M. catarrhalis*, with strains colonising an average of 2 months (Klingman *et al.*, 1995).

### 1.2.2 Seasonal and geographic differences

As is common for most respiratory pathobionts, *M. catarrhalis* carriage is significantly higher in winter, with this seasonal trend evident in all ages (Masaki *et al.*, 2011; Coughtrie *et al.*, 2018). Carriage also varies by geographical location. In a study of healthy 0-4 year olds, carriage prevalences of 58%, 42% and 31% were observed over the same period of time in Dutch, Angolan and Brazilian children respectively (Wolf *et al.*, 2000). Prevalence can also differ between regions of a country, as seen in young children from different regions of Indonesia (Dunne *et al.*, 2018). Furthermore, whilst strains isolated globally appear phenotypically homogenous (no matter the geographic or anatomical origin) (Catlin, 1990), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) suggests there may be geographical variation (Wolf *et al.*, 2000).

### 1.2.3 Co-colonisation

Viral colonisation and infection increase the likelihood of an individual becoming colonised by *M.* catarrhalis, with bacterial colonisation aided in numerous ways (Hament *et al.*, 1999; Nguyen *et al.*, 2012; Morris, Cleary and Clarke, 2017; DeMuri *et al.*, 2018). In particular, rhinovirus and adenovirus, have been associated with *M. catarrhalis* colonisation (Jacoby *et al.*, 2007). Current data for bacterial co-colonisation is mixed (Appendix A Table 1). Positive association between *M. catarrhalis* and *Streptococcus pneumoniae*, and *M. catarrhalis* and *Haemophilus influenzae* colonisation have been observed (Jacoby *et al.*, 2007; Bae *et al.*, 2012; Dunne *et al.*, 2012; Dunne *et al.*, 2018). However, there are studies that contradict this, showing no association between *M. catarrhalis* and *S. pneumoniae* (Jourdain et al., 2011) or no/negative association between *M. catarrhalis* and *H. influenzae* (Xu *et al.*, 2012). Similarly, some studies suggest a negative association between *M. catarrhalis* and *Staphylococcus aureus* colonisation (Dunne *et al.*, 2018) some suggest no association (Xu *et al.*, 2012). Nevertheless, *S. pneumoniae, H. influenzae* and *M. catarrhalis* do commonly co-colonise; this triad of co-colonisation is seen in 11% of samples (Jourdain *et al.*, 2011). Whilst numerous studies investigate co-carriage, only a subset investigates the co-carriage of *M. catarrhalis* with other pathogens.

## 1.3 *M. catarrhalis* and disease

### 1.3.1 Otitis Media

Otitis media (OM) is a group of inflammatory diseases of the middle ear which includes acute otitis media (AOM), otitis media with effusion (OME) and chronic suppurative otitis media (CSOM). One of the most common childhood infections worldwide, OM presents a major disease burden (Cripps, Otczyk and Kyd, 2005; Qureishi *et al.*, 2014; Global Burden of Disease Study 2013 Collaborators, 2015). Although death due to OM is rare (especially in the UK), there are roughly 21,000 deaths a year from OM complications (Schilder *et al.*, 2016). Complications can be extracranial (facial paralysis, mastoiditis, subperiosteal abscess) or intracranial (cerebral abscess, meningitis, encephalitis) of which intracranial complications have a 5-26% mortality rate, most commonly in children from low-income countries (Penido *et al.*, 2016).

AOM is an infection of the middle ear, defined as the presence of middle ear effusion and inflammation, with a rapid onset of symptoms including pain in the ear (otalgia), discharge (otorrhea) and fever (Cripps, Otczyk and Kyd, 2005; Danishyar and Ashurst, 2021). There are 709 million cases of AOM globally each year; 51% of which are in those aged 4 and under (Monasta *et al.*, 2012). Although AOM can occur at any age it is most common in 0-2 year olds (Danishyar and Ashurst, 2021) and is one of the most common childhood infections (Jacobs, 2017). By the age of three, 80% of children will have experienced at least one episode of AOM and by the age of 7, 40% will have suffered 6 or more recurrent cases (American Academy of Pediatrics Subcommittee on Management of Acute Otitis, 2004; Monasta *et al.*, 2012). Otitis prone children (those who get OM more than 4 times in a year or for 8 months or more) may suffer temporary or, in rare cases, permanent hearing loss, which can lead to speech and language delays (Teele *et al.*, 1990; Lang-Roth, 2014). A serious burden on health care systems, AOM is a common cause (some reports suggest the most common cause) of antibiotic use in children (American Academy of Pediatrics Subcommittee on Management of Acute Otitis, 2004; Murphy and Parameswaran, 2009; Monasta *et al.*, 2012); accounting for 13 million antibiotic prescriptions per year in the US alone (American Academy of Pediatrics Subcommittee on Management of Acute Otitis, 2004). Infection can be bacterial, viral or co-infection (Danishyar and Ashurst, 2021), with bacteria responsible for 65% of AOM cases (Kronman, Zhou and Mangione-Smith, 2014). *M. catarrhalis* is the third most common bacterial cause, after *S. pneumoniae* and *H. influenzae* (specifically non-typeable *H. influenzae*); however, some studies suggest figures for *H. influenzae* and *M. catarrhalis* are very similar (Kilpi *et al.*, 2001; Ruohola *et al.*, 2006; Murphy and Parameswaran, 2009; Ngo *et al.*, 2016; Danishyar and Ashurst, 2021). Since the introduction of pneumococcal conjugate

vaccines (PCV7, PCV13 and Hib), there has been an overall reduction in AOM. Yet *M. catarrhalis* is still a common cause, responsible for an increasing proportion of cases due to vaccine induced shifts in bacterial colonisation and infection (Revai *et al.*, 2006; Coker *et al.*, 2010). *M. catarrhalis* is significantly more prevalent in the NP of children with AOM who have had PCV compared to children who had OM prior to PCV implementation (Revai *et al.*, 2006; Lau *et al.*, 2015).

*M. catarrhalis* OM infection is strongly associated with NP colonisation (Faden *et al.*, 1991) with the NP acting as a reservoir of OM causing bacteria (Marchisio *et al.*, 2003). *M. catarrhalis* is commonly reported to cause 15-20% of paediatric AOM (Ren and Pichichero, 2016), with reports from the past two decades ranging from 7.6-30% of US cases (Grubb and Spaugh, 2010; Holder *et al.*, 2012) and 4.2-47% of European cases (Kilpi *et al.*, 2001; Ruohola *et al.*, 2006; Broides *et al.*, 2009; Sillanpää *et al.*, 2016; Marchisio *et al.*, 2017). Of the 25 million episodes of AOM in the US each year, *M. catarrhalis* causes 4-5 million episodes (Klein, 1994; Ruckdeschel *et al.*, 2008). In those with recurrent AOM the role of bacterial biofilms has been shown, however the pathogenesis of *M. catarrhalis* biofilms isn't fully understood (Hall-Stoodley *et al.*, 2006). Typically, AOM is either left to clear or treated with broad-spectrum antibiotics without the cause of infection being identified; therefore *M. catarrhalis* as a source of OM is considered to be underestimated (Perez and Murphy, 2017; National Institute for Health and Clinical Excellence, 2018). Treatment of OM (both acute and chronic) is often ineffective or not appropriate, which is an issue for the development of AMR (Qureishi *et al.*, 2014).

Unlike AOM, OME does not present with any inflammatory symptoms and is chronic (Cripps, Otczyk and Kyd, 2005). Characterised by fluid within the middle ear and commonly referred to as glue ear, OME is a common source of hearing impairment and loss (the most common cause in children) due to the pressure of fluid affecting vibration of the tympanic membrane. Half of OME cases result as a consequence of AOM (National Institute for Health and Care Excellence, 2021; Searight, Singh and Peterson, 2021). Whilst common in children, particularly 3-7 year olds; OME is less common in adults, with adult cases often associated with other underlying conditions such as paranasal sinus disease (Qureishi *et al.*, 2014). By the age of 10 years, 80% of children have experienced OME (Schilder *et al.*, 2016). The formation of bacterial biofilms plays a role in the persistence of bacterially induced chronic OM (Hall-Stoodley *et al.*, 2006). As is the case for AOM, *M. catarrhalis* is one of the most common bacterial causes of OME (Korona-Glowniak *et al.*, 2020).

CSOM is a chronic condition characterised by the inflammation of the middle ear and mastoid cavity, with symptoms such as recurrent/persistent otorrhea due to perforation of the tympanic membrane (Schilder *et al.*, 2016). Although there are at least 31 million cases of CSOM globally, 23% of these in children ages 4 and under (Monasta *et al.*, 2012; Morris, 2012), UK prevalence is <1% (National Institute for Health and Care Excellence). Commonly a complication of AOM (usually persistent AOM), CSOM causes hearing impairment in 60% of cases (Morris, 2012). Lack of or unsuccessful treatment can result in extra and intercranial complications from the spread of infection (National Institute for Health and Care Excellence). Whilst *Pseudomonas aeruginosa* and *S. aureus* are the most prominent causes, *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae* are reported as less common causes of CSOM (Hiremath, Mudhol and Vagrali, 2019).

### 1.3.2 Chronic Obstructive Pulmonary Disease (COPD)

The World Health Organization (WHO) estimates that 65 million people suffer from COPD (World Health Organization, 2016). An increasing cause of mortality, with death rates having doubled since 1970 (Jemal *et al.*, 2005), COPD is an umbrella term describing a number of long-term lung diseases including chronic bronchitis and emphysema. COPD results in irreversible damage to the airways ultimately impeding lung function. Symptoms include chronic increased bronchial secretions which obstructs the airways and generates a productive and persistent cough, hypertrophy and hyperplasia, deterioration and reduction of small airways, destructive enlargement of alveoli, inflammation, and a loss of elastic recoil in the lungs. Those with COPD have trouble breathing that worsens during activity i.e., walking, and are prone to frequent viral and bacterial infection (HSE; Chung and Adcock, 2008; Sethi and Murphy, 2008). In 2016 COPD was the 3rd highest cause of global mortality, having caused 3.04 million deaths (5.4% of deaths); an increase from 2000 (World Health Organization, 2018b). Not only debilitating, COPD is a major financial burden directly costing the UK £1,847 million in 2014; this includes NHS costs for both primary and secondary care, and private healthcare costs (Trueman D, 2017). Direct costs are associated with General Practitioner (GP) services and prescriptions and the hospitalisation of severe or exacerbated cases (Foster *et al.*, 2006). Causes of COPD include poor air quality/ environmental pollution, exposure to biomass combustion and genetic disposition; however, smoking is the biggest risk factor (Soriano *et al.*, 2017). Global mortality rates differ by country, region and economic status; ranging from 4.4 deaths per 100,000 in Japan to 130 deaths per 100,000 in China (Chung and Adcock, 2008). In 2016, COPD was not a top ten cause of death in low-income countries but was the fourth highest cause of death in lower-middle income countries (~40 deaths per 100,000). COPD was the third highest cause of death in upper-middle

countries (~45 deaths per 100,000) and the fifth highest cause of death in higher income countries (~45 deaths per 100,000) (World Health Organization, 2018b).

COPD is characterised by episodes where symptoms worsen, known as exacerbations, which can result in hospitalisation, respiratory failure and death (Sethi and Murphy, 2008). During exacerbation sputum production is increased, sputum becomes purulent, and breathing is laboured (far more than normal). Half of all exacerbations are caused by bacteria (Sethi and Murphy, 2008), in particular the acquisition of new bacterial strains (*M. catarrhalis*, *H. influenzae* and to a lesser extent S*. pneumoniae*) (Sethi *et al.*, 2002; Wilkinson *et al.*, 2017). Incidence of exacerbation and mortality increase in the winter. The seasonality exhibited is thought to be partly due to the higher prevalence of pathogens and RTI in the winter, particularly viruses that not only can cause exacerbation but predispose the respiratory tract (RT) to bacterial infection (Donaldson and Wedzicha, 2014; Morris, Cleary and Clarke, 2017; Wilkinson *et al.*, 2017). Seasonal increases of COPD exacerbations are seen in both the northern and southern hemisphere, but not tropical climates (Donaldson and Wedzicha, 2014). Whilst antibiotics are used to treat exacerbation of COPD, steroid inhalers and bronchodilators are widely used for the day-to-day management of COPD symptoms. Steroid inhalers assist with breathing difficulties via the reduction of inflammation and immune response (corticosteroids inhibit both innate and acquired immune function); however this leaves the airway vulnerable to infection. Those who use steroid inhalers are twice as likely to develop an infection (Karbasi-Afshar, Aslani and Ghanei, 2014; Brode *et al.*, 2017).

A previously underestimated and overlooked cause, *M. catarrhalis* is now recognised as the second most common bacterial cause of exacerbations in COPD, after NTHi (Murphy and Parameswaran, 2009; Wilkinson *et al.*, 2017; Perez and Murphy, 2019). Causing roughly 10-15% of all exacerbations, it accounts for up to 4 million exacerbations per year in the US alone (Murphy *et al.*, 2005b; Sethi and Murphy, 2008; Perez A C, 2018). The acquisition of new strains of *M. catarrhalis* is a key part of COPD exacerbation (Murphy and Parameswaran, 2009; Wilkinson *et al.*, 2017). Almost half of acquisitions of new strains of *M. catarrhalis* are associated with exacerbation (Murphy *et al.*, 2005b). Contrary to the known seasonality of carriage and RTI, *M. catarrhalis* is detected and associated with exacerbations year-round whilst NTHi is associated with an increased risk of exacerbation between October and March (Wilkinson *et al.*, 2017). However, *M. catarrhalis* COPD exacerbation tends to be more acute than NTHi infection. *M. catarrhalis* infection enhances airway inflammation by stimulating several neutrophil-related components during exacerbation, further contributing to COPD progression (Parameswaran *et al.*, 2009).

### 1.3.3 Respiratory tract infection

RTIs are a leading cause of mortality worldwide and a major burden on healthcare systems. Each year RTIs cause ~3 million deaths worldwide and are the most common cause of death in children aged four years and under (Black *et al.*, 2010; Lozano *et al.*, 2012; World Health Organization, 2018a). Most individuals in the UK will suffer some form of RTI each year, with 25% of the population seeking advice from their GP (Ashworth *et al.*, 2005; National Intitute for Health and Clinical Excellence, 2008). In UK general practice, 60% of all antibiotics prescribed are for RTI (National Institute for Health and Clinical Excellence, 2008a), at a cost of over £24 million in 2008 alone (National Institute for Health and Clinical Excellence, 2008b). The elderly are most at risk of bacterial infection due to immune senescence in later age (Buckley and Dorsey, 1970), whilst young children are also higher risk due to their undeveloped immunity/immune memory (Ejlertsen *et al.*, 1994b; Verduin *et al.*, 2002).

RTI is categorised as either upper or lower respiratory tract infection (URTI/LRTI). Both infections start with the colonisation of bacteria (or viruses) in the respiratory system. Bacteria need to be able to survive host clearance systems (mucus, cilia), acquire nutrients, reproduce, attach to the respiratory epithelium, and survive host immune defences and interactions with other microbes present in the RT. Bacteria are often carried harmlessly in the RT, so there is an aspect of opportunism for infection and disease to develop (Bosch *et al.*, 2013; Siegel and Weiser, 2015). When conditions are right, for example an imbalance in the dynamics between the host and microbial ecosystem, opportunistic pathogens previously asymptomatically carried or recently acquired, can cause RTI and sequela (Weiss-Salz, 2010; Bosch *et al.*, 2013; de Steenhuijsen Piters, Sanders and Bogaert, 2015; Li *et al.*, 2016; Man, de Steenhuijsen Piters and Bogaert, 2017). Healthy individuals (particularly children) act as reservoirs of opportunistic pathogens, facilitating their transmission to susceptible hosts i.e., infants, the elderly, the immunosuppressed and the unvaccinated (Bosch *et al.*, 2013; Siegel and Weiser, 2015).

*M. catarrhalis*, *S. pneumoniae* and *H. influenzae* are the most frequent bacterial causes of RTI (Murphy, Bakaletz and Smeesters, 2009; Bosch *et al.*, 2013). Reports show *M. catarrhalis* causes 14.1% of RTI in Poland (Kaczala *et al.*, 2008), 35% in Australia (Boyle *et al.*, 1991), and 11.4% of community-acquired RTI in Greece (Maraki and Papadakis, 2014).

### 1.3.4 Upper respiratory tract infection (URTI)

URTI is the most common illness worldwide, with ~17.2 billion incidences annually (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016). These infections

include the common cold, laryngitis, pharyngitis, tonsillitis, rhinitis and rhinosinusitis (National Institute for Health and Clinical Excellence, 2008a). Although mortality is rare, URTI is estimated to have caused 6000 deaths globally in 2016, a reduction on the 9000 deaths estimated for 2000 (World Health Organization, 2018a). A substantial economic burden, URTI directly cost £37 million in the UK in 2014 alone (Trueman D, 2017).

Infants, the elderly and the immunocompromised are most severely affected by URTI and complications, however URTI are common in all ages and populations (Eccles, 2002; National Intitute for Health and Clinical Excellence, 2008; de Steenhuijsen Piters, Sanders and Bogaert, 2015). URTI exhibits increased prevalence in late autumn and winter months; several reasons are suggested for this. The most commonly accepted is the crowding theory; in winter we change our behaviours and are confined inside more, allowing for increased transmission and spread of pathogens. An alternative theory suggests that exposure to the cold during the winter reduces the temperature in our airways and therefore the epithelium of the RT. This in turn decreases local immune defences such as leukocyte phagocytosis and mucociliary clearance (Eccles, 2002; Kaczala *et al.*, 2008). The latter is supported by the fact that even tropical climates (where colder weather doesn't necessarily result in people staying inside) show seasonal increases in URTI during colder months, showing the same seasonal trends as the northern hemisphere. Nevertheless, seasonal URTI do tend to be milder in tropical climates (Milam and Smillie, 1931; Mäkinen *et al.*, 2009).

#### 1.3.4.1 Sinusitis

Sinusitis is a frequent infection seen by GPs (Sande and Gwaltney, 2004) and is responsible for up to 10% of all URTI in infants (Blumer, 1998; Verduin *et al.*, 2002). However, sinusitis in children is thought to be under diagnosed due to the unspecific nature of symptoms and medical examination being of little use in young children (Verduin *et al.*, 2002). Nevertheless, sinusitis is much more common in adults than children, owing to children's under developed sinuses (Foden *et al.*, 2013); 6-15% of adults suffer from acute sinusitis annually (Bird *et al.*, 2013). Sinusitis is most commonly caused by viral infection. Of the bacterial cases, over 50% are caused by *S. pneumoniae* or *H. influenzae* (Sande and Gwaltney, 2004) with *M. catarrhalis* being the third most common bacterial cause accounting for 20-29% of bacterial cases (Sokol, 2001; Verduin *et al.*, 2002).

#### 1.3.4.2 Laryngitis

Laryngitis is most frequently seen in adolescence and adults. Bacteria are a less common cause than viruses (Tebruegge and Curtis, 2018), however *M. catarrhalis*, *H. influenzae, S. pneumoniae* and *S. aureus* are common bacterial causes (Thomas, Jette and Clary, 2017;

Tebruegge and Curtis, 2018). It is not standard practice to determine aetiology of laryngitis (Reveiz and Cardona, 2015); however prior research suggests *M. catarrhalis* is the most common bacterial cause, causing up to 55% of cases in otherwise healthy adults (Schalen *et al.*, 1980; Schalen *et al.*, 1985; Schalen *et al.*, 1993).

### 1.3.5 Lower respiratory tract infections (LRTI)

Globally there are ~291.8 million incidences of LRTI annually (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016). LRTI includes bronchitis, bronchiolitis, pneumonia and tracheitis, with pneumonia being one of the biggest causes of death in children aged 4 years and under (the biggest cause in 2008 resulting in 18% of deaths in this age range) (Black *et al.*, 2010; World Health Organisation, 2011). A substantial economic burden, LRTI directly cost the UK £797 million in 2014 alone (Trueman D, 2017).

In 2000, LRTI caused 3.3-3.4 million deaths globally, making them the third most common cause of death (6.4% of deaths) behind ischaemic heart disease and stroke (World Health Organization, 2018b;a). Despite the success of vaccines, LRTI was the fourth highest cause of mortality in 2016 (very closely following COPD) (World Health Organization, 2018c) causing 2.96 million deaths globally (5.2% of deaths) (World Health Organization, 2018a). The majority of deaths occur in middle and low income countries, where vaccines have a lower uptake and/or availability (Unger and Bogaert, 2017). In 2016 LRTI were the highest cause of death in low-income countries, causing ~75 deaths per 100,000. In comparison LRTI were the third highest cause of death in lower-middle income countries (~50 deaths per 100,000), the sixth highest cause in upper–middle income countries (~20 deaths per 100,000) and the sixth highest cause in high income countries (almost 40 deaths per 100,000) (World Health Organization, 2018b). This reduction of LRTI in both upper-middle and higher income countries is due to multiple factors, including better health care, availability of and access to vaccines and better education and awareness of communicable diseases.

LRTI are more common in late autumn and winter (Altizer *et al.*, 2006) and affects infants, the elderly or immunocompromised more commonly and severely (UK Department of Health and Social Care, 2012). *M. catarrhalis* is the third most common bacterial cause of LRTI, after *S. pneumoniae* and *H. influenzae* (Wirth et al., 2007), responsible for 11.5% of cases (Ramadan *et al.*, 2017). LRTI due to *M. catarrhalis* are generally infrequent in otherwise healthy adults (~1-3% of cases) but common in children and the elderly (McGregor *et al.*, 1998; Verduin *et al.*, 2002; Woodhead *et al.*, 2011). The majority of adults experiencing *M. catarrhalis* LRTI have a predisposing condition such as COPD, immunodeficiency, prior viral infection or cardiopulmonary illness (Murphy, 1996; McGregor *et al.*, 1998; Ramadan *et al.*, 2017).

#### 1.3.5.1 Bronchitis

Bacteria are responsible for 10% of cases of bronchitis; an infection and inflammation of the bronchi (Albert, 2010; Tapiainen *et al.*, 2016). Acute bronchitis is common in children and adults; roughly 6% of children (Fleming and Elliot, 2007) and 5% of adults (Wenzel and Fowler 2006) experience at least one case of acute bronchitis annually. Acute bronchitis is more prevalent in areas of low socioeconomic status, high industrialisation and/or high urbanisation (Fayyaz). *H. influenzae*, *S. pneumoniae and M. catarrhalis* are the most common bacterial causes of bronchitis (Fayyaz; Macfarlane *et al.*, 2001; Cuthbertson *et al.*, 2017); *S. pneumoniae* and *H. influenzae* account for 50% and 30% of cases respectively (Chi *et al.*, 2015). *M. catarrhalis* is considered the third most common cause of bronchopulmonary infections, including bronchitis (Sirwar *et al.*, 2013).

#### 1.3.5.2 Asthma

An estimated 339 million people suffer from asthma globally. Asthma caused almost 420,000 deaths in 2016, the majority of which occurred in low and low-middle income countries. Key risk factors for asthma include genetic predisposition and environmental factors including allergens, cigarette smoke and air pollution (World Health Organisation, 2020). The detection of *M. catarrhalis* is linked with an increased risk of the development and exacerbation of asthma (Alnahas *et al.*, 2017); *M. catarrhalis* is isolated from 17.8% of those suffering bronchial asthma (Sirwar *et al.*, 2013).

#### 1.3.5.3 Pneumonia

Globally in 2015 there were 138 million cases of pneumonia in children aged 4 and under (a reduction from the 178 million cases in 2000) and an estimated 921,000 deaths (McAllister *et al.*, 2019). It is unclear whether bacterial or viral pneumonia is more common (Tajima *et al.*, 2006), however concomitant viral-bacterial infection is frequent (~30% of cases) (Juven *et al.*, 2000; Cawcutt and Kalil, 2017).

*S. pneumoniae* used to account for up to 95% of bacterial pneumonia, however vaccines have significantly reduced this to 10-15% of cases. *H. influenzae* (particularly non-typeable) *S. aureus, M. catarrhalis* and *Pseudomonas aeruginosa* are other common causes, with *H. influenzae* and *M. catarrhalis* being particular risk factors associated with pneumonia in COPD patients (Musher and Thorner, 2014). *M. catarrhalis* is now the third most common cause of childhood (Jacobs, 2017) and community acquired pneumonia (Bamba *et al.*, 2006; Shi *et al.*, 2018); furthermore, studies suggest *M. catarrhalis* is a more prominent cause than *S. pneumoniae* (Bamba *et al.*, 2006; Hirai J, 2018). *M. catarrhalis* associated pneumonia tends to

result from co-infection, commonly with *H. influenzae* and/or *S. pneumoniae* (Bamba *et al.*, 2006; Sy and Robinson, 2010).

### 1.3.6 Invasive disease and nosocomial and other infection

Invasive disease is defined as the spread of infection into a normally sterile area of the body, such as the lining of the heart (endocarditis) (NHS, 2019), bloodstream (bacteraemia and septicaemia) (O'Connell K and Cafasso J, 2018) or central nervous system and cerebral spinal fluid (meningitis) (Hoffman and Weber, 2009). Concurrent respiratory illness is associated with 48–54% cases of bacteraemia (Shahani and Tavakoli Tabasi, 2015), whilst bacteraemia tends to precede meningitis. When a bacterial infection develops to meningitis, there is a fatality rate of up to 10% in children and 25% in adults (National Institute for Health and Care Excellence, 2020; Sharew *et al.*, 2020), while half of survivors develop long-term complications (Hoffman and Weber, 2009). The prevalence of both meningitis and septicaemia is now less common in the UK due to successful vaccine schedules; however there are still 3,200 cases of bacterial meningitis a year, of which *Neisseria meningitidis* is the most prominent cause (Tidy C, 2017). Vaccines have significantly reduced the incidence of bacterial meningitis globally; in Western countries, rates have reduced to an all-time low of 0.8 cases per 100,000 annually. However in areas such as Africa where vaccination is less common incidence rates remain higher at 10–40 cases per 100,000 people annually (Brouwer and van de Beek, 2018).

*M. catarrhalis* associated endocarditis has been observed (Stefanou *et al.*, 2000; Shahani and Tavakoli Tabasi, 2015); although rare cases are severe and often fatal (Neumayer *et al.*, 1999; Stefanou *et al.*, 2000). *M. catarrhalis* associated septicaemia (Wong and Ross, 1988; Westendorp *et al.*, 2005), bacteraemia (Collazos, de Miguel and Ayarza, 1992; Shahani and Tavakoli Tabasi, 2015) and meningitis (Daoud, Abuekteish and Masaadeh, 1996; Siwakoti *et al.*, 2019; Ventura *et al.*, 2020) is also rare with cases being sporadic and linked to prior condition such as pneumonia, endocarditis or being immunocompromised (Ioannidis *et al.*, 1995; Tolentino, 2007; Goldstein, Murphy and Parameswaran, 2009; Shahani and Tavakoli Tabasi, 2015; Funaki, Inoue and Miyairi, 2016). *M. catarrhalis* is less invasive than *S. pneumoniae* and *H. influenzae* with fewer cases of infection developing to invasive disease (Funaki, Inoue and Miyairi, 2016), although it is suggested that *M. catarrhalis* is an increasing source (Thorsson, Haraldsdottir and Kristjansson, 1998).

A source of hospital outbreak (Morgan *et al.*, 1992; Levy *et al.*, 2009; Warnke *et al.*, 2019), *M. catarrhalis* nosocomial infection has ranged from acute respiratory infection (Richards *et al.*,

1993) and bronchitis (Cook, Hecht and Snydman, 1989) to pneumonia (Patterson *et al.*, 1988; Berk and Verghese, 1989).

Whilst extremely rare, *M. catarrhalis* also causes keratitis (Das *et al.*, 2006; Mian and Malta, 2011), conjunctivitis (Whitcher and Cevallos, 2006), urogenital infection (Bhattacharyya S, 2017), cellulitis (Rotta and Asmar, 1994), mastoiditis (Leskinen and Jero, 2003) and septic arthritis (Olivieri *et al.*, 2004).

### 1.3.7 Epidemiology and pathogenesis of *M. catarrhalis* infection

Various studies have shown an increase in *M. catarrhalis* infection and disease. The increased implementation of conjugate vaccines and appearance of β-lactamase producing *M. catarrhalis* have been accredited with facilitating changes in bacterial carriage and disease patterns (Verduin *et al.*, 2002; Goldstein, Murphy and Parameswaran, 2009; Ramadan *et al.*, 2017). As with carriage, *M. catarrhalis* disease exhibits seasonal variation, globally, with the highest burden in the winter (Mbaki *et al.*, 1987; Hendley, Hayden and Winther, 2005; Verhaegh *et al.*, 2015; Liu *et al.*, 2018; Raveendran *et al.*, 2020). One study showed that 65% of *M. catarrhalis* associated LRTI occurred in the winter (Ramadan *et al.*, 2017). Seasonal differences in carriage and disease may be, in part, explained by the occurrence of increased adherence of *M. catarrhalis* to epithelial cells during winter months, as observed in the OP (Mbaki *et al.*, 1987; Wood, Johnson and McCormack, 1996). This linked to changes in expression of genes associated with iron acquisition, adherence, serum resistance and immune evasion (Spaniol *et al.*, 2011).

Infection and disease is a multifactorial process, reliant on numerous virulence factors, as described in Table 1. Such factors facilitate the adhesion of *M. catarrhalis* to host cells, the acquisition and utilisation of nutrients, the formation of biofilms and resistance of host immunity to name a few key functions, all of which allows for the survival of *M. catarrhalis* and the development and persistence of infection.

| Antigen | Acronym | Function | Further information |
|---|---|---|---|
| Lipooligosaccharide A, B and C | LOS A, B and C | Adherence to epithelial cells<br>Invasion of epithelial cells<br>Complement resistance | Endotoxins |
| Ubiquitous surface protein A1 | UspA1 | Adherence to epithelial cells and extracellular matrix (ECM) proteins<br>Complement resistance<br>Biofilm formation | A non-fimbrial adhesin, which mediates adhesion to epithelial cells through binding to carcinoembryonic antigen related cellular adhesion molecule 1 (CEACAM1), and ECM fibronectin and lectin.<br>Regulated by phase variation |
| Ubiquitous surface protein A2 | UspA2 | Adherence to ECM proteins<br>Complement resistance | A non-fimbrial adhesin which mediates adhesion through binding to ECM fibronectin and lectin.<br>Regulated by phase variation |
| Ubiquitous surface protein A2H | UspA2H | Adherence to epithelial cells<br>Complement resistance<br>Biofilm formation | A non-fimbrial adhesin which mediates adhesion to epithelial cells<br>Regulated by phase variation |
| *Catarrhalis* outer membrane protein B | CopB | Complement resistance<br>Mediates the acquisition iron from transferrin and lactoferrin | |
| Outer membrane protein CD | OMPCD | Adherence to epithelial cells<br>Adherence to mucins<br>Complement resistance via membrane stability | |
| Outer membrane protein E | OMPE | Putative nutrient acquisition<br>Complement resistance | Involved in the binding and transport of fatty acid and phospholipid (Schaar *et al.*, 2011a) |
| Outer membrane protein | OMPG1a | Putative copper transport | |
| Outer membrane proteinG1b | OMPG1b | Unknown | |
| *M. catarrhalis* immunoglobulin D binding outer membrane protein | MID/ Hag | Adherence to epithelial cells<br>Haemagglutination<br>IgD binding (non-immune related) | Also known as hemagglutinin (Hag)<br>Regulated by phase variation |
| *M. catarrhalis* adherence protein | McaP | Phospholipase<br>Esterase<br>Adherence to epithelial cells<br>(Timpe *et al.*, 2003; Lipski *et al.*, 2007) | |
| Outer membrane porin M35 | M35 | Nutrition transport<br>Aminopenicillin resistance | Important factor for growth in nutrient limited environments (Easton *et al.*, 2005; Easton *et al.*, 2008; Easton *et al.*, 2011) |

| Antigen | Acronym | Function | Further information |
|---|---|---|---|
| *Moraxella* haemagglutinin-like protein B1 | MhaB1 | Adherence to epithelial cells | A two-partner secretion (TPS) system comprising of MhaC (transporter), MhaB1 (exoprotein), and MhaB2 (exoprotein) (Plamondon, Luke and Campagnari, 2007; Fan *et al.*, 2012) |
| *Moraxella* haemagglutinin-like protein B2 | MhaB2 | Adherence to epithelial cells | |
| *Moraxella* haemagglutinin-like protein C | MhaC | Facilitates adherence to epithelial cells | |
| Transferrin Binding Protein A | TbpA | Transferrin binding | Required for the acquisition of iron, particularly in iron-limiting conditions |
| Transferrin Binding Protein B | TbpB | Transferrin binding | Required for the acquisition of iron, particularly in iron-limiting conditions |
| Lactoferrin binding protein A | LbpA | Lactoferrin binding | Lactoferrin binding protein essential for iron acquisition from human lactoferrin (Bonnah *et al.*, 1999) |
| Lactoferrin binding protein B | LbpB | Lactoferrin binding | Lactoferrin binding protein which facilitates the acquisition of iron from human lactoferrin (Bonnah *et al.*, 1999) |
| *Moraxella* surface protein 22 | Msp22 | Heme binding | Heme binding which is important for the use of exogenous heme (Tong and Guo, 2009) |
| *Moraxella* surface protein 75 | Msp75 | Putative succinic dehydrogenase | An enzyme key to intermediary metabolism and aerobic energy production (contributes to the aerobic electron-transport pathway to generate energy via oxidative phosphorylation) (Oyedotun and Lemire, 2004) |
| *Moraxella* surface protein 78 | Msp78 | Putative nitrate reductase | A key enzyme for the utilization of nitrate as a source of nitrogen for the synthesis of nitrogen-containing cellular compounds (Stouthamer, 1976) |
| Chelated iron ATP-binding cassette (ABC) transporter substrate binding protein (SBP) | AfeA | Ferric, ferrous, manganese and zinc ion binding | SBP of an ABC transporter which binds ferric, ferrous, manganese and zinc ions. Also plays a role in epithelial cell invasion (Murphy *et al.*, 2016a; Murphy *et al.*, 2017) |
| *M. catarrhalis* metallopeptidase-like Adhesin | McmA | Adhesion to epithelial cells<br>Biofilm formation (Lipski, Holm and Lafontaine, 2007) | |
| *M. catarrhalis* a cardiolipin synthase | MclS | Synthesis of cardiolipin (Buskirk and Lafontaine, 2014) | Cardiolipin is an important component of the inner mitochondrial membrane (Buskirk and Lafontaine, 2014) |

| Antigen | Acronym | Function | Further information |
|---|---|---|---|
| *M. catarrhalis* type IV pilin A | PilA | Adhesion to epithelial cells<br>Biofilm formation<br>Transformation (Luke *et al.*, 2007) | |
| *M. catarrhalis* type IV pilus biogenesis secretin | PilQ | Secretion of pilus filaments (Luke *et al.*, 2004) | Outer membrane secretin that forces out pilus filament (Luke *et al.*, 2004) |
| *M. catarrhalis* type IV pilus retraction NTPase | PilT | Nucleotide binding (Luke *et al.*, 2004) | Mediates pilin disassembly and retraction (Luke *et al.*, 2004) |
| *M. catarrhalis* type III restriction-modification system methyl-transferase | ModM | DNA methylation | Regulated by phase variation, the shifting of ModM expression affects the expression of certain genes. More research is required; however, it is expected the ModM regulates expression of virulence factors that are vital for disease progression. |
| Oligopeptide permease protein A | OppA | Peptide binding | A putative SBP of an ABC transporter, which binds peptides. It is an important nutritional virulence factor important for the utilisation of peptides (Murphy *et al.*, 2016a) |
| Substrate Binding Protein 2 | Sbp2 | Arginine uptake | |
| Opa like protein A | OlpA | Adhesion<br>Complement resistance (Brooks *et al.*, 2007; Bernhard *et al.*, 2014) | OMP that binds factor H contributing to serum resistance (Brooks *et al.*, 2007; Bernhard *et al.*, 2014) |
| Sulphate-binding protein CysP | CysP | Sulphate and thiosulfate ion binding | CysP is a sulphate binding protein of an ABC transporter system which has a nutritional role (Murphy *et al.*, 2016b) |
| Beta lactamase | β-lactamase | Antibiotic resistance | Production of β-lactamase facilitates resistance to β-lactam antibiotics |
| Hemin Utilization Protein | HumA | Heme binding (Furano and Campagnari, 2004) | Required for the acquisition of iron from heme (Furano and Campagnari, 2004) |

**Table 1. *M. catarrhalis* virulence factors**.

A list of virulence factors that have been identified for *M. catarrhalis*. Unless specifically referenced, data was obtained from (Blakeway *et al.*, 2017).

#### 1.3.7.1 Adhesion

An initial and key stage of *M. catarrhalis* colonisation (and therefore any subsequent infection and disease) is adhesion to host mucosal surfaces, of which there are numerous types (nasal, nasopharyngeal, lung). Adhesion is a multifactorial process, for which over 35 genes have been shown to play a role; with expression of some genes important for adhesion to pharyngeal cells, others important for adhesion to lung epithelial cells, but the majority appear to play a role in adhesion to multiple cell types (de Vries *et al.*, 2013). *M. catarrhalis* express numerous adhesins on the cell surface, as seen in Table 1. Adhesion is mediated by the binding of such adhesins to receptors on epithelial cells (i.e. the binding of UspA1 to CEACAM1) or through binding to ECM proteins (i.e. the binding of UspA2 to fibronectin and lectin) (de Vries *et al.*, 2009).

#### 1.3.7.2 Cell invasion

Following adhesion, cell invasion allows *M. catarrhalis* to avoid host defences and the effects of antimicrobial treatment. Cell invasion is thought to occur via micropinocytosis; a form of endocytosis resulting in nonspecific uptake into the cell (although more research is required), following which *M. catarrhalis* remains in a vacuole (Slevogt *et al.*, 2007). The expression of LOS and UspA1 regulate *M. catarrhalis* invasion of human epithelial cells, although the process and full set of genes/surface proteins involved are yet to be described fully (Slevogt *et al.*, 2007; Spaniol *et al.*, 2008). Whilst cell invasion does not appear to be strain specific, it does appear to rely on Rho-type GTPases regulation, and phosphoinositide 3-kinase dependent contractile mechanisms which close phagosomes (Araki *et al.*, 2003; Slevogt *et al.*, 2007).

#### 1.3.7.3 Biofilm formation

Biofilms are microbial communities surrounded by extracellular polysaccharides, and are a well-known virulence tool used by many species of bacteria to avoid host immunity and antibiotic treatment. They allow for persistent infection, the failure of treatment strategies and can facilitate the transmission of virulence factors between individual bacterial cells or between different bacterial species when biofilms are polymicrobial. Driven by environmental triggers or cues (including signals from other bacteria), biofilm formation starts with surface attachment, followed by the formation of micro-colonies surrounded by extracellular polysaccharides and then biofilm maturation. The last step in the cycle is the dispersal of the biofilm (Rabin *et al.*, 2015).

*M. catarrhalis* biofilm formation isn't yet fully understood, however numerous proteins are involved. UspA and MID/Hag are involved with the regulation of biofilm formation, with UspA1 and UspA2H positively affecting the formation of biofilms and MID/Hag negatively affecting formation (Pearson *et al.*, 2006; Verhaegh *et al.*, 2008). The formation of *M. catarrhalis* biofilm are a major contributor to chronic and recurrent OM, furthermore there is evidence to suggest that in multispecies biofilm, *M. catarrhalis* actually promotes growth and survival of other bacteria such as NTHi (Bair and Campagnari, 2019).

#### 1.3.7.4 Host Immune Evasion

The innate immune system is the body's first defence against pathogens. Rapid and non-specific in approach, it prevents invasion by pathogens in several ways. Firstly, the presence of physical barriers which block entry such as the skin and epithelial layer, secretion of mucus and presence of mucociliary to trap and clear pathogens from the airways, secretion of lysozyme to cause bacterial degradation and lysis, and acidic PH of certain areas to make them inhospitable/unattractive sites of colonisation. If a pathogen does manage to evade these defences, the innate immune system deploys an attack to remove any foreign cells/bodies to prevent the development of infection and disease (Institute for Quality and Efficiency in Health Care, 2006). Pattern Recognition Receptors (PPRs) including Toll Like Receptors (TLRs), recognise pathogens via Pathogen-Associated Molecular Patterns (PAMPs) e.g. cell wall lipopolysaccharides; activating immune cells such as phagocytes (macrophages, neutrophils and dendritic cells) which ingest and kill foreign cells and bodies (Akira, Uematsu and Takeuchi, 2006; Marshall *et al.*, 2018) and natural killer cells (lymphocytes) which cause the death of virally infected host cells via lysis or apoptosis (Vivier *et al.*, 2011). Furthermore, the production of cytokines and chemokines conscript more immune cells to sites of infection.

After a few days of infection (or vaccination) an antigen specific adaptive immune response is produced (Institute for Quality and Efficiency in Health Care, 2006). Antigen presenting cells (such as macrophages and dendritic cells) express fragments of pathogen antigens on their surface and present them to immature T cells (lymphocytes). When one of these unique T cells is able to bind to the antigen via the T-cell receptor, T cells start to differentiate into mature cytotoxic T cells (which destroy infected cells) and mature T helper cells (which activate bactericidal activity of macrophages and activate immature B cells). When these immature B cells come across matching antigens; they ingest them and secrete cytokines causing B cells to multiply and mature. Mature B cells produce antibodies which match the antigen, these antibodies then attach to the complementary antigens on bacterial cells marking them for death by neutralisation, phagocytosis, or the complement system. Memory

B cells remain to provide prompt protection against future infection (Marshall *et al.*, 2018). The complement system is a biochemical cascade, of which there are three pathways, which complements the action of phagocytes in clearing pathogens as seen in Figure 1.

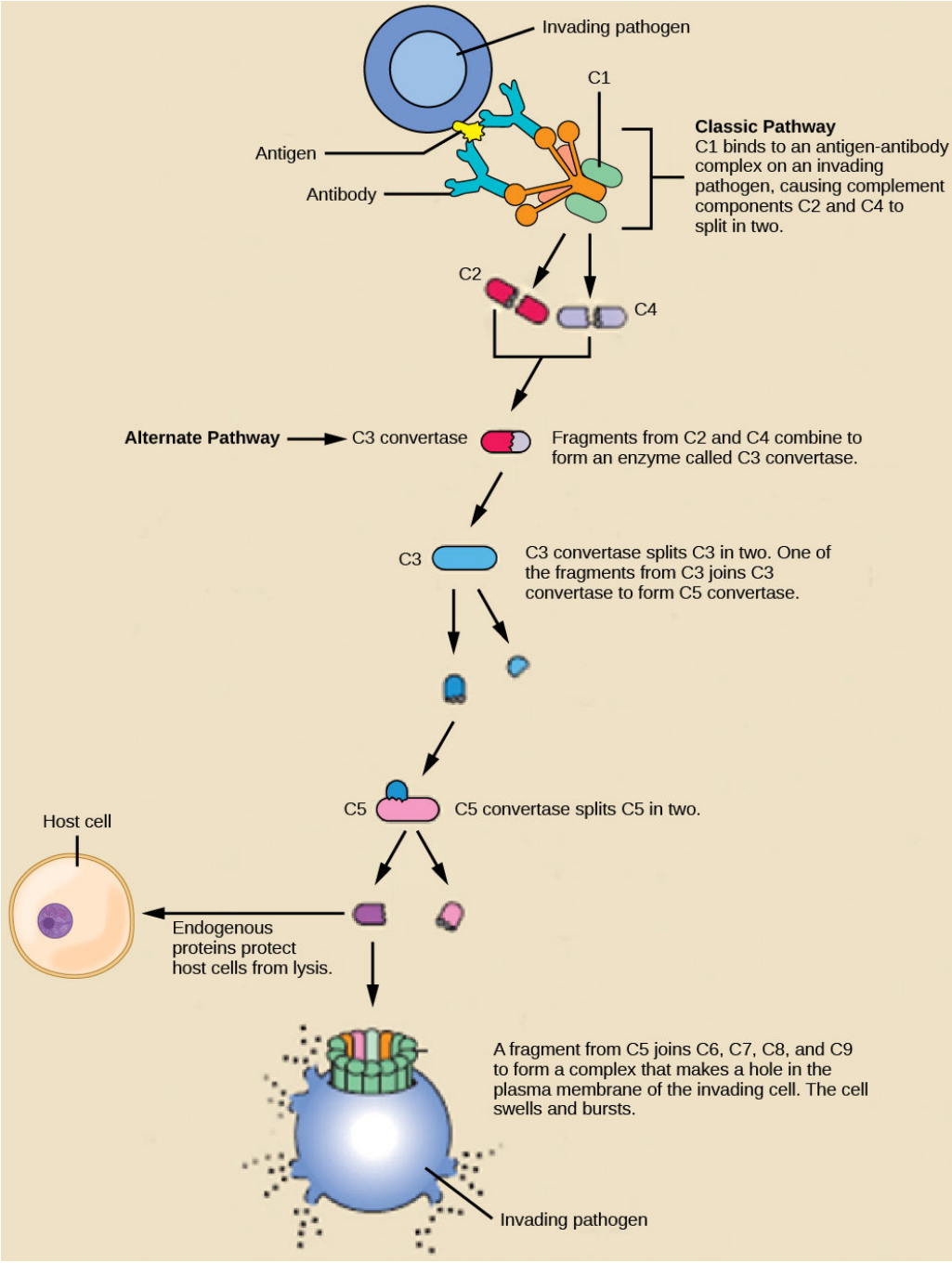



**Figure 1. Complement system**.

Describes the classic and alternate complement cascade pathways. There is of course a third pathway, in which mannose-binding lectin (MBL) binds to surface mannose, glucose, or other sugars with 3- and 4-OH groups, forming MASP (Mannose-binding lectin-Associated Serine Protease) 1 and 2 which are similar to C1 of the classical pathway. When this happens, these MASP1 and MASP-2 are activated to cleave (split) complement components C4 and C2 into two. The rest of the classical pathway is then followed (Ali *et al.*, 2012). Figure 1, has been quoted directly from https://courses.lumenlearning.com/wmbiology2/chapter/complement-system/.

*M. catarrhalis* expresses resistance to this complement system, counteracting the cascade in several ways. Firstly *M. catarrhalis* can bind to the C4b (part of the split C4) via UspA1 and UspA2, inhibiting the formation of C3 (Nordström *et al.*, 2004). Furthermore, UspA2 can bind to C3 and vitronectin which inhibits the alternative cascade pathway (Nordstrom *et al.*, 2005). UspA2 also has the capacity to attract vitronectin which binds C9 inhibiting the formation of the terminal complement complex, which is also known as the membrane attack complex (Singh *et al.*, 2010). Other antigens such as OMP CD, OMP E and CopB have been associated with complement resistance, however the mechanisms of such are yet to be described (de Vries *et al.*, 2009).

In addition to complement resistance, *M. catarrhalis* inhibits the pro-inflammatory response usually activated by TLR2 via the binding of UspA1 to the CEACAM1 receptor of host cells (de Vries *et al.*, 2009).

#### 1.3.7.5 Co-infection

*M. catarrhalis* commonly co-infects with other pathogens, particularly NTHi (Wark *et al.*, 2013; Perez and Murphy, 2019). In a 20-year study of COPD, of all the sputum samples containing *M. catarrhalis*, 26.7% also tested positive for NTHi, whilst *S. pneumoniae* and *P. aeruginosa* were present in 5.7% and 4.8% of samples respectively (Murphy *et al.*, 2005b; Perez A C, 2018). Furthermore, co-colonisation of *M. catarrhalis* with NTHi or *S. pneumoniae* increases the risk of AOM (Armbruster *et al.*, 2010; Ruohola *et al.*, 2013). This is attributed to the development of polymicrobial biofilms, which increase antimicrobial resistance and resistance to host immunity (Perez *et al.*, 2014). Reports suggest that ~40% of *M. catarrhalis* infections are associated with *S. pneumoniae* or *H. influenzae* (Raveendran *et al.*, 2020). Such co-infection is mutually beneficial. *M. catarrhalis* facilitates infection by and survival of other bacteria; firstly through the production of outer membrane vesicles, which bind to the third complement component (C3) providing protection from complement-dependent killing (Thuan Tong *et al.*, 2007). Secondly, the production of β-lactamase by *M. catarrhalis* supports the survival of bacteria normally susceptible to β-lactam antibiotics (Hol *et al.*, 1994; Schaar *et al.*, 2011b). Infection by and survival of *M. catarrhalis* is aided by the autoinducer-2 (AI-2) quorum signalling (QS) of certain co-infecting bacteria. *H. influenzae* AI-2 QS increases the survival of *M. catarrhalis* in the middle ear by promoting biofilm formation. This not only promotes bacterial persistence, but within such biofilms *M. catarrhalis* has been shown to protect otherwise susceptible *H. influenzae* from β-lactam antibiotics such as ampicillin, whilst otherwise susceptible *M. catarrhalis* were protected against antibiotics such as clarithromycin (Armbruster *et al.*, 2010). *S. pneumoniae* AI-2 QS increases *M. catarrhalis* NP colonisation and the spread of both bacteria to the middle ear (Perez *et al.*, 2014).

Furthermore, biofilms with *S. pneumoniae* can increase *M. catarrhalis*' resistance to azithromycin (Perez *et al.*, 2014). Such factors aid persistent infection and failure of medical treatment.

Viral infection aids bacterial colonisation and development of infection in numerous ways, including unveiling more adhesion sites, impairing immune responses, and causing cell/ tissue damage allowing the spread of bacteria. This enables bacterial infection to worsen clinical outcome and disease severity (Hament *et al.*, 1999; Nguyen *et al.*, 2012; Morris, Cleary and Clarke, 2017; DeMuri *et al.*, 2018). Viral and bacterial co-infection can be mutually beneficial; *M. catarrhalis* decreases antiviral innate immune responses by down-regulation of TLR3 (a viral recognition receptor) and therefore the expression of pro-inflammatory cytokines by inhibiting tumour suppressor protein P53. Furthermore, as P53 evokes apoptosis in virally infected cells it limits viral replication, such immune defence is therefore prevented during P53 inhibition (Heinrich *et al.*, 2016). *M. catarrhalis* and viral co-infection cause more severe exacerbation of COPD, with increased risk of hospitalisation of extended duration and impairment of lung function (Wark *et al.*, 2013; Perez and Murphy, 2019). Furthermore, the presence of human bocavirus, human rhinovirus, polyomaviruses, parainfluenza virus, adenovirus or RSV with *M. catarrhalis* in the NP has been associated with recurrent AOM (Wiertsema *et al.*, 2011). Chinchilla models show RSV and *M. catarrhalis* co-infection enhances migration of *M. catarrhalis* into the middle ear and development of OM (Brockson *et al.*, 2012). Whilst the presence of *M. catarrhalis* increases the risk of URTI or AOM, regardless of the presence or absence of viruses; the presence of both viruses and *M. catarrhalis* further increases the risk (Chonmaitree *et al.*, 2016).

Mechanisms of co-infection aren't yet fully understood, this is an area of continuing research, particularly for *M. catarrhalis* where research is limited.

## 1.4 Treatment of respiratory infections – Antibiotics

### 1.4.1 Antibiotic prescription

In light of increasing antibiotic resistance, there has been a concerted effort to reduce unnecessary or incorrect antibiotic prescription. When antibiotics are prescribed, broad-spectrum antibiotics are commonly given based on the type of infection, without confirmation of the cause (Verduin *et al.*, 2002). Community and National Institute for Health and Clinical Excellence (NICE) guidelines for antibiotic prescribing (summarised in Appendix A Table 2) provide an insight into the common treatment of infection, including infection caused by *M. catarrhalis*.

### 1.4.2 *M. catarrhalis* and antibiotic resistance

The production of β-lactamase is a leading source of resistance for *M. catarrhalis*. This enzyme digests β-lactam antibiotics rendering them ineffective, facilitating resistance to antibiotics such as penicillins, cephalosporins and cephamycins. The first β-lactam producing strains of *M. catarrhalis* emerged in 1976/77 (Malmvall, Brorsson and Johnsson, 1977; Percival *et al.*, 1977; Wallace *et al.*, 1989), appearing worldwide around the same time (Wolf *et al.*, 2000). It is now commonly reported that >98% of clinical isolates produce β-lactamase (Yamada, Arai and Saito, 2017; Król-Turmińska and Olender, 2018; Shi *et al.*, 2018; Raveendran *et al.*, 2020); although some studies report lower rates of 47.8% (Maraki and Papadakis, 2014) and 87.1% (Zhang *et al.*, 2016).

*M. catarrhalis* produces two distinct β-lactamases; BRO-1 and BRO-2 (McGregor *et al.*, 1998). They are chromosomally encoded, differing by 21-bp as a result of a deletion in the promoter region of BRO-2. Phenotypically the same, they have the same substrate and inhibition profile. It is surmised that BRO-1 evolved from BRO-2 (Wallace *et al.*, 1989; Bootsma *et al.*, 2000b; Schmitz *et al.*, 2002). Isolates that produce BRO-1 β-lactamase are more resistant/have a higher minimum inhibitory concentration (MIC) than those producing BRO-2 β-lactamase (Saito *et al.*, 2014; Yamada, Arai and Saito, 2017), since more β-lactamase is produced by BRO-1 than BRO-2 isolates (Wallace *et al.*, 1989). Isolates only produce one type of BRO β-lactamase (Ejlertsen and Skov, 1996); *BRO-1* is present in 90-97% of β -lactamase producing isolates, whilst *BRO-2* is present in <10% (Schmitz *et al.*, 2002; Yamada, Arai and Saito, 2017). A third BRO type has been identified, however there have been no further reports since its discovery (Christensen *et al.*, 1991). BRO β-lactamases have only been observed in *M. catarrhalis, M. lacunata* and *M. nonliquefaciens* (Wallace *et al.*, 1989) and there is no geographical difference in the prevalence of β-lactamase producing *M. catarrhalis* (Wolf *et al.*, 2000).

Hospital and community isolates show comparable levels of β-lactam resistance; *M. catarrhalis* strain and source of isolation appear to have no affect (McGregor *et al.*, 1998). High levels of resistance have been seen for penicillin (78%) and ampicillin (71%) (Gupta, Arora and Kundra, 2011); however, β-lactamase production does not render all β-lactam antibiotics ineffective. Second, third and fourth generation cephalosporins are still effective (Shi *et al.*, 2018). Susceptibility of *M. catarrhalis* to antibiotics tends to depend not just on β-lactamase production, but membrane permeability and variation of drug targets (Kyd, McGrath and Krishnamurthy, 2011). The amount of β-lactamase produced by *M. catarrhalis* is not affected by how much antibiotic is present, despite this β-lactamases can't simply be counteracted by increasing the amount of antibiotic. However, BRO β-lactamases are

inactivated by β-lactamase inhibitors, so *M. catarrhalis* infection can be effectively treated using β-lactam antibiotics in combination with a β-lactamase inhibitor (e.g. amoxicillin-clavulanic acid) (Jacobs, 2017).

Since the rapid acquisition of β-lactamase resistance (Khan *et al.*, 2010; Saito *et al.*, 2014) , global antimicrobial resistance of *M. catarrhalis* has remained relatively stable (Deshpande *et al.*, 2006; Sahm *et al.*, 2008). However, increased and high-level prevalence of macrolide-resistance has been observed in China and Japan (Murphy and Parameswaran, 2009; Wang *et al.*, 2011; Liu *et al.*, 2012; Liu *et al.*, 2015; Tang *et al.*, 2016; Kasai *et al.*, 2018). Mutations to the 23S rRNA gene and/or ribosomal L4 and L22 proteins confer such resistance (Kasai *et al.*, 2018). Low-level fluoroquinolone resistant clinical isolates have been identified in Japan and Poland, as a result of mutations to the *gyrA* and *gyrB* genes (Yamada and Saito, 2014; Król-Turmińska and Olender, 2018). Although, higher levels (25-50% depending on the fluoroquinolone antibiotic) have been seen in India (Raveendran *et al.*, 2020). Limited tetracycline resistance (<4%) has been reported in the Asia-Pacific region (Flamm *et al.*, 2012; Zhang *et al.*, 2016), the USA (Brown *et al.*, 1989) and Europe (Flamm *et al.*, 2012; Maraki and Papadakis, 2014); such resistance is encoded by *tetB* (Krol-Turminska, Olender and Bogut, 2020). Levels of sulphonamide resistance are unclear; some studies show high resistance to co-trimoxazole (83%) (Gupta, Arora and Kundra, 2011), others mid-low level resistance (32.3%) (Maraki and Papadakis, 2014), others show no resistance (Sirwar *et al.*, 2013). *M. catarrhalis* are innately resistant to trimethoprim (Ahmad *et al.*, 1984), clindamycin (Doern *et al.*, 1980) and vancomycin (Sweeney, Verghese and Needham, 1985) but generally remain mostly susceptible to macrolides, cephalosporins, rifampicin, fluoroquinolones, tetracyclines, amoxicillin-clavulanic acid, aminoglycoside and chloramphenicol (Gupta, Arora and Kundra, 2011; Sirwar *et al.*, 2013; Maraki and Papadakis, 2014; Zhang *et al.*, 2016; Król-Turmińska and Olender, 2018; Shi *et al.*, 2018). Innate resistance to vancomycin and clindamycin comes from the fact *M. catarrhalis* are Gram-negative and these antibiotics cannot effectively penetrate the outer membrane (Card *et al.*, 2015; Miller, 2016). Whilst trimethoprim resistance is a results of *M. catarrhalis*' chromosomally encoded dihydrofolate reductase (DHFR) being naturally insensitive to trimethoprim (Rudolf, 1982; Eliopoulos and Huovinen, 2001).

Interestingly AMR seems more prevalent in Asia; β-lactam MIC was higher in *M. catarrhalis* isolates from the Far East (Khan *et al.*, 2009). While macrolide and tetracycline resistance in American and European isolates was <1%, isolates from the Asian-Pacific region showed resistance rates of 7.6% and 3.2% for clarithromycin (a macrolide) and tetracycline respectively (Flamm *et al.*, 2012) and 75% for azithromycin (a macrolide) (Raveendran *et al.*,

2020). More data is need regarding the prevalence of resistance in clinical and carriage isolates from the UK.

## 1.5 Prevention of respiratory infections – Vaccines

RTIs cause millions of deaths a year; however mortality has reduced despite increases in population, particularly the at-risk population. This reduction is directly due to vaccination and indirectly to herd immunity afforded by successful vaccination schedules, particularly conjugate vaccines. Vaccines are widely regarded as the most effective intervention against infectious disease (Plotkin., 2004). Between 2005 and 2015, there was a 36.9% reduction in death from LRTI in children aged 0-4 years, whilst the overall reduction in death for all ages was 3.2% (Global Burden of Disease Study 2015 Lower Respiratory Infection Collaborators, 2017). With increasing levels of antibiotic resistance, the need for successful vaccine schedules is increasingly important in the fight against disease.

Conjugate vaccines are noteworthy as they evoke a T-cell dependent response, eliminating the carriage and disease of vaccine-type (VT) bacteria in those who have had the vaccine, even in children under 2 years of age. When vaccine coverage is high enough, the wider community is afforded protection through herd immunity. (Maiden and Spratt, 1999; Heath and McVernon, 2002; Ada and Isaacs, 2003; Kelly, Moxon and Pollard, 2004). They work by covalently attaching a bacterial capsular polysaccharide or protein to a carrier protein. On their own these capsular polysaccharides or proteins may have limited immunogenicity, only eliciting a T-cell independent immune response from B-cells. By attaching the polysaccharide to a highly immunogenic carrier protein, a T-cell dependent immune response is instigated and therefore immune memory and long term immunity (Kelly, Moxon and Pollard, 2004). Conjugate vaccines are considered the most successful vaccines against meningitis, sepsis and respiratory infection, due to the successes of the *H. influenzae* type b (Hib) conjugate vaccine, the meningococcal serogroup C (MenC) conjugate vaccine and PCV.

Hib is highly pathogenic; prior to vaccine implementation, Hib was the most common cause of childhood meningitis (Heath and McVernon, 2002; Brouwer, Tunkel and van de Beek, 2010), with global burden as high as 66 cases per 100,000 in 0-5 year olds. The Hib vaccine was added to the UK vaccination schedule in 1992 (Brouwer, Tunkel and van de Beek, 2010), prior to which incidence of Hib disease in young children was 21-44 cases per 100,000 depending on region (Heath and McVernon, 2002). Following vaccine introduction, incidence of Hib meningitis in 0-5 year olds fell to 0.6 cases per 100,000 (Brouwer, Tunkel and van de Beek, 2010), whilst England had no deaths from Hib in 2017 and 2018 (Public Health England, 2019a).

MenC disease mainly affects young children, teenagers and young adults. It has a mortality rate of 1 in 20 cases, however this figure is higher in teenagers and young adults (Oxford Vaccine Group). The MenC vaccine was introduced into the UK schedule in 1999, resulting in a 96% reduction in MenC meningococcal disease by 2020 in those immunised (Public Health England, 2021). In terms of UK mortality, there was a reduction from 78 child deaths in 1998/99 to 1 death in 2009 (Ali *et al.*, 2014).

Prior to the introduction of PCV, *S. pneumoniae* was the main cause of OM, causing 50% of cases (~7 million cases in the US annually) (Westerink, Schroeder and Nahm, 2012). In 2000 there were over 14 million cases of serious pneumococcal disease (World Health Organization). By 2002 *S. pneumoniae* was the 4$^{th}$ highest cause of mortality from infectious disease and in 2006 pneumococcal disease caused 1.5 million deaths, the majority of which were 0-4 year olds (World Health Organisation, 2007; O'Brien *et al.*, 2009). PCV7 offers protection against the 7 serotypes of *S. pneumoniae* most associated with disease or serotypes associated with antibiotic resistance (Clarke, 2006). Implementation to the vaccine schedule led to a 97% reduction of invasive pneumococcal disease (ID) in all ages by PCV7 VT *S. pneumoniae*. Following an update to PCV13, invasive pneumococcal disease caused by the additional 6 serotypes included in the vaccine reduced by 64% (Ladhani *et al.*, 2017).

## 1.6 *M. catarrhalis* vaccine development

In recent years the need for an efficacious vaccine against *M. catarrhalis* has been highlighted. High levels of disease (particularly OM) in children, risk of COPD exacerbation in adults, increasing prevalence in RTI, ineffectiveness of treatments for OM and COPD exacerbations and the threat of antibiotic resistance provide rationale for vaccine development. Vaccination could not only reduce the high levels of *M. catarrhalis* infection, but lower the financial burden of such infection and reduce levels of antibiotic prescribing. During OM, treatment with antibiotics is only slightly more effective than no treatment but can have adverse effects in up to 10% of children (Venekamp et al., 2013) showing how ineffective current treatment plans can be.

To be successful, vaccine candidates must elicit an antibody response, be expressed in sufficient levels during colonisation or infection, be expressed by a sufficient proportion of bacterial strains, be conserved amongst strains and ideally be present as a surface antigen (McMichael, 2000a; Perez A C, 2018). Researchers are gaining a better understanding of the pathogenesis of *M. catarrhalis*, however there is still much to learn. Similarly, little is known about the mechanisms of host immunity to *M. catarrhalis* (Perez and Murphy, 2017; Perez

and Murphy, 2019). From clinical vaccine trials for other pathogens and *M. catarrhalis* animal models it is surmised that antibodies, such as IgG, play an important role in host defences (Ren and Pichichero, 2016; Perez and Murphy, 2017; Perez and Murphy, 2019). *In vivo*, opsonizing antibodies engender enhanced clearance of *M. catarrhalis* in animal models, although their importance for protection in humans is yet to be confirmed (Easton *et al.*, 2011).

### 1.6.1 Progress so far

Vaccine candidates commonly undergo 1) identification of possible antigens, 2) confirmation of function, 3) raising of antibodies to the antigens to assess immune reactions, 4) assessment of the use of the antigen in animal vaccination models (McMichael and Green, 2003). However, ascertaining suitable vaccine candidates for *M. catarrhalis* is hindered by the lack of appropriate animal models. As *M. catarrhalis* is a human only pathogen, disease models in animals aren't ideal. Chinchillas are commonly used (Wang *et al.*, 2014; Perez and Murphy, 2017; Davidoss, Varsak and Santa Maria, 2018) with *M. catarrhalis* surviving between 1- 7 days (the former most common in OM models and the latter common in antigen research) (Perez and Murphy, 2017). The chinchilla otitis media model is the only model of disease that has any use for *M. catarrhalis* as the inner and middle ear of chinchillas are uniquely appropriate models for human otological disease (Apicella, 2009). Mouse pulmonary clearance models are used to test vaccine antigens, although *M. catarrhalis* is unable to survive longer than 24 hours in mouse airways (Perez and Murphy, 2017). A further limitation to mouse models is that mice do not develop disease, they simply clear the bacteria (Ruckdeschel *et al.*, 2009).

*M. catarrhalis* has no capsule and doesn't produce exotoxin, both are common vaccine targets. Therefore recent research has focussed on the identification of surface antigens; particularly outer membrane proteins (McMichael, 2000a). To date over 30 *M. catarrhalis* surface proteins have been investigated for their potential as vaccine targets with many showing promise (Table 2). The function of such candidates fit three key categories: nutrient acquisition, virulence factor and adhesion/colonisation. Whole cell vaccines have also been investigated using whole formalin killed *M. catarrhalis* (Kyd and Cripps, 1999); *in vitro* bactericidal assays showed the successful killing of >50% of bacteria, whilst immunisation of mice showed significantly increased bacterial clearance (Easton *et al.*, 2011). However, whole cell vaccines are inherently toxic so alternative vaccine candidates are preferred (McMichael and Green, 2003).

| Antigen | Potential as a vaccine target | References |
|---|---|---|
| LOS A, B and C | - high prevalence<br>- high levels of expression (if tested)<br>- highly conserved<br>- has a role in carriage and/or disease<br>- is a target of human antibodies such as IgG and IgA<br>- shows positive results in animal vaccine models with immunisation eliciting antibody responses and/or bacterial clearance | (Gu *et al.*, 1998; Yu and Gu, 2005; Yu and Gu, 2007; Ren *et al.*, 2011; Ren and Pichichero, 2016) |
| LbpA | | (Bonnah *et al.*, 1999; Yu *et al.*, 1999; McMichael, 2000a; Yassin, Amin and Attia, 2016; Blakeway *et al.*, 2017) |
| OMP CD and E | | (Murphy, Kirkham and Lesse, 1993; Hsiao, Sethi and Murphy, 1995; Bhushan *et al.*, 1997; Harabuchi *et al.*, 1998; Murphy *et al.*, 1999; McMichael, 2000b; Murphy *et al.*, 2000; Murphy *et al.*, 2003; Murphy *et al.*, 2005a; Liu, McMichael and Baker, 2007; Verhaegh *et al.*, 2008; Mitov, Gergova and Ouzounova-Raykova, 2010; Blakeway *et al.*, 2017) |
| OppA | | (Yang, Johnson and Murphy, 2011; Ren *et al.*, 2015; Murphy *et al.*, 2016a; Ren and Pichichero, 2016) |
| TbpA | | (Myers *et al.*, 1998; Yu *et al.*, 1999; Blakeway *et al.*, 2017) |
| UspA1, UspA2, UspA2H | | (Chen *et al.*, 1996; McMichael, 2000b; Meier *et al.*, 2002; Murphy *et al.*, 2005a; Mawas *et al.*, 2007; Verhaegh *et al.*, 2008; Ruckdeschel *et al.*, 2009; Su *et al.*, 2013; Ren and Pichichero, 2016; Blakeway *et al.*, 2017) |
| Msp22 | | (Ruckdeschel *et al.*, 2008; Ruckdeschel *et al.*, 2009; Ren *et al.*, 2015; Blakeway *et al.*, 2017) |
| Msp75 | | (Ruckdeschel *et al.*, 2008; Ruckdeschel *et al.*, 2009; Blakeway *et al.*, 2017) |
| CysP | | (Murphy *et al.*, 2016b; Blakeway *et al.*, 2017), |
| OMP M35 | | (Easton *et al.*, 2005; Easton *et al.*, 2008; Easton *et al.*, 2011; Blakeway *et al.*, 2017) |
| SBP2 | | (Otsuka *et al.*, 2014; Otsuka *et al.*, 2016; Blakeway *et al.*, 2017) |
| AfeA | | (Murphy *et al.*, 2016a; Blakeway *et al.*, 2017; Murphy *et al.*, 2017) |
| OMP G1a and G1b | | (Adlowitz *et al.*, 2004; Adlowitz *et al.*, 2005; Adlowitz *et al.*, 2006; Ren and Pichichero, 2016; Blakeway *et al.*, 2017) |
| OMP B1 | - high levels of expression<br>- highly conserved,<br>- highly prevalent in disease isolates and/or are a target of human antibodies<br>- not yet assessed in animal vaccination models. | (Sethi, Hill and Murphy, 1995; Mitov, Gergova and Ouzounova-Raykova, 2010), |
| Msp78 | | (Ruckdeschel *et al.*, 2008; Blakeway *et al.*, 2017) |
| McaP | | (Timpe *et al.*, 2003; Lipski *et al.*, 2007; Verhaegh *et al.*, 2008) |
| MID/Hag | - lower levels of conservation and/or expression<br>- has a role in carriage and/or disease | (Gjorloff Wingren *et al.*, 2002; Holm *et al.*, 2003; Mollenkvist *et al.*, 2003; Forsgren, Brant and Riesbeck, 2004; Bullard, Lipski and Lafontaine, 2005; Verhaegh *et al.*, 2008; LaFontaine *et al.*, 2009; Ren and Pichichero, 2016; Blakeway *et al.*, 2017) |

| Antigen | Potential as a vaccine target | References |
|---|---|---|
| OlpA | - further research is required | (Brooks *et al.*, 2007; Bernhard *et al.*, 2014; Blakeway *et al.*, 2017), |
| PilA | | (Luke *et al.*, 2007; Luke-Marshall, Sauberan and Campagnari, 2011; Blakeway *et al.*, 2017) |
| TbpB | | (Myers *et al.*, 1998; Blakeway *et al.*, 2017) |
| CopB | | (Aebi *et al.*, 1996; Aebi *et al.*, 1998; McMichael, 2000b; Murphy *et al.*, 2005a; Verhaegh *et al.*, 2008; Ren and Pichichero, 2016; Blakeway *et al.*, 2017) |
| Mha B1 and B2 | | (Balder *et al.*, 2007; Shaffer *et al.*, 2013; Blakeway *et al.*, 2017) |
| LbpB | | (Bonnah *et al.*, 1999; Yu *et al.*, 1999; McMichael, 2000b; Blakeway *et al.*, 2017) |
| McmA | Little research undertaken | (Lipski, Holm and Lafontaine, 2007) |

**Table 2. *M. catarrhalis* vaccine candidates**.

A brief summary of potential vaccine targets, as well as information as to whether they show promise as a vaccine target.

Further research is required before any antigens can progress to human trials or be ruled out. For instance, laboratory 10F3 (a CopB specific antibody) is only able to bind to 70-75% of *M. catarrhalis* isolates tested (Aebi *et al.*, 1998; McMichael, 2000b). However, CopB could still be an effective vaccine target, effective for 100% of isolates, if all serotypes of CopB are included in vaccine composition. And despite the observed low expression, research has confirmed MhaB1 and 2 are involved in attachment to host cells and are important for colonisation, so these proteins could still be valid vaccine targets. When vaccinating chinchillas with a polypeptide present in both MhaB1 and 2, the antibody response produced impeded *M. catarrhalis* colonisation (Balder *et al.*, 2007; Shaffer *et al.*, 2013). Lastly, many of the adjuvants used in animal models so far are not FDA approved (Perez A C, 2018), raising questions as to how effective the antigens will be with FDA approved adjuvants.

Whilst there is no vaccine specifically targeted to *M. catarrhalis*, a multi-component vaccine (OM-85) has been formulated to provide protection against 8 common RTI pathogens (including *H. influenzae, S. pneumoniae, Klebsiella pneumoniae, S. aureus and M. catarrhalis*) (Cantarutti *et al.*, 2021). This vaccine is a mixed bacterial lysate and has been shown to reduce the frequency and duration of RTIs, reduce risk of hospitalisation from exacerbation in COPD and reduce antibiotic prescription. Whilst registered for use in those aged one and over for the prophylaxis of recurrent RTIs, and despite its success in reducing infection, OM-85 isn't widely used and is only privately available (Arandjus *et al.*, 2006; Cantarutti *et al.*, 2021; Cao *et al.*, 2021).

### 1.6.2 Future vaccine research

Despite the identification of numerous potential vaccine targets, to date, no vaccine candidates have progressed to clinical trial for a *M. catarrhalis* specific vaccine (Perez and Murphy, 2017; Perez and Murphy, 2019). To help clarify the potential of suggested targets, a better understanding of the epidemiology of *M. catarrhalis* and the distribution and diversity of virulence factors and vaccine targets is required. Current data tends to be inconsistent and/or based on low sample numbers (Bootsma *et al.*, 2000a; Wirth *et al.*, 2007; Verhaegh *et al.*, 2008; Verhaegh *et al.*, 2011). For instance the prevalence of CopB is unclear; whilst some studies looking at disease isolates from children and adults found *CopB* to be present in 100% (n=174) of isolates (Verhaegh *et al.*, 2008), others show *CopB* to be present in only 53.8% (n=50/93) of disease isolates from patients of all ages and 0% (n=0/22) of carriage isolates (Mitov, Gergova and Ouzounova-Raykova, 2010). Furthermore, many of the studies looking at the expression and level of conservation of antigens have used small sample numbers i.e. when ascertaining level of conservation for M35 only 18 clinical isolates were used (Easton *et al.*, 2005). Similarly only 14 isolates were tested for conservation and 24 for expression of

*CopB* and only 2 isolates were tested for conservation and 8 for expression of *LpbA* (Blakeway *et al.*, 2017). Whilst such research has been vital for providing an insight into potential vaccine targets, increased sample size is required. As is the continued investigation of both carriage and disease isolates from people of all ages.

It is unknown whether pathogenesis is implicitly connected to a particular type, what the roles of additional subpopulations or strains of *M. catarrhalis* are in disease epidemiology, or indeed what can be considered as the gene repertoire for virulence (Blakeway *et al.*, 2017). For example, *uspa2* is vital for serum resistance, yet the gene is equally present in serum resistant and serum sensitive strains (Bootsma *et al.*, 2000a). Similarly, *mid* is present in at least 90% child RTI isolates, 91% adult RTI isolates and 80% of child carriage isolates, indicating no clear link between gene presence and carriage vs. disease (Mollenkvist *et al.*, 2003; Verhaegh *et al.*, 2008; Verhaegh *et al.*, 2011). This highlights the importance of looking at the genotypes and phenotypes of both disease and carriage isolates. As virulence is based on multiple factors, the balance between the harmless carriage of *M. catarrhalis* and the development of disease may be determined by the combination of the virulence genes present, differences in expression of these genes and environmental factors (Earl *et al.*, 2016). Understanding the prevalence and distribution of numerous virulence factors and their importance in carriage and disease, thus their potential use in vaccine development is vital. Furthermore, the expression of a number of antigens (Ups1A, UspA2, MID/Hag) are subject to phase variation, which means expression can be switched 'on' or 'off' as and when required (Ren and Pichichero, 2016). Ideal vaccine candidates are either, constantly and abundantly expressed, or candidates whose expression is highly associated with or essential for colonisation and/or disease. Therefore, understanding the regulation and expression of antigens subject to phase variation is favourable.

Another consideration is that the prevalence of certain antigens may vary in carriage versus disease, adults versus children, OM versus COPD exacerbation etc. Knowledge of the distribution and expression of antigens is therefore important; it would be ineffective to choose antigens expressed only by *M. catarrhalis* found in adults and not children (unless the vaccine is specifically formulated to provide protection for at risk adults), or candidates found only in isolates that cause sinusitis and no other type of infection. For example in a study of 195 isolates, UspA2 was shown to be more prevalent in isolates from children (95%, n=88/93) than adults (61%, n=53/87) (Verhaegh *et al.*, 2008) and therefore maybe an antigen more suitable for a child targeted vaccine.

The presence of various serotypes and epitopes may offer challenges for vaccine development and implementation. Firstly, any serotypes or epitopes included in the vaccine would need to have a high enough coverage of *M. catarrhalis* strains to be effective; it would be unproductive to develop a vaccine targeting just one serotype or epitope that only provides protection against 5% of isolates (unless these 5% caused the majority of disease cases). Secondly, there would be a need to consider and monitor vaccine induced mutation, which has the potential to drive the evolution of new serotypes and epitopes to evade vaccine induced immunity (as seen with capsule switching following PVC implementation) (Jefferies *et al.*, 2004). Furthermore, *M. catarrhalis* express epitopes capable of blocking complement resistant bactericidal activity (McMichael, 2000b). This could hinder the success of vaccines if they rely on the induction of bactericidal activity. Complement resistance is highly associated with disease, therefore surface proteins involved in this role would be a good target for vaccines. However, there are numerous factors involved in complement resistance making it difficult to pinpoint which would be vital for inclusion. It may be that all factors are required to truly inhibit complement resistance.

### 1.6.3 Considerations of vaccine development and implementation

The implementation of previous vaccines has highlighted important considerations when designing and implementing future vaccines against *M. catarrhalis*.

The use of conjugate vaccines alters the dynamics of bacterial colonisation, which is important as colonisation is often a prerequisite for disease. Conjugate vaccines are comprised of limited strain/serotype valency so influence changes in pathogen strain/serotype epidemiology, reducing disease burden by eradicating carriage of vaccine type (VT) strains; therefore, reducing transmission and incidence of infection in vaccinated and non-vaccinated groups. The introduction of Hib, MenC and PCV were subsequently followed by significant decreases in carriage and (Barbour *et al.*, 1995; Maiden *et al.*, 2008; Kaye, 2009; Devine *et al.*, 2017) disease caused by VT strains (Miller, Salisbury and Ramsay, 2001; Watt, Levine and Santosham, 2003; Ladhani *et al.*, 2013). However, as the circulation of VT strains decrease, the prevalence of other bacterial species and non-vaccine strain carriage and disease can increase (Ngo *et al.*, 2016; Devine *et al.*, 2017; Ladhani *et al.*, 2017). Following PCV implementation, decreases in VT carriage were paralleled by an increase in non-VT carriage or the carriage of vaccine-related serotypes (stains with a genotype of VT strains which had undergone capsule switching). This trend, but to a lesser extent, has also been seen in disease (Pai *et al.*, 2005a; Pai *et al.*, 2005b; Bechini, Boccalini and Bonanni, 2009; Weinberger, Malley and Lipsitch, 2011; Mulholland and Satzke, 2012; Devine *et al.*, 2017; Ladhani *et al.*, 2017). Invasive pneumococcal disease (IPD) by non-VTs has now doubled

(Galanis *et al.*, 2016). Similarly, as Hib and MenC carriage and disease decreased following vaccine implementation, NTHi and MenB carriage and disease increased (Cerquetti and Giufrè, 2016; Public Health England, 2017c;a;b). MenB became the most common cause of meningococcal disease in the UK and accounted for 58% of all invasive disease cases (418/724) between July 2014/and June 2015 (England, 2015). Although, since the introduction of the MenB vaccine in 2015, MenB cases have reduced certainly in children (Wise, 2016).

From birth the mucosal surface of the RT is colonised and a niche-specific microbiome is formed. Such communities are highly influenced by multiple environmental factors, and their composition and stability influence future respiratory health. From birth *Moraxella* spp increase in abundance and are one of the most prevalent species by the age of one year. From the age of 3 months *Moraxella* dominant communities are associated with ecological stabilisation and are linked to fewer RTI. Therefore, *Moraxella* spp may be important in the development of a stable respiratory microbiome in infants, playing a role in fending off the colonisation of more pathogenic bacteria and development of infection (Bosch *et al.*, 2017). Ordered stable microbial communities have been linked with a lack of RTI, whilst disordered communities with high bacterial turnover are associated with RTI (Coughtrie *et al.*, 2018). Furthermore, a nasal microbiome that is dominated by *M. catarrhalis* is associated with respiratory health in elderly populations (van den Munckhof *et al.*, 2020). Consequently, the potential impacts of a *M. catarrhalis* vaccine (particularly a conjugate vaccine that would impact colonisation) on the microbiome and respiratory health (particularly in infants and the elderly) needs investigation. Vaccines have the potential to disrupt ecological stabilisation allowing for the development of more frequent or more serious RTI in early life, a time when humans are arguably at their most vulnerable. Alternatively, *M. catarrhalis* could simply be replaced by another non-pathogenic *Moraxella* spp leaving the microbiome unaffected.

Vaccination can drive the mutation of VT bacteria as they attempt to avoid vaccine induced immunity. Examples include capsule switching by *S. pneumoniae* in response to PCV (Pai *et al.*, 2005b), and changes to *B. pertussis* as a result of the pertussis vaccine (van der Zee *et al.*, 1996; Mooi *et al.*, 1998; Mooi *et al.*, 1999; van Loo *et al.*, 1999; Mooi *et al.*, 2009). For vaccines to stay effective we must monitor the effect they are having on the epidemiology and genetic diversity of target bacteria and other pathogens.

For vaccine schedules to be truly effective, enough of the population or those at risk need to be vaccinated, both locally and globally. Despite reduction in disease following the implementation of the Hib vaccine, Hib caused 2% of child mortality in young children in

2008 (~200,000 deaths globally) due to limited vaccine use in some parts of the world. In 2007 only 41% of children worldwide had access to the Hib vaccine, with cost preventing implementation in poorer countries; fortunately, this has increased to 72% (Girard *et al.*, 2005; Brouwer, Tunkel and van de Beek, 2010; World Health Organization, 2013;2019).

It is also essential that the right groups or populations are targeted for vaccination. Despite the initial success of the MenC vaccine and overall reduction in disease, US data from 2008 showed that MenC still accounted for 31% of meningococcal disease (38/124 cases in the Active Bacterial Core Surveillance Areas alone) (Centers for Disease Control and Prevention, 2009; Brouwer, Tunkel and van de Beek, 2010). To further reduce UK MenC disease, a MenC booster vaccine was required and introduced for teenagers aged 13-14 and university students aged 19-25 who were still at risk. Furthermore, in response to an increase in meningococcal disease by non-VT (particularly MenW), the single valent MenC vaccine given to these groups was updated to a multi-valent vaccine (MenACWY) (Oxford Vaccine Group; Public Health England, 2018).

The points above illustrate the important challenges faced during future vaccine development and implementation. Evidently, as new strain-specific vaccines are introduced, strain mutation and species/type replacement will increase the circulation of non-VT bacteria which may reduce effectiveness at preventing morbidity and mortality; however, this would be dependent on the virulence of the replacement strains (Jefferies *et al.*, 2011). Active surveillance of respiratory pathogens is therefore vital to ensure effective and appropriate action against emerging/increasing non-VT bacteria is maintained.

## 1.7 Carriage studies

### 1.7.1 The need for surveillance of respiratory disease

It is important to monitor infection and disease to ensure effective treatments are successfully targeted, and to increase our knowledge to inform future disease prevention strategies. Surveillance is important as such information can be used to pre-empt potential public health threats i.e., epidemics. It can also be used to monitor the direct effect of changes in policy i.e., the introduction of a new vaccine.

Effective infectious disease control requires consistent monitoring of disease trends, this includes the prevalence of morbidity and mortality, the causative agents and those affected. Inconsistent surveillance means fluctuations and patterns of disease may be missed or causes underestimated. Not only should species of microbial agent be monitored; so should the

subtype and the emergence of new microbial agents including variants of common pathogens with increased pathogenesis e.g., increased antimicrobial resistance (AMR). As RTI is a major cause of morbidity and mortality, it is an area in particular need of surveillance (Horstmann, 1974).

### 1.7.2 The need for surveillance of microbial carriage

Carriage is often a prerequisite for disease; pathogens need to be carried and present in the community to cause infection and disease (Hackett *et al.*, 2002; Smith-Vaughan *et al.*, 2006; Vu *et al.*, 2011; Simell *et al.*, 2012). Carriage studies are the surveillance tool used to investigate microbial carriage, focussing on particular pathogens or the emergence of new threats, and to establish and monitor the epidemiology of pathogens. It is important to monitor the prevalence of microbial carriage and to see who commonly carries which microbes to ascertain who might be most at risk of disease. Additionally, knowledge of geographical and chronological distributions of microbes is important for understanding strain transmission and circulation, which can be important for understanding disease development or when considering implementation of disease control strategies such as vaccine schedules (Trotter and Gay, 2003). For example, the unexpected appearance of an unknown strain of coronavirus (severe acute respiratory syndrome coronavirus 2 also known as covid-19) left governments worldwide unprepared and therefore on the back foot in terms of limiting transmission and developing successful prevention strategies. Studies of influenza carriage and disease provide an example of successful monitoring programmes which highlighted the need for yearly influenza vaccination at the start of winter and annual updates to the vaccine due to viral antigenic shift (Cox and Subbarao, 2000; Houser and Subbarao, 2015). Longitudinal or annual studies are best for the collection of this kind of information as they allow for the identification of temporal and seasonal fluctuations and trends. The benefit of looking at carriage and disease is that, with disease surveillance alone you only get half the picture e.g., you can miss the emergence of strains with high pathogenic potential.

Studies have shown that carriage is a predictor of disease; therefore, carriage is a useful measure of potential disease burden (Simell *et al.*, 2012). Monitoring trends of carriage e.g., changes in the prevalence and distribution of bacteria can be used to pre-empt future disease patterns. For instance, if there is an increase in the carriage of certain species/type of bacteria, a subsequent increase in disease caused by that species/type could be expected. During the Paediatric post pneumococcal conjugate vaccine study (R&D No. RHM CHI0678 and REC No. 14/NS/1064), serotypes 3 and 15a were seen to significantly increase in carriage in 0-4year olds, following this there was increased prevalence of IPD in the elderly

caused by these serotypes (data unpublished). Monitoring carriage allows prophylactic strategies to be implemented to reduce the impact before increases in disease are experienced e.g., the promotion of vaccines to increase uptake in at risk populations.

Combined with disease incidence data, carriage studies can show relative risk of carriage of different bacteria (i.e., when and how often bacterial carriage leads to disease) and how successful vaccine implementation is (i.e., how have vaccines affected the carriage and disease caused by VT and non-VT bacteria). For instance, conjugate vaccines should reduce carriage of and disease from VT bacteria. If conjugate VT bacteria are still being seen in the community this highlights a failing. Either the vaccine isn't totally effective or maybe the vaccine coverage isn't high enough to afford herd immunity and reduce bacterial carriage for certain VT bacteria. In order to establish the full effect of vaccines, the carriage of both VT and non-VT bacteria should be observed, providing useful information for vaccine updates and maintaining public confidence in the vaccine. Bacteria can evolve in response to the evolutionary pressures of vaccines and antibiotics. Carriage studies facilitate surveillance, so we know what's occurring on a population level (changes in the prevalence of different bacteria and changes to the microbial ecosystem/ecological niche as a whole), but also at a bacterial or genetic level (Weiss-Salz, 2010; Croucher *et al.*, 2011).

Carriage isolates can also be compared with disease isolates to help identify mechanisms of pathogenicity that allow certain strains to be so adept at causing disease (Yazdankhah *et al.*, 2004). Whilst the comparison of healthy and disease states helps determine factors that allow asymptomatic carriage to develop into disease. For instance, studies have shown that diversity of RT microbiome is associated with ecosystem stability, which in turn allows for stability between the host immune system and the microbial ecosystem (Fernandez *et al.*, 2000; Erb-Downward *et al.*, 2011). Dysbiosis and/or a takeover by one bacterial species, is associated with disease. COPD and respiratory disease states are both associated with low biodiversity (Erb-Downward *et al.*, 2011). Furthermore, investigating bacterial carriage and disease can also help provide information on the invasiveness of certain bacteria; if certain bacteria are highly prevalent in carriage but rarely seen in disease, they have low pathogenic potential. Alternatively, if bacteria are rarely seen in carriage but cause high disease rates, they must be highly pathogenic. Carriage studies are also used to monitor and detect potential associations that lead to bacterial carriage i.e., does viral colonisation lead to higher carriage of bacteria.

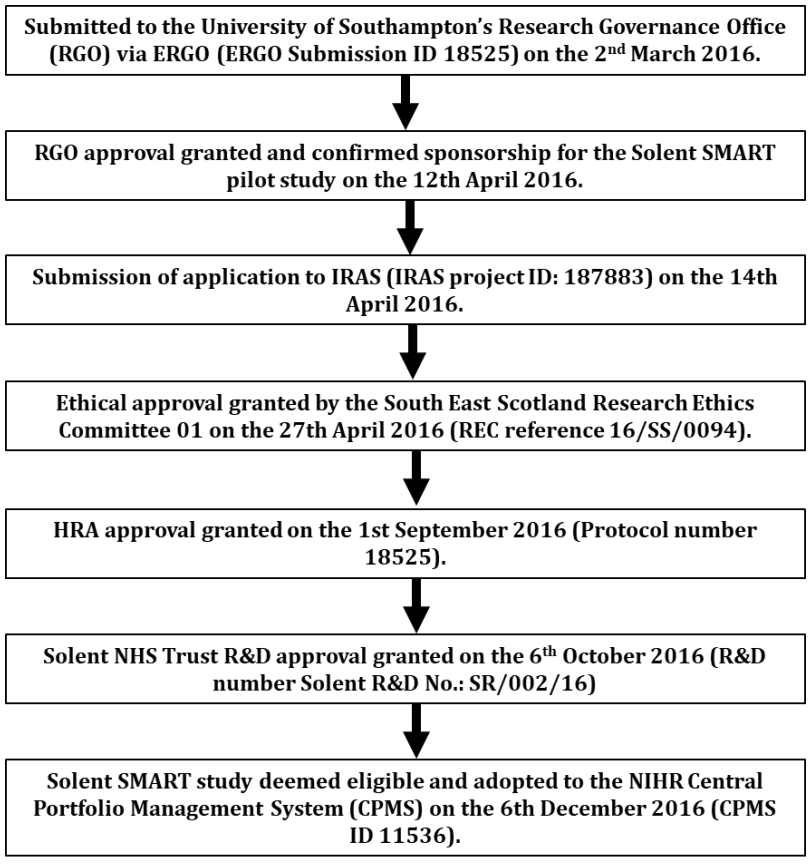
## 1.8 Study rationale

As described, *M. catarrhalis* is a common cause of RTI, being particularly associated with OM in children and COPD exacerbations in adults (Murphy *et al.*, 2005b; Ngo *et al.*, 2016). In an ageing population with distinct health challenges e.g., those living in care/nursing home residents and/or sufferers of COPD, this is particularly pertinent and therefore there is a pressing need to investigate *M. catarrhalis* carriage in these vulnerable cohorts. Moreover, there is data to suggest increasing carriage and burden in disease. With no current vaccine and rising concerns of antibiotic resistance a better understanding of the epidemiology of *M. catarrhalis* in addition to its genetic diversity, distribution of virulence factors and potential vaccine candidates, and risk factors for carriage and disease is required. Thus, the aim of this thesis is to help understand the epidemiology of *M. catarrhalis*. In this respect, a study (Solent SMART Study) was designed and implemented to facilitate the isolation of *M. catarrhalis* from members of the public and the investigation of the epidemiology of this organism. The materials and methods in Chapter 2 give an overview of the study and subsequent analysis, as well as a discussion of chosen methodology and the alternative methods considered. Chapter 3 investigates recruitment of participants and isolation of *M. catarrhalis*. The epidemiology of *M. catarrhalis* is then investigated throughout the rest of the thesis. Chapter 4 looks at the carriage of *M. catarrhalis*, including sites of and risk factors for colonisation including co-colonisation with other bacterial pathogens, and chapter 5 investigates prevalence of AMR. Chapter 6 utilises *M. catarrhalis* isolates from the Solent SMART Study and an additional study to investigate changes in the carriage and AMR of *M. catarrhalis* over a 12-year period (October 2008 –March 2020).

# Chapter 2 Materials and methods

## 2.1 Study approvals

Prior to undertaking clinical research in the UK, approval must be obtained. The approval required is based on the type of research, the involvement of human participants and the requirements of participation. The Solent SMART Study was undertaken by researchers at the University of Southampton (UoS) and sponsored by the UoS, therefore prior approval and agreement of sponsorship was required from the UoS Research Governance Office. Such approvals were obtained via the University's Ethics and Research Governance Online (ERGO) system. To ensure ethical standards of research and to safeguard the rights and well-being of research participants, Research Ethics Committee (REC) approval was required. As this study involved NHS/HSC sites, Human Research Authority (HRA) approval was also required. REC and HRA approvals were obtained by the submission of research applications via the integrated research application system (IRAS). The IRAS submission was further used to request and assess eligibility for National Institute for Health Research (NIHR) Clinical Research Network (CRN) Portfolio adoption. Studies deemed eligible and entered to the NIHR portfolio management system gain access to additional study support. As the study was undertaken within the Solent NHS Trust, R&D approval was also sought to confirm Trust capacity and capability for such research.



All study documentation (i.e., participant information sheets, consent forms and questionnaires) can be found in Appendix B.

## 2.2 Study outline

A large community based URT microbiology carriage study, the Solent SMART Study, was undertaken over a period of 18 months (excluding follow on laboratory and data analysis). Recruitment occurred across two winter time-points: October 2016 to March 2017 and October 2017 to March 2018. The purpose of the study was to undertake a bacteriological examination of the URT, with a focus on *M. catarrhalis*, to determine community carriage levels of common respiratory pathogens. In addition, the prevalence of antibiotic resistance in recovered isolates was determined.

### 2.2.1 Solent SMART Study aims

The primary aims of the study were:

1. To recruit 200 children aged 0-4 years, 340 children and adolescents aged 5-16 years, 540 adults aged 17-49 years, 540 adults aged 50+ years and 140 care/nursing home residents.
2. To sample the URT, using a cross-sectional community sampling approach, in order to characterise the epidemiology of common respiratory pathogens, with particular focus on *M. catarrhalis*.
3. To generate novel epidemiology data for these same pathogens in an understudied community cohort; those residing in care/nursing homes.

Secondary aims were to demonstrate the feasibility of recruiting in community and care/nursing homes settings when obtaining healthcare professional-administered swabs, and to generate data to inform sample size calculations for future studies.

The specific objectives of this thesis were to a) determine the carriage prevalence of *M. catarrhalis*, in people of all ages, b) determine risk factors associated with carriage of *M. catarrhalis*, c) as a sub-study determine the carriage prevalence of *M. catarrhalis* in care/nursing home residents and participants with COPD, d) determine the prevalence of AMR in the community, care/nursing homes and participants with COPD.

## 2.3 Recruitment and informed consent

This study recruited members of the public across all age groups whilst they attended participating Solent NHS Trust site or sites working in partnership with Solent NHS Trust. Solent NHS Trust site included GP's, health centres and hospital campuses, which participants could be attending for any number of reasons either for themselves or to accompany a friend or family member. To gain samples representative of carriage throughout the entire community, anyone of any age who was deemed capable of providing informed consent was given the opportunity to participate. Members of the research team approached individuals as they attended such sites. Those willing to participate, or considering participating, were provided with a participant information sheet (PIS). Various versions of the PIS were written in the English language, each tailored to different ages/levels of literacy, to support the procurement of informed consent by facilitating participant's understanding of the study and requirements of participation. Following an opportunity to read the PIS and ask any questions; those willing to take part were asked to provide informed consent, via the written completion of a consent form, in accordance with Good Clinical Practice (GCP). Participants were also asked to complete a short questionnaire requesting basic demographic data and information regarding recent RTI status, recent use of antibiotics, long term illness status, vaccination status, smoking status (adults only) and day care attendance (young children only).

Consent for participants aged 0-16 was tailored to age. Parents/guardians were completely responsible for providing informed consent and completing the questionnaire for participants aged 0-10 years. However, at the age of 11 participants were deemed cognitively capable of being part of the decision-making process. Therefore, 11-16 year olds were asked to provide assent alongside parent consent. Participants between the ages of 11-16 were given their own PIS to read, allowing for their informed inclusion in the decision-making process and involvement in determining whether to participate. Parents/guardians were also provided with a copy of the PIS and asked to assist in the accurate completion of the questionnaire.

Some children were recruited from schools in the Solent Trust region. A cover letter and PIS (and where relevant questionnaire and consent form) were either given to pupils to give to their parents or sent directly to parents. This was done approximately a week before the research team visited the school to undertake recruitment and sampling. Dependent on the age of the participants and agreement with the school, either pupils returned to school with a signed consent form and were swabbed during a school day or consent was provided and the

swabs taken during a parents evening. The contact details of the research team were listed on the cover letter and PIS to ensure any questions could be asked prior to consent being given.

A cohort of participants from care/nursing homes were also recruited. A cover letter and PIS were sent to the care/nursing home manager. Following approval from the manager to visit the home, the research team attended to undertake recruitment and sampling. Only those capable of providing informed consent were approached. Where possible, information was sought from home staff as to individual's level of mental capacity in relation to providing informed consent. Those thought to have capacity were approached and provided with study information. A trained mental health and older person's research nurse confirmed capacity, based on the Mental Capacity Act 2005, before informed consent was taken.

Informed consent was provided for every participant. Entry to this study was voluntary and anyone who wished to withdraw could do so at any point. All participants took part on one occasion with no follow-up.

### 2.3.1 Inclusion criteria

Any individual of any age willing to participate and able to provide informed consent, or whose parent/guardian were willing to provide informed consent, could take part. The research team were responsible for assessing whether individuals, or their parents/guardians, were capable of providing informed consent. If it was believed informed consent was not possible (i.e., due to a lack of understanding of the study and the requirements of participation), the decision was made to exclude the individual from the study. All members of the research team were fully trained in recruitment and informed consent and had undertaken NIHR GCP training prior to the start of the study.

### 2.3.2 Exclusion criteria

Exclusion criteria are characteristics/aspects which make an individual unsuitable to participate in the study. For the Solent SMART Study this was limited to:

- Individuals unable to provide informed consent; in the interest of maintaining ethical standards only adults with the capacity to provide informed consent at the time of participation could do so.

- Individuals with obstructed nasal passages; all participants were required to have unobstructed nasal passages to allow NP and nose swabs to be taken.

- Recruitment was restricted to one person per family to avoid bias from family members that may have similar bacterial carriage patterns.

## 2.4 Swabbing

To fully survey bacterial carriage in the URT, each participant was asked to provide a nasopharyngeal (NP), oropharyngeal (OP) and lower nose/nasal swab sample. All swabs were taken by trained members of the research team; SOPs for swabbing are included in Appendix B.

Paediatric NP samples were taken using Transwab® Pernasal Amies with Charcoal (Medical wire ID MW173C). Paediatric nasal and OP samples were taken using Deltalab Sterile Amies Charcoal Transport Swabs (Medline scientific code 300285 or 300281/1). Adult samples were all taken using Deltalab Sterile Amies Charcoal Transport Swabs (NP samples using Medline scientific code 300281/1, nasal and OP samples using Medline scientific code 300285).

## 2.5 Anonymisation and recruitment logs

To keep data anonymous, via link anonymisation, swabs and questionnaires were only labelled with the study number, swab type and the time the swab was taken. Only the consent forms (which were also labelled with the study number) had any participant identifiable information, and this was for the purposes of consent only. As various HCP/researchers could be recruiting at the same time at different sites, a single study numbering system was avoided to prevent duplication. The study number was therefore based on the swabber; starting with their initials followed by the number of participants that researcher had recruited to the study i.e., DM001, DM002, DM003, etc.

When swabs and questionnaires arrived at the laboratory, they were assigned a laboratory number to help track the number of samples received and to counteract any issues from the erroneous duplication of study numbers. The laboratory number started at SS0001 and increased sequentially for each participant recruited (SS for Solent SMART).

To help keep track of study numbers, a recruitment log was designed to be completed during recruitment. The log acted as a record of who recruited where and when, when participants were recruited (useful for NIHR portfolio uploads) and detailed the study number and laboratory number for each participant.

## 2.6 Transportation of samples

Swabs were transported to the laboratory research team (along with consent forms, questionnaires, and recruitment logs), in a Versapak Insulated Pathology Medical Carrier in accordance with UN3373 biological safety regulations. The carrier was zip-tied for security, due to the presence of participant identifiable information on the consent forms and biological matter on the swabs. Specific transport swabs, containing Amies with charcoal, were used to ensure viability of bacteria. Taxis were used for transportation to ensure laboratory receipt and processing of swabs within 48 hours.

## 2.7 Microbiological processing

All swabs were processed within 24-48 hours of being taken. For culture-dependent microbiology, swabs were streaked onto a selection of multi-purpose media (designed for the cultivation of a wide variety of bacteria) and selective media (designed for the culture of specific bacteria) to allow for the detection of *M. catarrhalis, S. pneumoniae, H. influenzae, N. meningitidis* and *S. aureus*. Columbia blood agar with horse blood (CBA, Oxoid PB0122), Columbia blood agar with chocolated horse blood (CHOC, Oxoid PB0124), Columbia blood agar with colistin and nalidixic acid (CNA, Oxoid PB0308), Columbia agar with chocolated horse blood and bacitracin (BACH, Oxoid PB0220) and lysed GC selective agar (GC, Oxoid, PB0962) were used. Discs containing 5µg optochin laboratory grade antibiotic (Oxoid, DD0001), were placed on the CNA plates to determine sensitivity to optochin; a known method for the confirmation of *S. pneumoniae*. All plates were incubated for 24-48 hours at 37°C in 5% $CO_2$ and examined for the presence of *M. catarrhalis, S. pneumoniae, H. influenzae, N. meningitidis* and *S. aureus* using standard colony morphology.

*M. catarrhalis* were initially identified by standard colony morphology; non-haemolytic colonies on multi-purpose CBA and CHOC plates, which stay as complete colonies when pushed across agar and are opaque, flat, smooth, dry, 1-3mm in diameter after 24 hours of incubation and appear grey/white on blood agar or pinkish-brown on chocolated agar (Public Health England, 2015b). Suspected *M. catarrhalis* isolates were confirmed as Gram-negative and oxidase, tributyrin and DNAse positive (Public Health England, 2015b). Tributyrin hydrolysis was used to differentiate *Moraxella spp.* from *Neisseria* spp. Moraxella *spp.* test positive for tributyrin hydrolysis whereas *Neisseria spp* are negative. DNAse production was used to differentiate *M. catarrhalis* from other *Moraxella* spp; *M. catarrhalis* tests positive (Catlin and Cunningham, 1964; Christensen, Gadeberg and Bruun, 1986; Mannion, 1989; Singh *et al.*, 1997; Public Health England, 2015b). Oxidase testing was undertaken by transferring a small amount of pure bacterial growth onto an oxidase strip

(Oxoid, 10331743). Oxidase-positive isolates turned the oxidase strip a dark blue instantly. Tributyrin testing was undertaken by placing a tributyrin strip (Sigma-Aldrich, 75744-300) into a 1 ml turbid suspension of pure bacterial growth (made by adding suspected *M. catarrhalis* which has been streaked and grown on CBA to1 ml of saline). Tributyrin–positive isolates turned yellow after incubation at 37°C for 24 hours. DNase testing was undertaken by transferring pure bacterial growth onto DNAse/methyl green plates (VWR, EOLAPP0560). Plates were incubated at 37°C for 24 hours. DNase-positive isolates were identified by the presence of clear zones immediately around the bacterial growth.

*S. pneumoniae* were identified as α-haemolytic colonies of 1-2mm with a characteristic draughtsman/red blood cell shape (although some serotypes are mucoid), that are sensitive to optochin and can grow on selective CNA plates. Suspected *S. pneumoniae* isolates were confirmed as Gram-positive diplococci (Public Health England, 2014b).

*H. influenzae* were identified as colonies which appear small, grey, round, convex and possibly iridescent which can grow on selective BACH plates. Suspected *H. influenzae* were confirmed as Gram-negative and oxidase positive (methodology as described for *M. catarrhalis*). Final confirmation was undertaken by X and V testing. The surface of a Blood Agar Base (BAB, Oxoid PO1046) plate was inoculated with a suspension of suspected *H. influenzae* culture in sterile distilled water. X (Oxoid, DD0003), V (Oxoid, DD0004) and XV (Oxoid, DD0005) factor discs were placed on the plate at equal distances from each other. Plates were incubated at 37°C with 5% $CO_2$ for 24 hours. Isolates which grew around the X+V disc, but not the X or V discs were verified as *H. influenzae* (Public Health England, 2015a).

*N. meningitidis* were identified as colonies which appear smooth, round, moist, uniform, large and grey/brown in colour and can grow on the GC agar. Suspected *N. meningitidis* were confirmed as Gram-negative cocci and oxidase positive (methodology as described for *M. catarrhalis*) (Public Health England, 2015c). Final confirmation of suspected N. meningitidis isolates was achieved by performing API NH testing. API NH strips (Biomerieux, 10400) were inoculated with a 5ml turbid suspension of each suspect isolate in saline, in accordance with manufacturer's instructions.

*S. aureus* were identified as colonies which appear opaque and golden in colour which can grow on CNA plates. Suspected *S. aureus* were confirmed as Gram-positive cocci and coagulase positive (Public Health England, 2014a). Coagulase activity was assessed for each isolate using a Pastorex Staph Plus Kit (Bio-Rad, 56353). Small amounts of pure bacterial growth were mixed with the negative control and test reagents (containing monoclonal

antibodies). The presence of an agglutination reaction was indicative of a coagulase-positive colony, and therefore isolates were confirmed as *S. aureus*.

Confirmed bacterial isolates were sub-cultured from primary plates and the subsequent pure growth was frozen at -80°C in skim milk, tryptone, glucose, and glycerin storage medium (STGG) for future analysis.

## 2.8 Antibiotic resistance testing

All *M. catarrhalis* isolates were phenotypically tested for antibiotic resistance using antibiotic discs. Firstly, 10µL (a suspension of cells in liquid STGG) of each isolate was plated onto CBA (Oxoid, PB0122). Plates were incubated for 24 hours at 37°C in 5% $CO_2$. Pure colonies were added to 1ml of saline to get an inoculum of 0.5 McFarland. A sterile swab was used to spread this inoculum over Mueller Hinton F plates (Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD, Oxoid PB1229A). Antibiotic discs, four per plate, were added and plates were incubated at 37°C in 5% $CO_2$ for 18 hours (±2 hours). Each *M. catarrhalis* isolate was tested with amoxicillin-clavulanic acid 2-1ug (CT0538B), cefotaxime 5ug (CT0407B) and ceftriaxone 30ug (CT0417B) (cephalosporins), erythromycin 15ug (CT0020B) (macrolide), tetracycline 30ug (CT0054B) (tetracycline), ciprofloxacin 5ug (CT0425B) (fluoroquinolone) and meropenem 10ug (CT0774B) (carbapenem) (all from Oxoid). European committee on antimicrobial susceptibility testing (EUCAST) breakpoints were used to assess susceptibility and resistance. *H. influenzae* ATCC49766 was used as a control, in accordance with EUCAST guidelines.

**Figure 2. *M. catarrhalis* AMR plate**.

Figure 2 shows two isolates of M. catarrhalis, one of which shows susceptibility to antibiotics (left isolate) and the other shows antibiotic resistance (right isolate) as determined by the size of the halo of no growth around the antibiotic disc.

## 2.9 Sampling and statistical power

The study initially aimed to recruit 200 children aged 0-4 years, 340 children and adolescents aged 5-16 years, 340 adults aged 17-49 years, 340 adults aged 50+ years and 140 care/nursing home residents. Adult recruitment was later increased to 540 adults aged 17-49 years, 540 adults aged 50+ years (as discussed in section 2.12.1).

Target recruitment figures were based on statistical sample size calculations and expected carriage prevalence based on findings from previous studies, primarily:

- Analysis of the microbial community of the upper respiratory tract to support the development of effective vaccine policy (Bupa SMART Study); REC number 11/SC/0518.
- Paediatric post pneumococcal conjugate vaccine study (Southampton Pneumococcal Carriage Study); REC numbers 06/Q1704/105 and 14/NS/1064.

Worldwide carriage prevalence of *S. pneumoniae* in healthy young children (those aged 0-4 years) can be as high as 90% in healthy children (Adegbola *et al.*, 2014), 86% for *M. catarrhalis*, 85% for *H. influenzae* (Thors *et al.*, 2016b) and 23% for *S. aureus* (Adegbola *et al.*, 2014; Coughtrie *et al.*, 2018). However carriage in the UK and Western countries is more commonly reported at ~32% for *S. pneumoniae* (Coughtrie *et al.*, 2014; Devine *et al.*, 2017), ~30% for *M. catarrhalis*, ~19% for *H. influenzae* and ~10% for *S. aureus* (Regev-Yochay *et al.*, 2009; Gamblin *et al.*, 2013; Coughtrie *et al.*, 2014; Giufrè *et al.*, 2015; Slack, 2015). Carriage in older children and adults is lower (as low as 1-3%) (Verduin *et al.*, 2002; Coughtrie *et al.*, 2014; Coughtrie *et al.*, 2018). Based on sample size/power calculations (Appendix A Tables 3-7) performed by UoS statistician Ho Ming Yuen, the recruitment of 340 participants allowed for the detection of a carriage prevalence as high as 16% (with 95% CI width of 4% allowing an error rate of 5% to occur during culturing) or as high as 30% (with 95% CI width of 5% allowing an error rate of 5% to occur during culturing), both sufficient for the expected range of bacterial carriage in participants aged five years and over. Lower recruitment was required for those aged 0-4 years due to access to additional data drawn from a similar population of young children in the south of England available from the Southampton Pneumococcal Carriage Study. Such additional data from the Southampton Pneumococcal Carriage Study was described and used in chapter 6.

In addition, the study aimed to recruit 140 care/nursing home residents. There is limited data available on the recruitment of participants from this setting or the bacterial URT carriage in this cohort (particularly for *M. catarrhalis*). However, recruiting 140 participants allowed for the detection of a carriage prevalence as high as 10% (with 95% CI width of 5% allowing an error rate of 5% to occur during culturing). More importantly it allowed for the collection of initial carriage and antibiotic resistance data, informed sample size requirements for future studies and supports assessment into the feasibility of recruiting from this cohort.

Recruitment targets were not met for child age groups during October 2016 to March 2017 (as discussed in Chapter 3); therefore, the study was extended for a second time-point during October 2017 to March 2018. Recruitment targets were consequently increased to 540 for adults aged 17-49 years and 540 for adult's aged 50+ years (as discussed in sections 2.12.1) increasing recruitment of new participants in those age groups with the lowest bacterial carriage. Recruiting 540 participants allowed for the detection of a carriage rate as high as 5% (with 95% CI width of 2% allowing an error rate of 5% to occur during culturing) or 30% (with 95% CI width of 4% allowing an error rate of 5% to occur during culturing).

## 2.10 Statistical analysis

Swab positivity was calculated for each swab site (NP, OP and nose) in order to ascertain the extent of detection of *M. catarrhalis* for each site.

Swab positivity (%) =

(Number of swabs positive for bacteria of interest/total number of swabs*) × 100

*Only one swab type/site was taken into consideration at a time.

Prevalence of *M. catarrhalis* carriage was calculated first as an overall and then according to anatomical site, age group, gender, recent RTI status, recent use of antibiotics, long term illness status, diabetes status, vaccination status, smoking status, ethnicity, and day care attendance to understand the effect of these variables on carriage.

Carriage prevalence (%) =

(Frequency of bacterial isolation/total number of participants swabbed*) × 100

*Only one swab type/site was taken into consideration at a time.

Co-colonisation prevalence (%) =

(Number of samples >1 bacterial species / total number of samples) × 100

Whilst carriage prevalences were determined by anatomical site, estimate of true carriage (a Boolean variable defined by whether a person carries a bacterium or not, regardless of the site or number of sites of carriage) was also determined.

True carriage (%) =

(Number of participants positive for *M. catarrhalis*/total number of participants swabbed) × 100

Confidence Intervals (CI)

CI was determined using the formulae below and confirmed using http://www.sample-size.net/confidence-interval-proportion/

$$CI = \hat{p} \pm Z^* \sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$$

CI = confidence interval

ˆp = the proportion of the population positive for event or outcome (i.e., the proportion of those carrying *M. catarrhalis*)

Z*= the critical value of the z-distribution

n = the sample size

Univariate logistic regression (IBM SPSS statistics version 28) was used for modelling outcome using binary or categorical variables (Harrell, 2001; Harrell, 2015). Relationship or association between a dependent variable (i.e., carriage of *M. catarrhalis*) and an independent categorical variable (i.e., age, ethnicity, gender, carriage of *M. catarrhalis* in another site, carriage of another bacteria) was measured. Pearson's chi squared ($X^2$) or Fischer's exact tests (FET) was used to determine whether a relationship or association between an independent and a dependent variable was statistically significant, as demonstrated by a P-value of <0.05. $X^2$ is more powerful when there is a lot of data, when the expected value in each cell is greater than 5. When one or more cells had a value lower than 5 (as identified in SPSS version 28), FET was used as applying an approximation method ($X^2$) is insufficient (Kim, 2017). In cases of significant association, an odds ratio (OR) was calculated and used to understand the effect of the independent variable on the dependent variable (McNamee, 2005). Formulae for $X^2$, FET and OR are shown below:

$$\chi^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

$O_i$ = observed value

$E_i$ = expected value

**FET =** $$p = \frac{\binom{a+b}{a}\binom{c+d}{c}}{\binom{n}{a+c}} = \frac{\binom{a+b}{b}\binom{c+d}{d}}{\binom{n}{b+d}} = \frac{(a+b)!\,(c+d)!\,(a+c)!\,(b+d)!}{a!\;b!\;c!\;d!\;n!}$$

p = P-value

a, b, c, d = frequency values from the contingency table

n = total frequency

$$\textbf{OR} = \frac{\textbf{(a/c)}}{\textbf{(b/d)}}$$

| | | Carriage of *M. catarrhalis* | |
|---|---|---|---|
| | | Yes | No |
| Exposure / disease / condition / characteristic | Yes | A | b |
| | No | C | d |

(Exposure i.e., smoking, disease i.e., COPD, characteristic i.e., gender or ethnicity)

So

$$\textbf{OR} = \frac{\textbf{Odds of the event in exposed group}}{\textbf{Odds of the event in non-exposed group}}$$

## 2.11 Biases, confounding and interaction

Whilst planning and undertaking a study there is a need to consider random error, biases, confounding and interaction as these can produce false results such as a statistically significant associations where there are none or an erroneous absence of association (type 1 and type 2 error).

Bias, a systematic error resulting in an incorrect estimate of a result or association, is a consequence of flawed methodology. There are two key types of bias; information bias (includes observer bias, interviewer bias, recall bias, social desirability bias, performance bias and detection bias) and selection bias (includes sampling bias and allocation bias). The latter is a systematic difference between those who participate in the study and those who do not, which affects generalisability of results (Vetter and Mascha, 2017; Barratt H, 2018). To limit bias in the Solent SMART Study the following measures were put in place:

- Development of protocols for standardised collection, measurement and interpretation of samples and information.
- Use of standardised questionnaires
- Quality control and calibration of consumables and instruments used
- Training of researchers to ensure non-leading answering of questionnaires
- Assuring participants that the correct questionnaire answer is the best answer, and should participants not know it is perfectly acceptable to 'select do not know'
- Assuring the confidentiality of data obtained as part of the study
- Not limiting who can participate (where possible, in accordance with ethical regulations and considerations)
- Recruiting from multiple sites

Confounding occurs when an observed association is inaccurate because the result also correlates with another risk factor/variable different to that being investigated. Confounding can result in an observed association where there is none, no observed association when a true association exists or an under/overestimate of association (negative/positive confounding). During study design, confounding was accounted for by designing a comprehensive questionnaire which can be used ascertain potential confounding variables. During analysis confounding variables were controlled for using stratification. This allowed association between variable and result to be examined within different strata of the confounding variable, for example by age. If there is little difference, then the strata variable isn't confounding. Multivariable analysis is another way of accounting for confounders. Statistical modelling such as multivariate logistic regression analysis is used to control for more than one confounder at a time and was therefore undertaken using the entire culture dataset in SPSS version 28.

Interaction occurs when the extent of an association between two variables varies according to a third variable. Interaction isn't statistically adjusted for; instead, association is determined for multiple levels of the third variable. This was undertaken where necessary.

## 2.12 Discussion

Development of methodology for the Solent SMART Study was based on experience of carriage studies. The Solent SMART Study built upon approaches used in the Bupa SMART Study (REC number 11/SC/0518) and the Southampton Pneumococcal Carriage Study (REC numbers 06/Q1704/105 and 14/NS/1064).

Whilst sections 2.1-2.11 outline the design of the Solent SMART Study; this section (2.12) discusses chosen methodology as well as the consideration of alternative methods. There were many factors to consider in terms of study design. Ultimately, any chosen methodology must balance effective bacterial isolation, anatomical separation (e.g., in infants it is difficult to separately sample NP and nasal cavities effectively given the proximity of the two sites), being appropriately invasive and ensuring sufficient recruitment for the accurate achievement of study aims.

Techniques used to sample bacteria and most likely site of colonisation are both important factors for effective bacterial isolation. There are numerous sites from which respiratory pathogens can be sampled, the most common being the nasal cavity, NP, oral cavity, and OP (Coughtrie *et al.*, 2014). Other sites including the bronchi can be sampled; however, this requires more invasive techniques such as a bronchial lavage (BAL) (Maskell *et al.*, 2003; Collins *et al.*, 2014). Sampling numerous sites may be necessary to fully investigate bacterial carriage and co-carriage; the URT is made of distinct anatomical sites, each with its own unique environmental conditions and factors e.g., epithelial cell type, each acting as a niche for different microbial communities (Man, de Steenhuijsen Piters and Bogaert, 2017). Furthermore, there are many different techniques used to sample respiratory pathogens. These include nose blowing (Leach *et al.*, 2008), swabbing (Bøe *et al.*, 1964) (nasal (Leach *et al.*, 2008; Coughtrie *et al.*, 2014), NP (Coughtrie *et al.*, 2014), mouth and OP swabbing (Rapola *et al.*, 1997; Coughtrie *et al.*, 2014)), nasal and NP washes (Lieberman *et al.*, 2006; Abdullahi *et al.*, 2007), NP aspirates (Rapola *et al.*, 1997) and sputum sampling (Wilkinson *et al.*, 2017).

Recruitment is also an important factor that needs to be tailored for the research question, in terms of who to recruit, where to recruit from and when. For example, if looking at the effect of vaccines (including herd immunity) on a community, a representative subset of the entire population should be sampled; whilst both VT and non-VT bacteria should be sampled so

secondary effects are observed. Alternatively, if the research question is more resolute/specific i.e., *what is the prevalence of a certain bacterial species in young children?* then only that bacteria and a representative sample of 0-4 year olds would need to be sampled. The chosen methodology must be appropriate to achieve study aims as discussed here.

### 2.12.1 Recruitment age groups

Four age groups were used for recruitment: 0-4, 5-16, 17-49 and 50+ years. Age groupings were chosen, and recruitment targets determined for each, to ensure recruitment was representative of the population being sampled (high recruitment across all ages) and effective isolation of bacteria for the achievement of study aims. Furthermore, these age groups streamlined the process of recruitment (in terms of consent) and allowed for accurate collection of data (in terms of the completion of questionnaires). The 0-4 age group was chosen as young children have higher bacterial carriage. The 5-16 age group was chosen as those of this age are classed as children for the purposes of consent. Additionally, bacterial carriage in this age group is expected to be higher than that seen in adults but lower than that seen in young children, making it logical to group these ages when determining recruitment targets. There were multiple reasons for choosing the 17-49 and 50+ year's recruitment age groups. Firstly, participants in both age groups are classed as adults for the purposes of consent. However, vaccination history differs between the two groups due to the years in which certain vaccines were introduced or the age at which people are eligible for certain vaccines. Having these two groups made it feasible to have separate questionnaires targeted to age, based on the potential vaccine history. Furthermore, the 17-49 age group was selected over a 17-64 group (as other studies have used) as 17-49 is a narrower age range, allowing for recruitment that is more representative of the age distribution of the population being sampled. If a 17-64 age group was used recruitment would have been proportionally low whilst recruitment for a 65+ group would be proportionally high when compared to the age distribution of the general population.

The Solent SMART Study aimed to recruit a total of 1360 participants: 200 aged 0-4 years, 340 aged 5-16 years, 340 aged 17-49 years, 340 aged 50+ years and 140 care/nursing home residents. Recruitment targets were based on sample size calculations using predicted bacterial carriage prevalence from previous carriage studies. During the first recruitment time-point, recruitment targets were not met for child age groups. Therefore, the study was extended for a second time-point which was carried out during the same seasonal period the following year (ensuring seasonal fluctuations hadn't affected carriage prevalence). Adult targets were also increased, the primary reason being to validate the recruitment of

children from the second time-point. By comparing carriage data from the high recruitment of adults from time-points one and two, any fluctuation in carriage between the years could be detected and accounted for. Should carriage from both time-points be statistically comparable, this would validate the grouped analyses of all child data from both time-points. Secondly as carriage is low in adults and adult recruitment was found to be easy, it was beneficial to take the opportunity of the second time-point to increase recruitment and sampling in this group to increase statistical power. Targets were increased to allow for the recruitment of an additional 200 participants aged 17-49 and 200 aged 50+ years.

### 2.12.2 Sampling methodology

Various sampling methodologies were considered including nose blowing, nasal and NP washes, NP aspirates and sputum sampling. Nose blowing was ruled out as children under two years of age cannot blow their nose, whilst bacterial detection is lower for nose blowing when compared to nose swabbing (Leach *et al.*, 2008). Studies suggest nasal and NP washes can be more sensitive for bacterial detection than swabbing (Abdullahi *et al.*, 2007), whilst swabbing (nasal and NP) and NP aspirates are comparable to each other for the isolation of *M. catarrhalis, S. pneumoniae* and *S. aureus* (Carville *et al.*, 2007). Although washes and aspirates have been used on children (Hendley, Hayden and Winther, 2005) and adults, they aren't recommended for children particularly those under five, due to the level of participant cooperation required (Gritzfeld *et al.*, 2011). In particular, aspirate collection is not suggested for healthy children (Rapola *et al.*, 1997), therefore these methods were unsuitable for this study. Sputum sampling is also unsuitable for this study as it is only used in cases of infection thus is inapposite for investigating bacterial carriage. Although used in research, bronchial lavage (BAL) (Maskell *et al.*, 2003; Collins *et al.*, 2014) is less commonly performed, certainly on healthy members of the public. This due to the invasive nature of BAL, making this procedure unsuitable in many cases; firstly, because it is unethical to be more invasive than is necessary to achieve the study aims, and secondly because BALs can only be performed in a clinical setting. All alternative methods described above (bar nose blowing) require specialised training and equipment, have limited use in community-based sampling due to the need for a suitable clinical setting, and are impractical for recruitment and sampling of bacterial carriage in participants of all ages as they are often not well tolerated by children. Such methods were therefore discounted.

Swabbing was determined the best method to sample bacterial carriage for the Solent SMART Study. Swabbing is quick, easy, non-invasive and low cost, making it ideal when aiming for high recruitment. Furthermore, swabbing can be used to sample people of all ages and swabs can be taken in any room; there is no requirement for a clinical setting, allowing for

recruitment within the wider community. In addition, swabbing is the gold standard for the detection of multiple bacteria being isolated as part of this study (van den Bergh et al., 2012) and is considered a standard method for carriage studies (Satzke *et al.*, 2013).

### 2.12.3 Anatomical swabbing sites

The Solent SMART Study aimed to sample the URT to determine carriage prevalence of common respiratory pathogens, with a focus on *M. catarrhalis*. Therefore, sampling of the NP, OP and nose was determined necessary to fully investigate bacterial carriage and to determine *M. catarrhalis* epidemiology (Man, de Steenhuijsen Piters and Bogaert, 2017).

NP swabs are considered the gold standard for the detection of *S. pneumoniae* from the NP, and this is also the recommended sampling site for *M. catarrhalis* (van den Bergh et al., 2012). Despite this, a gold standard sampling site for the detection of *M. catarrhalis* remains unclear (Satzke *et al.*, 2013), thus the sampling of multiple sites offered the opportunity to compare swabbing methods to determine the most effective for *M. catarrhalis* isolation. Nasal swabbing has been proposed as an alternative to NP swabbing, although prevalence of *M. catarrhalis* (and other bacteria) from the Bupa SMART study was lower in participant self-taken nasal swabs compared to health care professionally administered NP swabs (2.5% and 4.5% respectively). However, differences in prevalence of *M. catarrhalis* from participant self-swabbing (S-S) and Health Care Professional (HCP) swabbing of the mouth (13.7% and 27.4% respectively) indicates such disparities could be due to differences in study methodology. Potential differences in the age distribution of participants from S-S and HCP groups was not a factor, highlighting the need for a study which obtains nasal and NP swabs from the same participant, so carriage in the nose and NP can definitively be compared. NP and OP are the optimal sample sites for *H. influenzae* isolation and nasal swabs are considered the standard for *S. aureus* isolation (van den Bergh *et al.*, 2012), whilst OP swabs are the standard for *N. meningitidis* isolation (Jordens et al., 2002) although no gold standard is established (Basta *et al.*, 2013).

### 2.12.4 Recruitment methodology

Whilst HCP swabbing was chosen, participant S-S was also considered. The key factor was that taking NP swabs was deemed only feasible with HCP/researcher swabbing due to the more invasive nature of this swab type. Whilst people have become more familiar with S-S over the course of the covid-19 pandemic, it is felt that HPC swabbing may still be required to ensure the NP, rather than the upper nasal area, is truly being sampled. Further justification for HCP/researcher swabbing was that it permitted the standardisation of swab taking. All swabs taken to the same high standard by trained individuals gives comparable and reliable

results. With participant S-S (certainly when using postal swab packs like the Bupa SMART study) no researcher is present and despite providing instructions, the quality of swabs cannot be guaranteed. It is also not possible to be certain of the length of time between swabs being taken and received by the laboratory. It may be that despite instructions, participants could take several days/weeks to post swabs to the laboratory, affecting accuracy of bacterial detection due to a reduction in the viability of bacteria over time.

Furthermore, with HCP/researcher swabbing, a member of the study team can be present at all times to answer participant's questions and offer support, which can encourage participation and ensure informed consent is always provided. When using participant S-S, if potential participants do not fully understand the study, they may be unwilling to contact researchers for clarification. This could result in a decision not to take part, participating without truly providing informed consent or participating but incorrectly completing the consent form/questionnaire, which (unless planned for and approved by REC) could mean samples can't be used.

The Bupa SMART study (Coughtrie, 2012; Coughtrie *et al.*, 2014; Coughtrie *et al.*, 2018) highlighted several considerations when implementing HCP swabbing. Firstly, it resulted in much lower recruitment (314 participants versus 1260 S-S participants recruited during the same period). Consequently, HCP swabbing had a much lower recruitment success rate (8.2% for those aged ≥5 years and 2.9% for those aged 0-4 years) than S-S (27.8% for those aged ≥5 years and 16.1% for those aged 0-4 years). Furthermore, HCP swabbing mainly recruited elderly or very young participants, with 65% of those recruited being aged either 0-4 or 65+ years. Therefore, sampling was not representative of the community with the 2011 Office of National Statistics Census showing that only 24% of the Hampshire population is aged 0-4 or 65+ years. Furthermore, if carriage is lower in those aged 5-64 years then low recruitment in these ages may prevent accurate carriage prevalence being established. However, the low and disproportionate recruitment seen with HCP swabbing was likely due to the method of recruitment, which relied on participants attending their GP for a research appointment. Participant S-S gained much higher recruitment, arguably because swab packs were sent to potential participants meaning taking part was much more convenient and didn't rely on availability for and of appointments. For HCP swabbing to be successful for the Solent SMART Study, recruitment had to be carried out differently to the method used for the Bupa SMART study. Recruitment practices for the Southampton Pneumococcal Carriage Study were therefore considered (and chosen) as the better approach for this study; approaching potential participants at sites they were already attending and inviting them to participate whilst there.

The Solent NHS Trust research team were enlisted to undertake recruitment and swabbing for the Solent SMART Study, as part of a collaboration. As a team of highly trained researchers, with community links, NHS infrastructure and insurances they offered access to a wide number of services and community sites across Hampshire and the Isle of Wight. The Solent NHS Trust research team made high recruitment feasible due to the number of HCP/researchers available to recruit. Their input also meant there was no time or research burden on NHS services or staff at the sites attended, meaning short staffing, and overburdening of the NHS system had no impact on the study (an issue experienced for both S-S and HCP recruitment during the Bupa SMART study).

To increase recruitment/participation rates, the possibility of sending out a study invitation letter and PIS to those attending planned appointments at NHS sites was considered, however was decided against. This method was tested and deemed unsuccessful during the Southampton Pneumococcal Carriage study. Furthermore, Solent SMART Study recruitment wasn't limited to those with appointments nor was it restricted to one site or specific times or days. Therefore, sending letters simply wasn't practical.

### 2.12.5 Transportation methodology

Taxis were used for transportation to ensure prompt delivery to the laboratory. The availability of research staff to collect/deliver swabs could not be guaranteed, hospital sample transportation links could not guarantee swabs would get to the laboratory within 48 hours of being taken, and because samples were biological and the consent forms and questionnaires contained sensitive data, secure yet economically viable transportation was required.

### 2.12.6 Questionnaire design

Age specific questionnaires were written to obtain background information, which could offer a better understanding of factors influencing bacterial carriage. Questionnaire design was developed based on those tried and tested as part of the Bupa SMART and Southampton Pneumococcal Carriage studies. To inform future study design participants were asked whether they would take part in the study again and why, and to confirm why they didn't have all swabs taken if they had refused any. There was also a notes section so swabbers could state if any swabs had been taken improperly and why e.g., participant was too young to get an effective OP swab or participant pulled away during NP. This provided a better understanding when analysing the bacterial growth and informed on the feasibility of undertaking each swab type.

The use of age tailored questionnaires streamlined the participation process and allowed for more accurate completion with participants only having to answer questions of relevance. For example, children were not asked if they smoke, simply if there are exposed to second hand smoke (SHS). Similarly, 0-16 year olds were asked if they had received PCV-13, MenB and Fluenz vaccinations, 17-49 year olds were asked if they had received MenC vaccination (to target university students) and those aged 50+ were asked about the pneumococcal polysaccharide vaccine (PPV23) (targeted to at risk adults or those aged 65+years).

### 2.12.7 Laboratory methodology

There are two main types of laboratory technique used for bacterial identification from respiratory samples, namely culture dependent and culture independent methods. The Solent SMART Study used culture dependent bacterial identification for multiple reasons. Firstly, although no guidelines for *M. catarrhalis* exist, for other respiratory pathogens such as *S. pneumoniae* culture is the gold standard for the detection from swab samples (O'Brien, Nohynek and World Health Organization Pneumococcal Vaccine Trials Carriage Working Group, 2003; Satzke *et al.*, 2013). Furthermore, clinical diagnostic laboratories routinely use culture for the identification of bacterial pathogens, validating its use. Lastly, whilst culture methods only show those bacteria which are of potential clinical relevance, as only viable/live bacteria are identified, it also allows for phenotypic testing of AMR. Culture methodology followed Public Health England (PHE) standard practice, which is stringently regulated and modified as required. DNAse plates were included as a confirmation for *M.* catarrhalis, as per a PHE update and findings from the Bupa study which highlighted misidentification of *N. meningitidis* as *M. catarrhalis*.

STGG and cryogenic beads (Micobank ™, Pro-Lab Diagnostics, UK) are common bacterial storage methods; both were tested as part of the Southampton Pneumococcal Carriage Study. However, STGG is more effective for long-term storage (O'Brien *et al.*, 2001; Charalambous *et al.*, 2003; Kaijalainen *et al.*, 2004) and was therefore chosen as the method of storage for the Solent SMART Study.

The swabs used in this study were of Clinical Laboratory Standards Institute (CLSI) Approved Standard M40-A2 (Clinical Laboratory Standards Institute, 2014). This ensures that bacterial numbers won't reduce by more than 3 $\log_{10}$ under at refrigeration and ambient temperature (2-30$^{0}$C), or increase by more than 1 $\log_{10}$ at refrigeration temperature, during a 48-hour holding period; as guaranteed by compliance tests. Therefore, swabs were processed within 48 hours of being taken to ensure reliable and accurate recovery of bacteria.

Molecular methodology was considered as an alternative to culture-based bacterial identification because culture methodology relies on bacteria being viable. This restricts the amount of time swabs have to be delivered to the laboratory, which requires consideration when organising deliveries and planning for the associated cost. Also, it can be difficult to isolate and/or identify bacteria present at lower concentrations when using culture methodology. The benefit of PCR is it is highly sensitivity making it significantly more effective for the detection of bacteria (Aly *et al.*, 2012; Baysallar *et al.*, 2013; Coughtrie *et al.*, 2018). Aly et al found culture methods identified *S. pneumoniae* and *M. catarrhalis* in 10% and 12% of samples respectively. Whilst PCR found *S. pneumoniae* and *M. catarrhalis* in 36% and 44% of samples respectively (Aly *et al.*, 2012). Furthermore, qPCR can be used to assess the abundance of each bacterial species. However, the detection of nucleic acids to identify bacterial species is likely to falsely inflate carriage prevalence due to the detection of dead bacterial cells (Rogers *et al.*, 2008). Although, this can be moderated by using quantitative PCR and having a cut off where bacteria aren't included if the DNA yield is below a specified threshold. Alternatively, by using rRNA based methods such as rRNA targeted reverse transcription PCR, only viable bacteria are detected (Matsuda *et al.*, 2007). However, culture dependent bacterial identification was chosen based on the merits of this method, as previously discussed.

### 2.12.8 Patient and Public Involvement (PPI)

PPI is a key aspect of research, which allows members of the public to get involved often providing researchers with insight and advice relevant the design and implementation of studies. PPI provides many benefits when planning a study such as ensuring the study is acceptable to potential participants and ensuring study documents are understandable and written in lay language. The study documents used in the Solent SMART Study were written based on PPI advice gained during the setup and undertaking of the Bupa SMART and Southampton Pneumococcal Carriage studies. Study design was similarly based on previous PPI advice as well as personal experience of recruitment. Solent SMART Study documents were tested, prior to approval, on members of the public randomly approached and asked for feedback. The Solent NHS research team, as a team with extensive experience of recruitment and face to face patient involvement, were also asked to provide feedback.

# Chapter 3 Recruitment and isolation of *M. catarrhalis* from the Solent SMART Study

## 3.1 Introduction

Carriage studies are a valuable surveillance tool used to investigate bacterial carriage and to determine and monitor the epidemiology of pathogens. The WHO defines epidemiology as "the study of the distribution and determinants of health-related states or events (including disease), and the application of this study to the control of diseases and other health problems" (World Health Organization). In terms of bacterial carriage this means determining which bacteria are carried where (body site, geographical location), by who (what ages, gender, ethnicity), how often and in what context. As carriage is often a prerequisite for disease, investigating carriage can provide insight for understanding disease and disease control. To answer specific questions regarding *M. catarrhalis* epidemiology, a cross-sectional population-based observational URT carriage study (Solent SMART Study) was undertaken over two time-points, providing a large collection of *M. catarrhalis* isolates. The Solent SMART Study was an all-age carriage study, with an added focus on care/nursing home residents, a cohort with limited prior data related to *M. catarrhalis*, and those with COPD, a cohort where the acquisition of *M. catarrhalis* is a risk factor for exacerbation. This chapter reports the study outcomes in terms of participant recruitment and isolation of *M. catarrhalis*.

## 3.2 Chapter aims and objectives

Aim: Having designed and undertaken a community-based carriage study (Solent SMART Study), this chapter aims to evaluate recruitment and investigate swabbing methodology.

Hypothesis: NP swabs are the most effective swab type for isolating *M. catarrhalis* from the URT.

Specific Objectives were to:

- Assess overall recruitment and study success, by comparing age stratified recruitment and the recruitment of at-risk groups/cohorts of interest.
- To compare swabbing methods to determine the most effective swab type for isolating *M. catarrhalis* in the URT.

## 3.3 Results

### 3.3.1 Overview of recruitment

**Recruitment**

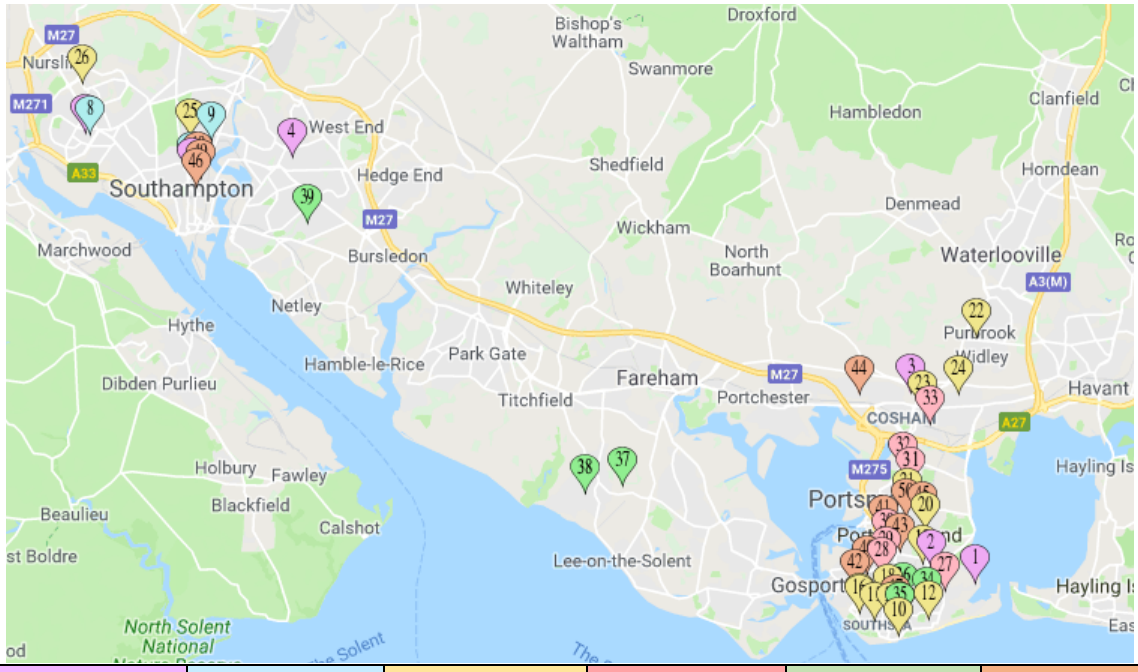
In total 1701 participants were recruited to the Solent SMART Study. During the first time-point, all recruitment targets for the adult age groups were obtained, however recruitment of care/nursing home residents and children was less successful (Table 3). By the end of recruitment time-point 2, recruitment targets were met for all age groups, except for care/nursing home residents.

| Age range/cohort | Recruitment time-point one n (%) | Recruitment time-point two n (%) |
|---|---|---|
| 0-4 | 161/200 (80.5) | 200/200 (100) |
| 5-16 | 152/340 (44.7) | 341/340 (100.3) |
| 17-49 | 340/340 (100) | 540/540 (100) |
| 50+ | 340/340 (100) | 541/540 (100.3) |
| Care/nursing home residents | 79/140 (56.4) | - |
| Total | 1071/1360 (78.8) | 1701/1760 (96.7) |

**Table 3. Recruitment targets and achievements for both time-points**.

**Recruitment sites**

Recruitment was undertaken at wide variety of locations across the Solent region (covering both Southampton and Portsmouth) to reduce potential sampling biases. Figure 3 shows that recruitment took place in 51 different sites (point 38 covers two sites; Crofton Hammond Infants and Crofton Hammond Infants Junior School). Care/nursing homes were only recruited from in time-point one (as discussed later in this thesis). The majority of other sites were attended during both time-points, with the exception of Somerstown Children's Centre, Highbury Community and Children's Centre and Swanmore College which were only visit in time-point one. Landport Children's Centre, Cumberland Infant School, Priory School, St Mary's Fire Station COPD Clinic, St Swithuns Church and St Albans Church were only attended in time-point two.




| Hospital/Health Centre | | GP Surgery | | Care/Nursing Home | | Children's Centre | | School | | Other | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | St James Hospital | 14 | Alton Manor Care Home | 27 | Milton Park Children's Centre | 40 | Portsmouth Civic offices | | | | |
| 2 | St Mary's Community Health Campus | 15 | Braemar Care Home | 28 | Somerstown Children's Centre | 41 | Buckland Community Centre | | | | |
| 3 | Queen Alexandra Hospital | 16 | Queen Anne Lodge Nursing Home | 29 | Landport Children's Centre | 42 | Portsmouth Dental Academy | | | | |
| 4 | Bitterne Health Centre | 17 | Wansbeck House Care Home | 30 | Buckland Children's Centre | 43 | Eastney Methodist Church | | | | |
| 5 | Royal South Hants Hospital | 18 | Home of Comfort Nursing Home | 31 | Battenberg Avenue Clinic | 44 | Paulsgrove Community Centre | | | | |
| 6 | Adelaide Health Centre | 19 | Hartford Court Care Home | 32 | Northern Parade Children's Centre | 45 | St Albans Church | | | | |
| 7 | Nicholstown GP Surgery | 20 | Mary Rose Manor Nursing Home | 33 | Highbury Community and Children's Centre | 46 | St Mary's Fire Station COPD Clinic | | | | |
| 8 | Adelaide GP Surgery | 21 | Meadow House Care Home | 34 | Cumberland Infant School | 47 | St Swithuns Church | | | | |
| 9 | Portswood GP Surgery | 22 | Latham Lodge Nursing Home | 35 | Craneswater Junior School | 48 | Homeless Health Care | | | | |
| 10 | Regency Nursing Home | 23 | Cosham Court Nursing Home | 36 | Priory School | 49 | Graham Road Community Centre | | | | |
| 11 | Summerland's Care Home | 24 | Hamilton House Care Home | 37 | Crofton Secondary School | 50 | Lorraine Lambe hairdressing | | | | |
| 12 | Seaview Residential Care Home | 25 | Mayflower Court Care Home | 38 | Crofton Hammond Schools | | | | | | |
| 13 | St Ronan's Nursing and Residential Care Home | 26 | Neptune Court Residential and Sheltered Living | 39 | Swanmore College | | | | | | |

**Figure 3. Map of Solent SMART Study recruitment sites.**

Markers show the location of each recruitment site, with numbers indicting the name of the site and colour highlighting the type of site. Map created at www.mapcustomizer.com © Copyright 2014-2020 Ursus Software, LLC.

Participants were recruited from six different hospital or health centres; however, within these settings, multiple clinics and services were targeted to reduce recruitment/site bias. At St Mary's Community Health Campus, recruitment was undertaken at the Spinnaker ward, research department, St Mary's health promotion room, Portsmouth enablement centre and the outpatient department. The outpatient department provides services including phlebotomy, podiatry, dermatology, physiotherapy, diabetic eye screens, mental health, respiratory clinics, day surgery, ultrasound, obstetrics and gynaecology. At St James' Hospital, recruitment was only undertaken at podiatry clinics due to the limited number of services run from this site. The rehabilitation block was the only site for recruitment at Queen Alexandra Hospital; however, this gave access to pulmonary rehabilitation, physiotherapy and hydrotherapy services. At Royal South Hants Hospital, sexual health and pulmonary rehab clinics were sources of recruitment. Sexual health was a source of copious recruitment for the first time-point, however changes in clinic practice significantly reduced success during the second time-point. At Adelaide Health Centre, participants were recruited from muscular skeletal, podiatry and MRI services in addition to pulmonary rehab clinics. The services listed are visited by a broad range of people, allowing for community wide recruitment. Furthermore, recruitment was not patient focussed; any visitors to these sites were approached for recruitment, including friends and family of patients. Numerous non-NHS sites were also recruited from to reduce the recruitment bias of recruiting exclusively from health care settings. Such sites included children's centre, schools, colleges, Portsmouth civic offices, community centres and a hairdresser. Table 4 shows that over 40% of recruitment was undertaken at non-NHS sites or was of non-NHS service users.

| | **Recruitment** | |
|---|---|---|
| **Age group** | Non-patient recruitment - n (%) | Recruitment from non-NHS sites - n (%) |
| 0-4 | 191 (95.5) | 190 (95.0) |
| 5-16 | 336 (98.8) | 331 (97.4) |
| 17-49 | 133 (24.6) | 100 (18.5) |
| 50+ | 67 (12.4) | 53 (9.8) |
| All | 778 (48.0) | 676 (41.7) |

**Table 4. Summary of non-NHS recruitment**.
This table shows recruitment of non-patients and recruitment from non-NHS sites (excludes care home data) as a number and percentage of recruitment total by age range and overall recruitment.

**Representative Sampling**

The aim of recruitment was to obtain a representative sample of the community (or the most representative sample possible), and therefore to isolate *M. catarrhalis* representative of

carriage throughout the community. Figure 3 shows recruitment from a variety of sites, whilst Figures 4 and 5 show recruitment of a wide distribution of ages was obtained, including those under-represented in previous studies or cohorts previously not sampled as part of *M. catarrhalis* research.

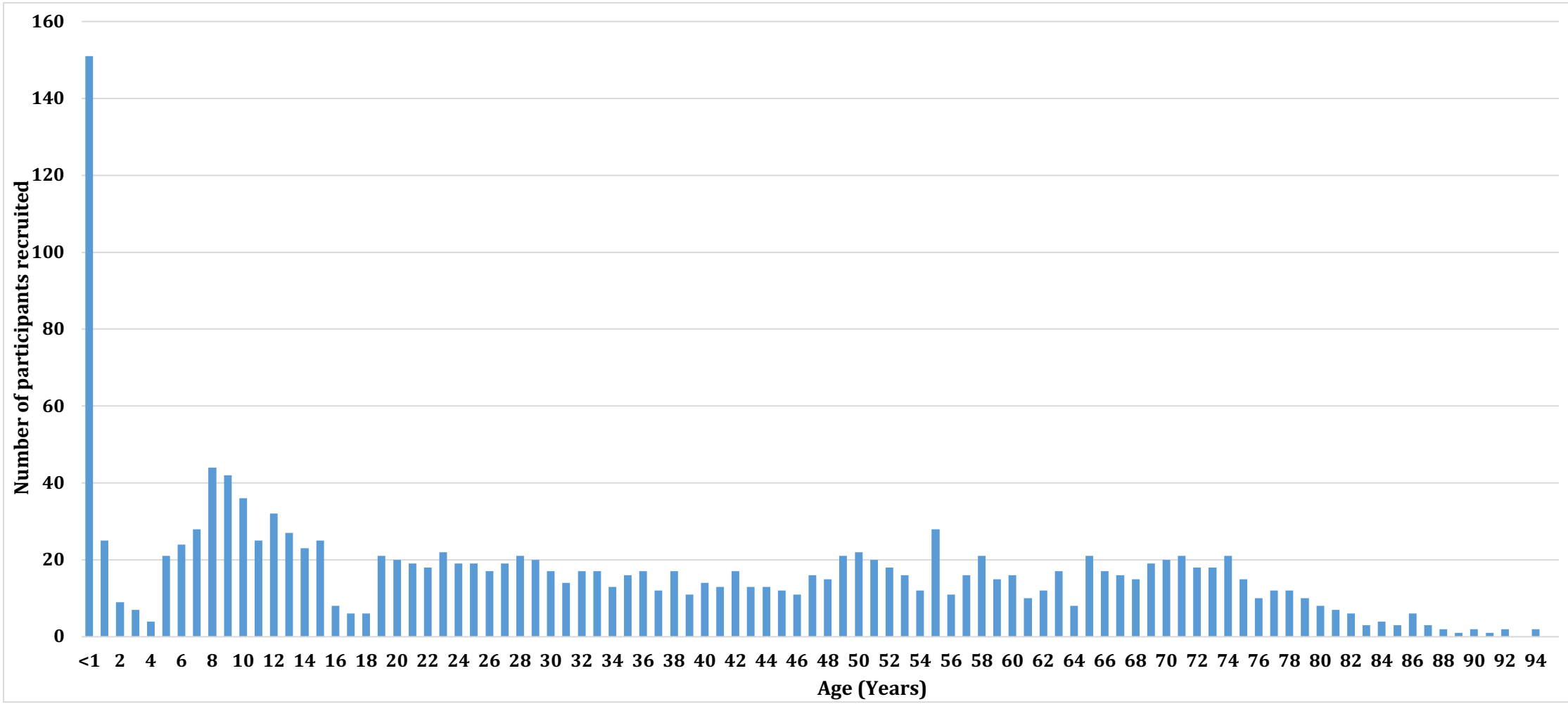



**Figure 4. Age distribution of all participants recruited to the Solent SMART Study**.

Each bar shows how many participants recruited belong to each year of age.

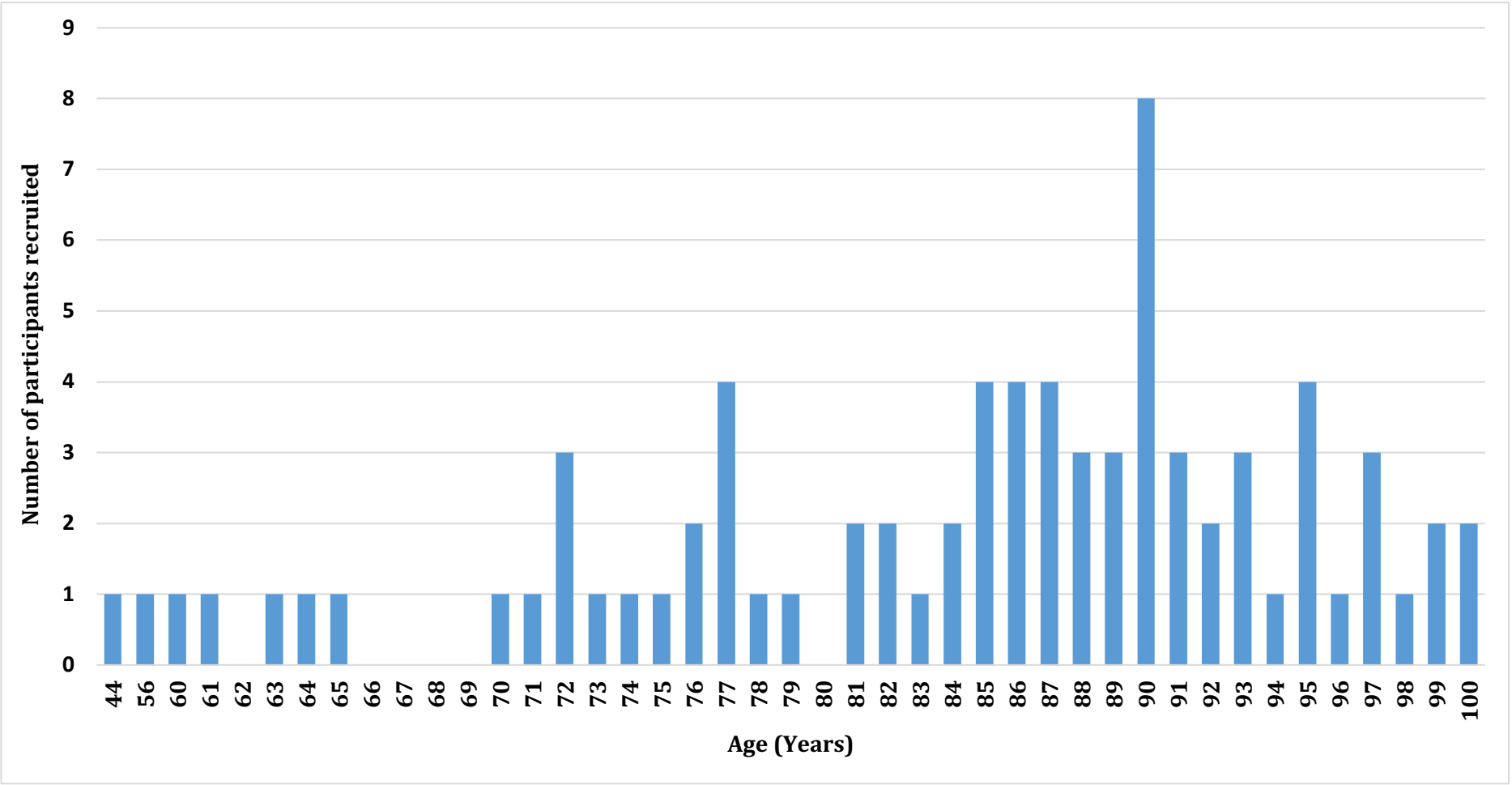



**Figure 5. Age distribution of participants recruited from care/nursing homes to the Solent SMART Study**.

Each bar shows how many participants recruited belong to each year of age.

| | | 0-4 | 5-16 | 17-49 | 50+ |
|---|---|---|---|---|---|
| **Proportion of the community (%)** | 2016 population estimates | 6.1 | 13.9 | 43.4 | 36.6 |
| | Total recruitment minus care/nursing homes | 12.4 | 21.0 | 33.3 | 33.3 |
| | Total recruitment with care/nursing homes | 11.8 | 20.0 | 31.8 | 36.4 |

**Table 5. Age distribution of study recruitment versus Office for National Statistics (ONS) 2016 population estimates**.

Table 5 shows the age distribution of participants recruited to the study appears to be comparable to that of the local Southampton, Fareham and Portsmouth community. Based on ONS 2016 population data, community demographics are reported in terms of the percentage of the community in Southampton, Fareham and Portsmouth which fall into recruitment age groups 0-4, 5-16, 17-49 and 50+ years (Office for National Statistics, 2020). The percentage of Solent SMART Study recruitment for each age group is also reported to highlight whether sampling was representative of the community being sampled. A t-test of the mean was undertaken (T = 0.000, P=1.000) showing that whilst the community population and study population are similar, the similarity was not statistically significant.

The overall distribution of males and females in the UK is 49.1% and 50.9% respectively (Office for National Statistics, 2012), which is comparable to the overall recruitment to the study (Figure 6). However, when looking at recruitment age groups, recruitment of males for the 17-49 group is low. Care/nursing home recruitment was heavily skewed to the recruitment of women, which fits ONS data and my experience having attended most of the recruitment sessions, showing the ratio of males to females in care/nursing homes is approximately 1:3 (Office for National Statistics, 2014).

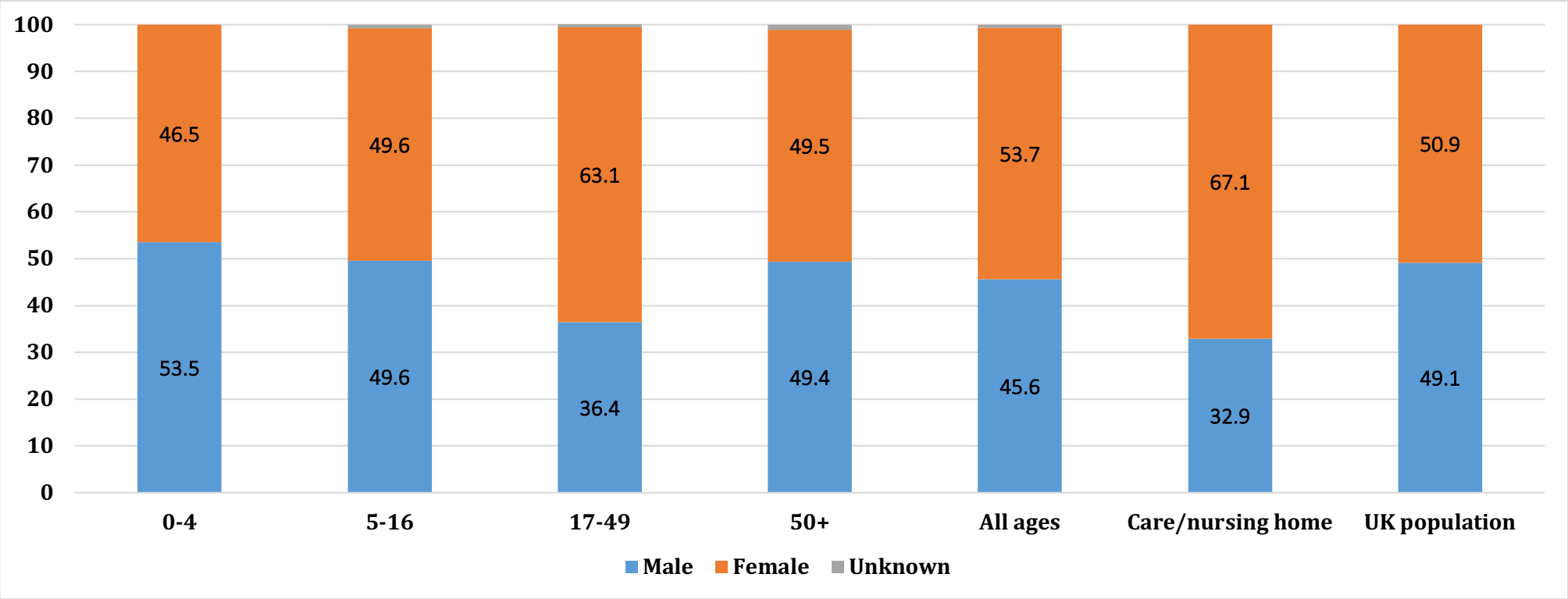



**Figure 6. Percentage of males and females recruited to the Solent SMART Study**.

The distribution of each gender recruited is displayed for age groups 0-4, 5-16, 17-49, 50+ years and care/nursing home residents. Data for community participants was also displayed as a whole under 'all ages'. The overall distribution of males and females in the UK is bases on 2016 census data is also shown.

| | | Total recruitment | |
|---|---|---|---|
| | | General study population - n (%) | Care/nursing homes - n (%) |
| **Total Study Recruitment** | | 1622 | 79 |
| **Age** | Mean | 35.1 | 84.1 |
| | Min | 0.02 | 44 |
| | Max | 94 | 100 |
| | Median | 32 | 87 |
| | IQR | 46 | 14 |
| | 0-4 | 200 (12.3) | 0 (0) |
| | 5-16 | 341 (21.0) | 0 (0) |
| | 17-49 | 540 (33.3) | 1 (1.3) |
| | 50+ | 541 (33.4) | 77 (97.5) |
| | unknown | 0 | 1 (1.3) |
| **Gender** | Male | 739 (45.6) | 26 (32.9) |
| | Female | 871 (53.7) | 53 (67.1) |
| | Unknown/missing | 12 (0.7) | 0 (0) |
| **Recent antibiotic use #** | Yes | 223 (13.8) | 27 (34.2) |
| | No | 1394 (85.9) | 49 (62.0) |
| | Unknown/missing | 5 (0.3) | 3 (3.8) |
| **Recent RTI #** | Yes | 705 (43.5) | 25 (31.6) |
| | No | 907 (55.9) | 53 (67.1) |
| | Unknown/missing | 10 (0.6) | 1 (1.3) |
| **Vaccination status Δ** | Up to date | 1437 (88.6) | 51 (64.6) |
| | Not up to date | 68 (4.2) | 7 (8.9) |
| | Unknown/missing | 117 (7.2) | 21 (26.6) |
| **Received annual flu vaccination** | Yes | 661 (40.8) | 50 (63.3) |
| | No | 875 (53.9) | 23 (29.1) |
| | Unknown/missing | 86 (5.3) | 3 (3.8) |
| **Smoker (17 year olds + only)** | Cigarette/cigar only | 164 (15.2) | 4 (5.1) |
| | E-cigarette only | 38 (3.5) | 0 (0) |
| | Cigarette/cigar and E- | 35 (3.2) | 2 (2.5) |
| | Non smoker | 828 (76.7) | 73 (92.4) |
| | Unknown/missing | 15 (1.4) | 0 (0) |
| **Long term illness** | Yes | 560 (34.5) | 57 (72.1) |
| | No | 1032 (63.6) | 19 (24.1) |
| | Unknown/missing | 30 (1.9) | 3 (3.8) |
| **Ethnicity** | African | 32 (2) | 0 (0) |
| | American | 12 (0.7) | 0 (0) |
| | South-East Asian | 60 (3.7) | 0 (0) |
| | European | 1375 (84.8)*a | 79 (100)*b |
| | Eastern Mediterranean | 34 (2.1) | 0 (0) |
| | Western Pacific | 9 (0.6) | 0 (0) |
| | Mixed | 70 (4.3) | 0 (0) |
| | Unknown/missing | 30 (1.8) | 0 (0) |

**Table 6. Participant characteristics**.

# Recent is defined as within a month of participation; *a 93.7% of which were white British; *b 100% of which were white British; Δ in line with the UK immunisation schedule; WHO defined regions.

Characteristics of those recruited to the study are reported in Table 6. It highlights the number (n) and percentage of participants recruited that were aged 0-4, 5-16, 17-49 and 50+ years. Whilst the interquartile range (IQR) for the general study population was 46, quartile 1 was 11 and quartile 3 was 57, the IQR for care home residents was 14, quartile 1 was 77 and quartile 3 was 91. Gender and other data self-reported via the questionnaire in terms of the number and percentage of participants that fall into each group/category is also presented. Unknown/missing encompasses when participants selected 'don't know', 'would rather not say' or refused to provide an answer. The mean and median age in Hampshire in the 2011 census was 41.1 and 42 respectively. Of those with a long-term illness, 260 of these were respiratory related illness or conditions (mostly asthma or COPD), whilst 82 participants had diabetes. Of the 82 participants with diabetes, 21 had a comorbidity of long-term respiratory illness/condition. Recent is defined as within a month of being recruited and swabbed.

**Reproducibility**

Whilst the overall study design is reproducible, if the study was poorly tolerated or regarded by participants, the design may require improvement. However, this was not an issue as 89.6% of all participants (excluding care-home data) indicted that they would take part in this or a similar study in the future (Figure 7). Therefore, the study can be considered reproducible, even with repeated swabbing of the same participants.

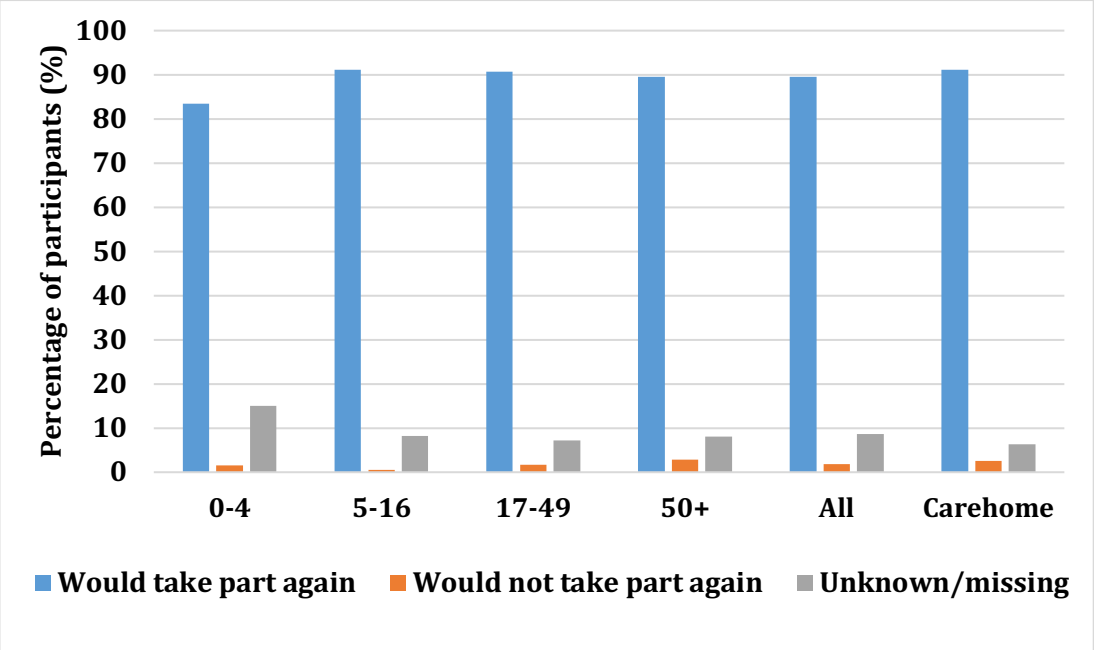



**Figure 7. Summary of the proportion of participants that would take part again.**

### 3.3.2 Comparison of swabbing methods to determine the most effective swab type for isolating *M. catarrhalis* in the URT

The isolation of *M. catarrhalis* from NP, OP and nasal swabs was investigated to test whether NP swabs are the most effective swab type for isolating *M. catarrhalis* in the URT. More *M. catarrhalis* was isolated from NP swabs than either the OP or nasal swabs; 110 were isolated from NP swabs, 20 from OP swabs and 98 from nasal swabs. Swab positivity was calculated for each swab type in order to ascertain the extent of detection of *M. catarrhalis* for each swab type. NP swabs had a 6.5% (CI: 5.4-7.8%) positivity for *M. catarrhalis*, whilst OP swabs had a 1.2% (CI: 0.7-1.9%) positivity and nose swabs had a 5.9% (CI: 4.8-7.1%) positivity. Therefore, should only one swab be administered during future research, taking the NP swab is the most effective and results in less *M. catarrhalis* carriage being missed. However, Figure 8 shows that by only taking one type of swab not all carriage is accounted for. By only taking an NP swab 24.1% of isolates could be missed, only taking an OP swab means 86.2% of isolates could be missed and only taking a nose swab means 67.6% of isolates could be missed.

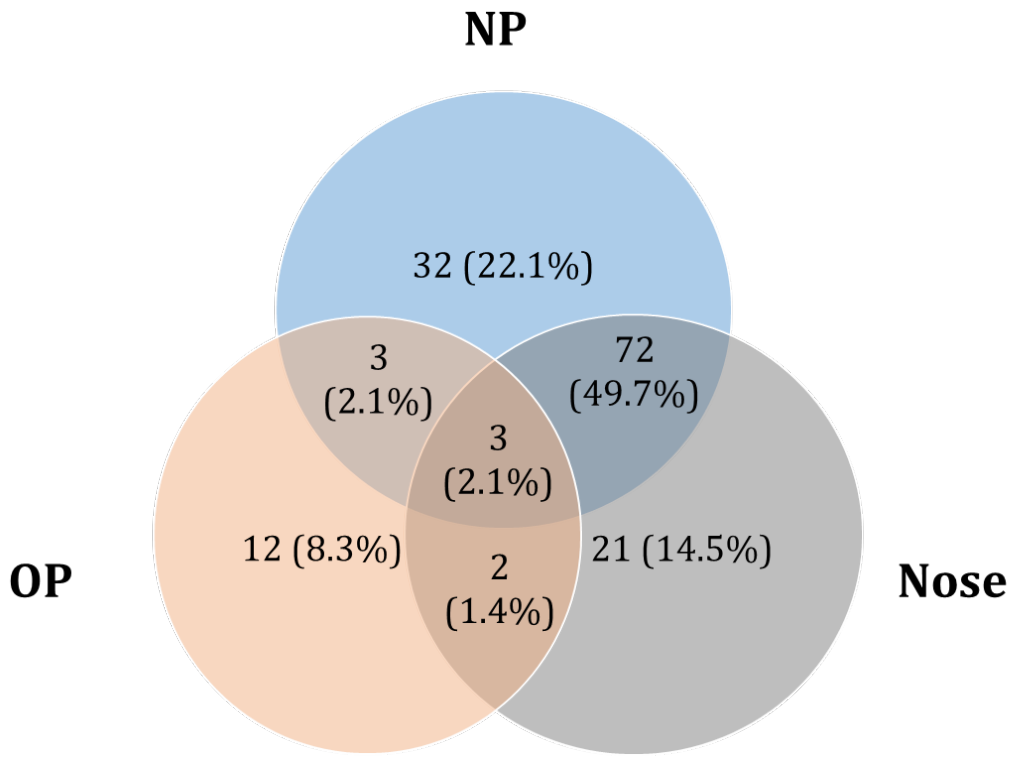



**Figure 8. Carriage of *M. catarrhalis*.**

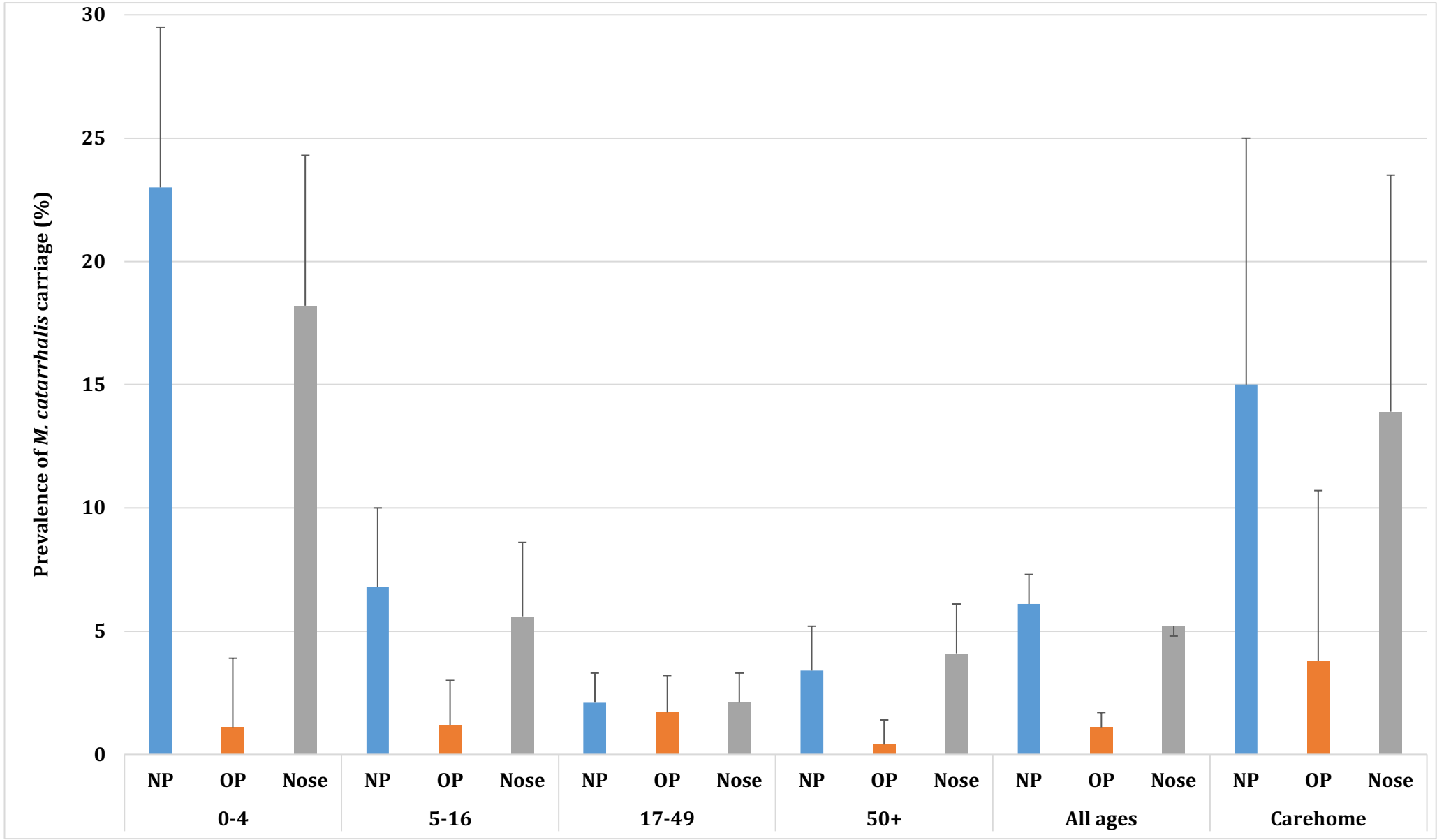
Illustrates the number and percentage of *M. catarrhalis* which were isolated from just the NP, OP or nose in addition to multiple anatomical sites; includes all *M. catarrhalis* isolates including those from care home residents.

Using $X^2$, carriage site shows a statistically significant association (P=<0.001, df 2) with the carriage of *M. catarrhalis*. NP carriage is most associated with carriage; using the NP as a

reference swab site, carriage in the OP and nose are less likely with OR of 0.165 and 0.881 respectively. When investigating carriage at multiple sites, the carriage of *M. catarrhalis* in the NP and OP, the NP and nose and the OP and nose all have a statistically significant association (P=<0.01, df = 1). If a participant carries *M. catarrhalis* in the NP, they are likely to carry it in the OP (OR 6.499) or nose (OR 152.389) and vice versa. If a participant carries *M. catarrhalis* in the OP, they are likely to carry it in the nose (OR 5.628) and vice versa.

When carriage by site was analysed for each age range (Figure 9), the NP swab showed higher *M. catarrhalis* carriage then the OP and Nose swab for all ages except 50+. For those aged 50+ the nasal swab does seem to detect slightly more *M. catarrhalis* than the NP, however this difference is not significant (P=0.777).

Adding further to discussions on the effectiveness of different swab types, as anticipated it was not possible to obtain OP swabs from children aged 0-4 years due to their strong gag reflexes and/or inability or unwillingness to hold their mouth open as required (as noted on the questionnaires). Therefore, OP swabs taken from these young children were actually mouth swabs, which could have affected the carriage of *M. catarrhalis* seen in this age group. Nevertheless, Figure 9 shows equally low carriage of *M. catarrhalis* in the OP of all age groups, suggesting inability to take OP swabs may not have affected the detection of *M. catarrhalis* in young children, unless of course child OP carriage is higher than other age groups.


Prevalence of M. catarrhalis carriage (%)
30
25
20
15
10
5
0
NP
OP
Nose
0-4
5-16
17-49
50+
All ages
Carehome

| | | *M. catarrhalis* carriage prevalence | Binomial exact 95% CI | Number of *M. catarrhalis* isolated |
|---|---|---|---|---|
| 0-4 | NP carriage | 23.0 | 17.4-29.5 | 46 |
| | OP carriage | 1.1 | 0.1-3.9 | 2 |
| | Nose carriage | 18.2 | 13.1-24.3 | 36 |
| 5-16 | NP carriage | 6.8 | 4.3-10.0 | 23 |
| | OP carriage | 1.2 | 0.3-3.0 | 4 |
| | Nose carriage | 5.6 | 3.4-8.6 | 19 |
| 17-49 | NP carriage | 2.1 | 0.9-3.3 | 11 |
| | OP carriage | 1.7 | 0.8-3.2 | 9 |
| | Nose carriage | 2.1 | 0.9-3.3 | 11 |
| 50+ | NP carriage | 3.4 | 2.0-5.2 | 18 |
| | OP carriage | 0.4 | 0.05-1.4 | 2 |
| | Nose carriage | 4.1 | 2.6-6.1 | 22 |
| All ages (excluding care home) | NP carriage | 6.1 | 5.0-7.3 | 98 |
| | OP carriage | 1.1 | 0.6-1.7 | 18 |
| | Nose carriage | 5.2 | 2.8-4.8 | 88 |
| Care home | NP carriage | 15.0 | 8.1-25.0 | 12 |
| | OP carriage | 3.8 | 0.8-10.7 | 3 |
| | Nose carriage | 13.9 | 7.2-23.5 | 11 |

**Figure 9. NP, OP and nasal carriage prevalences of *M. catarrhalis* by age groups**.

### 3.3.3 Comparison of carriage from time-point one and two

In order to ensure carriage from both time-points are comparable and can therefore be analysed together, carriage prevalence from adults from time-point one and two were investigated to ensure they were comparable. Only adult data was considered as this was the only age group in which considerable recruitment was undertaken during both time-points.

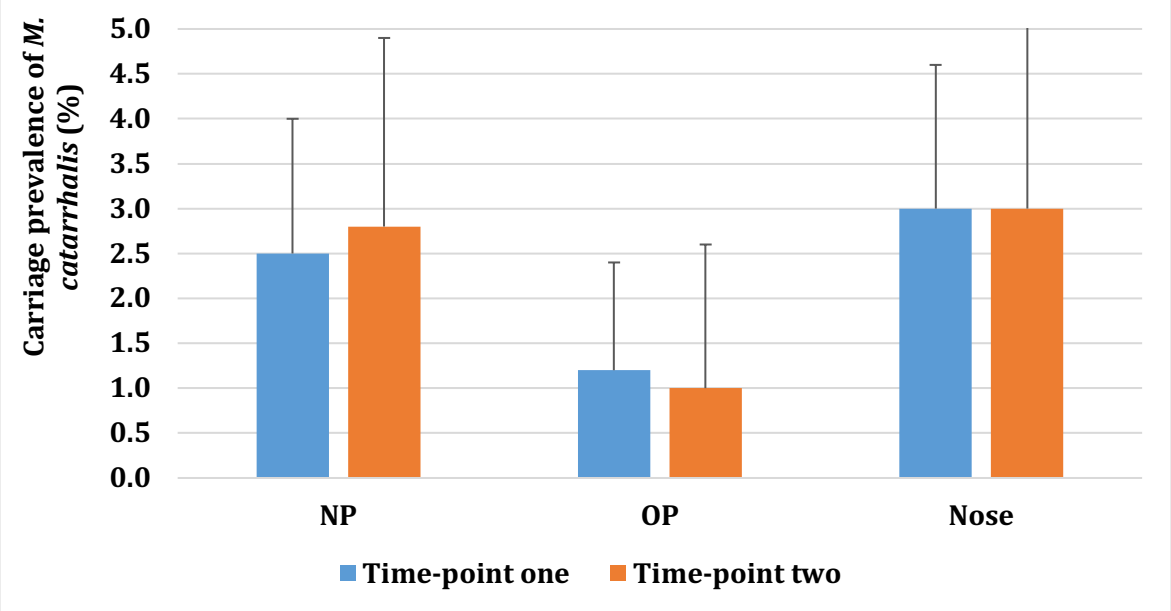



**Figure 10. Comparison of NP, OP and nasal carriage prevalences of *M. catarrhalis* from adult participants from time-point one and two.**

The carriage prevalence of M. catarrhalis in those aged 17 years and over from time-point one was 2.5% (CI: 1.5-4.0%) in the NP, 1.2% (CI: 0.5-2.4%) in the OP and 3.0% (CI: 1.8-4.6%) in the nose. Comparatively, the carriage prevalence of M. catarrhalis in those aged 17 and over from time-point two was 2.8% (CI: 1.4-4.9%) in the NP, 1.0% (CI: 0.3-2.6%) in the OP and 3.0% (CI: 1.6-5.2%) in the nose. Figure 10 shows results from time-point one and two were comparable, suggesting no fluctuations in carriage between the two years. Using X2, M. catarrhalis carriage in the NP, OP and nose showed no significant association with time-point. Therefore, data from time-points one and two were analysed together.

## 3.4 Discussion

Within this chapter, the aim was to assess recruitment and investigate the isolation of *M. catarrhalis* at different sites. A collection of *M. catarrhalis* were isolated from people of all ages and the most effective swabbing site was assessed. As the swab that isolated the highest number of *M. catarrhalis*, NP swabbing was determined to be the most effective for the detection and isolation of *M. catarrhalis* in the URT. Furthermore, NP carriage, above other

carriage, has been linked most to disease (more so OM than COPD) (Faden *et al.*, 1991; McGregor *et al.*, 1998). Therefore, if NP carriage is more clinically significant, sampling the carriage here may be of greater interest for public health. When carriage is broken down by age, the NP swab had a greater swab positivity for 0-4 year olds, 5-16 year olds and care home residents. For 17-49 year olds, NP and nasal swab positivity was the comparable. For those aged 50+, nasal swabs had the greatest swab positivity although this was not significantly higher than the swab positivity of NP swabs. Isolation of *M. catarrhalis* is much lower from OP than the NP or nose for all groups. $X^2$ and OR show carriage in the NP, OP and nose is associated (particularly NP and nasal carriage), so perhaps the use of less invasive nasal swabbing is a sufficient proxy for NP swabbing and should be used if the research environment calls for it. However as stated, if only one swab type is taken, whether NP or nose, carriage will be missed.

Following refinement of certain practical aspects (i.e., which schools could be recruited from), the study design serves as a blueprint for future studies, providing an effective and successful way to obtain HCP samples whilst recruiting high numbers of participants of all ages. Additionally, numerous affiliations and partnerships were created as part of the study, an invaluable resource for future research. Community recruitment targets were achieved (excluding care-homes) and recruitment was shown to be representative of the community sampled, with recruitment obtained from a variety of locations reducing sampling bias. This discussion further considers overall recruitment and study success.

### 3.4.1 Recruitment

Study design led to the successful recruitment of participants of all ages and the achievement of community recruitment targets, including the increased recruitment targets for those aged 17-49 and 50+ years. Recruitment targets for care/nursing home residents were not achieved within the first time-point, however. This was primarily due to recruitment being limited to those with the capacity to provide informed consent. A high proportion of residents in many care/nursing homes did not have capacity (data not collected). Recruitment was also hindered by the number of care/nursing homes that were approached and agreed to act as a host/site for the study. The Solent NHS Trust team had links with several Portsmouth homes; however, it was difficult to obtain research agreements with new care homes with no prior relationship within the timeframe of the first time-point. Recruitment in this cohort was then discontinued for the second time-point in the interest of a care/nursing home specific study being set-up.

Recruitment of children was delayed in time-point one as introductions and partnership had to be established with Sure Start centres and schools before recruitment could be undertaken. Furthermore, the need for recruitment to be accountable to the Solent NHS Trust for EDGE (an online programme for the real-time management of clinical research inclusive of study recruitment) and portfolio accruals meant it was unclear what schools could be approached. Towards the end of time-point one it was confirmed that the Solent NHS Trust cover all schools in Hampshire and the Isle Wight for speech and language therapy, so any school could be approached. This simplified recruitment for time-point two. A consideration for future research is to establish partnerships and connections with potential recruitment sites (including schools and care homes) before the recruitment period starts; delays in responses set-back and prevented recruitment in some sites.

Another factor that hampered recruitment of children, particularly 0-4 year olds, was the low number of staff able to swab this age group. Recruitment of 0-4 year olds was reliant on just two part-time team members. A hindrance to the recruitment of 5-10 year old children, albeit it an expected aspect of GCP guidelines, was the requirement for parents to be present; this meant recruitment mainly occurred on parents' evenings giving limited recruitment opportunities. Delays in responses from some schools meant parents' evenings and therefore recruitment opportunities were missed. For older children, an invitation letter, PIS, consent forms and questionnaire could be sent to parents for signing and then participation could be completed (if they and the child had consented) in their absence whilst the child was at school. Although successful, it did rely on the child remembering to bring in the signed consent form and the school agreeing with researchers attending during school hours.

**Recruitment locations**

A major strength of this study was that recruitment occurred in a large number of sites, including numerous non-NHS sites. Recruiting at multiple Solent NHS Trust sites and sites working in partnership with the Trust maximised recruitment potential, as multiple HCP/researchers could recruit at various locations at any time during the recruitment period. This reduced recruitment bias which would have been caused by recruiting in one/ few locations or during the same limited time. Furthermore, such methodology reduced the recruitment bias of recruiting exclusively from NHS sites. To limit bias further, recruitment at NHS sites wasn't restricted to patients, anyone including friends and family of those attending appointments could participate.

Undertaking recruitment at numerous sites and types of site facilitated the procurement of samples as representative of the Solent community as possible. Of course, no matter where

sampling occurs, there may be some selection/recruitment bias resulting from the systematic difference between those who chose to participate and those who didn't. Whilst every measure was taken to reduce or limit bias, there will always be some aspect of bias in a study so the sample recruited/data collected will never be completely representative of the general population.

### 3.4.2 Summary of study benefits and weaknesses

There were many benefits of the Solent SMART Study. Firstly, recruitment was as representative of the community as possible (i.e., all ages, ethnicities …) therefore, the study obtained bacterial samples as representative as possible of carriage throughout the community. The achievement of recruitment targets (including increased 17+ recruitment) highlights the successful study design, whilst the study proved it feasible of gaining high recruitment of participants of all ages, when using community-based HCP sampling methods. The study also provided evidence that it is not possible to obtain OP swabs in young children (as assumed) and that NP swabs are the most effective swab type for isolating *M. catarrhalis* from the URT, in children and the elderly especially.

The study generated novel epidemiology data in elderly care/nursing home residents. Studies in this cohort have commonly focused on one bacterial species in particular (*S. aureus* or *Streptococcus* are common focuses) (Barr *et al.*, 2007; Milne *et al.*, 2011; Cummins *et al.*, 2012; Elias *et al.*, 2013; Jans *et al.*, 2013; Lee *et al.*, 2013; Budimir *et al.*, 2014; Datta *et al.*, 2014; Gibson *et al.*, 2014; Bellini *et al.*, 2015; Jallad *et al.*, 2015; Jump and Donskey, 2015; Willemsen *et al.*, 2015; Dandachi *et al.*, 2016; Ismail *et al.*, 2016; Batina, Crnich and Dopfer, 2017; Becker and Diel, 2017; Kolsek-Sustersic *et al.*, 2017; Rodrigues *et al.*, 2017; da Silveira *et al.*, 2018; Kwetkat *et al.*, 2018)) whilst *M. catarrhalis* carriage has not been investigated. As a major cause of exacerbation in COPD there is certainly a need to investigate *M. catarrhalis* carriage in this vulnerable cohort, who often suffer from COPD. The study successfully recruited 79 participants from care/nursing homes and highlighted a particularly high prevalence of *M. catarrhalis*. However, by limiting participation to those with mental capacity to consent it is possible this could have caused a selection bias if for example patients with dementia are more or less likely to be carrying *M. catarrhalis*. The study also recruited 105 participants with COPD. Carriage of *M. catarrhalis* in these cohorts was investigated more in chapter 4.

This study also provides data on the homeless; an understudied cohort at risk of RTI and an overlooked part of the population. An initial insight into the carriage of *M. catarrhalis* is given in Chapter 4. There have been very few studies that have investigated bacterial carriage or

disease in the homeless, and those that do commonly focus on one pathogen such as Streptococcus (Mosites *et al.*, 2019), Tuberculosis (von Streit *et al.*, 2019), *S. aureus* (Conceicao *et al.*, 2019) or a bacteria that has caused an outbreak of infection in the homeless community (Bubba *et al.*, 2019). *M. catarrhalis* carriage and disease have not been investigated in the homeless. Unfortunately, having only recruited a small sample number (n=23) no further analysis will be undertaken on this subset. Therefore, further research would be of benefit in the future as this cohort is reported to have a high prevalence of chronic respiratory diseases (such as bronchitis, asthma and COPD) (Usatine *et al.*, 1994) with respiratory disease frequently associated with death (Ly *et al.*, 2019). To this end, the recruitment of homeless participants in the Solent SMART Study does inform on the feasibility of future research in this cohort. Recruitment was difficult as potential participants were cautious of researchers and suspicious of sampling, whilst the Homeless Health Centre was small and hard to recruit in. Recruitment of this cohort is more difficult in winter, unless approaching out on the streets instead of at a homeless health hub or shelter. This is because liveable street space can be hard to come by so the homeless are reluctant to move as they risk losing their safe space, so are less likely to attend any homeless health/support sites so are missed if recruitment is restricted to such a site.

Despite the successes of the study, there were a several potential weaknesses. One of the aims of the Solent SMART Study, as outlined in the methods, was to inform sample size calculations for future studies. This study will provide data on carriage prevalence and thus information such as how many people need to be recruited to gain x number of isolates. However, the study failed to obtain data on recruitment success rates; no data was collected on the number of people approached vs the number of people who consented to take part. A form to collect such data was made, however, it wasn't completed during recruitment. Nevertheless, with this study design and recruitment strategy, such information is arguably unnecessary.

A weakness of the study was the need for recruitment to be accountable to the Solent NHS Trust for EDGE and portfolio accruals, to ensure the Trust were recognised for their recruitment efforts in terms of CRN recruitment figures and subsequent research funding allocations. To an extent this limited where recruitment could take place. Recruitment could not be undertaken at certain community sites, such as supermarkets, as those recruited may not be Solent NHS Trust service users which would make portfolio and EDGE accruals difficult. Such restrictions could be avoided in future studies, for example by having University led recruitment or requesting details of participant's GP as part of the questionnaire and allocating recruitment to the applicable NHS Trust.

Another aspect that could be described as a weakness is that fact the PIS was only written in English, perhaps excluding some from participating? Though the use of a non-English PIS, without a researcher that also speaks that language could, if not careful, lead to someone taking part without truly being able to give informed consent as the research may not be fully aware if they don't understand the information provided. Having a braille version would have been a more inclusive approach, nevertheless the lack of such version did not exclude anyone from taking part. Researchers happily read the PIS and described the study to those with impaired sight, or indeed those who were illiterate, and assisted with the completion of study documents.

The wording of parts of the questionnaire could have been improved. In particular, the smoking questions could have been streamlined, whilst the answer options for ethnicity should have been more comprehensive to make analysis more straightforward. For example, questions 9 and 10 could have been condensed into one:

**9. Do you smoke cigarettes and/or cigars?**

Yes ☐ No ☐ Would rather not say ☐

**10. Do you smoke e-cigarettes?**

Yes ☐ No ☐ Would rather not say ☐

To

**. Do you smoke?**

Cigarettes ☐ Cigars ☐ E-cigarettes ☐ Nothing ☐

Would rather not say ☐

In terms of ethnicity quite a few participants were unsure how they fit into the options / didn't fit so completed the 'other not listed' option. As a result, there were a lot of ununiformed or ambiguous answers which made analysis more complicated. As a result, WHO defined regions were used when analysing ethnicity rather than the standard ethnicity classifications. Such issues were not flagged when such ethnicities were used in prior studies, most likely due the majority of participates being white British making answering more straight-forward. More extensive PPI involvement could have helped improve the questionnaire design to make it more efficient. An alternative way to avoid such issues would have been to gain approval to access participant's medical records to confirm ethnicity, as well as vaccine status, long term health status, use of antibiotics etc.

Lastly, to try and ascertain reproducibility of the study, participants were asked if they would take part in this or a similar study in the future. However, such data could be more a measure of acceptability at the time of the study, rather than a measure of reproducibility as it only captures intentions which may not be good predictors of future studies. Nevertheless, all aspects of the study design are physically reproducible and perhaps in the wake of the covid-19 pandemic swabbing might even be better tolerated/accepted, which could have a positive effect on recruitment. Of course reproducibility can also refer to the ability of independent researchers to come to similar results and conclusions either by re-running the same analysis strategy on the data or when replicating the study. Statistical analysis was robust, therefore reanalysis should provide comparable results. Similarly, whilst replicating the study may not yield the same exact carriage rates, it is hoped that overall trends and associations would be observed or even significance clarified where sample size was perhaps inhibitory.

# Chapter 4 Investigation of the epidemiology of *M. catarrhalis* and risk factors associated with *M. catarrhalis* carriage

## 4.1 Introduction

Increasing interest in the development of vaccines against *M. catarrhalis* necessitates a better understanding of carriage and disease epidemiology; both to inform vaccine development and implementation strategies. The current body of knowledge for *M. catarrhalis* is largely based on old studies or isolates from small/limited cohorts and disease cases, with data often being contradictory. Little is published on the potential risk factors of *M. catarrhalis* carriage, and the majority of what is published centres on *M. catarrhalis* in young children (Verhaegh *et al.*, 2010) resulting in a paucity of data from teenagers and adults. Furthermore, comparisons of data or accurate meta-analysis can be difficult due to lack of data from certain cohorts and differences in methodologies between studies.

Presented here is a collection of *M. catarrhalis* isolated as part of the Solent SMART Study. This chapter seeks to establish carriage prevalence across all ages and to define the epidemiology of *M*. catarrhalis in the context of potential risk factors. Vaccination history, disease status, use of antibiotics, smoking status, ethnicity, and bacterial co-carriage are investigated for a possible association with *M. catarrhalis* carriage. Many of these factors have been identified as being associated with increased human RT colonisation by common pathobionts such as *S. pneumoniae*, *H. influenzae* and *S. aureus*. For example, smoking is considered a risk factor for *S. pneumoniae*, *M. catarrhalis* and *S. aureus*, having been positively associated with the carriage of these bacteria (Bogaert *et al.*, 2004; Greenberg *et al.*, 2006; Brook and Gober, 2007; Farida *et al.*, 2014). Likewise, SHS is positively associated with the carriage of *S. pneumoniae* (Lee *et al.*, 2010) and *S. aureus* (Bogaert *et al.*, 2004), in addition to meningococcal carriage and paediatric invasive disease (Lee *et al.*, 2010). Research suggests exposure to tobacco smoke may be associated with increased carriage of *M. catarrhalis* in children (Bakhshaee *et al.*, 2012; Fadlyana *et al.*, 2018) and the elderly (Kurtti *et al.*, 1997), however more research is required. In addition, recent/concurrent RTI has been associated with the carriage of pathobionts (Mackenzie *et al.*, 2010; Fadlyana *et al.*, 2018), with some evidence of an association with carriage of *M. catarrhalis* (Fadlyana et al., 2018). Male gender (Mackenzie *et al.*, 2010; Drayß *et al.*, 2019; Zanella *et al.*, 2019), lack of diabetes, annual flu vaccination, COPD (Zanella *et al.*, 2019) and antibiotic use (Gisselsson-Solen *et al.*, 2014) have

also been linked to increased carriage of common pathobionts such as *S. pneumoniae*, *H. influenzae* and *S. aureus*. Specifically male gender and lack of diabetes, have been associated with *S. pneumoniae* carriage (Mackenzie *et al.*, 2010; Drayß *et al.*, 2019; Zanella *et al.*, 2019), lack of annual flu vaccination and having COPD are risk factors for *H. influenzae* (Zanella *et al.*, 2019) whilst prior antibiotic use is a risk factor for *H. influenzae* and *S. aureus* and potentially *M. catarrhalis* (Gisselsson-Solen *et al.*, 2014; Zanella *et al.*, 2019). Furthermore, there is an overall lack of data regarding the impact of smoking e-cigarettes on bacterial carriage and respiratory disease. With the growing popularity of e-cigarette smoking (Action on Smoking and Health, 2016) and increasing reports of their ill effects on respiratory health (Schier *et al.*, 2019), looking at the impact of these cigarettes on *M. catarrhalis* is both novel and of importance for public health.

In this chapter carriage and co-carriage was determined overall (independent of site) and then by individual sample site. It is known that the nostrils/anterior nares (nose) are lined with keratinised squamous epithelium and sebum-producing glands, which enriches the growth of lipophilic skin colonisers such as *Staphylococcus spp*, *Moraxella spp* and *Streptococcus spp*. However, the NP is lined with a stratified squamous epithelium interspersed by respiratory epithelial cells, and is therefore host to a more diverse microbial community, which further includes *Haemophilus spp*. The OP is lined by a non-keratinized stratified squamous epithelium and hosts the most diverse microbial community, which includes *Neisseria spp* (Man, de Steenhuijsen Piters and Bogaert, 2017). Therefore, carriage and co-carriage may vary depending on site.

Those with COPD are a cohort where the acquisition of *M. catarrhalis* is a risk factor for exacerbation (Wilkinson *et al.*, 2017). Therefore, gaining a better understanding of the carriage of *M. catarrhalis* in this group, and the risk factors of such carriage is beneficial. Similarly, care/nursing home residents are a cohort with no prior data related to *M. catarrhalis*. As a cohort who often suffer from COPD and frequent RTI, there is certainly a need to investigate *M. catarrhalis* carriage in this vulnerable group.

## 4.2 Chapter Aims and Objectives

Aim: Examine the epidemiology of *M. catarrhalis* and identify risk factors associated with carriage.

Hypothesis 1: The prevalence of *M. catarrhalis* is higher in children and the elderly.

Objectives

- To determine the overall carriage prevalence of *M. catarrhalis* in the community.

- To determine the carriage prevalence of *M. catarrhalis* by anatomical site, in specific age groups and cohorts of interest; namely elderly care/nursing home residents and those with COPD.

Hypothesis 2: The presence of *S. pneumoniae* and / or *H. influenzae* results in an increased carriage of *M. catarrhalis*.

Objectives

- To determine the prevalence and impact of bacterial co-colonisation with *M. catarrhalis.*
- To determine whether co-carriage of *M. catarrhalis* with other respiratory pathogens occurs more with in specific age groups and cohorts of interest; namely elderly care/nursing home residents in addition to those with COPD.

Hypothesis 3: Smoking is associated with an increased prevalence of *M. catarrhalis* with differential risks according to type of smoking.

Objectives

- To compare carriage of *M. catarrhalis* in non-smokers, cigarette/cigar smokers and e-cigarette smokers.
- To identify other risk factors associated with carriage of *M. catarrhalis*.

## 4.3 Methods

In this chapter, the prevalence of *M. catarrhalis* carriage was investigated by anatomical site and by age:

Carriage prevalence in the NP/OP/nose (%) =

(Frequency of *M. catarrhalis* isolation in the NP, OP or nose/total number of participants who had their NP or OP or Nose swabbed) × 100

Carriage prevalence in the NP/OP/nose of those in a certain age group or cohort (%) =

(Frequency of *M. catarrhalis* isolation in the NP, OP or nose of those in a certain age group or cohort /total number of participants in that age group or cohort who had their NP, OP or nose swabbed) × 100

True carriage was also determined as an overall (analysis included data from all participants) and then by age group.

True carriage (%) =

(Frequency of participants positive for *M. catarrhalis*/total number of participants swabbed) × 100

Co-colonisation was calculated as a percentage of *M. catarrhalis* isolated, and was determined by site and by age group/cohort:

Co-colonisation prevalence with bacteria of interest in the NP, OP or nose (%) =

(Number of *M. catarrhalis* isolated with bacteria of interest from the same NP, OP or nose swab/ total number of *M. catarrhalis* isolated from NP, OP or nose swabs) × 100

Co-colonisation in a certain age group or cohort (%) =

(Number of *M. catarrhalis* isolated with bacteria of interest in a certain age group or cohort / total number of *M. catarrhalis* isolated from that age group or cohort) × 100

Co-colonisation in the NP. OP or nose in a certain age group or cohort (%) =

(Number of *M. catarrhalis* isolated with bacteria of interest from the same NP, OP or nose swab in a certain age group or cohort / total number of *M. catarrhalis* isolated from NP, OP or nose swabs in that age group or cohort) × 100

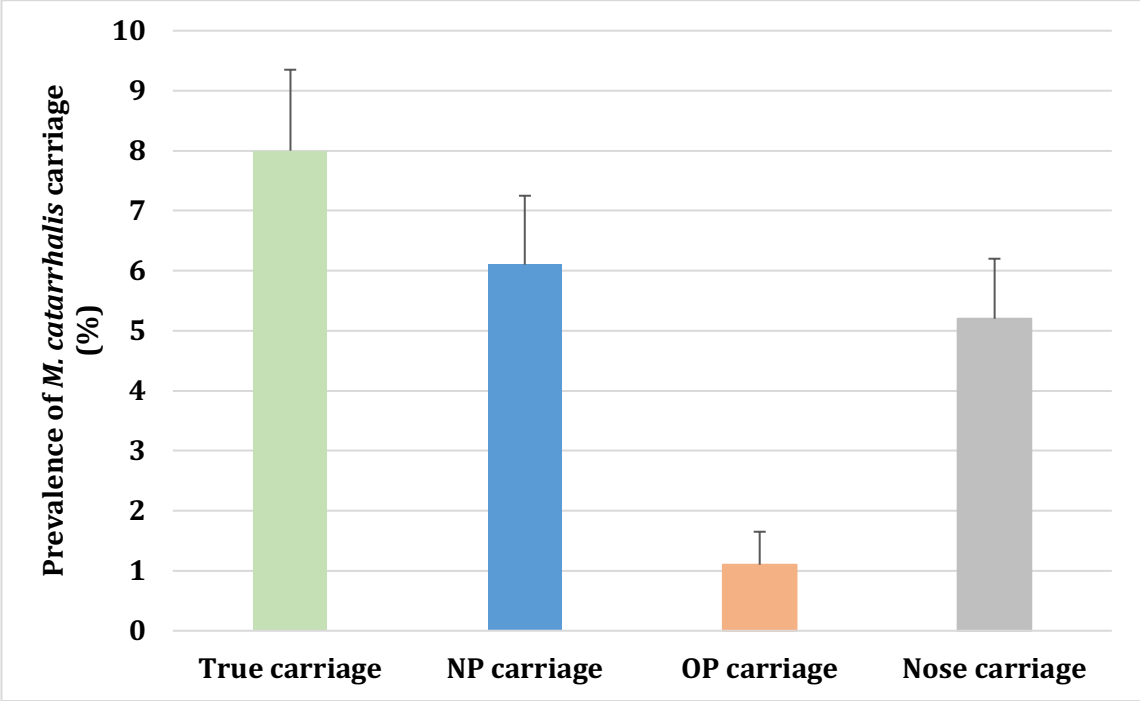
Prevalence of *M. catarrhalis* carriage was calculated by gender, recent RTI status, recent use of antibiotics, long term illness status, diabetes status, vaccination status, smoking status, ethnicity, and day care attendance to understand the effect of these variables on carriage.

Data was then investigated using logistic regression analysis in SPSS version 28. Univariate logistic regression modelling is a method used for modelling binary outcomes (Harrell, 2015). It measures the relationship/association between a dependent variable (i.e., carriage of *M. catarrhalis*, yes vs no) and a set of independent variables (age, ethnicity, gender, recent RTI, antibiotic use). $X^2$ or FET were used to determine whether an association/relationship between an independent and a dependent variable was significant, as demonstrated by the P-value. In cases of significant association, an odds ratio (OR) was calculated and used to understand the effect of the independent variable on the dependent variable (McNamee, 2005). Univariate logistic regression modelling was also undertaken on data stratified by participant swabbing site and/age group. To counteract confounding, multivariate logistic regression analysis of the entire culture dataset was also undertaken.

## 4.4 Results

### 4.4.1 Carriage prevalence of *M. catarrhalis*

The true carriage prevalence of *M. catarrhalis* in community-based participants in this study was identified as 8.0%. This excludes data from care/nursing home residents, which this study classes as a separate cohort of interest and not community data. When considering site of carriage, *M. catarrhalis* was most commonly found in the NP (Figure 11). *M. catarrhalis* is carried in the NP, OP and nose of 6.1%, 1.1% and 5.2% of community-based participants, respectively.




| | *M. catarrhalis* carriage prevalence | Binomial exact 95% CI | Number of *M. catarrhalis* isolated |
|---|---|---|---|
| True carriage | 8.0 | 6.7-9.4 | 130 |
| NP carriage | 6.1 | 5.0-7.3 | 98 |
| OP carriage | 1.1 | 0.6-1.7 | 18 |
| Nose carriage | 5.2 | 2.8-6.4 | 88 |

**Figure 11. Prevalence of *M. catarrhalis* carriage.**

Illustrates the true prevalence of *M. catarrhalis* in addition to the carriage prevalence in the NP, OP and nose of community-based participants of all ages; excludes data from care/ nursing home residents.

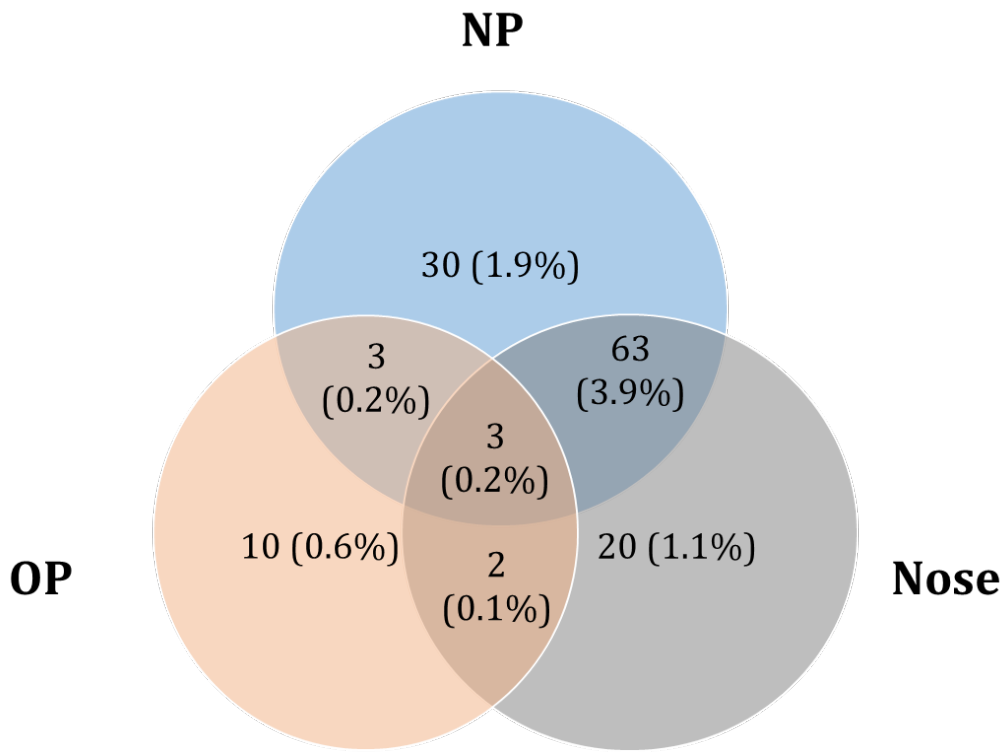



**Figure 12. Carriage of *M. catarrhalis*.**

Illustrates the number and percentage of participants carrying *M. catarrhalis* in just their NP, OP or nose in addition to those with carriage at multiple anatomical sites; excludes data from care/ nursing home residents.

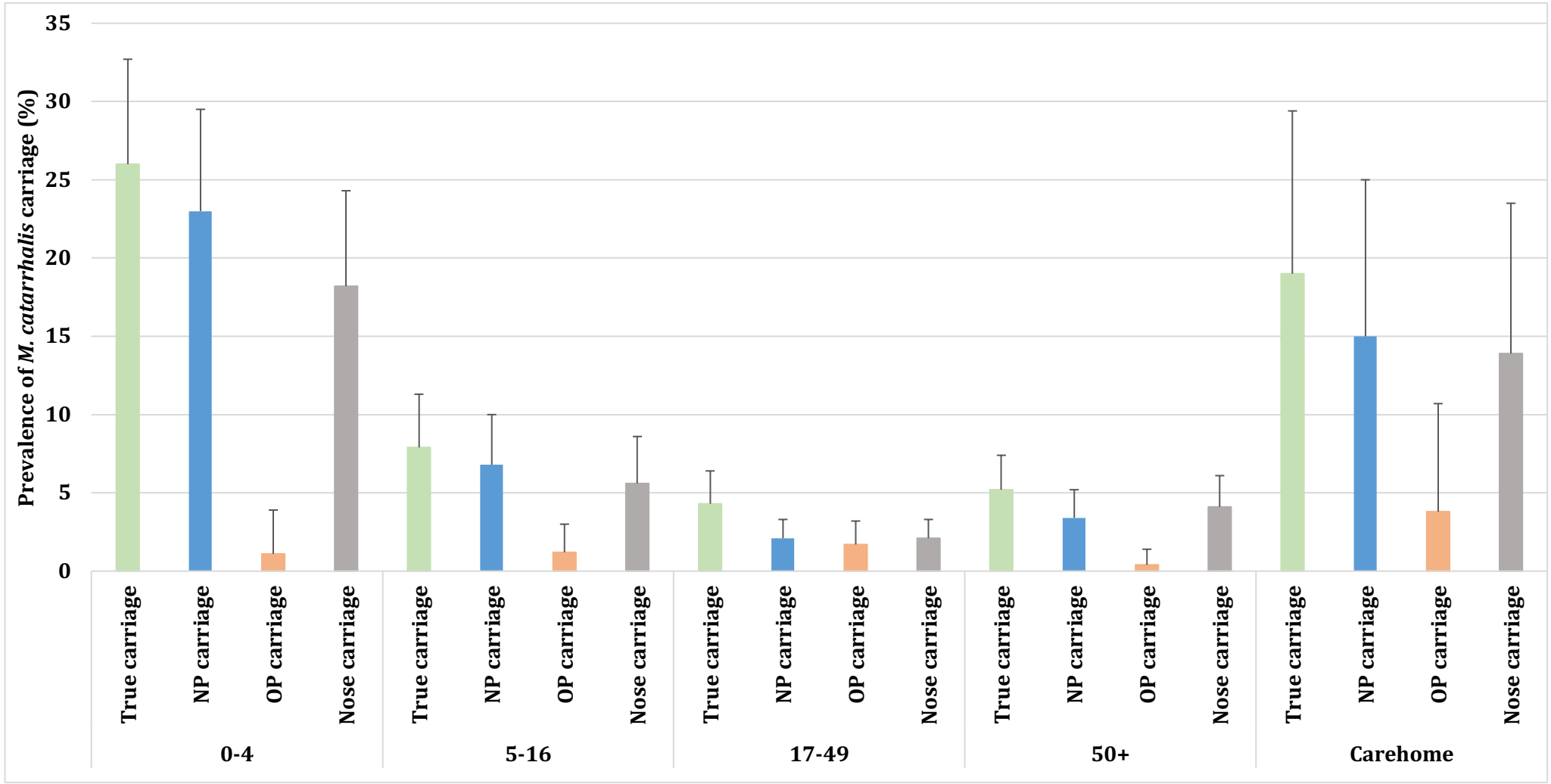
### 4.4.2 Age-stratified prevalence of *M. catarrhalis* carriage

**Carriage in community-based participants and care/nursing home residents.**

*M. catarrhalis* is most commonly carried in 0-4 year olds and care/nursing home residents (Figure 13). Using $X^2$, *M. catarrhalis* carriage was significantly associated with age group/cohort (P=<0.001, df =4). Using the 0-4 year group as a reference, those aged 5-16, 17-49, 50+ and those in care homes are less likely to carry *M. catarrhalis* with ORs of 0.293, 0.122, 0.165 and 0.768 respectively.

Using $X^2$, overall *M. catarrhalis* carriage in the NP (P=<0.001, df =4) and nose (P= <0.001 df =4) was shown to be significantly associated with age. However, there was no significant association between OP carriage and age (P=0.060, df =4).

Further consideration of age and carriage site shows that older children aged 5-16 are significantly less likely to carry *M. catarrhalis* in the NP and nose (P=<0.001; OR=0.242 and P=0.01; OR=0.278 respectively) than children aged 0-4. Adults aged 17-49 are significantly less likely to carry *M. catarrhalis* in the NP and nose (P=<0.001; OR=0.070 and P=0.01; OR=0.098 respectively) than children aged 0-4. Adults aged 50+ are significantly less likely to carry *M. catarrhalis* in the NP and nose (P=<0.001; OR=0.115 and P=0.01; OR=0.199 respectively) than children aged 0-4. Care home residents are significantly less likely to carry *M. catarrhalis* in the NP and nose (P=<0.001; OR=0.600 and P=0.01; OR=0.763 respectively) than children aged 0-4.


Prevalence of M. catarrhalis carriage (%)
35
30
25
20
15
10
5
0
True carriage
NP carriage
OP carriage
Nose carriage
0-4
5-16
17-49
50+
Carehome

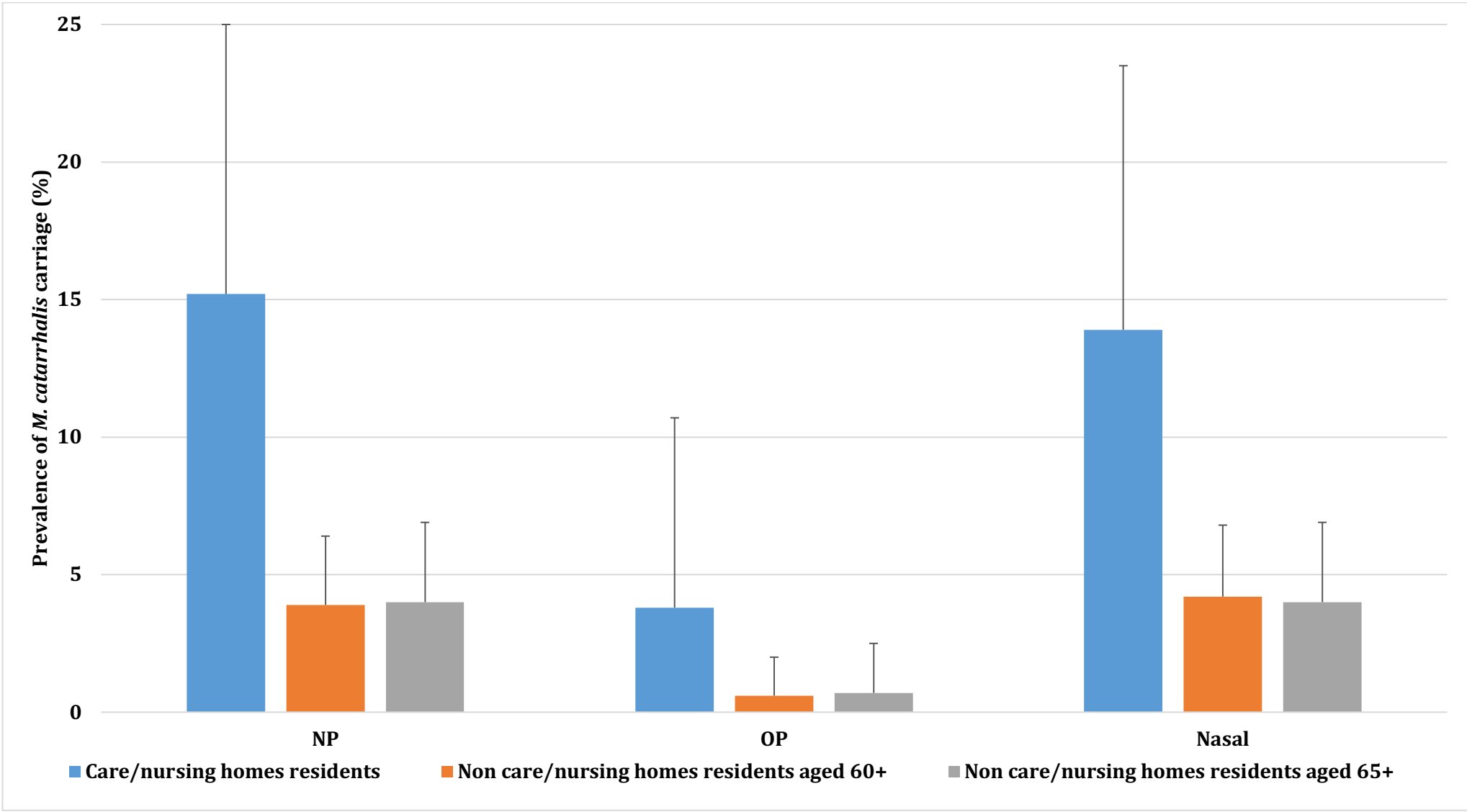
| | | *M. catarrhalis* Carriage prevalence | Binomial exact 95% CI | Number of *M. catarrhalis* isolated |
|---|---|---|---|---|
| 0-4 | True carriage | 26.0 | 20.1-32.7 | 52 |
| | NP carriage | 23.0 | 17.4-29.5 | 46 |
| | OP carriage | 1.1 | 0.1-3.9 | 2 |
| | Nose carriage | 18.2 | 13.1-24.3 | 36 |
| 5-16 | True carriage | 7.9 | 5.3-11.3 | 27 |
| | NP carriage | 6.8 | 4.3-10.0 | 23 |
| | OP carriage | 1.2 | 0.3-3.0 | 4 |
| | Nose carriage | 5.6 | 3.4-8.6 | 19 |
| 17-49 | True carriage | 4.3 | 2.7-6.4 | 23 |
| | NP carriage | 2.1 | 0.9-3.3 | 11 |
| | OP carriage | 1.7 | 0.8-3.2 | 9 |
| | Nose carriage | 2.1 | 0.9-3.3 | 11 |
| 50+ | True carriage | 5.2 | 3.5-7.4 | 28 |
| | NP carriage | 3.4 | 2.0-5.2 | 18 |
| | OP carriage | 0.4 | 0.05-1.4 | 2 |
| | Nose carriage | 4.1 | 2.6-6.1 | 22 |
| Care home | True carriage | 19.0 | 11.0-29.4 | 15 |
| | NP carriage | 15.0 | 8.1-25.0 | 12 |
| | OP carriage | 3.8 | 0.8-10.7 | 3 |
| | Nose carriage | 13.9 | 7.2-23.5 | 11 |

**Figure 13. True carriage prevalence and carriage prevalence of *M. catarrhalis* in the NP, OP and nose by age group**.

It is unclear whether the high carriage of *M. catarrhalis* in care/nursing home residents is due to the higher age distribution of residents or the home environment itself. This was therefore investigated (Figure 14). Although the age range of care/nursing home residents was 44-100, only two participants were under the age of 60 years, so an age range of 60+ years was included as a community comparison for further analysis. Because most participants were aged 65+ years (n= 73, 92.4%), a 65+ age range was also included in Figure 14. This highlighted the minimal difference in *M. catarrhalis* carriage prevalence between 60+ and 65+year olds.

Care/nursing home residency was shown to be significantly associated with *M. catarrhalis* carriage (P=<0.001, df 1, OR 4.440). When considering the different swab types, there was a significant association between care/nursing home residency and NP (P= <0.001, OR 4.753), OP (P=0.043, OR 7.026) and nasal (P=0.002, OR 3.975) carriage of *M. catarrhalis*.

As community based participants aged 75 and over could arguably be described as more similar to the care home population, this community age range was also compared to the care home population. Again care/nursing home residency was shown to be significantly associated with *M. catarrhalis* carriage (P=<0.001, df 1, OR 5.221). When considering the different swab types, there was a significant association between care/nursing home residency and NP (P= 0.002, OR 6.328) and nasal (P=0.002, OR 8.654) carriage of *M. catarrhalis*. Care home residency was not significantly associate with OP carriage (P= 0.073), likely due to the low sample size.

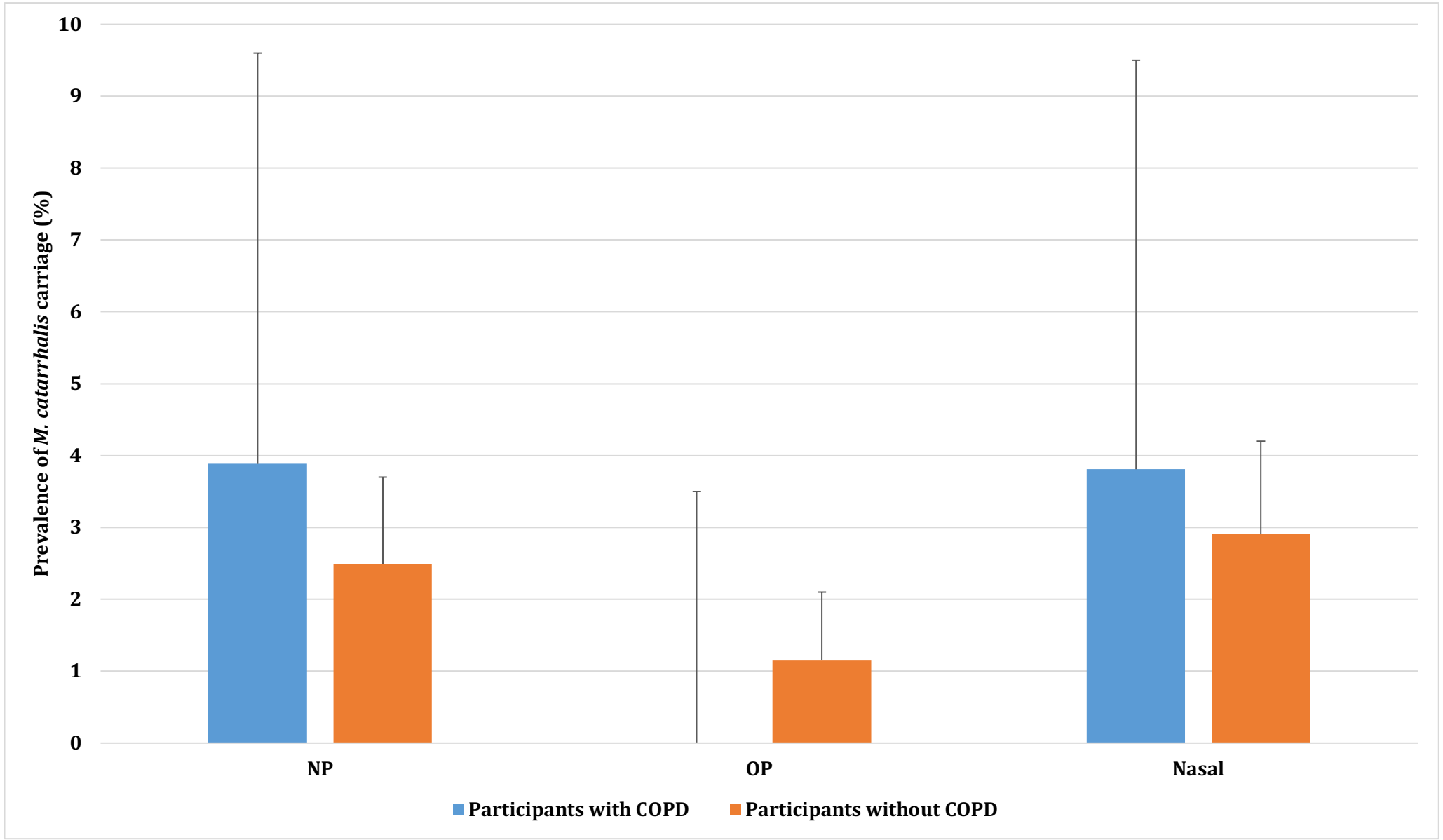

25
20
15
10
5
0
Prevalence of M. catarrhalis carriage (%)
NP
OP
Nasal
Care/nursing homes residents
Non care/nursing homes residents aged 60+
Non care/nursing homes residents aged 65+

| | | NP | | | | OP | | | | nasal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a nasal swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| Care/nursing homes residents | 79 | 79 | 12 | 15.2 | 8.1-25.0 | 79 | 3 | 3.8 | 0.8-10.7 | 79 | 11 | 13.9 | 7.2-23.5 |
| Community participants aged 60+ | 364 | 360 | 14 | 3.9 | 2.1-6.4 | 352 | 2 | 0.6 | 0.1-2.0 | 360 | 15 | 4.2 | 2.4-6.8 |
| Community participants aged 65+ | 301 | 297 | 12 | 4.0 | 2.1-6.9 | 292 | 2 | 0.7 | 0.1-2.5 | 298 | 12 | 4.0 | 2.1-6.9 |

**Figure 14. Prevalence of *M. catarrhalis* in care/nursing home residents and community-based elderly participants**.

**COPD**

COPD patients were another cohort of interest as *M. catarrhalis* is a common cause of exacerbation in this group. Those with COPD had a higher carriage prevalence of *M. catarrhalis* than those of the same age range without COPD (Figure 15). However, $X^2$ shows differences are not statistically significant; COPD is not significantly associated with *M. catarrhalis* carriage (P=0.097, df 1, OR 0.583). When considering the different swab sites, again there was no significant association between NP (P=0.213), OP (P=0.645) or nose (P=0.282) carriage of *M. catarrhalis* and COPD status.


10
9
8
7
6
5
4
3
2
1
0
Prevalence of M. catarrhalis carriage (%)
NP
OP
Nasal
Participants with COPD
Participants without COPD

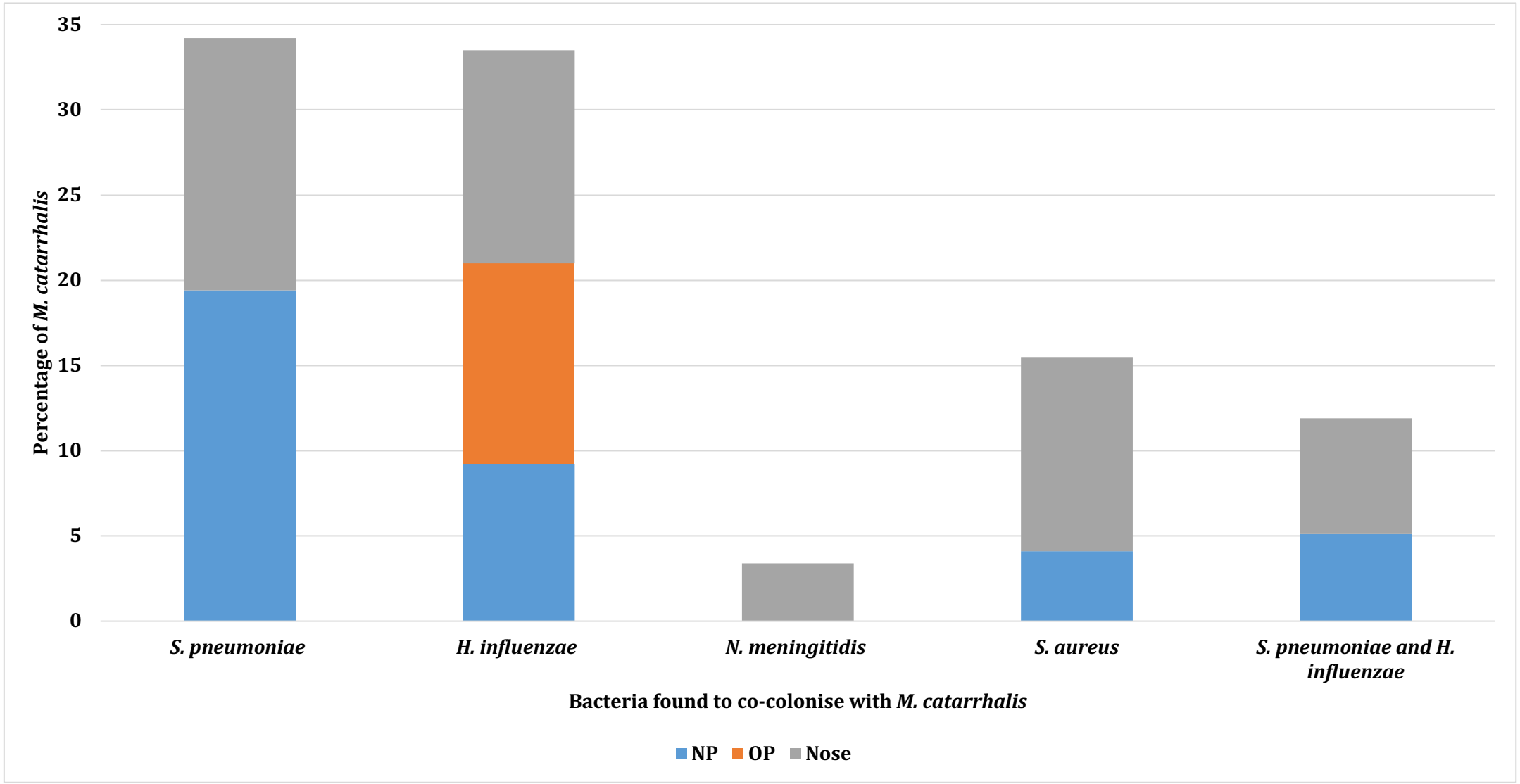
| | | NP | | | | OP | | | | nasal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a nasal swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| COPD | 106 | 103 | 4 | 3.9 | 1.1-9.6 | 105 | 0 | 0.0 | 0.0-3.5 | 105 | 4 | 3.8 | 1.0-9.5 |
| without COPD | 969 | 965 | 24 | 2.5 | 1.6-3.7 | 949 | 11 | 1.2 | 0.6-2.1 | 964 | 28 | 2.9 | 1.9-4.2 |

**Figure 15. Carriage prevalence of *M. catarrhalis* in participants aged 17+ with and without COPD**.

Ages 17-49 and 50+ years were analysed together as there were only 2 participants with COPD in the 17-49 age range, which was too low to provide any statistically reliable data. Excludes data from care/nursing home residents.

### 4.4.3 *M. catarrhalis* co-colonisation

Of the 228 *M. catarrhalis* isolated as part of this study, 202 were isolated from community-based participants whilst 26 were isolated from care/nursing home residents. Only 3.9% (n=1) of the isolates obtained from care home residents were found to co-colonise, compared to 27.7% (n=56) of community isolates. The single co-colonising *M. catarrhalis* isolated from care/nursing home residents was found to co-colonise with *S. aureus*. The bacteria most found to co-colonise with *M. catarrhalis* isolated from community-based participants was *S. pneumoniae* and *H. influenzae* (Figure 16). A statistically significant association was found between the community carriage of *M. catarrhalis* and *S. pneumoniae* (P= <0.001, df = 1, OR 5.908), *M. catarrhalis* and *H. influenzae* (P= <0.001, df = 1, OR 3.397) and *M. catarrhalis* and *S. aureus* (P= <0.001, df = 1, OR 0.470). No significant relationship was found between *M. catarrhalis* and *N. meningitidis* (P= 0.219). No statistical analysis was undertaken for care/nursing home co-colonisation due to the lack of data.


35
30
25
20
15
10
5
0
Percentage of M. catarrhalis
S. pneumoniae
H. influenzae
N. meningitidis
S. aureus
S. pneumoniae and H. influenzae
Bacteria found to co-colonise with M. catarrhalis
NP
OP
Nose

| | *M. catarrhalis* isolated from the NP (N= 98) | | | *M. catarrhalis* isolated from the OP (N = 17) | | | *M. catarrhalis* isolated from the nose (N = 88) | | |
|---|---|---|---|---|---|---|---|---|---|
| *M. catarrhalis* co-colonisation with: | Number co-colonising | Co-carriage prevalence | Binomial exact 95% CI | Number co-colonising | Co-carriage prevalence | Binomial exact 95% CI | Number co-colonising | Co-carriage prevalence | Binomial exact 95% CI |
| *S. pneumoniae* | 19 | 19.4 | 12.1-28.6 | 0 | 0 | 0.0-19.5 | 13 | 14.8 | 8.2-24.2 |
| *H. influenzae* | 9 | 9.2 | 4.3-16.7 | 2 | 11.8 | 1.5-36.4 | 11 | 12.5 | 6.5-21.5 |
| *N. meningitidis* | 0 | 0 | 0.0-3.7 | 0 | 0 | 0.0-19.5 | 3 | 3.4 | 0.7-9.7 |
| *S. aureus* | 4 | 4.1 | 1.1-10.1 | 0 | 0 | 0.0-19.5 | 10 | 11.4 | 4.8-18.7 |
| *S. pneumoniae* and *H. influenzae* | 5 | 5.1 | 1.7-11.5 | 0 | 0 | 0.0-19.5 | 6 | 6.8 | 2.6-14.4 |

**Figure 16. Co-colonisation of *M. catarrhalis* with other bacterial pathogens**.

The percentage of the *M. catarrhalis* isolated which were found to co-colonise with *S. pneumoniae*, *H. influenzae*, *N. meningitidis, S. aureus* or both *S. pneumoniae* and *H. influenzae* at each site of swabbing is reported.

#### 4.4.3.1 Co-colonisation at different swab sites

**Co-carriage in the NP**

Of the 98 *M. catarrhalis* isolated from the NP, 32.7% (CI: 23.5-42.9%) were co-colonised with other bacteria of interest. When investigating NP co-carriage, a statistically significant positive association (and therefore increased risk of carriage) was found between the carriage of *M. catarrhalis* and *S. pneumoniae* (P= <0.001, df = 1, OR 5.868) and *M. catarrhalis* and *H. influenzae* (P= 0.009, OR 2.863). A statistically significant negative association (and therefore decreased risk of carriage) was seen between *M. catarrhalis* and *S. aureus* (P= <0.001, df = 1, OR 0.220).

**Co-carriage in the OP**

Of the 17 *M. catarrhalis* isolated from the OP, 11.8% (CI: 1.5-36.4%) were co-colonised with other bacteria of interest. When investigating OP co-carriage, no significant relationship was found between *M. catarrhalis* and any other bacteria; *M. catarrhalis* and *S. pneumoniae* (P= 1.000), *M. catarrhalis* and *H. influenzae* (P= 0.227). *M. catarrhalis* and *N. meningitidis* (P= 1.000) and *M. catarrhalis* and *S. aureus* (P= 1.000).

**Co-carriage in the nose**

Of the 88 *M. catarrhalis* isolated from the nose, 42.1% (CI: 31.6-53.1%) were co-colonised with other bacteria of interest. When investigating nasal co-carriage, a statistically significant positive association was found between the carriage of *M. catarrhalis* and *S. pneumoniae* (P= <0.001, OR 6.401), *M. catarrhalis* and *H. influenzae* (P= <0.001, OR 8.400), and *M. catarrhalis* and *N. meningitidis* (P= 0.001, OR 27.375). A statistically significant negative association was seen between *M. catarrhalis* and *S. aureus* (P= 0.016, df 1, OR 0.467).

#### 4.4.3.2 Co-colonisation in different ages

**Co-carriage in those aged 0-4 years**

Of the 84 *M. catarrhalis* isolated from 0-4 year olds, 40.5% (CI: 29.9-51.8%) were co-colonised with other bacteria of interest. When investigating co-carriage in 0-4 year olds, a statistically significant positive association was found between the carriage of *M. catarrhalis* and *S. pneumoniae* (P= <0.001, df = 1, OR 3.349) and *M. catarrhalis* and *H. influenzae* (P= <0.001, df = 1, OR 2.921).

**Co-carriage in those aged 5-16 years**

Of the 46 *M. catarrhalis* isolated from 5-16 year olds, 28.3% (CI: 16.0-43.5%) were co-colonised with other bacteria of interest. When investigating co-carriage in 5-16 year olds, a

statistically significant positive association was found between the carriage of *M. catarrhalis* and *S. pneumoniae* (P= 0.011, OR 3.707).

**Co-carriage in those aged 17-49 years**

Of the 31 *M. catarrhalis* isolated from those aged 17-49 year olds, 16.1% (CI: 5.5-33.7%) were co-colonised with other bacteria of interest. When investigating co-carriage in 17-49 year olds, a no statistically significant association was found, likely due to the low number of incidences.

**Co-carriage in those aged 50+ years**

Of the 42 *M. catarrhalis* isolated from 50+ year olds, 7.1% (CI: 1.5-19.5%) were co-colonised with other bacteria of interest. When investigating co-carriage in 50+ year olds, no statistically significant association was found, likely due to the low number of incidences.

#### 4.4.3.3 Co-colonisation in COPD patients and care/nursing home residents

No significant association was seen in the co-carriage of *M. catarrhalis* with any of the other bacteria isolated, in both the care home and COPD cohort; likely due to the low level of bacterial isolation in these groups (Figures 17 and 18).

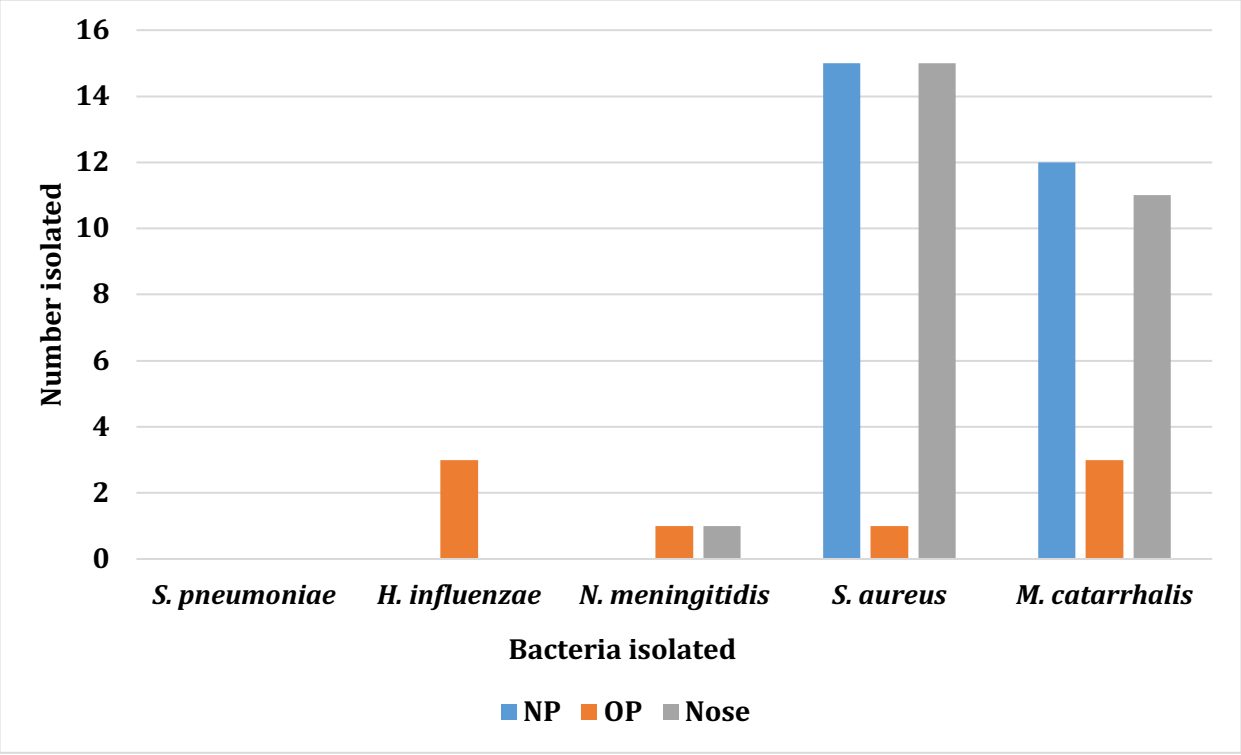



**Figure 17. Number of bacteria isolated from care/nursing home residents**.

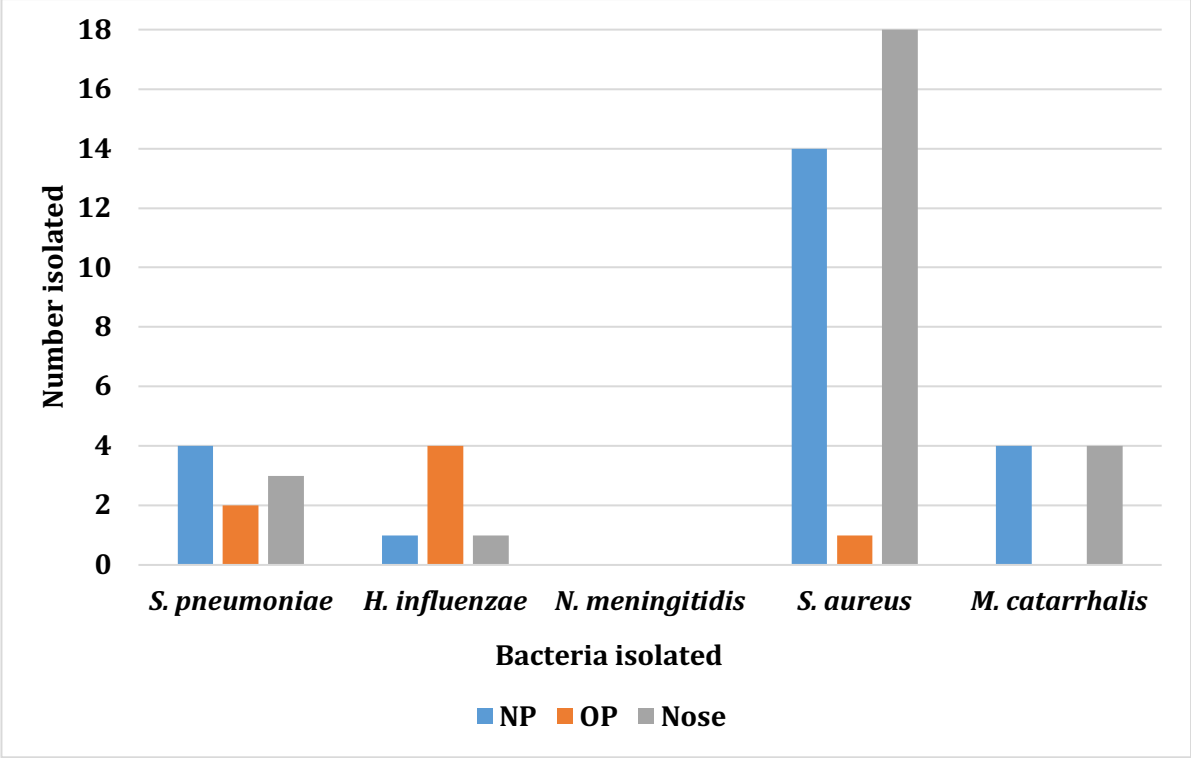
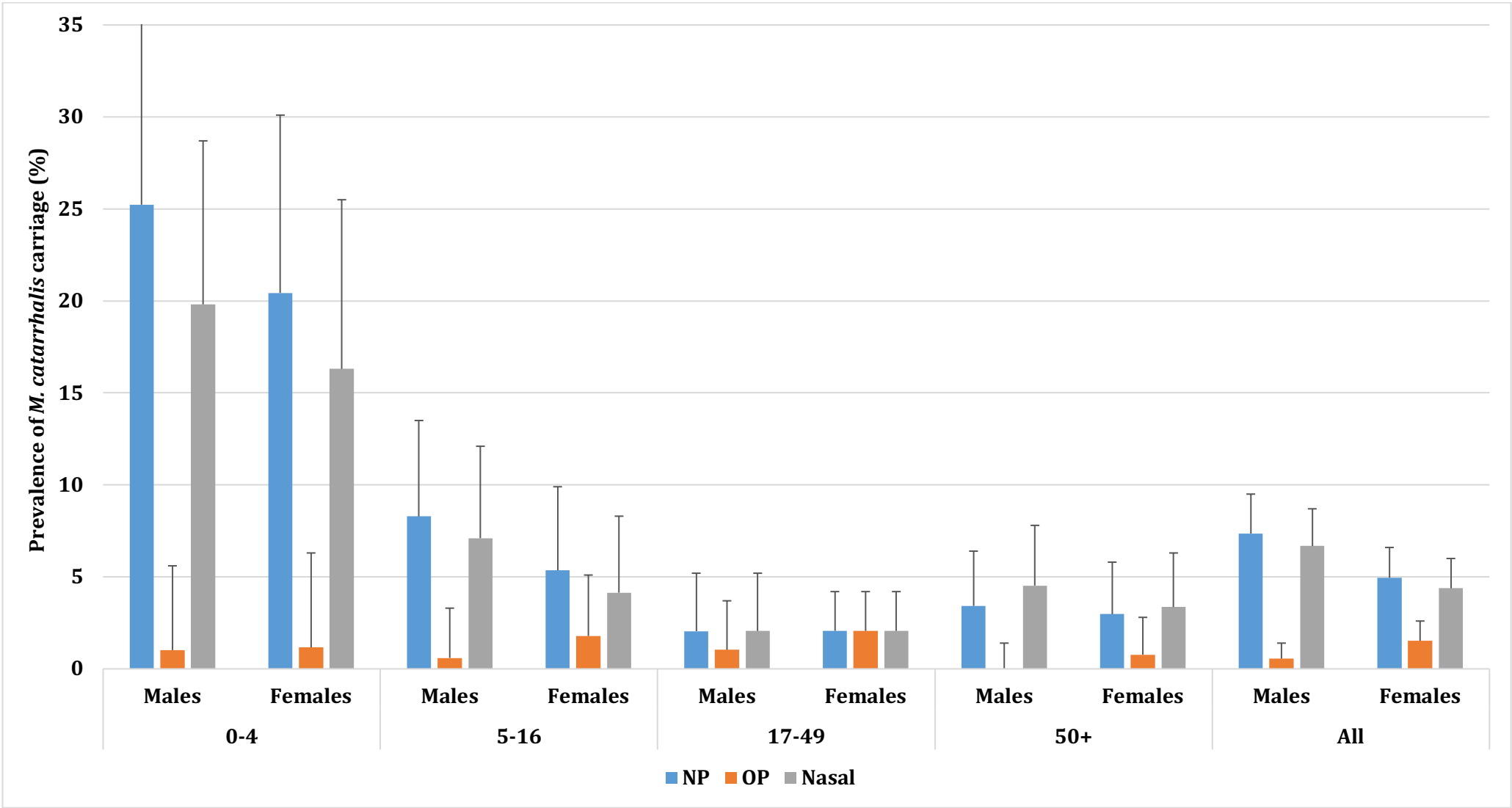



**Figure 18. Number of bacteria isolated from participants with COPD.**

### 4.4.4 Determining risk factors that may be associated with of the carriage of *M. catarrhalis*

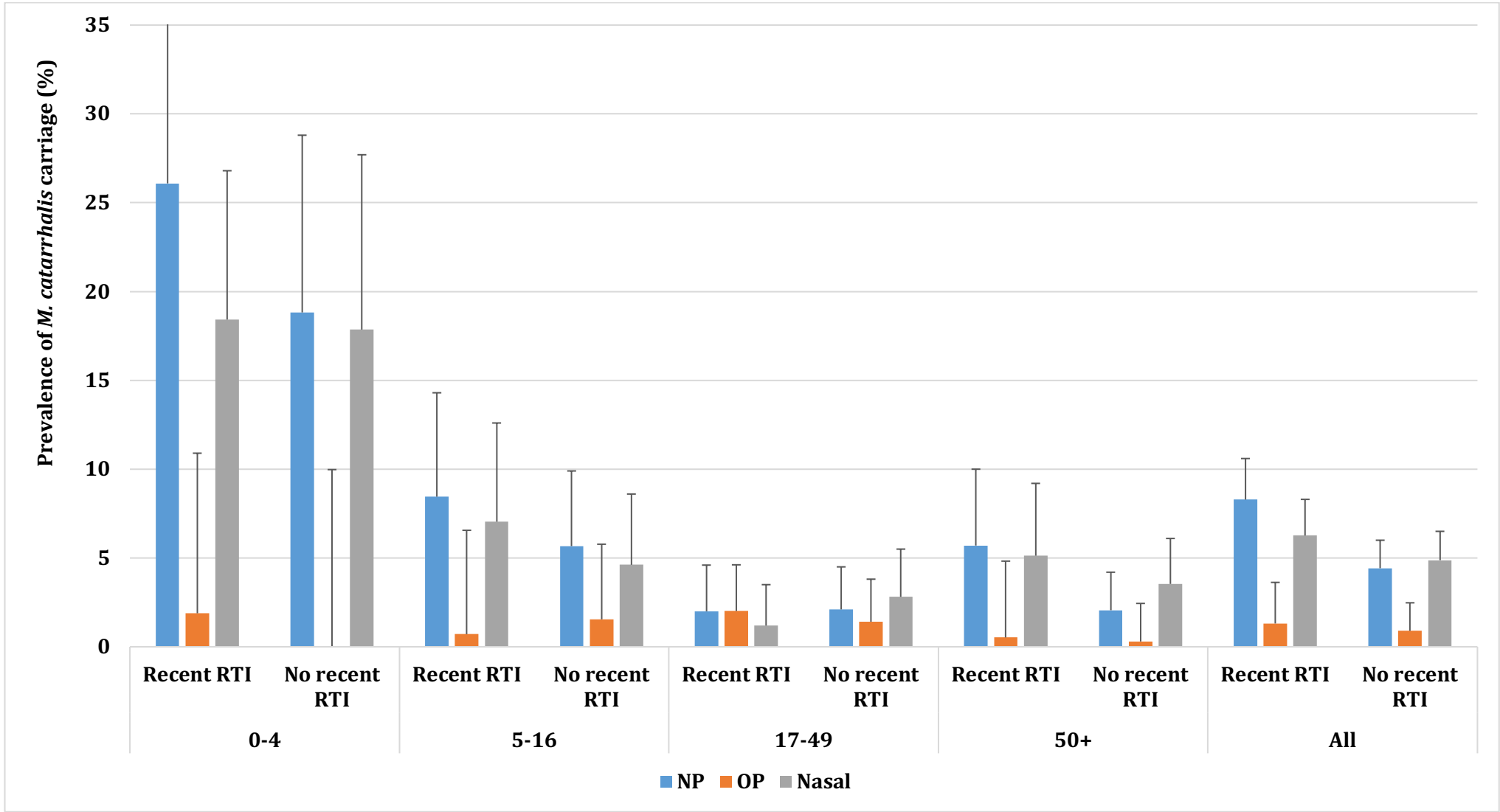
In section 4.4.2, age was established as a risk factor for *M. catarrhalis* carriage, as was care/nursing home residency. In this section gender, vaccination history, disease status, use of antibiotics, smoking history, ethnicity, and nursery attendance are investigated as potential risk factors for the carriage of *M. catarrhalis.*


Prevalence of M. catarrhalis carriage (%)
35
30
25
20
15
10
5
0
Males
Females
Males
Females
Males
Females
Males
Females
Males
Females
0-4
5-16
17-49
50+
All
NP
OP
Nasal

| | | | NP | | | | OP | | | | Nose | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a nasal swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| 0-4 | Males | 107 | 107 | 27 | 25.2 | 17.3-36.4 | 98 | 1 | 1.0 | 0.0-5.6 | 106 | 21 | 19.8 | 12.7-28.7 |
| | Females | 93 | 93 | 19 | 20.4 | 12.8-30.1 | 86 | 1 | 1.2 | 0.0-6.3 | 92 | 15 | 16.3 | 9.4-25.5 |
| 5-16 | Males | 169 | 169 | 14 | 8.3 | 4.6-13.5 | 168 | 1 | 0.6 | 0.0-3.3 | 169 | 12 | 7.1 | 3.7-12.1 |
| | Females | 169 | 168 | 9 | 5.4 | 2.5-9.9 | 168 | 3 | 1.8 | 0.4-5.1 | 169 | 7 | 4.1 | 1.7-8.3 |
| 17-49 | Males | 196 | 195 | 4 | 2.1 | 0.6-5.2 | 193 | 2 | 1.0 | 0.1-3.7 | 194 | 4 | 2.1 | 0.6-5.2 |
| | Females | 340 | 338 | 7 | 2.1 | 0.8-4.2 | 339 | 7 | 2.1 | 0.8-4.2 | 339 | 7 | 2.1 | 0.8-4.2 |
| 50+ | Males | 268 | 263 | 9 | 3.4 | 1.6-6.4 | 262 | 0 | 0.0 | 0.0-1.4. | 265 | 12 | 4.5 | 2.4-7.8 |
| | Females | 269 | 269 | 8 | 3.0 | 1.3-5.8 | 260 | 2 | 0.8 | 0.1-2.8 | 268 | 9 | 3.4 | 1.6-6.3 |
| All | Males | 740 | 734 | 54 | 7.4 | 5.6-9.5 | 721 | 4 | 0.6 | 0.2-1.4 | 734 | 49 | 6.7 | 5.0-8.7 |
| | Females | 871 | 868 | 43 | 5.0 | 3.6-6.6 | 853 | 13 | 1.5 | 0.8-2.6 | 868 | 38 | 4.4 | 3.1-6.0 |

**Figure 19. Carriage prevalence of *M. catarrhalis* in males and females by age**.

When investigating the carriage *M. catarrhalis* from all swab types, no significant association was found between gender and the carriage of *M. catarrhalis* (P= 0.077). When investigating carriage from individual swab types, again there was no significant association (P=0.088, 0.194 and 0.405 for NP, OP and nasal carriage respectively).

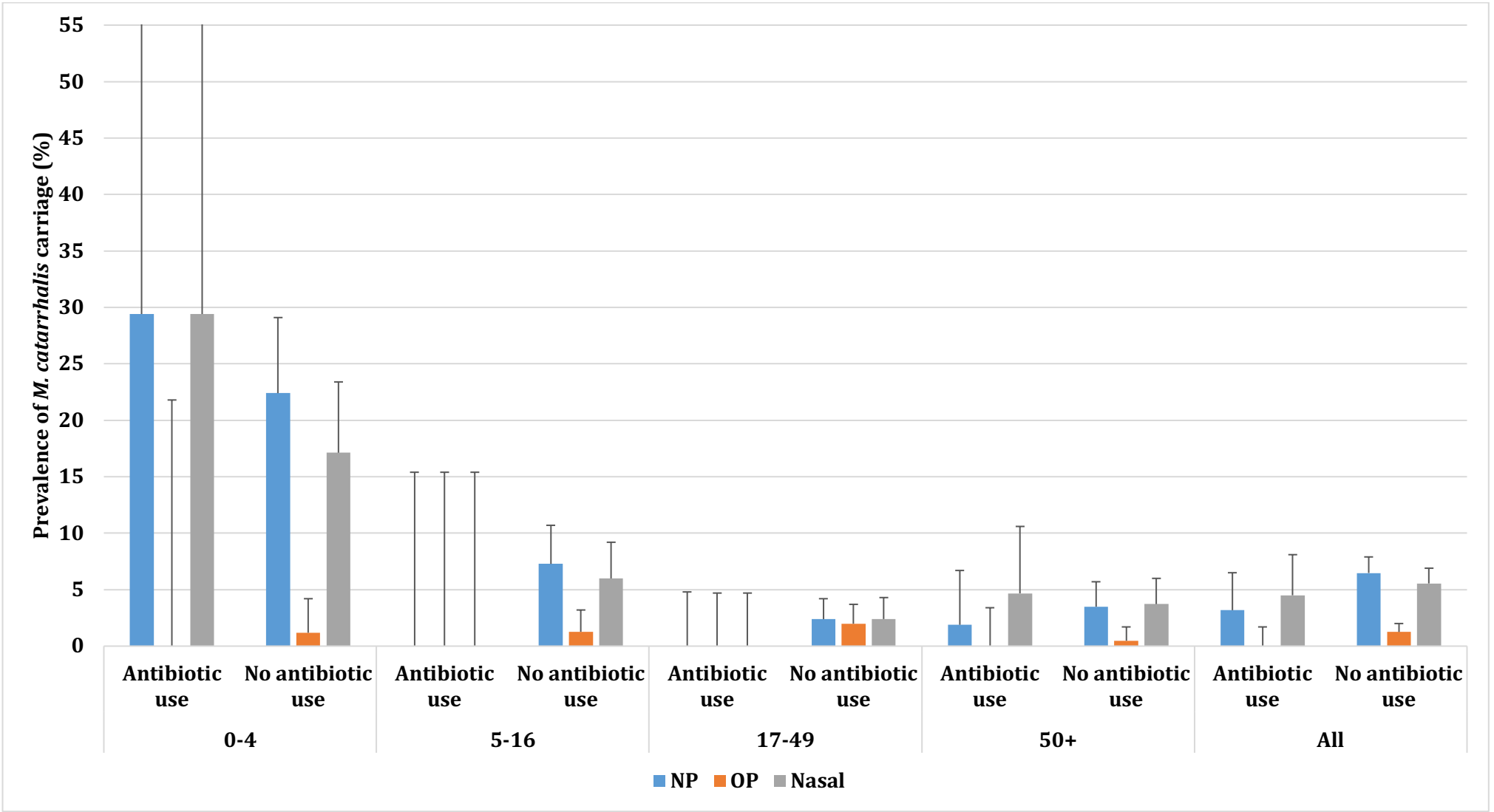

Prevalence of M. catarrhalis carriage (%)
35
30
25
20
15
10
5
0
Recent RTI
No recent RTI
0-4
5-16
17-49
50+
All
NP
OP
Nasal

| | | | NP | | | | OP | | | | Nose | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a Nose swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| 0-4 | Recent RTI | 115 | 115 | 30 | 26.1 | 18.3-35.1 | 106 | 2 | 1.9 | 0.2-6.7 | 114 | 21 | 18.4 | 11.8-26.8 |
| | No Recent RTI | 85 | 85 | 16 | 18.8 | 11.2-28.8 | 78 | 0 | 0.0 | 0.0-4.6 | 84 | 15 | 17.9 | 10.4-27.7 |
| 5-16 | Recent RTI | 142 | 142 | 12 | 8.5 | 4.4-14.3 | 141 | 1 | 0.7 | 0.0-3.9 | 142 | 10 | 7.0 | 3.4-12.6 |
| | No Recent RTI | 195 | 194 | 11 | 5.7 | 2.9-9.9 | 194 | 3 | 1.5 | 0.3-4.5 | 195 | 9 | 4.6 | 2.1-8.6 |
| 17-49 | Recent RTI | 252 | 250 | 5 | 2.0 | 0.7-4.6 | 248 | 5 | 2.0 | 0.7-4.6 | 251 | 3 | 1.2 | 0.3-3.5 |
| | No Recent RTI | 285 | 285 | 6 | 2.1 | 0.8-4.5 | 283 | 4 | 1.4 | 0.4-3.6 | 284 | 8 | 2.8 | 1.2-5.5 |
| 50+ | Recent RTI | 196 | 193 | 11 | 5.7 | 2.9-10.0 | 191 | 1 | 0.5 | 0.0-2.9 | 195 | 10 | 5.1 | 2.5-9.2 |
| | No Recent RTI | 342 | 340 | 7 | 2.1 | 0.8-4.2 | 332 | 1 | 0.3 | 0.0-1.7 | 339 | 12 | 3.5 | 1.8-6.1 |
| All | Recent RTI | 705 | 700 | 58 | 8.3 | 6.4-10.6 | 686 | 9 | 1.3 | 0.6-2.5 | 702 | 44 | 6.3 | 4.6-8.3 |
| | No Recent RTI | 907 | 904 | 40 | 4.4 | 3.2-6.0 | 887 | 8 | 0.9 | 0.4-1.8 | 902 | 44 | 4.9 | 3.6-6.5 |

**Figure 20. Carriage prevalence of *M. catarrhalis*, by age group, in those who have recently had a RTI and those who have not**.

* those who have recently had a RTI is defined as those who have a RTI or have had an RTI within a month prior to participating.

There is a significant association between recent RTI and *M. catarrhalis* carriage (P= 0.009,) with not having had an infection having an OR of 0.625. When considering the different swab types, there was a significant association between recent RTI and NP carriage of *M. catarrhalis* (P= 0.011, OR = 0.515). No significant association was determined between recent RTI and OP carriage of *M. catarrhalis* (P=0.523) and recent RTI and nasal carriage of *M. catarrhalis* (P= 0.405).


Prevalence of M. catarrhalis carriage (%)
55
50
45
40
35
30
25
20
15
10
5
0
Antibiotic use
No antibiotic use
0-4
5-16
17-49
50+
All
NP
OP
Nasal

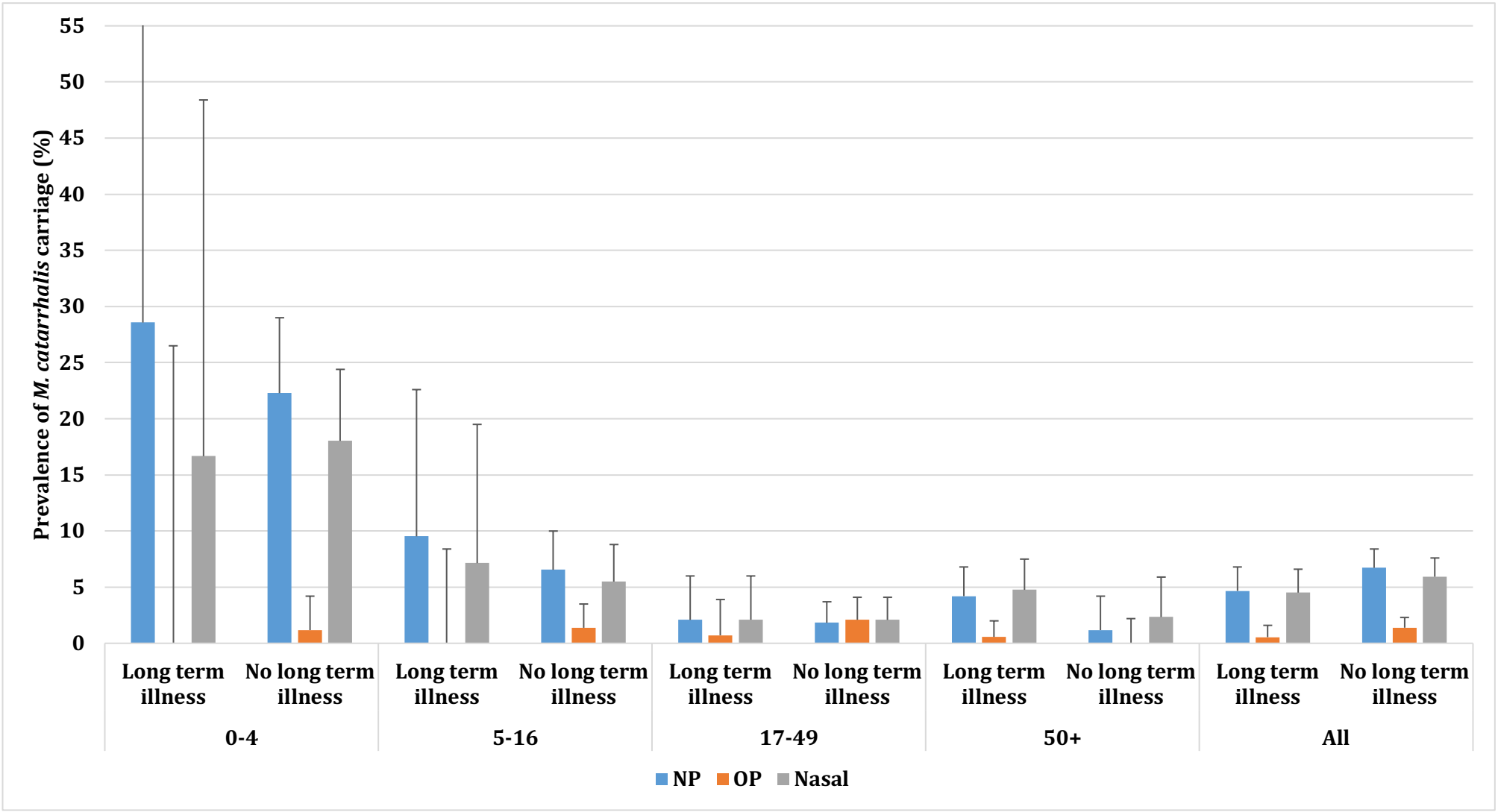
| | | | NP | | | | OP | | | | nasal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a nasal swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| 0-4 | Antibiotic use | 17 | 17 | 5 | 29.4 | 10.3-56.0 | 15 | 0 | 0.0 | 0.0-21.8 | 17 | 5 | 29.4 | 10.3-56.0 |
| | No antibiotic use | 183 | 183 | 41 | 22.4 | 16.6-29.1 | 169 | 2 | 1.2 | 0.1-4.2 | 181 | 31 | 17.1 | 11.9-23.4 |
| 5-16 | Antibiotic use | 22 | 22 | 0 | 0.0 | 0.00-15.4 | 22 | 0 | 0.0 | 0.0-15.4 | 22 | 0 | 0.0 | 0.00-15.4 |
| | No antibiotic use | 317 | 316 | 23 | 7.3 | 4.7-10.7 | 315 | 4 | 1.3 | 0.3-3.2 | 317 | 19 | 6.0 | 3.7-9.2 |
| 17-49 | Antibiotic use | 77 | 75 | 0 | 0.0 | 0.00-4.8 | 77 | 0 | 0.0 | 0.0-4.7 | 76 | 0 | 0.0 | 0.00-4.7 |
| | No antibiotic use | 460 | 460 | 11 | 2.4 | 1.2-4.2 | 454 | 9 | 2.0 | 0.9-3.7 | 459 | 11 | 2.4 | 1.2-4.3 |
| 50+ | Antibiotic use | 107 | 106 | 2 | 1.9 | 0.2-6.7 | 106 | 0 | 0.0 | 0.0-3.4. | 107 | 5 | 4.7 | 1.5-10.6 |
| | No antibiotic use | 434 | 430 | 15 | 3.5 | 2.0-5.7 | 420 | 2 | 0.5 | 0.1-1.7 | 430 | 16 | 3.7 | 2.1-6.0 |
| All | Antibiotic use | 223 | 220 | 7 | 3.2 | 1.3-6.5 | 220 | 0 | 0.0 | 0.0-1.7. | 222 | 10 | 4.5 | 2.2-8.1 |
| | No antibiotic use | 1394 | 1389 | 90 | 6.5 | 5.2-7.9 | 1358 | 17 | 1.3 | 0.7-2.0 | 1387 | 77 | 5.6 | 4.4-6.9 |

**Figure 21. Carriage prevalence of *M. catarrhalis*, by age group, in those who have recently had antibiotics and those who have not**.

* those who have recently had antibiotics are those who have had antibiotics within a month of participating.

Recent antibiotic use and *M. catarrhalis* carriage show a statistically significant association (P= 0.013, OR 0.570). When considering swab type, no statistically significant association was determined for recent antibiotic use and NP carriage of *M. catarrhalis* (P= 0.055), OP carriage of *M. catarrhalis* (P=0.196) and recent antibiotic use and nasal carriage of *M. catarrhalis* (P= 0.220).

Analysis was redone for the 0-4 age group, which clearly showed a different pattern to all other age group. A non-significant association was shown (P=0.289; OR 1.566).

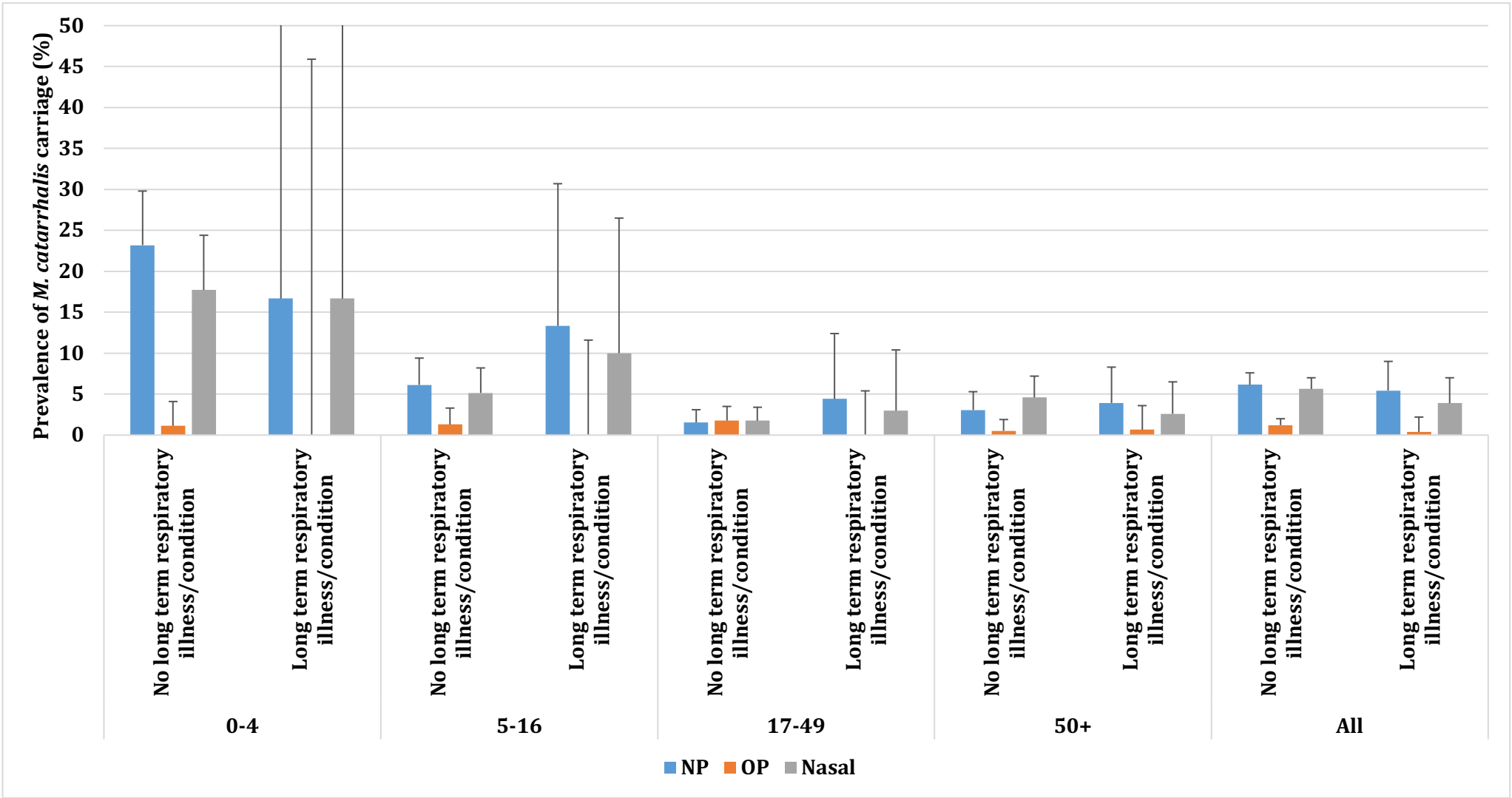

Prevalence of M. catarrhalis carriage (%)
55
50
45
40
35
30
25
20
15
10
5
0
Long term illness
No long term illness
0-4
5-16
17-49
50+
All
NP
OP
Nasal

| | | | NP | | | | OP | | | | nasal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a nasal swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| 0-4 | Long-term illness | 14 | 14 | 4 | 28.6 | 8.4-58.1 | 12 | 0 | 0.0 | 0.0-26.5 | 12 | 2 | 16.7 | 2.1-48.4 |
| | No long-term illness | 184 | 184 | 41 | 22.3 | 16.5-29.0 | 170 | 2 | 1.2 | 0.1-4.2 | 183 | 33 | 18.0 | 12.8-24.4 |
| 5-16 | Long-term illness | 42 | 42 | 4 | 9.5 | 2.7-22.6 | 42 | 0 | 0.0 | 0.0-8.4 | 42 | 3 | 7.1 | 1.5-19.5 |
| | No long-term illness | 291 | 290 | 19 | 6.6 | 4.0-10.0 | 289 | 4 | 1.4 | 0.4-3.5 | 291 | 16 | 5.5 | 3.2-8.8 |
| 17-49 | Long-term illness | 144 | 144 | 3 | 2.1 | 0.4-6.0 | 141 | 1 | 0.7 | 0.0-3.9 | 143 | 3 | 2.1 | 0.4-6.0 |
| | No long-term illness | 385 | 383 | 7 | 1.8 | 0.7-3.7 | 382 | 8 | 2.1 | 0.9-4.1 | 384 | 8 | 2.1 | 0.9-4.1 |
| 50+ | Long-term illness | 360 | 357 | 15 | 4.2 | 2.4-6.8 | 351 | 2 | 0.6 | 0.1-2.0 | 357 | 17 | 4.8 | 2.8-7.5 |
| | No long-term illness | 172 | 170 | 2 | 1.2 | 0.1-4.2 | 166 | 0 | 0.0 | 0.0-2.2 | 171 | 4 | 2.3 | 0.6-5.9 |
| All | Long-term illness | 560 | 557 | 26 | 4.7 | 3.1-6.8 | 546 | 3 | 0.5 | 0.1-1.6 | 554 | 25 | 4.5 | 2.9-6.6 |
| | No long-term illness | 1032 | 1027 | 69 | 6.7 | 5.3-8.4 | 1007 | 14 | 1.4 | 0.8-2.3 | 1029 | 61 | 5.9 | 4.6-7.6 |

**Figure 22. Carriage prevalence of *M. catarrhalis*, by age group, in those who have a long-term illness and those who do not**.

Long-term illness and *M. catarrhalis* carriage appear to have a statistically significant association (P= 0.032, OR 0.686). Considering swab type, a statistically significant association was found between long-term illness and NP carriage of *M. catarrhalis* (P= 0.048, OR 0.680), but no significant association was determined for OP (P=0.380) or nasal (P= 0.520) carriage of *M. catarrhalis***.** However, Figure 22 suggests this trend isn't true for all ages. As the long-term illness diagnosed in children are likely to be very different to those in adults, these age groups were analysed separately. In children (0-16 year olds) long-term illness and *M. catarrhalis* carriage did not have a statistically significant association (P= 0.579, OR 1.105). Considering swab type, no statistically significant association was found between long-term illness and NP (P= 0.739, OR 1.153) OP (P=1.000, OR 0.000) and nasal (P= 1.000, OR 0.746) carriage of *M. catarrhalis*. In adults (those aged 17 years and over) long-term illness and *M. catarrhalis* carriage did not have a statistically significant association (P= 0.19, OR 1.578). However when swab type was considered there was a statistically significant association was found between long-term illness and NP carriage of *M. catarrhalis* (P= <0.01, OR 2.255). Long-term illness and OP (P=0.327, OR=0.411) or nasal (P=0.215, OR1.877) carriage were not significantly associated.

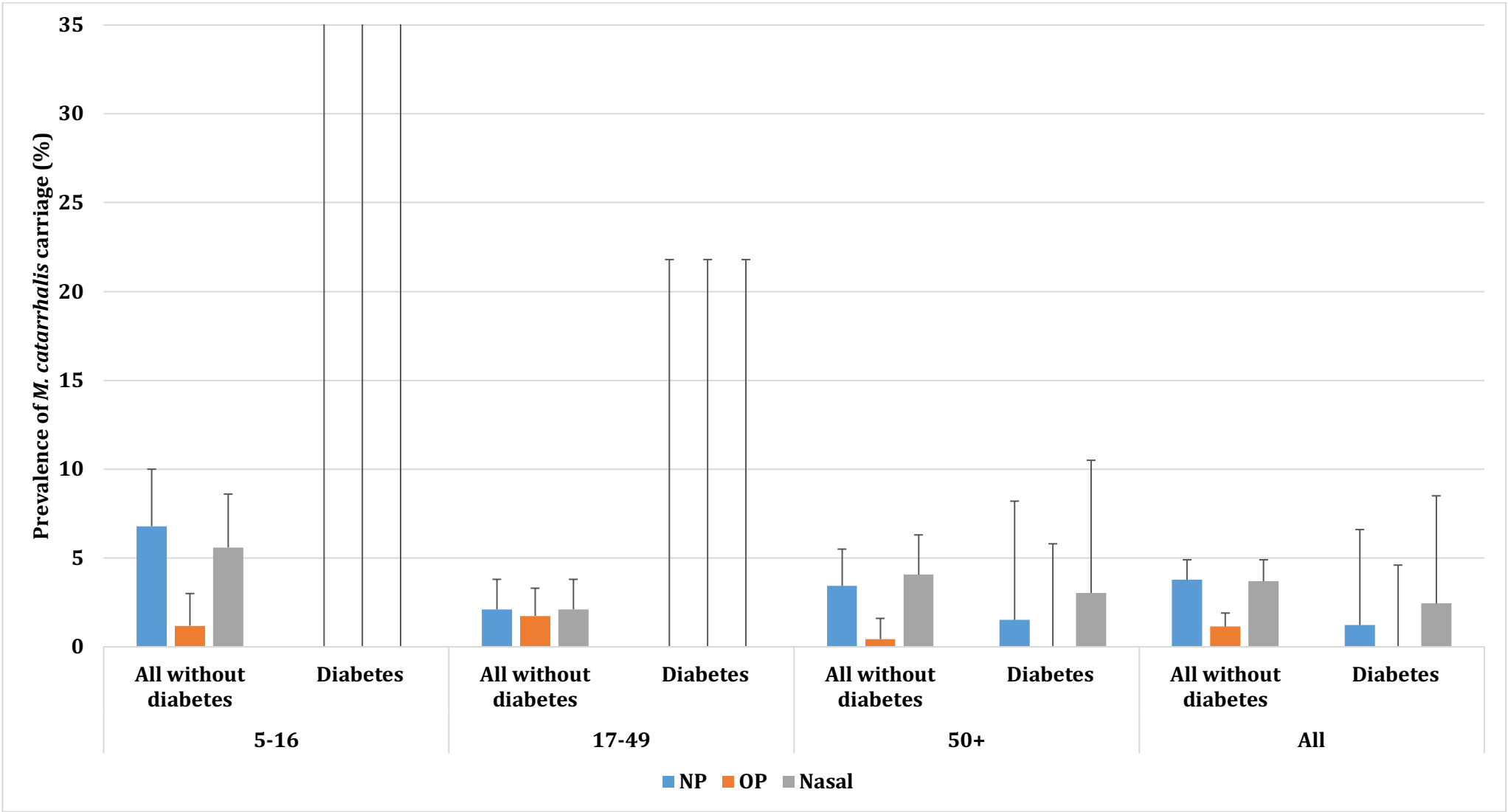
To further investigate the impact of long-term illness, analysis was done focussing on long-term respiratory illness/condition, followed by diabetes.


Prevalence of M. catarrhalis carriage (%)
50
45
40
35
30
25
20
15
10
5
0
No long term respiratory illness/condition
Long term respiratory illness/condition
0-4
5-16
17-49
50+
All
NP
OP
Nasal

| | | | NP | | | | OP | | | | nasal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a nasal swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| 0-4 | No long-term respiratory illness or condition | 194 | 194 | 45 | 23.2 | 17.5-29.8 | 178 | 2 | 1.1 | 0.1-4.1 | 192 | 34 | 17.7 | 12.8-24.4 |
| | Long-term respiratory illness/condition | 6 | 6 | 1 | 16.7 | 0.4-64.1 | 6 | 0 | 0.0 | 0.00-45.9. | 6 | 1 | 16.7 | 0.4-64.1 |
| 5-16 | No long-term respiratory illness or condition | 311 | 310 | 19 | 6.1 | 3.7-9.4 | 309 | 4 | 1.3 | 0.4-3.3 | 311 | 16 | 5.1 | 3.0-8.2 |
| | Long-term respiratory illness/condition | 30 | 30 | 4 | 13.3 | 3.8-30.7 | 30 | 0 | 0.0 | 0.00-11.6 | 30 | 3 | 10.0 | 2.1-26.5 |
| 17-49 | No long-term respiratory illness or condition | 457 | 455 | 7 | 1.5 | 0.6-3.1 | 452 | 8 | 1.8 | 0.8-3.5 | 456 | 8 | 1.8 | 0.8-3.4 |
| | Long-term respiratory illness/condition | 68 | 68 | 3 | 4.4 | 0.9-12.4 | 67 | 0 | 0.0 | 0.00-5.4 | 67 | 2 | 3.0 | 0.4-10.4 |
| 50+ | No long-term respiratory illness or condition | 394 | 392 | 12 | 3.1 | 1.6-5.3 | 382 | 2 | 0.5 | 0.1-1.9 | 392 | 18 | 4.6 | 2.7-7.2 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Long-term respiratory illness/condition | 156 | 153 | 6 | 3.9 | 1.5-8.3 | 153 | 1 | 0.7 | 0.0-3.6 | 154 | 4 | 2.6 | 0.7-6.5 |
| All | No long-term respiratory illness or condition | 1356 | 1351 | 83 | 6.1 | 4.9-7.6 | 1321 | 16 | 1.2 | 0.7-2.0 | 1351 | 76 | 5.6 | 4.5-7.0 |
| | Long-term respiratory illness/condition | 260 | 257 | 14 | 5.4 | 3.0-9.0 | 256 | 1 | 0.4 | 0.0-2.2 | 257 | 10 | 3.9 | 1.9-7.0 |

**Figure 23. Carriage prevalence of *M. catarrhalis*, by age group, in those who have a long-term respiratory illness/condition and those who do not**.

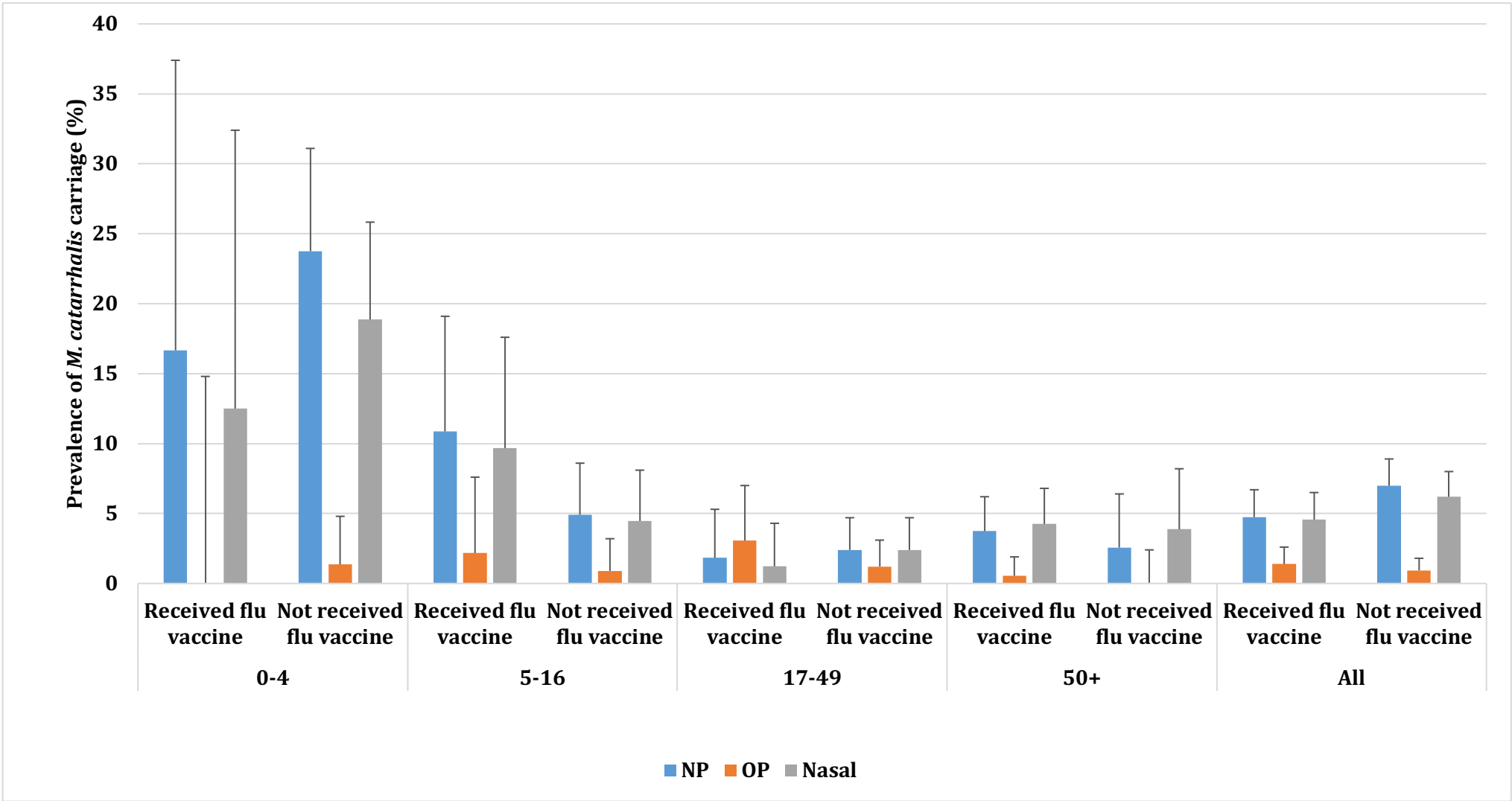

Prevalence of M. catarrhalis carriage (%)
35
30
25
20
15
10
5
0
All without diabetes
Diabetes
5-16
17-49
50+
All
NP
OP
Nasal

| | | | NP | | | | OP | | | | nasal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a nasal swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| 5-16 | Participants without diabetes | 339 | 339 | 23 | 6.8 | 4.4-10.0 | 338 | 4 | 1.2 | 0.3-3.0 | 340 | 19 | 5.6 | 3.4-8.6 |
| | Diabetes | 1 | 1 | 0 | 0.0 | 0.00-97.5 | 1 | 0 | 0.0 | 0.00-97.5 | 1 | 0 | 0.0 | 0.0-97.5 |
| 17-49 | Participants without diabetes | 523 | 521 | 11 | 2.1 | 1.1-3.8 | 517 | 9 | 1.7 | 0.8-3.3 | 521 | 11 | 2.1 | 1.1-3.8 |
| | Diabetes | 15 | 15 | 0 | 0.0 | 0.00-21.8 | 15 | 0 | 0.0 | 0.00-21.8 | 15 | 0 | 0.0 | 0.0-21.8 |
| 50+ | Participants without diabetes | 472 | 467 | 16 | 3.4 | 2.0-5.5 | 461 | 2 | 0.4 | 0.1-1.6 | 468 | 19 | 4.1 | 2.5-6.3 |
| | Diabetes | 66 | 66 | 1 | 1.5 | 0.0-8.2 | 62 | 0 | 0.0 | 0.00-5.8. | 66 | 2 | 3.0 | 0.4-10.5 |
| All | Participants without diabetes | 1334 | 1327 | 50 | 3.8 | 2.8-4.9 | 1316 | 15 | 1.1 | 0.6-1.9 | 1329 | 49 | 3.7 | 2.7-4.9 |
| | Diabetes | 82 | 82 | 1 | 1.2 | 0.0-6.6 | 78 | 0 | 0.0 | 0.00-4.6. | 82 | 2 | 2.4 | 0.3-8.5 |

**Figure 24. Carriage prevalence of *M. catarrhalis*, by age group, in those with and without diabetes**.

Long-term respiratory condition showed no significant association with the carriage of *M. catarrhalis* (P=0.170, df 1). Considering swab type, no significant association with long-term respiratory condition and the carriage of *M. catarrhalis* in the NP (P=0.647, df 1), OP (P=0.502) or nose (P=0.292, df 1).

Diabetes showed no significant association with the carriage of *M. catarrhalis* (P= 0.367, df 1). Considering swab type, no significant association with diabetes and the carriage of *M. catarrhalis* in the NP (P=0.717), OP (P=1.000) or nose (P=1.000).

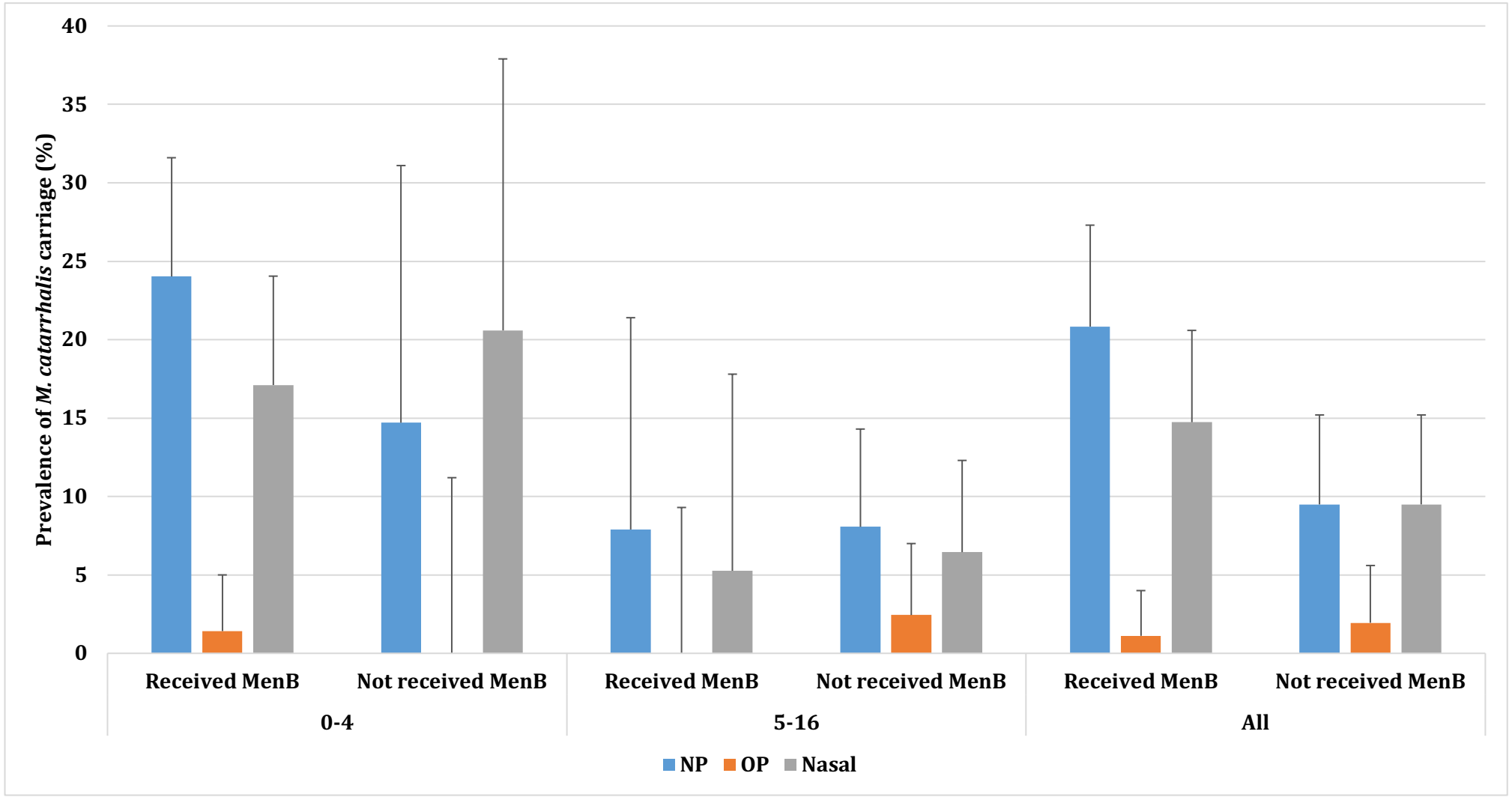

Prevalence of M. catarrhalis carriage (%)
40
35
30
25
20
15
10
5
0
Received flu vaccine
Not received flu vaccine
0-4
5-16
17-49
50+
All
NP
OP
Nasal

| | | | NP | | | | OP | | | | nasal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a nasal swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| 0-4 | Received flu vaccine | 24 | 24 | 4 | 16.7 | 4.7-37.4 | 23 | 0 | 0.0 | 0.0-14.8 | 24 | 3 | 12.5 | 2.7-32.4 |
| | Not received flu vaccine | 160 | 160 | 38 | 23.8 | 17.4-31.1 | 148 | 2 | 1.4 | 0.2-4.8 | 159 | 30 | 18.9 | 13.11-25.83 |
| 5-16 | Received flu vaccine | 93 | 92 | 10 | 10.9 | 5.3-19.1 | 92 | 2 | 2.2 | 0.3-7.6 | 93 | 9 | 9.7 | 4.5-17.6 |
| | Not received flu vaccine | 224 | 224 | 11 | 4.9 | 2.5-8.6 | 223 | 2 | 0.9 | 0.1-3.2 | 224 | 10 | 4.5 | 2.2-8.1 |
| 17-49 | Received flu vaccine | 165 | 164 | 3 | 1.8 | 0.4-5.3 | 163 | 5 | 3.1 | 1.0-7.0 | 164 | 2 | 1.2 | 0.2-4.3 |
| | Not received flu vaccine | 335 | 334 | 8 | 2.4 | 1.0-4.7 | 331 | 4 | 1.2 | 0.3-3.1 | 334 | 8 | 2.4 | 1.0-4.7 |
| 50+ | Received flu vaccine | 379 | 374 | 14 | 3.7 | 2.1-6.2 | 369 | 2 | 0.5 | 0.1-1.9 | 376 | 16 | 4.3 | 2.5-6.8 |
| | Not received flu vaccine | 156 | 156 | 4 | 2.6 | 0.7-6.4 | 153 | 0 | 0.0 | 0.0-2.4 | 155 | 6 | 3.9 | 1.4-8.2 |
| All | Received flu vaccine | 661 | 654 | 31 | 4.7 | 3.2-6.7 | 647 | 9 | 1.4 | 0.6-2.6 | 657 | 30 | 4.6 | 3.1-6.5 |
| | Not received flu vaccine | 875 | 874 | 61 | 7.0 | 5.4-8.9 | 855 | 8 | 0.9 | 0.4-1.8 | 872 | 54 | 6.2 | 4.7-8.0 |

**Figure 25. Carriage prevalence of *M. catarrhalis*, by age group, in those who have received the annual flu vaccine and those who have not**.

Receipt of the annual flu vaccination was significantly associated with decreased *M. catarrhalis* carriage (P=<0.001, OR 0.882). When considering swab types, receipt the annual flu vaccination was significantly associated with NP (P=<0.001, OR 0.810) and nasal (P= 0.015, OR 0.861) carriage of *M. catarrhalis*. No significant association was seen between receiving the annual flu vaccination and OP carriage (P=0.783).

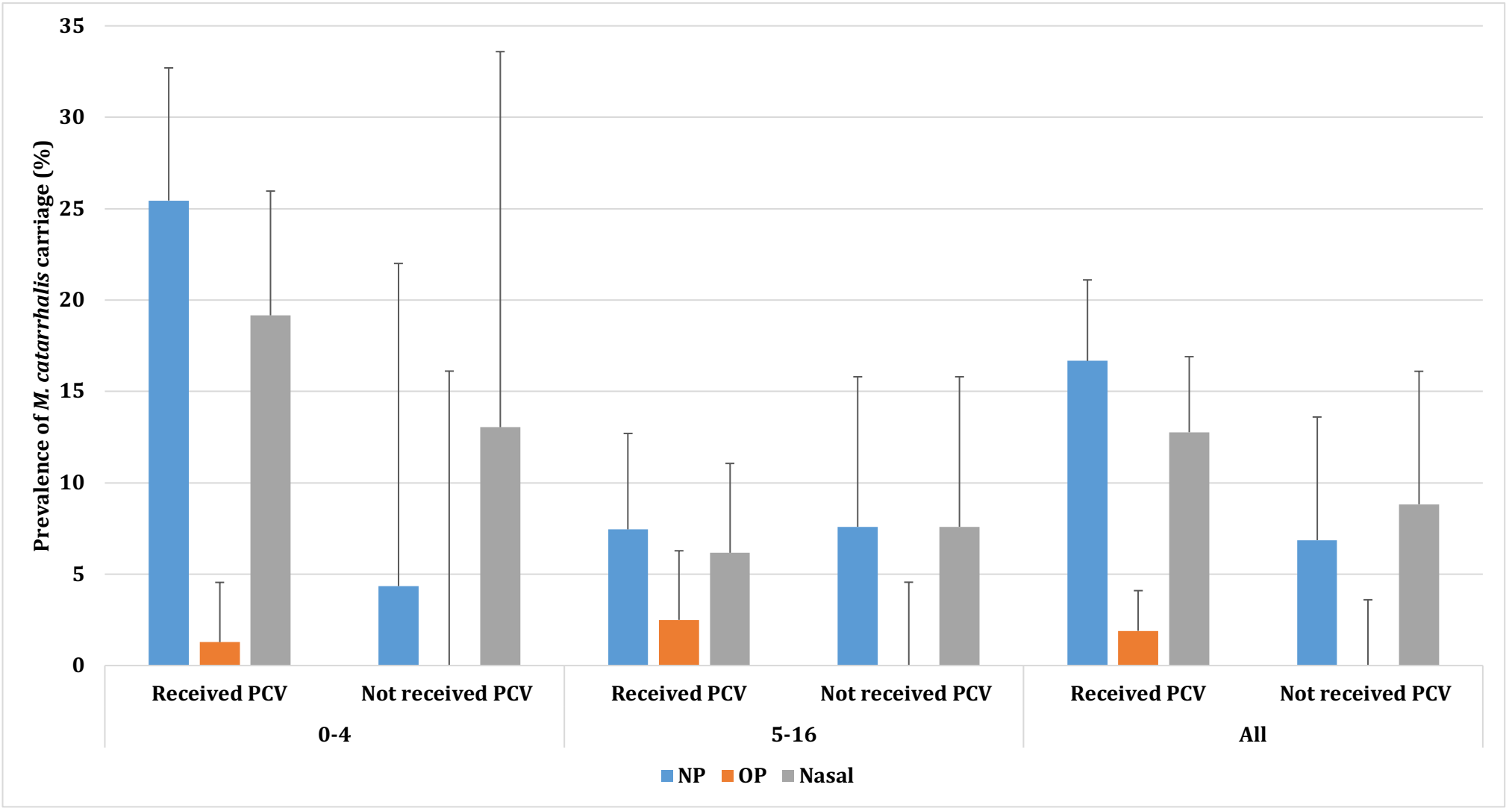
Whilst those who had received the flu vaccine and were 0-4 clearly had lower carriage, it is less clear cut for other ages. In fact, 5-16 year olds who had received the vaccine had higher carriage. Therefore, analysis was redone according to age group, whilst there was no significant association for any age group (presumably due to lower sample number) receipt of the flu vaccine is associated (not significantly) with reduced carriage in 0-4 year olds (P=0.446, or 0.553), and increased carriage in those aged 5+ (P=0.268, OR 1.310).


40
35
30
25
20
15
10
5
0
Prevalence of M. catarrhalis carriage (%)
Received MenB
Not received MenB
Received MenB
Not received MenB
Received MenB
Not received MenB
0-4
5-16
All
NP
OP
Nasal

| | | | NP | | | | OP | | | | nasal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a nasal swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| 0-4 | Received MenB | 154 | 154 | 37 | 24.0 | 17.5-31.6 | 141 | 2 | 1.4 | 0.2-5.0 | 152 | 26 | 17.1 | 11.49-24.05 |
| | Not received MenB | 34 | 34 | 5 | 14.7 | 5.0-31.1 | 31 | 0 | 0.0 | 0.00-11.2 | 34 | 7 | 20.6 | 8.7-37.9 |
| 5-16 | Received MenB | 38 | 38 | 3 | 7.9 | 1.7-21.4 | 38 | 0 | 0.0 | 0.00-9.3 | 38 | 2 | 5.3 | 0.6-17.8 |
| | Not received MenB | 124 | 124 | 10 | 8.1 | 3.9-14.3 | 123 | 3 | 2.4 | 0.5-7.0 | 124 | 8 | 6.5 | 2.8-12.3 |
| All | Received MenB | 192 | 192 | 40 | 20.8 | 15.3-27.3 | 179 | 2 | 1.1 | 0.1-4.0 | 190 | 28 | 14.7 | 10.02-20.59 |
| | Not received MenB | 158 | 158 | 15 | 9.5 | 5.4-15.2 | 154 | 3 | 1.9 | 0.4-5.6 | 158 | 15 | 9.5 | 5.4-15.2 |

**Figure 26. Carriage prevalence of *M. catarrhalis*, by age group, in those who have received the MenB vaccine and those have not**.

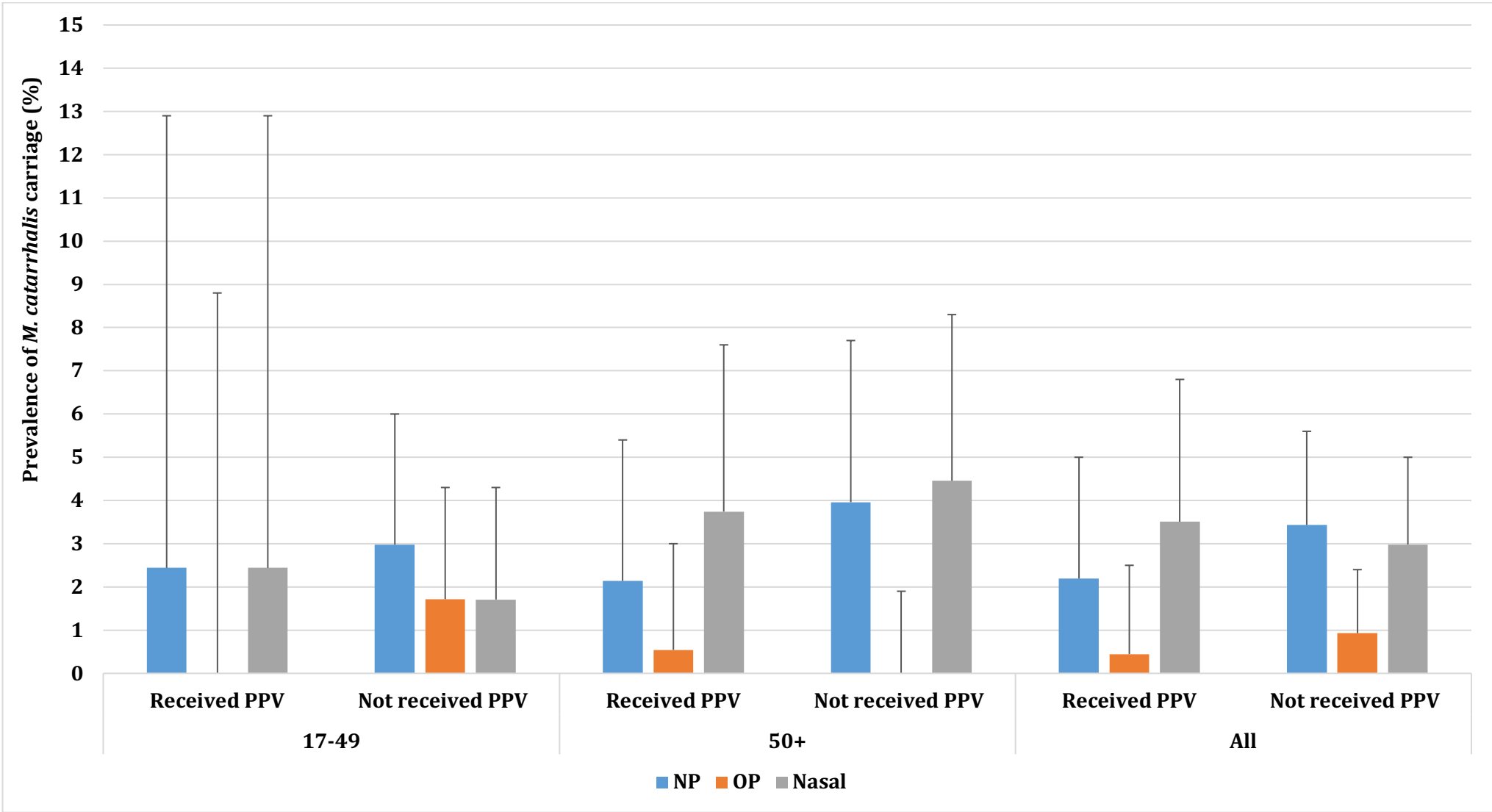
Receipt of the MenB vaccine was significantly associated with *M. catarrhalis* carriage (P= <0.001, OR 1.803). When considering swab type, receipt of the MenB vaccine was significantly associated with NP (P= 0.002, OR 2.462). Receipt of the MenB vaccine was not associated with OP carriage (P= 0.610) nor nasal carriage (P= 0.117) of *M. catarrhalis*. Only those aged 0-16 were included in the analysis as these are the only ages who would have received the vaccine, and inclusion of other age groups could lead to the unnecessary inclusion of confounding factors


35
30
25
20
15
10
5
0
Prevalence of M. catarrhalis carriage (%)
Received PCV
Not received PCV
Received PCV
Not received PCV
Received PCV
Not received PCV
0-4
5-16
All
NP
OP
Nasal

| | | | NP | | | | OP | | | | nasal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a nasal swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| 0-4 | Received PCV | 169 | 169 | 43 | 25.4 | 19.1-32.7 | 156 | 2 | 1.3 | 0.16-4.55 | 167 | 32 | 19.2 | 13.49-25.96 |
| | Not received PCV | 23 | 23 | 1 | 4.3 | 0.1-22.0 | 21 | 0 | 0.0 | 0.00-16.11 | 23 | 3 | 13.0 | 2.77-33.59 |
| 5-16 | Received PCV | 162 | 161 | 12 | 7.5 | 3.9-12.7 | 160 | 4 | 2.5 | 0.69-6.28 | 162 | 10 | 6.2 | 3.00-11.06 |
| | Not received PCV | 79 | 79 | 6 | 7.6 | 2.8-15.8 | 79 | 0 | 0.0 | 0.00-4.56 | 79 | 6 | 7.6 | 2.84-15.80 |
| All | Received PCV | 331 | 330 | 55 | 16.7 | 12.8-21.1 | 316 | 6 | 1.9 | 0.7-4.1 | 329 | 42 | 12.8 | 9.4-16.9 |
| | Not received PCV | 102 | 102 | 7 | 6.9 | 2.8-13.6 | 100 | 0 | 0.0 | 0.0-3.6 | 102 | 9 | 8.8 | 4.1-16.1 |

**Figure 27. Carriage prevalence of *M. catarrhalis*, by age group, in those who have received the PCV and those have not**.

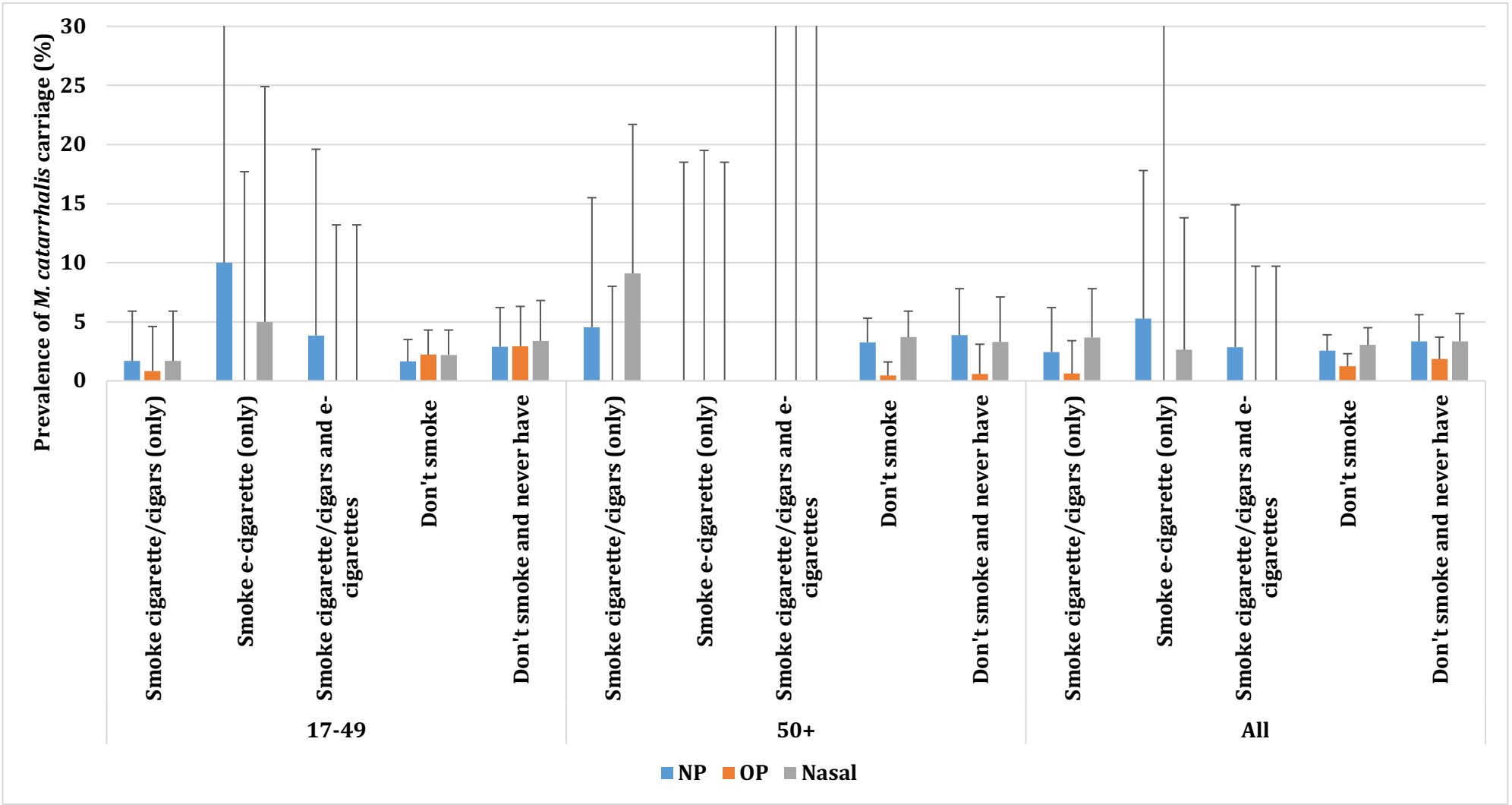
Receipt of the PCV vaccine was significantly associated with *M. catarrhalis* carriage (P =<0.001, OR 1.837). When considering swab type, receipt of the PCV vaccine was significantly associated with NP (P= 0.020, OR= 2.363) *M. catarrhalis* carriage. Receipt of the PCV vaccine was not associated with OP carriage or and nasal of *M. catarrhalis* (P= 0.379 and 0.069 respectively). Only those aged 0-16 were included in the analysis as these are the only ages who would have received the vaccine, and inclusion of other age groups could lead to the unnecessary inclusion of confounding factors.


Prevalence of M. catarrhalis carriage (%)
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Received PPV
Not received PPV
Received PPV
Not received PPV
Received PPV
Not received PPV
17-49
50+
All
NP
OP
Nasal

| | | | NP | | | | OP | | | | nasal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a nasal swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| 17-49 | Received PPV | 41 | 41 | 1 | 2.4 | 0.1-12.9 | 40 | 0 | 0.0 | 0.00-8.8 | 41 | 1 | 2.4 | 0.1-12.9 |
| | Not received PPV | 236 | 235 | 7 | 3.0 | 1.2-6.0 | 233 | 4 | 1.7 | 0.5-4.3 | 234 | 4 | 1.7 | 0.5-4.3 |
| 50+ | Received PPV | 190 | 187 | 4 | 2.1 | 0.6-5.4 | 184 | 1 | 0.5 | 0.0-3.0 | 187 | 7 | 3.7 | 1.5-7.6 |
| | Not received PPV | 202 | 202 | 8 | 4.0 | 1.7-7.7 | 197 | 0 | 0.0 | 0.00-1.9 | 202 | 9 | 4.5 | 2.1-8.3 |
| All | Received PPV | 231 | 228 | 5 | 2.2 | 0.7-5.0 | 224 | 1 | 0.4 | 0.0-2.5 | 228 | 8 | 3.5 | 1.5-6.8 |
| | Not received PPV | 438 | 437 | 15 | 3.4 | 1.9-5.6 | 430 | 4 | 0.9 | 0.3-2.4 | 436 | 13 | 3.0 | 1.6-5.0 |

**Figure 28. Carriage prevalence of *M. catarrhalis*, by age group, in those who have received the PPV and those have not**.

Receipt of the PPV vaccine was not significantly associated with *M. catarrhalis* carriage (P=0.848). When considering swab type, receipt of the PPV vaccine was not significantly associated with *M. catarrhalis* carriage in the NP (P=0.518), OP (P=0.521) or nose (P=0.868). Only those aged 17+ were included in the analysis as these are the only ages who would have received the vaccine, and inclusion of other age groups could lead to the unnecessary inclusion of confounding factors.


Prevalence of M. catarrhalis carriage (%)
30
25
20
15
10
5
0
Smoke cigarette/cigars (only)
Smoke e-cigarette (only)
Smoke cigarette/cigars and e-cigarettes
Don't smoke
Don't smoke and never have
Smoke cigarette/cigars (only)
Smoke e-cigarette (only)
Smoke cigarette/cigars and e-cigarettes
Don't smoke
Don't smoke and never have
Smoke cigarette/cigars (only)
Smoke e-cigarette (only)
Smoke cigarette/cigars and e-cigarettes
Don't smoke
Don't smoke and never have
17-49
50+
All
NP
OP
Nasal

| | | | NP | | | | OP | | | | nasal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a nasal swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| 17-49 | Smoke cigarette/cigars (only) | 119 | 119 | 2 | 1.7 | 0.2-5.9 | 119 | 1 | 0.8 | 0.0-4.6 | 119 | 2 | 1.7 | 0.2-5.9 |
| | Smoke e-cigarette (only) | 20 | 20 | 2 | 10.0 | 1.2-31.7 | 19 | 0 | 0.0 | 0.00-17.7 | 20 | 1 | 5.0 | 0.1-24.9 |
| | Smoke cigarette/cigars and e-cigarettes | 26 | 26 | 1 | 3.8 | 0.1-19.6 | 26 | 0 | 0.0 | 0.00-13.2 | 26 | 0 | 0.0 | 0.0-13.2 |
| | Don't smoke | 365 | 365 | 6 | 1.6 | 0.6-3.5 | 360 | 8 | 2.2 | 1.0-4.3 | 363 | 8 | 2.2 | 1.0-4.3 |
| | Those who don't smoke anything and never have | 208 | 208 | 6 | 2.9 | 1.1-6.2 | 204 | 6 | 2.9 | 1.1-6.3 | 207 | 7 | 3.4 | 1.4-6.8 |
| 50+ | Smoke cigarette/cigars (only) | 45 | 44 | 2 | 4.5 | 0.6-15.5 | 44 | 0 | 0.0 | 0.00-8.0 | 44 | 4 | 9.1 | 2.5-21.7 |
| | Smoke e-cigarette (only) | 18 | 18 | 0 | 0.0 | 0.00-18.5 | 17 | 0 | 0.0 | 0.00-19.5 | 18 | 0 | 0.0 | 0.0-18.5 |
| | Smoke cigarette/cigars and e-cigarettes | 9 | 9 | 0 | 0.0 | 0.00-33.6 | 9 | 0 | 0.0 | 0.00-33.6 | 9 | 0 | 0.0 | 0.0-33.6. |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Don't smoke | 463 | 459 | 15 | 3.3 | 1.8-5.3 | 450 | 2 | 0.4 | 0.1-1.6 | 460 | 17 | 3.7 | 2.2-5.9 |
| | Those who don't smoke anything and never have | 181 | 181 | 7 | 3.9 | 1.6-7.8 | 175 | 1 | 0.6 | 0.0-3.1 | 181 | 6 | 3.3 | 1.2-7.1 |
| All | Smoke cigarette/cigars (only) | 164 | 163 | 4 | 2.5 | 0.7-6.2 | 163 | 1 | 0.6 | 0.0-3.4 | 163 | 6 | 3.7 | 1.4-7.8 |
| | Smoke e-cigarette (only) | 38 | 38 | 2 | 5.3 | 0.6-17.8 | 36 | 0 | 0.0 | 0.00-60.2 | 38 | 1 | 2.6 | 0.1-13.8 |
| | Smoke cigarette/cigars and e-cigarettes | 35 | 35 | 1 | 2.9 | 0.1-14.9 | 35 | 0 | 0.0 | 0.00-9.7 | 35 | 0 | 0.0 | 0.00-9.7 |
| | Don't smoke | 828 | 824 | 21 | 2.5 | 1.6-3.9 | 810 | 10 | 1.2 | 0.6-2.3 | 823 | 25 | 3.0 | 2.0-4.5 |
| | Those who don't smoke anything and never have | 389 | 389 | 13 | 3.3 | 1.8-5.6 | 379 | 7 | 1.8 | 0.7-3.7 | 388 | 13 | 3.4 | 1.8-5.7 |

**Figure 29. Carriage prevalence of *M. catarrhalis*, by age group, in those who smoke cigarettes/cigars, e-cigarettes, both cigarettes/cigars and e-cigarettes or do not smoke**.

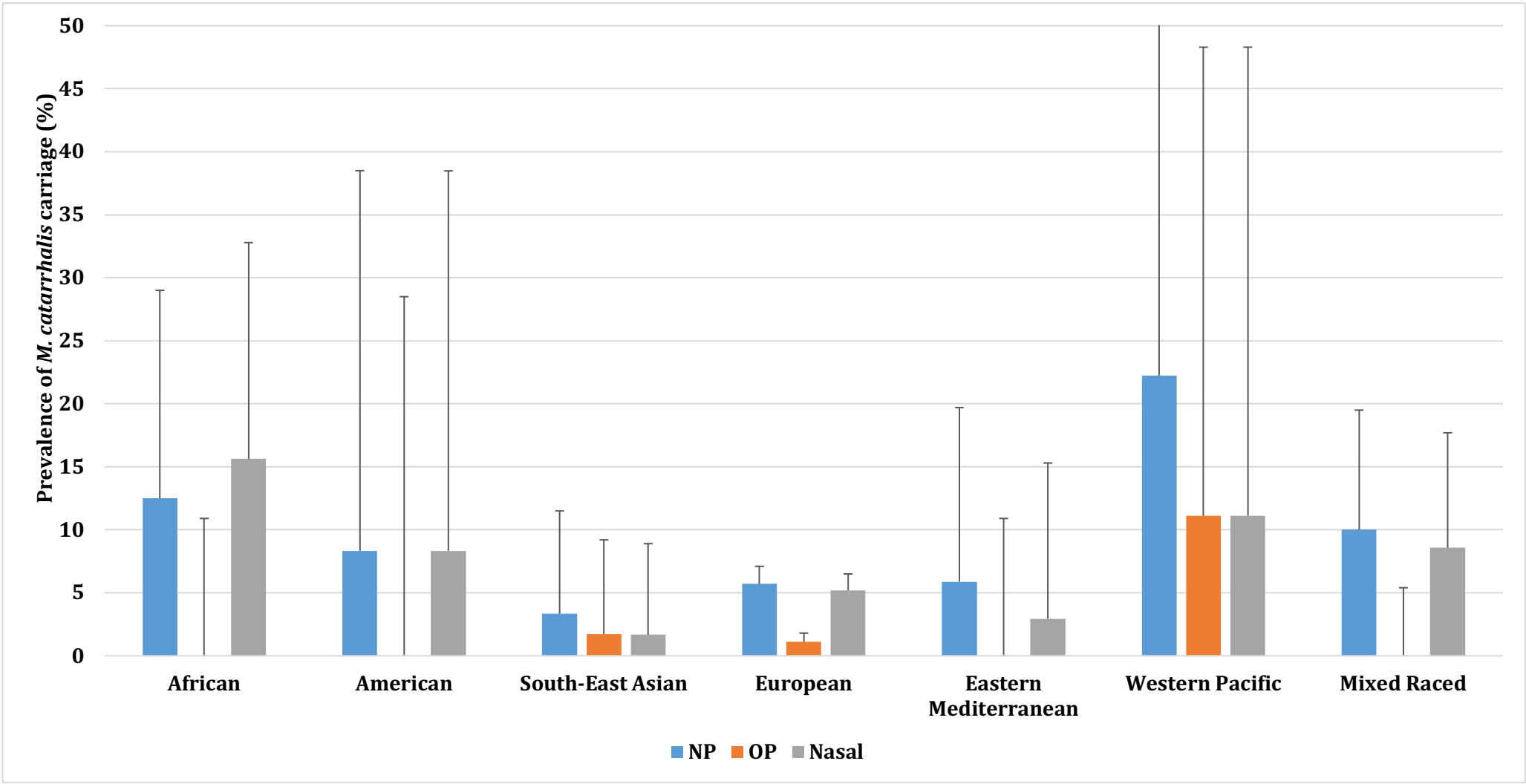
Smoking was not associated with the carriage of *M. catarrhalis*:

| | **Smokes cigarettes or cigars** | **Smokes e-cigarettes** |
|---|---|---|
| Association with *M. catarrhalis* carriage; p value (differential factor) | P=0.828 | P=0.899 |
| Association with carriage of *M. catarrhalis* in the NP; p value (differential factor) | P=1.000 | P= 0.556 |
| Association with carriage of *M. catarrhalis* in the OP; p value (differential factor) | P=0.715 | P=1.000 |
| Association with carriage of *M. catarrhalis* in the nose; p value (differential factor) | P=1.000 | P=0.787 |

**Table 7. Association between smoking and carriage of *M. catarrhalis* in those aged 17+.**

However, exposure to second hand (SH) cigarette/cigar smoke and SH e-cigarette smoke are both significantly associated with carriage (P=<0.001). With no exposure to SH cigarette/cigar smoke acting as the reference, having occasional, weekly and daily exposure had an OR of 0.456, 0.661, and 0.276 respectively. With no exposure to SH e-cigarette smoke acting as the reference, having occasional, weekly, and daily exposure had an OR of 0.547, 0.562 and 0.381 respectively. Interestingly, exposure to SHS was prevalent in community-based participants; 23.6% (n=383) and 18.6% (n=301) were occasionally (~once a month) exposed to cigarette/cigar smoke and e- cigarette smoke respectively. Whereas 20.5% (n=333) and 12% (n=195) were exposed to cigarette/cigar smoke and e- cigarette smoke respectively on a weekly-daily basis.

27.4% of 17-49 year olds (n=148) stated they smoked cigarettes/cigars (n=119 of which only smoke cigarettes/cigars) and 8.5% (n=46) stated they smoke e-cigarettes (n=20 of which only smoke e-cigarettes). Whilst only 10.0% (n=54) of those aged 50+ stated they smoke cigarettes/cigars (n=45 of which only smoke cigarettes/cigars) and 5.2% (n=28) stated they smoke e-cigarettes (n=18 of which only smoke e-cigarettes). The average of such figures for smoked cigarettes/cigars is in line with PHE data for smoking prevalence in Portsmouth and Southampton which shows an average of 16.6% of the adult population smoke (Public Health England, 2019b).

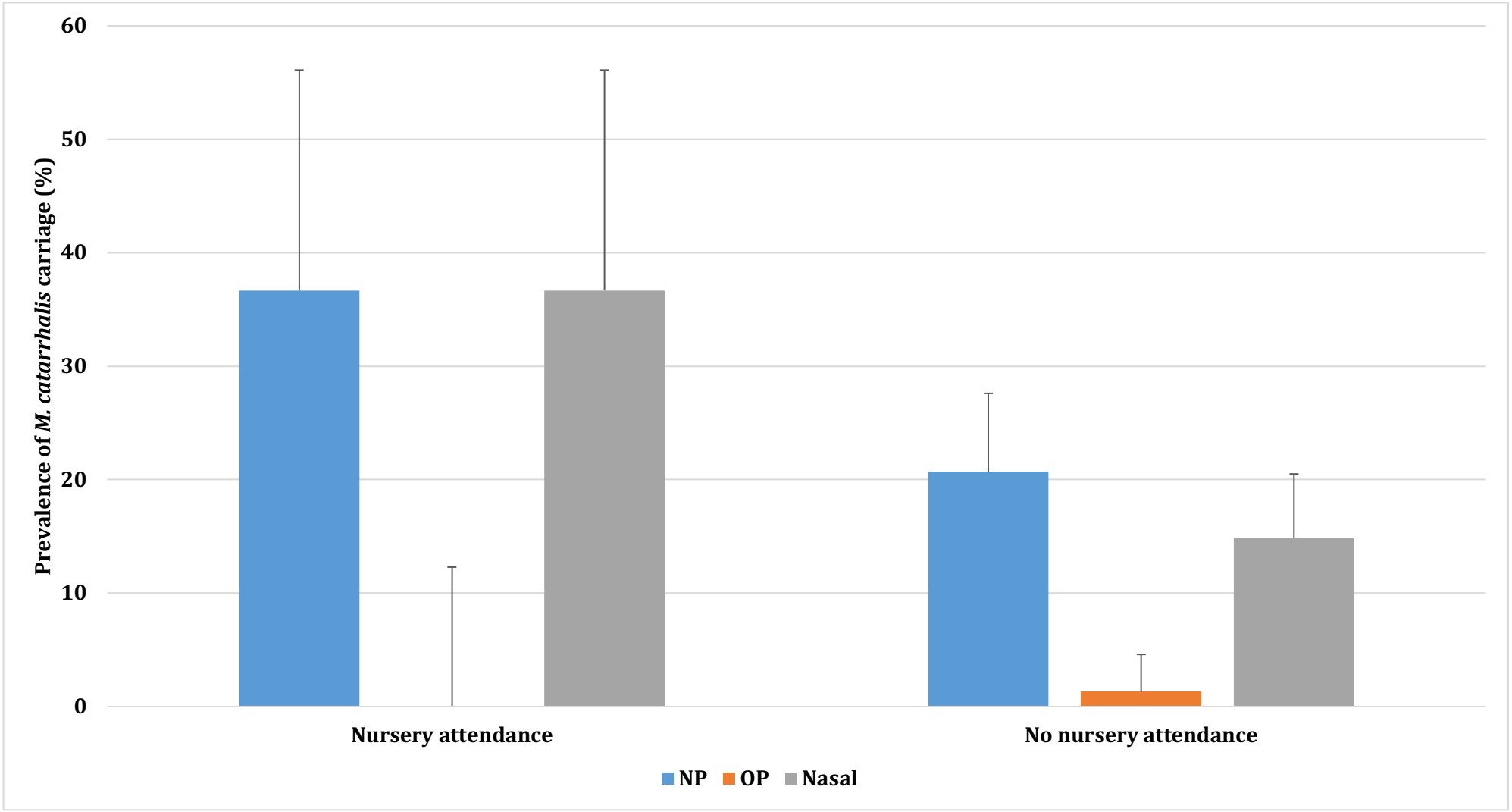

Prevalence of M. catarrhalis carriage (%)
50
45
40
35
30
25
20
15
10
5
0
African
American
South-East Asian
European
Eastern Mediterranean
Western Pacific
Mixed Raced
NP
OP
Nasal

| | | | NP | | | | OP | | | | nasal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a nasal swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| All | African | 32 | 32 | 4 | 12.5 | 3.5-29.0 | 32 | 0 | 0.0 | 0.00-10.9 | 32 | 5 | 15.6 | 5.28-32.79 |
| | American | 12 | 12 | 1 | 8.3 | 0.2-38.5 | 11 | 0 | 0.0 | 0.0-28.5 | 12 | 1 | 8.3 | 0.21-38.48 |
| | South-East Asian | 60 | 60 | 2 | 3.3 | 0.4-11.5 | 58 | 1 | 1.7 | 0.0-9.2 | 60 | 1 | 1.7 | 0.0-8.9 |
| | European | 1375 | 1367 | 78 | 5.7 | 4.5-7.1 | 1345 | 15 | 1.1 | 0.6-1.8 | 1367 | 71 | 5.2 | 4.1-6.5 |
| | Eastern Mediterranean | 34 | 34 | 2 | 5.9 | 0.7-19.7 | 32 | 0 | 0.0 | 0.0-10.9 | 34 | 1 | 2.9 | 0.1-15.3 |
| | Western Pacific | 9 | 9 | 2 | 22.2 | 2.8-60.0 | 9 | 1 | 11.1 | 0.3-48.3 | 9 | 1 | 11.1 | 0.3-48.3 |
| | Mixed Race | 70 | 70 | 7 | 10.0 | 4.1-19.5 | 66 | 0 | 0.0 | 0.0-5.4 | 70 | 6 | 8.6 | 3.2-17.7 |

**Figure 30. Carriage prevalence of *M. catarrhalis* by ethnicity**.

Ethnicity and *M. catarrhalis* carriage were shown to have a statistically significant association (P= 0.014). Using African ethnicity as the reference, American, Southeast Asian, European, Eastern Mediterranean, Western Pacific and mixed ethnicity had an OR of 0.569, 0.220, 0.398, 0.293, 1.681, and 0.638 respectively. However, when swab type was considered no significant association was determined for NP (P= 0.132), OP (P= 0.367) or nose (P= 0.088) carriage, possibly as sample sizes were too small.

All care/nursing home residents of European ethnicity (in fact all were white British), therefore looking at ethnicity as a risk factor in this cohort was not possible.


60
50
40
30
20
10
0
Prevalence of M. catarrhalis carriage (%)
Nursery attendance
No nursery attendance
NP
OP
Nasal

| | | NP | | | | OP | | | | nasal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total number of participants | Number that had a NP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had an OP swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) | Number that had a nasal swab | Number of *M. catarrhalis* isolated | Carriage prevalence (%) | 95% CI (%) |
| Children that attend nursery | 30 | 30 | 11 | 36.7 | 19.9-56.1 | 28 | 0 | 0.0 | 0.0-12.3 | 30 | 11 | 36.7 | 19.9-56.1 |
| Children that do not | 169 | 169 | 35 | 20.7 | 14.9-27.6 | 155 | 2 | 1.3 | 0.2-4.6 | 168 | 25 | 14.9 | 9.4-20.5 |

**Figure 31. Carriage prevalence of *M. catarrhalis* in children who attend nursery and those who do not**.

Nursery attendance was significantly associated with *M. catarrhalis* carriage (P= 0.010, OR 2.322), as seen in Figure 31. When swab type was considered, there was a significant association between nursey attendance and nasal carriage (P= 0.013, OR 3.335). There was no significant association between nursery attendance and *M. catarrhalis* NP (P= 0.128) or OP (P=1.000) carriage. Only those aged 0-4 were included in the analysis as these are the only ages who would attend nursery, and inclusion of other age groups could lead to the unnecessary inclusion of confounding factors.

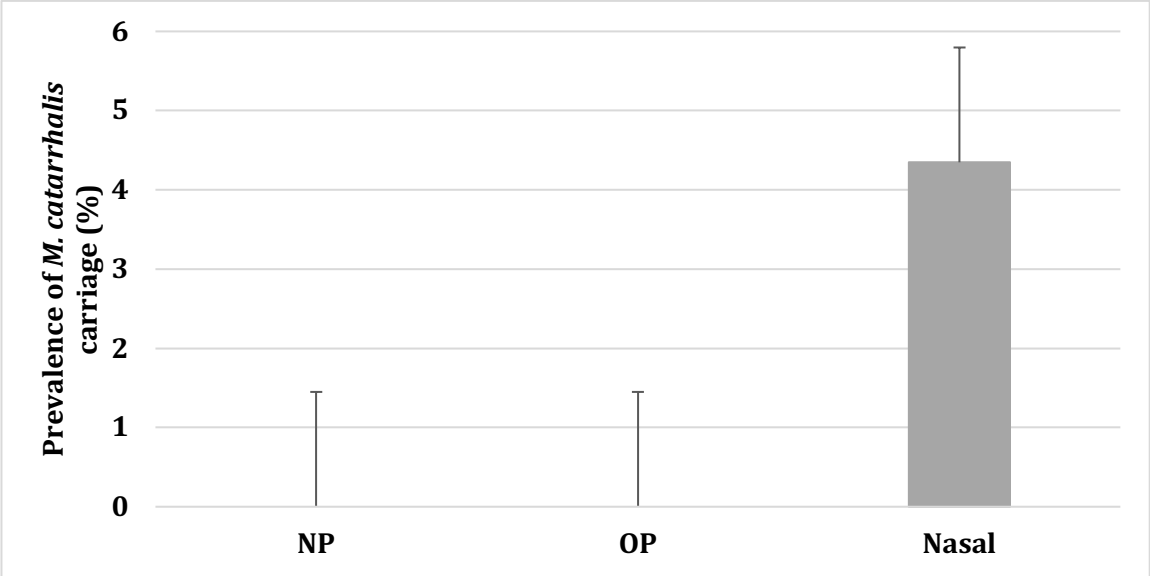



**Figure 32. Carriage prevalence of *M. catarrhalis* in those who are homeless**.

Homeless participants showed a 0.0% (CI: 0.0-14.8%) carriage prevalence of M. catarrhalis in their NP or OP, whilst 4.3% (CI: 0.1-22.0%, n=1) carried M. catarrhalis in their nose. Whilst data is presented for the participants that were homeless (Figure 32), no further analysis was undertaken; limited recruitment meant sample number was too low to detect statistical significance.

### 4.4.5 Multivariate analysis

Sample site and age of participant are obvious variables that may have a confounding effect, such confounding was controlled for using stratification. This allowed the association between each variable and the carriage of *M. catarrhalis* to be examined within different age groups and/or by sites of isolation. However, there are several issues that arise from combating confounding in this way. By stratifying the data set according to age and/or swab site, sample size is reduced and can result in too few cases to obtain reliable results or to determine significance. Statistical modelling (multivariable regression analysis) avoids this issue and is used to control for more than one confounder at the same time.

A multivariate model was designed in which all variables were included for analysis, Table 8 presents the outputs as well as the results of univariate analysis for comparison.

| | | Univariate analysis | | | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sig. | Exp(B) | 95% C.I.for EXP(B) | | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
| | | | | Lower | Upper | | | Lower | Upper |
| Swab site | NP | **<0.001** | Reference category | | | **0.000** | Reference category | | |
| | OP | **<0.001** | 0.165 | 0.098 | 0.277 | **0.000** | 0.149 | 0.090 | 0.246 |
| | Nose | 0.405 | 0.881 | 0.655 | 1.187 | 0.603 | 0.924 | 0.685 | 1.246 |
| Age Range | 0-4 | **<0.001** | Reference category | | | **0.000** | Reference category | | |
| | 17-49 | **<0.001** | 0.122 | 0.08 | 0.186 | **0.000** | 0.175 | 0.082 | 0.376 |
| | 5-16 | **<0.001** | 0.293 | 0.201 | 0.427 | **0.001** | 0.359 | 0.191 | 0.674 |
| | 50+ | **<0.001** | 0.165 | 0.112 | 0.242 | **0.000** | 0.182 | 0.079 | 0.417 |
| | Care home | 0.269* | 0.768 | 0.48 | 1.226 | 0.726 | 0.855 | 0.356 | 2.054 |
| Microbial carriage | *S. pneumoniae* | **<0.001** | 5.908 | 3.911 | 8.925 | **0.001** | 2.322 | 1.402 | 3.846 |
| | *H. influenzae* | **<0.001** | 3.397 | 2.124 | 5.432 | **0.001** | 1.366 | 0.757 | 2.467 |
| | *N. meningitidis* | 0.274 | 1.938 | 0.592 | 6.347 | **0.013** | 5.378 | 1.433 | 20.186 |
| | *S. aureus* | **0.009** | 0.470 | 0.266 | 0.831 | **0.001** | 0.398 | 0.225 | 0.702 |
| Received a pneumococcal vaccine | No | **0.000** | Reference category | | | 0.941 | Reference category | | |
| | Yes | **0.000** | 2.364 | 1.677 | 3.334 | 0.481 | 0.854 | 0.551 | 1.325 |
| | Don't know | 0.348 | 0.813 | 0.528 | 1.252 | 0.422 | 0.837 | 0.542 | 1.293 |
| | Rather not say/no answer | 0.998 | 0.000 | 0.000 | | 0.998 | 0.000 | 0.000 | |
| | Too young | 0.998 | 0.000 | 0.000 | | 0.997 | 0.000 | 0.000 | |
| Vaccine status up to date in accordance with UK schedule | No | 0.160 | Reference category | | | **0.016** | Reference category | | |
| | Yes | 0.064 | 3.758 | 0.925 | 15.267 | 0.099 | 2.513 | 0.841 | 7.511 |
| | Don't know | 0.401 | 1.952 | 0.410 | 9.297 | **0.006** | 5.385 | 1.633 | 17.756 |
| | Rather not say/no answer | 0.999 | 0.000 | 0.000 | | 0.999 | 0.000 | 0.000 | |
| | Too young | 0.145 | 4.405 | 0.601 | 32.299 | 0.169 | 6.148 | 0.462 | 81.849 |
| Received the MenB vaccine | No | **0.000** | Reference category | | | 0.591 | Reference category | | |
| | Yes | **0.000** | 2.785 | 2.000 | 3.878 | 0.642 | 0.876 | 0.500 | 1.533 |
| | Don't know | 0.283 | 0.806 | 0.543 | 1.195 | 0.285 | 0.764 | 0.467 | 1.251 |

| | | Univariate analysis | | | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sig. | Exp(B) | 95% C.I.for EXP(B) | | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
| | | | | Lower | Upper | | | Lower | Upper |
| | Rather not say/no answer | 0.078 | 0.562 | 0.296 | 1.066 | 0.547 | 0.800 | 0.387 | 1.653 |
| | Too young | 0.595 | 1.478 | 0.350 | 6.247 | 0.297 | 4.200 | 0.282 | 62.437 |
| Received an annual flu vaccine | No | **0.000** | Reference category | | | 0.678 | Reference category | | |
| | Yes | 0.432 | 0.882 | 0.644 | 1.207 | 0.163 | 1.291 | 0.902 | 1.850 |
| | Don't know | 0.961 | 0.983 | 0.506 | 1.911 | 0.760 | 0.905 | 0.476 | 1.720 |
| | Rather not say/no answer | 0.999 | 0.000 | 0.000 | | 0.998 | >100 | 0.000 | |
| | Too young | **0.000** | 3.297 | 2.086 | 5.212 | 0.947 | 0.980 | 0.543 | 1.768 |
| Recently had a cold | No | **0.000** | Reference category | | | **0.038** | Reference category | | |
| | Yes | **0.000** | 1.965 | 1.481 | 2.606 | **0.011** | 3.712 | 1.359 | 10.141 |
| | Rather not say/no answer | 0.998 | 0.000 | 0.000 | | 0.998 | 0.000 | 0.000 | |
| Recently had the flu | No | 0.880 | Reference category | | | 0.716 | Reference category | | |
| | Yes | 0.412 | 0.708 | 0.310 | 1.616 | 0.414 | 1.533 | 0.550 | 4.273 |
| | Don't know | 0.999 | 0.000 | 0.000 | | 0.999 | 0.000 | 0.000 | |
| Recently had an ear infection | No | 0.233 | Reference category | | | 0.134 | Reference category | | |
| | Yes | 0.088 | 0.180 | 0.025 | 1.291 | 0.134 | 0.206 | 0.026 | 1.625 |
| Recently had a chest infection | No | 0.342 | Reference category | | | 0.950 | Reference category | | |
| | Yes | 0.143 | 0.603 | 0.306 | 1.187 | 0.950 | 0.970 | 0.380 | 2.480 |
| Had a recent RTI | Yes | **0.014** | Reference category | | | 0.355 | Reference category | | |
| | No | **0.001** | 0.625 | 0.471 | 0.829 | 0.150 | 2.142 | 0.759 | 6.044 |
| | Don't know | 0.999 | 0.000 | 0.000 | | 0.999 | 0.000 | 0.000 | |
| Recently used antibiotics | No | **0.023** | Reference category | | | **0.003** | Reference category | | |
| | Yes | **0.029** | 0.570 | 0.344 | 0.943 | 0.208 | 0.715 | 0.425 | 1.204 |
| | Rather not say/no answer | 0.112 | 3.362 | 0.753 | 15.008 | **0.002** | 14.427 | 2.629 | 79.170 |

| | | Univariate analysis | | | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sig. | Exp(B) | 95% C.I.for EXP(B) | | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
| | | | | Lower | Upper | | | Lower | Upper |
| Ethnicity | African | **0.015** | Reference category | | | 0.105 | Reference category | | |
| | American | 0.484 | 0.569 | 0.117 | 2.768 | 0.612 | 0.636 | 0.110 | 3.660 |
| | South East Asian | **0.014** | 0.220 | 0.066 | 0.733 | 0.089 | 0.327 | 0.090 | 1.187 |
| | European | **0.010** | 0.398 | 0.197 | 0.804 | 0.083 | 0.496 | 0.224 | 1.096 |
| | Eastern Mediterranean | 0.072 | 0.293 | 0.077 | 1.116 | 0.439 | 0.570 | 0.137 | 2.370 |
| | Western Pacific | 0.421 | 1.681 | 0.475 | 5.952 | 0.130 | 3.210 | 0.708 | 14.549 |
| | Mixed race | 0.320 | 0.638 | 0.263 | 1.548 | 0.364 | 0.632 | 0.234 | 1.704 |
| | Rather not say/no answer | 0.197 | 0.450 | 0.133 | 1.515 | 0.612 | 0.653 | 0.126 | 3.393 |
| Gender | Male | 0.112 | Reference category | | | 0.684 | Reference category | | |
| | Female | 0.040 | 0.743 | 0.560 | 0.987 | 0.384 | 0.877 | 0.654 | 1.177 |
| | Rather not say/no answer | 0.829 | 1.171 | 0.278 | 4.941 | 1.000 | 5.476 | 0.000 | |
| Has a long-term illness | No | 0.061 | Reference category | | | 0.642 | Reference category | | |
| | Yes | **0.020** | 0.686 | 0.499 | 0.943 | 0.338 | 1.211 | 0.819 | 1.790 |
| | Don't know | 0.197 | 4.130 | 0.479 | 35.583 | 0.482 | 2.277 | 0.230 | 22.599 |
| | Rather not say/no answer | 0.951 | 1.033 | 0.373 | 2.858 | 0.629 | 0.691 | 0.154 | 3.092 |
| Currently attends nursery | No | **0.000** | Reference category | | | 0.208 | Reference category | | |
| | Yes | **0.000** | 4.260 | 2.535 | 7.158 | 0.076 | 1.837 | 0.937 | 3.600 |
| | Rather not say/no answer | 0.999 | 0.000 | 0.000 | | 0.999 | 0.000 | 0.000 | |
| Exposed to SH cigar/cigarette smoke | Never | **0.000** | Reference category | | | 0.184 | Reference category | | |
| | On occasion | **0.000** | 0.456 | 0.306 | 0.679 | 0.134 | 0.677 | 0.406 | 1.128 |
| | Once a week | 0.146 | 0.661 | 0.378 | 1.156 | 0.376 | 1.411 | 0.658 | 3.026 |
| | Daily | **0.000** | 0.276 | 0.145 | 0.527 | 0.108 | 0.543 | 0.258 | 1.143 |

| | | Univariate analysis | | | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sig. | Exp(B) | 95% C.I.for EXP(B) | | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
| | | | | Lower | Upper | | | Lower | Upper |
| | No answer | 0.998 | 0.000 | 0.000 | | 0.998 | 0.000 | 0.000 | |
| Exposed to SH e-cigarette smoke | Never | **0.008** | Reference category | | | 0.773 | Reference category | | |
| | On occasion | **0.006** | 0.547 | 0.356 | 0.838 | 0.978 | 0.993 | 0.575 | 1.714 |
| | Once a week | 0.117 | 0.562 | 0.274 | 1.156 | 0.330 | 0.609 | 0.224 | 1.652 |
| | Daily | **0.022** | 0.381 | 0.167 | 0.869 | 0.323 | 0.629 | 0.250 | 1.578 |
| | No answer | 0.997 | 0.000 | 0.000 | | 0.997 | 0.000 | 0.000 | |
| Smokes cigars/cigarettes | No | 0.912 | Reference category | | | 1.000 | Reference category | | |
| | Yes | 0.667 | 0.871 | 0.465 | 1.631 | 0.987 | 1.006 | 0.514 | 1.966 |
| | Rather not say/no answer | 0.999 | 0.000 | 0.000 | | 0.999 | 0.000 | 0.000 | |
| Smokes e-cigarettes | No | 0.909 | Reference category | | | 0.992 | Reference category | | |
| | Yes | 0.662 | 0.797 | 0.288 | 2.205 | 0.900 | 0.932 | 0.309 | 2.805 |
| | Rather not say/no answer | 0.998 | 0.000 | 0.000 | | 0.998 | 0.000 | 0.000 | |
| Has COPD | No | 0.125 | Reference category | | | 0.905 | Reference category | | |
| | Yes | 0.140 | 0.583 | 0.285 | 1.194 | 0.655 | 1.175 | 0.578 | 2.388 |
| | Rather not say/no answer | 0.168 | 2.824 | 0.645 | 12.370 | 0.998 | >100 | 0.000 | |

**Table 8. Overview of univariate and multivariate analysis using all data**.

*When carriage in care home residents was compared to the community-based participant population of the same age, residency was actually significantly associated with carriage (P=<0.001, df 1, OR 4.440).

** When receipt of PCV and PPV were analyses separately in comparison to community-based participants of the correct age, receipt of PCV was significantly associated with carriage (P= 0.029, OR 1.837) although receipt of PPV was not (P= 0.569, OR 0.831). When receipt of PVC was analysed in a multivariate model using only child participants, no significant association was found confirming the results in Table 8 to be accurate.

*** When long term illness was assessed separately in children and adults, long-term illness and *M. catarrhalis* carriage did not have a statistically significant association (P= 0.579, OR 1.105) in children (0-16 year olds) nor in adults (those aged 17 years and over) (P= 0.19, OR 1.578).

## 4.5 Discussion

In the interest of updating the current body knowledge for *M. catarrhalis*, this chapter aimed to establish carriage prevalence across all ages and in cohorts of interest, and to define the epidemiology of *M*. *catarrhalis* in the context of potential risk factors. Such data is valuable for informing vaccine development and implementation strategies.

### 4.5.1 Univariate analysis

Care/nursing home residency was a significant risk factor for *M. catarrhalis* carriage. Residents were over four times more likely to carry *M. catarrhalis* than those of the same age based in the community. This is perhaps expected as residents are elderly with reducing immunity (immunosenescence) (Haq and McElhaney, 2014). Furthermore bacterial carriage and respiratory infection spread easily and quickly in care home settings due to prolonged close contact between residents and carers (Phipps *et al.*, 2019), whilst residents are often frail or have co-morbidities and therefore prone to bacterial carriage and disease.

The presence of *S. pneumoniae* and *H. influenzae* is associated with an increased likelihood of *M. catarrhalis* carriage by five and three times respectively. Whilst the presence of *S. aureus* reduces the risk of *M. catarrhalis* carriage by almost 50%.

Previous research has shown that viral carriage and infection increase the likelihood of an individual becoming colonised and infected by *M. catarrhalis* (Hament *et al.*, 1999; Nguyen *et al.*, 2012; Morris, Cleary and Clarke, 2017; DeMuri *et al.*, 2018). However univariate analysis showed that receipt of an annual flu vaccine reduced the likelihood of *M. catarrhalis* carriage. When broken down by age, receipt of the flu vaccine may actually be associated with reduced carriage in 0-4 year olds (P=0.446, or 0.553), and increased carriage in those aged 5+ (P=0.268, OR 1.310). It is unclear if this is due to the differences in vaccine received; a Live Attenuated Influenza Vaccine (LAIV) in the form of a nasal spray is given to children and an injectable inactivated influenza vaccine is given to adolescents and adults. It is possible that LAIV nasal spray affects the microbiome of the airway, resulting in a reduction in the carriage of *M. catarrhalis* (if so, this is likely to be a short-term affect and detected because swabbing occurred over the same time flu vaccines are given). Although other studies have suggested increases in colonisation and replication in the NP and middle ear by bacteria (*S. aureus*, *S. pneumoniae* and *H. influenzae*) (Smith and Huber, 2018), including *M. catarrhalis* (Thors *et al.*, 2016a) following LAIV. There is no published data for the effects of the inactivated influenza

vaccine on bacterial carriage. To truly look at the association of the different flu vaccines and *M. catarrhalis* carriage, the study should have confirmed what vaccine was given.

Long-term illness/condition was associated with an increased risk of *M. catarrhalis* NP carriage in adults, this possibly linked to frequent or long-term medicine use, such as antibiotics or statins affecting the microbiome of the NP (Graziano *et al.*, 2015). Whilst those who have not reported a recent RTI were almost half as likely to carry *M. catarrhalis* (OR 0.608), therefore recent RTI is a risk factor for the carriage of *M. catarrhalis*. This should perhaps be expected given the association of *M. catarrhalis* carriage and disease, and the association of *M. catarrhalis* carriage and various other pathobionts including *S.* pneumoniae, H*. influenzae* and numerous viruses.

Overall, recent use of antibiotics was significantly associated with reduced carriage of *M. catarrhalis*. However, Figure 21 shows that children aged 0-4 who received antibiotics within a month of participation, showed increased carriage in the NP and nose. The majority of antibiotic prescriptions in children (74%) are for RTI (O'Brien *et al.*, 2015), and the first line antibiotics commonly prescribed are amoxicillin or penicillin V (Williams *et al.*, 2018). As β-lactams, *M. catarrhalis* are resistant to these antibiotics; therefore, the increased carriage seen here is the likely result of *M. catarrhalis* filling the niche left by eradicated bacterial species following antibiotic treatment. Amoxicillin is the most commonly prescribed antibiotic for RTI's in children, and the majority (94%) of the young children participants who had recently taken antibiotics confirmed they had recently had an RTI or confirmed use of amoxicillin. Doxycycline is the most commonly prescribed for RTI's for adults and the elderly and the majority (65%) of participant who had recently used antibiotics had also recently had an RTI or confirmed use of doxycycline (Dekker, Verheij and van der Velden, 2015). *M. catarrhalis* are susceptible to tetracyclines such as doxycycline, possibly explaining the reduction in carriage seen the adult age groups. Of course the reduction in carriage in those aged 5-16 of course remains unexplained.

Receipt of PCV was associated with increased *M. catarrhalis* carriage, with carriage almost twice as likely in those vaccinated. These results are supported by data from Greenland showing that *M. catarrhalis* carriage increased significantly among vaccinated children (Navne *et al.*, 2017), although other research reports *M. catarrhalis* carriage to be unaffected (Spijkerman *et al.*, 2012). Increases may be explained by *M. catarrhalis* filling the niche once filled by VT S. pneumoniae, or as a result of vaccine induced changes in respiratory microbiome dynamics and colonisation patterns. Or perhaps positive interactions between *M. catarrhalis* and certain non-vaccine serotype *S. pneumoniae* are responsible (Navne *et al.*,

2016). Other studies have reported a similar phenomenon where by vaccination with one species affects the carriage of another; receipt of the measles-yellow fever vaccine shown to significantly reduce carriage of *H. influenzae* and *S. pneumoniae* (Bottomley *et al.*, 2015).

Receipt of the MenB vaccine was also shown to be associated with increased *M. catarrhalis* carriage. A consideration regarding the impact of such vaccines on *M. catarrhalis* carriage, is that vaccination history was based on completion of the questionnaire. Some participants/ parents of participants have stated that all scheduled vaccines were received but then said that the MenB and PCV vaccines were not, even though they are part of the schedule. Perhaps analysis should be redone with the answers researchers believe are correct, i.e., if the questionnaire states all scheduled vaccines have been received and the participant is of correct age, then analysis should be done with the assumption that PCV and MenB vaccines were indeed received? However, such re-analysis could introduce bias. With hindsight, the better approach would have been to obtain consent to check GP records. Parents are unlikely to remember the details of which vaccines have been given, and the lack of ability to confirm is a limitation of the methodology.

Nursery attendance almost doubles the likelihood of *M. catarrhalis* carriage, particularly in the nose, which was the only site to have a significant association when individual sites were investigated. This may be expected as children have a higher exposure to circulating bacteria due to close interaction with a range of other young children. Studies have shown nursery attendance to be a risk factor for the carriage of other pathobionts; even having a sibling who attends day-care is a risk factor for *S. pneumoniae* carriage (Navne *et al.*, 2017).

Smoking was not associated with *M. catarrhalis* carriage, which contrasts the findings of prior research which suggested smoking was associated with increased carriage of *M. catarrhalis.* However such research comprised a low sample number (only 40 adults, 20 smokers and 20 non-smokers), thus results are perhaps less reliable (Brook and Gober, 2007) SHS from cigars/cigarettes and of e-cigarettes was associated with a reduction in carriage, although it is unclear why. Published data has shown that SHS is positively associated with the carriage of *S. pneumoniae* (Lee *et al.*, 2010) and *S. aureus* (Bogaert *et al.*, 2004), significantly associated with increased risk of meningococcal carriage and disease in children and adolescents (Lee *et al.*, 2010; Kusel, Timm and Lockhart, 2013). Whilst there is limited research on the effects of smoking and SHS on *M. catarrhalis* carriage, especially for e-cigarettes, the data here does contradict prior research which suggests tobacco smoke exposure is associated with increased carriage of *M. catarrhalis* in children (Bakhshaee *et al.*, 2012; Fadlyana *et al.*, 2018) and the elderly (Kurtti *et al.*, 1997).

Carriage is often a prerequisite for disease; therefore, the factors tested and shown to be associated with increased carriage of *M. catarrhalis* could also be risk factors for disease. This is something to consider when implementing and designing vaccine policies. Certainly, vaccines focused to those in care homes would be of interest for public health. Nevertheless, the need to also look at disease cases and isolates has been highlighted. For example, whilst COPD was not a risk factor for *M. catarrhalis* carriage, the acquisition of new strains of *M. catarrhalis* is a key part of COPD exacerbation (Murphy and Parameswaran, 2009; Wilkinson *et al.*, 2017). Therefore, epidemiology of carriage and disease, and the transition between the two requires further understanding. A further consideration is that perhaps asymptomatic carriers are less likely to develop disease but can pass on to others in the same environment.

### 4.5.2 Multivariate analysis

Multivariate analysis was undertaken to counteract confounding variables. Analysis suggests that only site, age, microbial co-carriage, vaccination status, recent/concurrent cold and recent use of antibiotics were associated with the carriage of *M. catarrhalis*. NP and 0-4 years were the site and age group most associated with carriage. *S. pneumoniae*, H*. influenzae and N. meningitidis* were associated with an increased likelihood of carriage by 2.3, 1.4 and 5.4 times respectively. *S. aureus* was associated with a 60% reduction in the likelihood of *M. catarrhalis* carriage. Recent/concurrent cold was associated with increased likelihood of *M. catarrhalis* carriage (almost 4 times more likely), although other RTI (flu, ear infection and chest infection) was not. It is unclear whether this is a true distinction or due to the lower proportion of participants that had these other RTIs and therefore lower detection of *M. catarrhalis*. In total, 566 participants had a recent/concurrent cold (of which 9.9%, 1.9% and 8.0% carried *M. catarrhalis* in the NP, OP and nose respectively). However only 65 participants had flu (of which 4.6%, 0% and 4.6% carried *M. catarrhalis* in the NP, OP and nose respectively), 41 had an ear infection (of which 2.1, 0% and 0% and carried *M. catarrhalis* in the NP, OP and nose respectively) and 126 had a chest infection (of which 4.8%, 0% and 4.0% carried *M. catarrhalis* in the NP, OP and nose respectively). No recent use of antibiotics was also associated with increased likelihood of carriage.

It is possible that during univariate analysis, age acted as a confounder, suggesting a significant association between PCV and MenB vaccination and carriage where the perhaps there was none. Equally it is possible that recent antibiotic use was a confounder causing the association seen for LTI.

# Chapter 5 Antimicrobial resistance of community *M. catarrhalis* isolates

## 5.1 Introduction

With few new antibiotics being developed and decreasing susceptibility to those available, antibiotic resistance is an increasing issue (Conly and Johnston, 2005). The use of vaccines helps reduce the prevalence of infection, indirectly reducing the use of antibiotic and emergence and/or spread of AMR (Micoli *et al.*, 2021). Furthermore, vaccines such as the PCV target not only the most pathogenic serotypes, but those associated with AMR. The reduction in carriage of such serotypes directly reduces the prevalence of AMR and spread of AMR genes (Andrejko *et al.*, 2021). However, when bacterial infection does occur, we are reliant on antibiotics; and with no vaccine available for *M. catarrhalis* antibiotics are the only defence available. Therefore, this chapter aimed to investigate prevalence of AMR in *M. catarrhalis* isolated from the community and care home residents as part of the Solent SMART Study. Especially as UK AMR data for this bacterium is limited. This chapter also aimed to investigate prevalence of AMR in association with factors such as vaccine status and health status. This chapter will then reflect on the level of AMR exhibited by *M. catarrhalis*, as well as the impact of this and any association between AMR and such independent variables on public health. Furthermore, with access to data from *M. catarrhalis* isolated from clinical cases, prevalences of AMR in carriage and disease isolates were compared. Disease data was provided by the Public Health England laboratory at University Hospital Southampton (UHS) and represents all *M. catarrhalis* isolated in the laboratory from cases of disease over the same period as the Solent SMART Study.

## 5.2 Chapter aims and objectives

Aim: To characterise the prevalence of antibiotic resistance in *M. catarrhalis* carried in the community.

Hypothesis: Antibiotic resistance is highly prevalent in *M. catarrhalis* carried in the community.

Specific Objectives

- To determine the overall prevalence of antibiotic resistance (of those antibiotics most commonly prescribed for respiratory infection) in *M. catarrhalis* isolated from community-based participants.

- To determine the prevalence of antibiotic resistance (of those antibiotics most commonly prescribed for respiratory infection) in *M. catarrhalis* isolated from in specific age groups and cohorts of interest.

## 5.3 Methods

Phenotypic AMR testing was undertaken using antibiotic discs in accordance with EUCAST. Antibiotics chosen for testing were selected due to clinical relevance (based on community and NICE guidelines for the prescription of antibiotics for RTI and complications; Appendix A Table 2) and the availability of EUCAST guidance and breakpoints. For instance, *M. catarrhalis* isolates were not tested for penicillin as isolates express β-lactamase and therefore resistance is assumed. Per methods section 2.8, *M. catarrhalis* isolates were plated onto CBA, following 24 hours, growth was added to 1ml of saline to get an inoculum of 0.5 McFarland. This was spread onto Mueller Hinton F plates. Antibiotic discs, four per plate, were added and plates were incubated at 37°C in 5% CO2 for 18 hours (±2 hours). Each isolate was tested with amoxicillin-clavulanic acid (co-amoxiclav) 2-1ug, cefotaxime 5ug and ceftriaxone 30ug (cephalosporins), erythromycin 15ug (macrolide), tetracycline 30ug (tetracycline), ciprofloxacin 5ug (fluoroquinolone) and meropenem 10ug (carbapenem). European committee on antimicrobial susceptibility testing (EUCAST) breakpoints were used to assess susceptibility and resistance.

The prevalence of AMR amongst *M. catarrhalis* was determined as a percentage of all isolates:

Prevalence of AMR (%) =

(Frequency of resistant *M. catarrhalis*/total number of *M. catarrhalis* tested) × 100

This was done individually for each antibiotic tested and shown as a total and then by anatomical site and age. A percentage of isolates resistant to 1, 2, 3+ antibiotics was also calculated:

(Frequency of *M. catarrhalis* resistant x number of antibiotics/total number of *M. catarrhalis* tested) × 100

Association between AMR of *M. catarrhalis* and age, ethnicity, health status, recent AMR use was investigated. An OR was calculated and used to understand the effect of such variables on AMR (McNamee, 2005). $X^2$ or FET was used to determine whether a relationship or association between an independent variable and AMR was statistically significant, as demonstrated by a P-value of $< 0.05$.

## 5.4 Results

Overall, resistance was low; 28.2% (n=62) of isolates were resistant to at least one antibiotic, 14.6% (n=32) of isolates were resistant to at least two antibiotics, 9.6% (n=21) of isolates were resistant to at least three antibiotics, 4.6% (n=10) of isolates were resistant to at least four antibiotics and 1.8% (n=4) of isolates were resistant to at least five antibiotics. All isolates were sensitive to at least three of the antibiotics tested.

### 5.4.1 AMR of *M. catarrhalis* isolated from community participants

Most resistance was seen for ciprofloxacin, with 30.9% (n=60) of isolates being resistant (Figure 33). 14.4% (n=28) of isolates were resistant to chloramphenicol, 10.3% (n=20) were resistant to meropenem, 5.7% (n=11) were resistant to tetracycline, 3.6% (n=7) were resistant to erythromycin, 1% (2) were resistant to cefotaxime and 0.5% (1) were resistant to ceftriaxone. None of the isolates were resistant to co-amoxiclav.

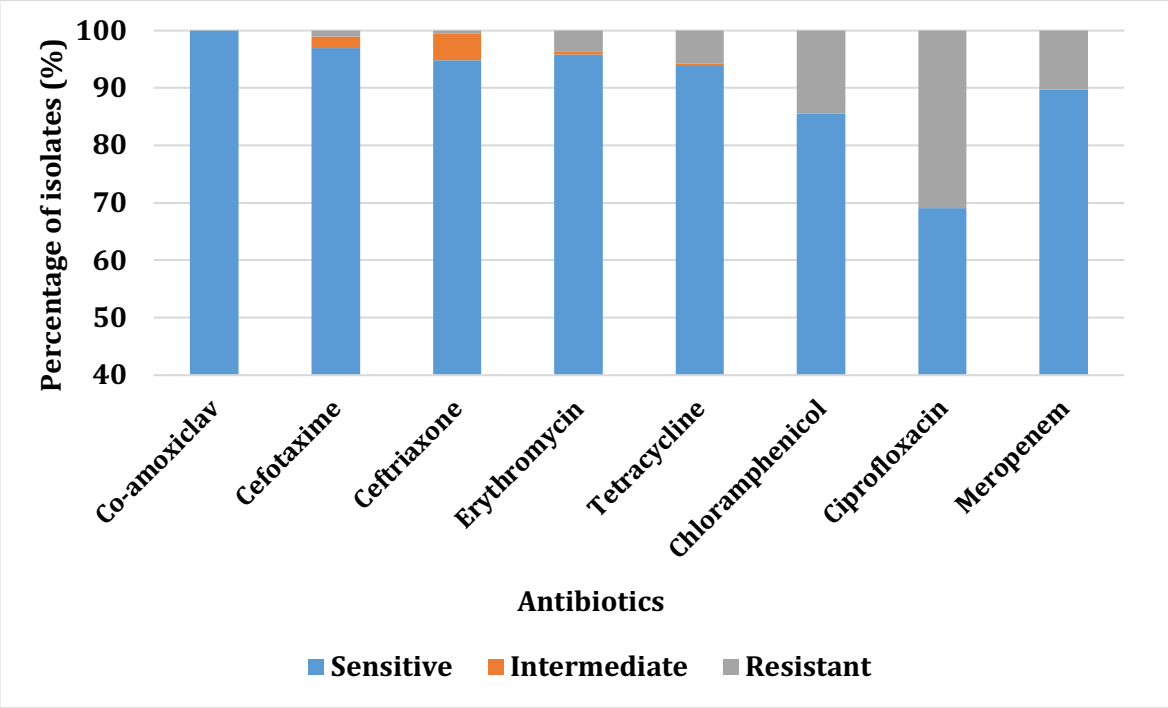
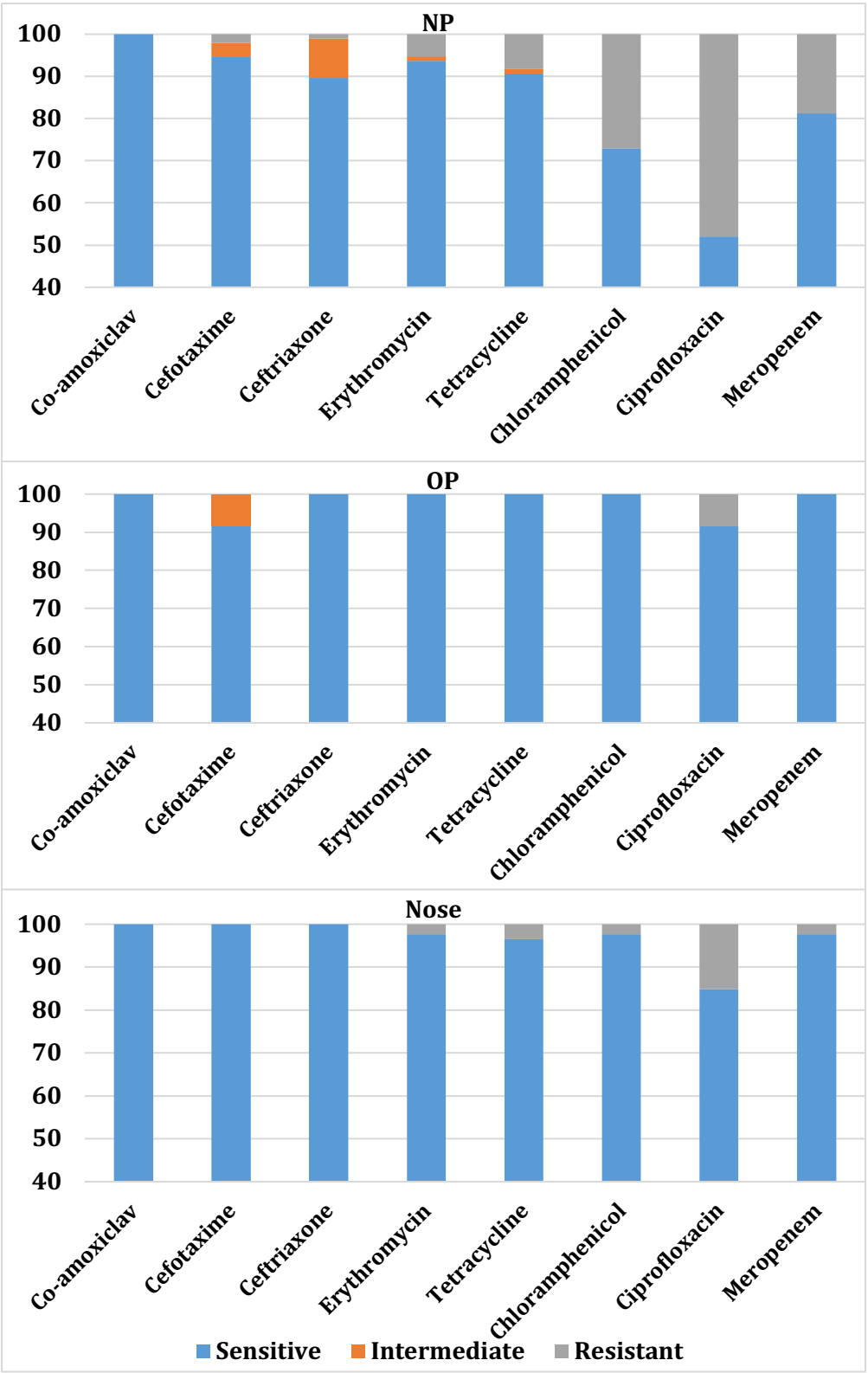



**Figure 33. AMR of *M. catarrhalis* isolated from community-based participants.**

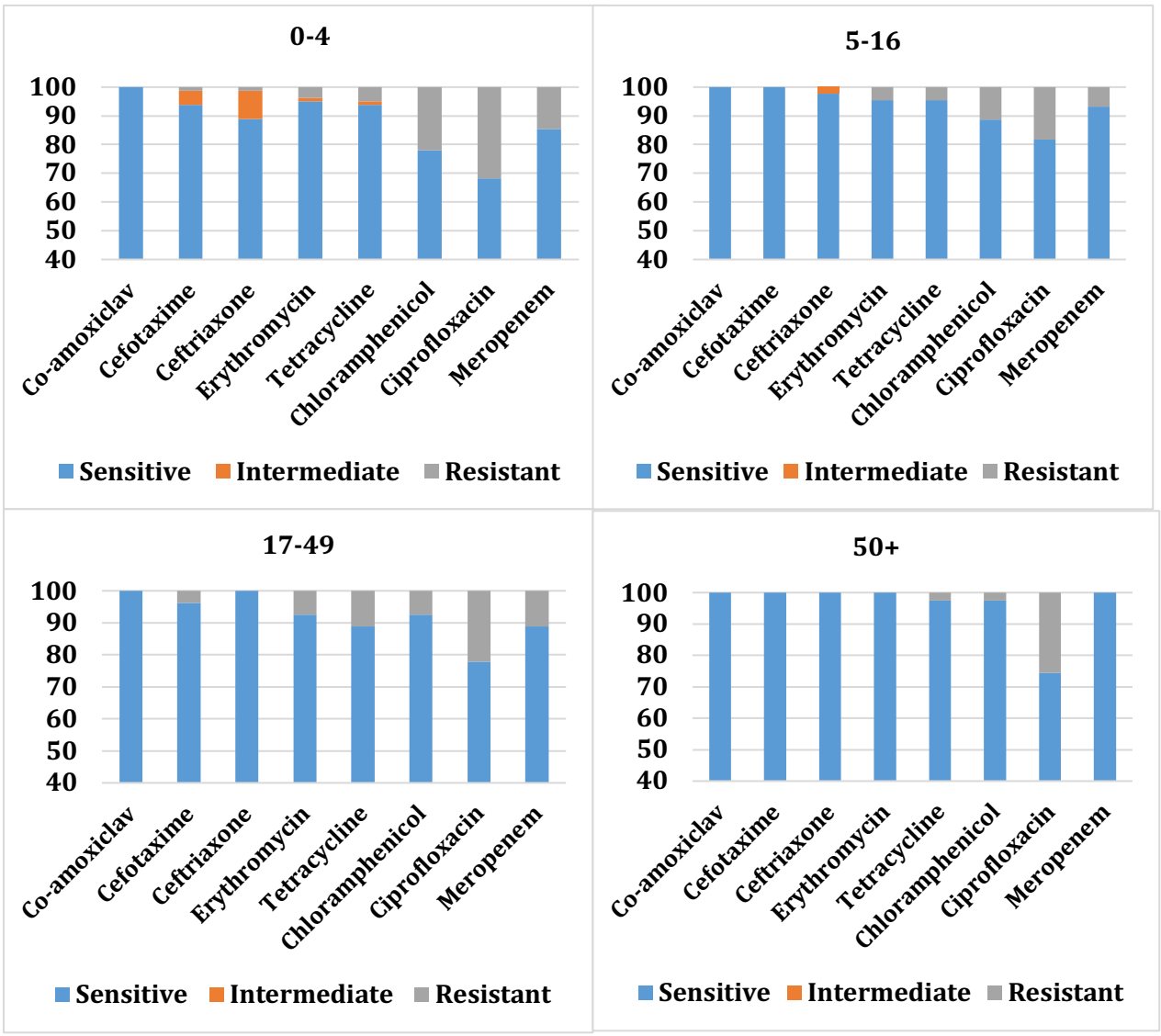
When looking at AMR by site (Figure 34), *M. catarrhalis* isolated from the NP displayed a higher prevalence of AMR than those isolated from other sites. Using $X^2$, chloramphenicol, ciprofloxacin and meropenem resistance was significantly more likely in *M. catarrhalis* isolated from the NP (data in Table 10).




| | Prevalence of resistance (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Co-amoxiclav | Cefotaxime | Ceftriaxone | Erythromycin | Tetracycline | Chloramphenicol | Ciprofloxacin | Meropenem |
| NP | 0.0 | 2.1 | 1.0 | 5.2 | 8.3 | 27.1 | 47.9 | 18.8 |
| OP | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 8.3 | 0.0 |
| Nose | 0.0 | 0.0 | 0.0 | 2.3 | 3.5 | 2.3 | 15.1 | 2.3 |

**Figure 34. AMR of *M. catarrhalis* isolated from community-based participants by site.**

When looking at AMR by age group (Figure 35), ciprofloxacin was most prevalent in all age groups. *M. catarrhalis* isolated from those aged 0-4 showed higher prevalence of resistance for the majority of antibiotics. However, when using $X^2$, age group was only statistically significantly associated with chloramphenicol resistance with 0-4 year olds showing statistically higher prevalence (data in Table 10).




| | Prevalence of resistance (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Co-amoxiclav | Cefotaxime | Ceftriaxone | Erythromycin | Tetracycline | Chloramphenicol | Ciprofloxacin | Meropenem |
| 0-4 | 0.0 | 1.2 | 1.2 | 3.7 | 4.9 | 22.0 | 31.7 | 14.6 |
| 5-16 | 0.0 | 0.0 | 0.0 | 4.5 | 4.5 | 11.4 | 18.2 | 6.8 |
| 17- | 0.0 | 3.7 | 0.0 | 7.4 | 11.1 | 7.4 | 22.2 | 11.1 |
| 50+ | 0.0 | 0.0 | 0.0 | 0.0 | 2.6 | 2.6 | 25.6 | 0.0 |

**Figure 35. AMR of M. catarrhalis isolated from community-based participants by age group.**

### 5.4.2 AMR of *M. catarrhalis* isolated from care home residents

Consistent with *M. catarrhalis* isolated from community-based participants, isolates from care home residents showed the most resistance to ciprofloxacin, with 34.6% (n=9) of isolates being resistant. However only 7.7% (n=2) of isolates were resistant to chloramphenicol, 7.7% (n=2) were resistant to meropenem and 3.8% (n=1) were resistant to tetracycline. None of the isolates were resistant to co-amoxiclav, cefotaxime, ceftriaxone and erythromycin.

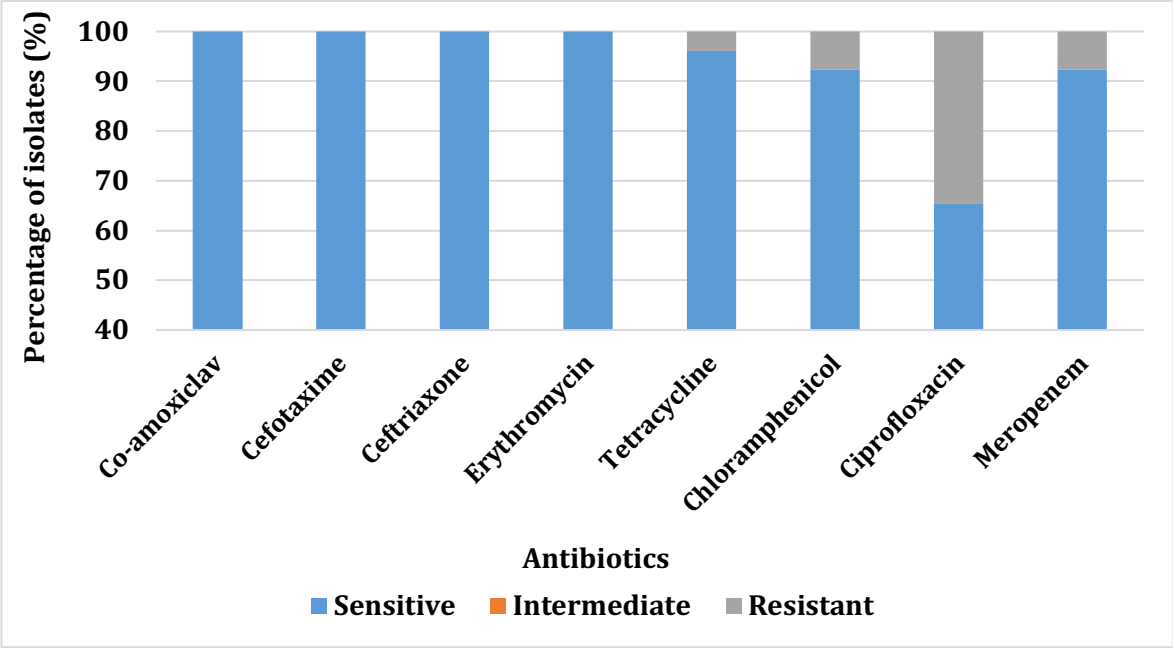



**Figure 35. AMR of *M. catarrhalis* isolated from care home residents.**

### 5.4.3 AMR of *M. catarrhalis* isolated from participants with COPD

Consistent with *M. catarrhalis* isolated from other community-based participants and care home residents, isolates from those with COPD showed the most resistance to ciprofloxacin, with 25.0% (n=2) of isolates being resistant. However, resistance was not shown for any other antibiotics.

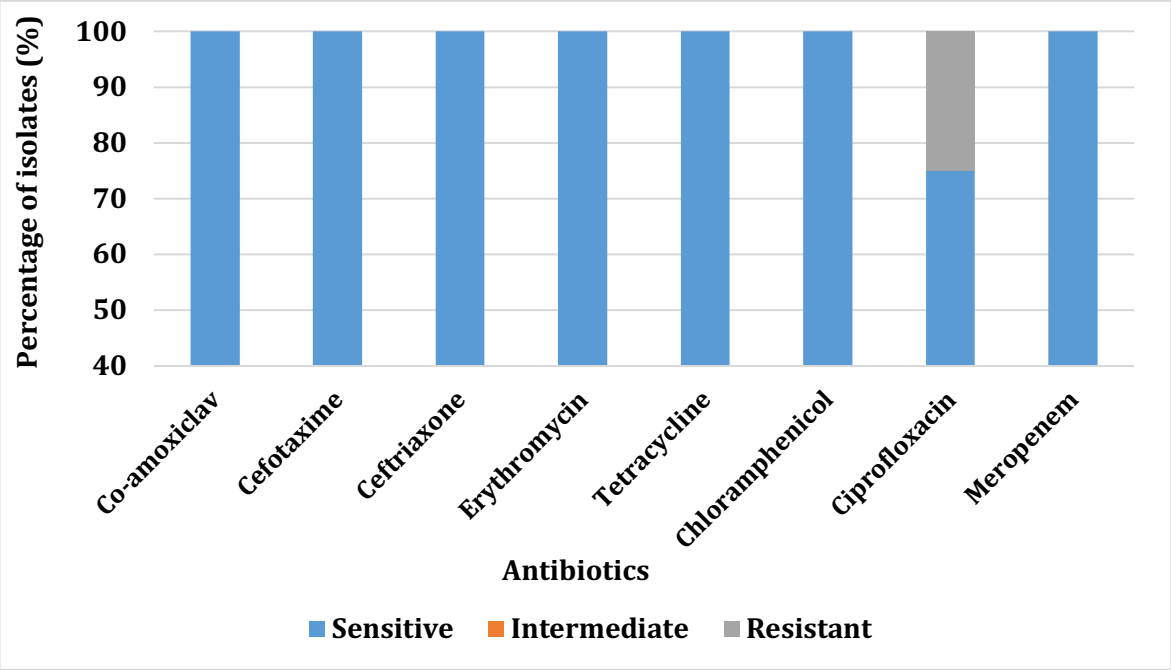



**Figure 36. AMR of *M. catarrhalis* from participants with COPD**.

### 5.4.4 AMR and associated risk factors

Co-amoxiclav, cefotaxime and ceftriaxone were not tested due to low levels of resistance (none, two and one isolate/s respectively). Using $X^2$, swab site was significantly associated with chloramphenicol, ciprofloxacin and meropenem resistance. Age was also significantly associated with chloramphenicol resistance. Data shown in Table 9. OR was further used to describe the association, as seen in Table 10. Isolates of *M. catarrhalis* found in the NP were most associated with AMR, although AMR of OP isolates was not significant, possibly due to the low number of isolates. Only isolates from those ages 0-4 and 50+ were significantly associated with chloramphenicol AMR.

| | Co-amoxiclav/ Cefotaxime/Ceftriaxone | Erythromycin | Tetracycline | Chloramphenicol | Ciprofloxacin | Meropenem |
|---|---|---|---|---|---|---|
| Swab site | N/A | P=0.671 | P=0.346 | **P= <0.001** | **P= <0.001** | **P= <0.001** |
| Age group | N/A | P=0.417 | P=0.676 | **P=0.026** | P=0.426 | P=0.072 |
| Co- carriage with *S. pneumoniae* | N/A | P= 0.276 | P=1.000 | P=0.147 | P=0.667 | P=0.089 |
| Co- carriage with *H. influenzae* | N/A | P= 1.000 | P= 0.606 | P= 0.084 | P= 0.203 | P= 0.230 |
| Co- carriage with *N. meningitidis* | N/A | P= 1.000 | P= 1.000 | P= 1.000 | P= 0.564 | P= 1.000 |
| Co- carriage with *S. aureus* | N/A | P= 0.067 | P= 0.152 | P= 0.399 | P= 1.000 | P= 0.624 |
| Receipt of all scheduled vaccines | N/A | P= 1.000 | P= 1.000 | P= 0.873 | P= 0.830 | P= 0.649 |
| Receipt of the pneumococcal vaccine | N/A | P= 0.509 | P= 0.100 | P= 0.151 | P= 0.787 | P= 0.395 |
| Receipt of the MenB vaccine | N/A | P= 1.000 | P= 0.486 | P= 0.397 | P= 0.442 | P= 0.456 |
| Receipt of the annual flu vaccine | N/A | p= 0.424 | P= 0.912 | P**= 0.048** | P= 0.239 | P= 0.102 |
| Recent cold | N/A | P= 0.719 | P= 0.766 | P= 0.157 | P= 0.879 | P= 0.483 |
| Recent flu | N/A | P= 1.000 | P= 1.000 | P= 1.000 | P= 0.664 | P= 1.000 |
| Recent ear infection | N/A | P= 1.000 | P= 1.000 | P= 1.000 | P= 1.000 | P= 1.000 |
| Recent chest infection | N/A | P= 1.000 | P= 1.000 | P= 1.000 | P= 1.000 | P= 1.000 |
| No recent RTI | N/A | P= 0.703 | P= 0.546 | P= 0.319 | P= 0.879 | P= 0.814 |
| Recent antibiotic use | N/A | P= 0.596 | P= 0.654 | P= 0.809 | P= 0.901 | P= 1.000 |
| Ethnicity | N/A | P= 1.000 | P= 0.780 | P= 0.620 | P= 0.117 | P= 0.939 |
| Gender | N/A | P= 0.481 | P= 0.791 | P= 0.478 | P= 1.000 | P= 1.000 |
| Long-term illness | N/A | P= 0.522 | P= 1.000 | P= 0.263 | P= 0.371 | P= 0.206 |
| Nursery attendance | N/A | P= 0.532 | P= 0.608 | P= 1.000 | P= 0.617 | P= 0.436 |
| SHS - cigar and cigarette | N/A | P= 0.669 | P= 0.0394 | P= 0.193 | P= 0.117 | P= 0.252 |
| SHS – E-cigarette | N/A | P= 0.490 | P= 1.000 | P= 0.553 | P= 0.787 | P= 0.526 |
| Smokes - cigar and cigarette | N/A | P= 1.000 | P= 0.528 | P= 1.000 | P= 0.745 | P= 1.000 |
| Smokes – E-cigarette | N/A | P= 1.000 | P= 1.000 | P= 0.568 | P= 1.000 | P= 0.444 |
| COPD | N/A | P= 1.000 | P= 0.528 | P= 1.000 | P= 0.862 | P= 1.000 |

**Table 9. Results of $X^2$ analysis for potential risk factors for AMR**.

| Antibiotics | Variable | X2 | | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower | Upper |
| Chloramphenicol | Swab site | P= <0.001 | NP | **0.002** | Reference | | |
| | | | OP | 0.998 | 0.000 | 0.000 | |
| | | | Nose | **0.000** | 0.069 | 0.016 | 0.298 |
| | Age group | P=0.026 | 0-4 | **0.052** | Reference | | |
| | | | 17-49 | 0.104 | 0.280 | 0.060 | 1.296 |
| | | | 5-16 | 0.141 | 0.449 | 0.154 | 1.306 |
| | | | 50+ | **0.023** | 0.092 | 0.012 | 0.718 |
| | | | care home | 0.116 | 0.292 | 0.063 | 1.353 |
| Ciprofloxacin | Swab site | P= <0.001 | NP | **0.000** | Reference | | |
| | | | OP | **0.026** | 0.096 | 0.012 | 0.759 |
| | | | Nose | **0.000** | 0.197 | 0.096 | 0.404 |
| Meropenem | Swab site | P= <0.001 | NP | **0.014** | Reference | | |
| | | | OP | 0.998 | 0.000 | 0.000 | |
| | | | Nose | **0.003** | 0.109 | 0.025 | 0.482 |

**Table 10. Results of univariate analysis for potential risk factors for AMR**.

When multivariate analysis was carried out, the only variables associated with AMR were as follows:

Chloramphenicol

Swab site (NP and nose); isolates from the NP are most associated with resistance to chloramphenicol. When NP isolates are used as a reference category, nasal isolates are less associated with an OR of 0.006 (P= <0.001). There was no significant association for OP isolates.

Co-carriage with *S. pneumoniae*; isolates found to co-colonise with *S. pneumoniae* were over 10 times more likely to be resistant to chloramphenicol (P= 0.052, OR 10.492).

Ciprofloxacin

Swab site (NP, OP and nose); isolates from the NP are most associated with resistance to chloramphenicol. When NP isolates are used as a reference category, nasal and OP isolates are less associated with ORs of 0.062 (P= 0.021) and 0.121 (P= <0.001) respectively.

Meropenem

Swab site (NP and nose); isolates from the NP are most associated with resistance to chloramphenicol. When NP isolates are used as a reference category, nasal isolates are less associated with an OR of 0.006 (P= <0.001).

### 5.4.5 AMR in carriage vs disease isolates

*M. catarrhalis* isolated from disease and processed in the PhD laboratory at UHS are routinely tested for resistance to ampicillin/ amoxicillin, co-amoxiclav, doxycycline, chloramphenicol and ciprofloxacin. Almost all disease isolates were resistant to ampicillin/ amoxicillin (99.4% ). Interestingly when comparing disease and carriage isolates, AMR was more prevelant in carriage isolates (Figure 38). Carriage isolates were associated with a higher assocciation to AMR than disease isolates. When carriage was used as a reference category; disease isolates were less likley to be resistant to chloramhenicol, ciprofloxacin and tetracycline each having an OR of 0.02 (P= <0.001), 0.008 (P= 0.001) and 0.169 (P= 0.007) respectively. Comparable to carriage isolates, all (but one) disease isolates were sensitive to at least three of the antibiotics tested. The final isolate was however sensitive to two of the antibiotics tested.

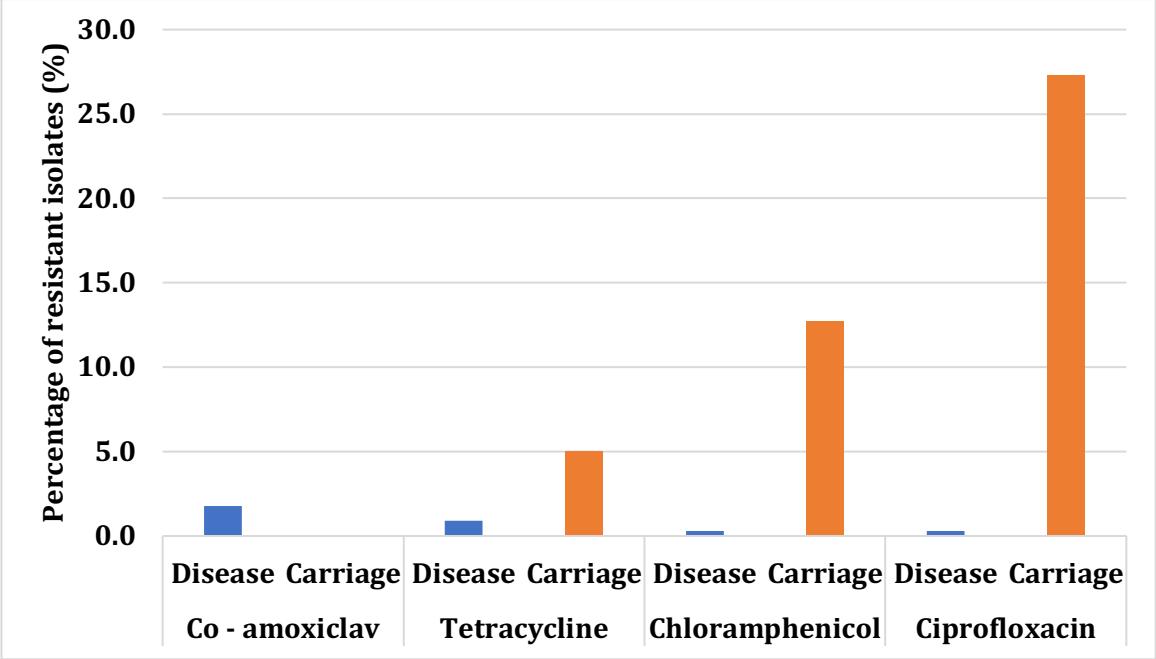



**Figure 37. AMR of carriage and disease *M. catarrhalis* isolates**.
Carriage AMR presented here includes isolates from community and care home participants.

## 5.5 Discussion

Only isolates from those ages 0-4 and 50+ years were significantly associated with resistance to chloramphenicol, this possibly associated with the increased prevalence of RTI and therefore antibiotic prescription in these group. Chloramphenicol is most commonly prescribed topically for conjunctivitis. This pattern fits as young children and the older adults are most at risk for bacterial conjunctivitis (Janine Robus, 2020). However, if this explanation is correct it is surprising that isolates from care home residents didn't have a significant association. Significance may not have been reached due to the lower number of isolates obtained from care home residents as a result of lower recruitment in this group.

Prevalence of resistance is low in both carriage and disease. What is of concern is that resistance to co-amoxiclav appears to be emerging, with 1.8% of disease isolates expressing resistance. Co-amoxiclav is commonly used for bacteria, including *M. catarrhalis*, which show resistance to β-lactam antibiotics. Fortunately, resistance to co-amoxiclav is clearly not a key aspect of disease due to its low prevalence in disease isolates. Likewise, isolates show high levels of sensitivity to other antibiotics so multi-drug resistance is not yet a concern for *M. catarrhalis.* Although, results for erythromycin can be used to determine susceptibility to azithromycin, clarithromycin and roxithromycin suggesting almost 4% prevalence of resistance to these antibiotics too.

All isolates of *M. catarrhalis* were included in analysis. Even when *M. catarrhalis* was carried in multiple anatomical sites, which arguably could unjustly inflate levels of resistance or sensitivity, as the assumption could be that the *M. catarrhalis* isolated from multiple sites are all the same strain. However, when looking at the AMR profiles of the *M. catarrhalis* isolated from multiple locations, they often had different profiles suggesting different strains may be residing in different anatomical sites. Of the 52 cases where *M. catarrhalis* is found in multiple anatomical locations in the same participant, 33 cases showed the isolates to have different AMR profiles (Appendix A Table 8). From those 33 cases, the majority of NP isolates seem to show more resistance than the isolates found in other sites (not true for all cases). This could perhaps be due to NP carriage having a longer duration therefore allowing *M. catarrhalis* to gain resistance via horizontal gene transfer from other bacterial species co-colonising at the site? Perhaps it is linked to the specific environment or microbial make-up of the NP niche? There are many reasons we can speculate on, but further investigation would be of benefit.

Surprisingly results show AMR is more associated with carriage than disease isolates, but are results comparable? Methodology used here is the same as that used by the PHE laboratory at UHS. However, whilst carriage isolates were tested with tetracycline the disease isolates were tested with doxycycline (both of which are tetracyclines). Isolates susceptible to tetracycline are also susceptible to doxycycline and minocycline, but some resistant to tetracycline may be susceptible to minocycline and/or doxycycline. Therefore, disease and carriage resistance to tetracyclines are not quite comparable. Results of the other antibiotics are however comparable.

There are several ways to test AMR; culture techniques include E-tests, disc diffusion and broth micro-dilution (Singh *et al.*, 2012), whilst molecular techniques include PCR and bioinformatic analysis on sequencing data. As the study utilised culture dependent bacterial identification, AMR was tested phenotypically using culture techniques. Phenotypic evidence is beneficial as the presence of an AMR gene found during molecular testing doesn't guarantee resistance is expressed. Various culture dependent AMR testing methods were considered. Micro-dilution can be time consuming as dilutions need to be made up, giving room for error, especially when a large number of isolates are being tested and the results accumulated and compared. Disc diffusion and E-testing are simpler and less laborious, particularly when media is bought in. Both methods are equally effective (Singh *et al.*, 2012), however disc diffusion is more cost effective (at the time of testing E-tests cost £3.71 each, whilst discs are only £0.02 each). The limitation of using disc diffusion is that the EUCAST break points are limited for some bacteria/antibiotics, which means there are many bacteria/antibiotics for which resistance cannot be assessed due to the lack of quantitative

cut off to define resistance versus sensitivity. *M. catarrhalis* is one of the bacteria for which there are many antibiotics with no breakpoints; however all clinically relevant antibiotics had breakpoints. Discs diffusion adequately confirmed resistance or sensitivity levels for *M. catarrhalis* and informs methodology design of future studies. Additionally, disc diffusion has been well standardised and is easily reproducible. Although a minimum inhibitory concentration (MIC) isn't established, disc diffusion allowed for the fulfilment of study aims and objectives (Jorgensen and Ferraro, 2009; Singh *et al.*, 2012).

# Chapter 6 Changes in carriage of *M. catarrhalis* and prevalence of AMR over time

Whilst so far this thesis has characterised the epidemiology of *M. catarrhalis* isolated as part of the Solent SMART Study, this chapter takes advantage of isolates collected as part of the Southampton Pneumococcal Carriage study. The Southampton Pneumococcal Carriage Study is a longitudinal study which provides over 10 years' worth of data for *M. catarrhalis* in young children. Data from both studies were used to investigate changes in carriage and AMR overtime, using $X^2$ test for trend. Calculations were carried out using IBM SPSS statistics version 28 and statistical significance assumed for P-values of <0.05. This chapter further reflects on the impact of changes (or lack of changes) in the prevalence of *M. catarrhalis* carriage and AMR, and the implications for the wider community and public health.

## 6.1 Chapter aims and objectives

Aim: To investigate the changes in the prevalence of *M. catarrhalis* carriage and AMR using supplementary data from another study.

Hypothesis: The prevalence of *M. catarrhalis* has increased over the past 12 years.

Objectives:

- To determine whether the prevalence of *M. catarrhalis* has increased over a 12 year period using supplementary data from the Southampton Pneumococcal Carriage Study.
- To determine if the prevalence of antibiotic resistance in *M. catarrhalis* isolated from young children (0-4 year olds), has increased over a 12 year period using supplementary isolates from the Southampton Pneumococcal Carriage Study.

## 6.2 Method

The Southampton Pneumococcal Carriage Study is a longitudinal cross-sectional study. Recruitment took place between October and March each year, from October 2006 onwards; although isolation of *M. catarrhalis* started from October 2008. Children aged 4 years and under were recruited as they visited the Paediatric Outpatients department at UHS. From October 2017 recruitment also took place at Solent NHS Trust sites and sites working in partnership with the Solent NHS Trust. As participants were aged 4 years and under; the parents or legal guardian were asked to provide informed consent for their child's

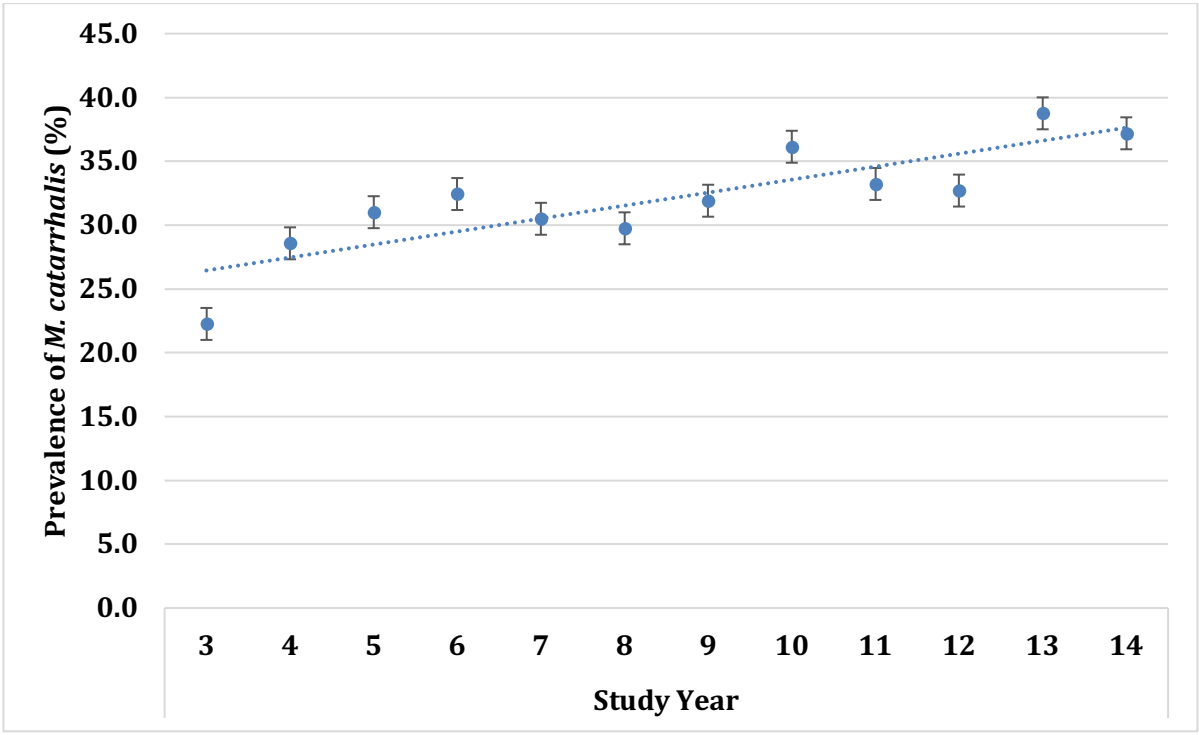
participation in the study. Informed consent was taken after having time to read the PIS provided and the opportunity to question study personnel. Entry to the study was voluntary.

NP swabs were taken to sample the URT of up to 1,000 children aged 4 years and under in each year of the study. Swabs were taken from children who are already attending/visiting the Paediatric Outpatients department or Solent NHS Trust sites and sites working in partnership with the Solent NHS Trust. Participating children either had an appointment at the site they are recruited or will be the relative of a child with an appointment. Swabbing was performed following a modified World Health Organisation (WHO) protocol (O'Brien, Nohynek and World Health Organization Pneumococcal Vaccine Trials Carriage Working, 2003; Satzke *et al.*, 2013). Swabs were collected by either a trained healthcare professional (i.e., a research nurse, health care assistant, medical student, doctor) or a trained member of the study team (i.e., clinical trials assistants) and submitted to the project research team for selective bacterial isolation and further analysis such as AMR testing. Swabs were taken solely for research purposes and were anonymised immediately. Swabs were processed (plated onto multi-purpose and selective media) within 48 hours. The remaining bacterial contents of the swabs was stored in STGG microbial storage media for the future analysis of bacterial DNA only. Following incubation for 24-48 hours at 37°C in 5% CO2 plates were examined for the presence of S. *pneumoniae, S. aureus, H. influenzae, N. meningitidis and M. catarrhalis*. Bacteria were identified in line with the methodology described in chapter 2.7 (although DNase testing was brought in later years and retrospectively done for earlier years).

## 6.3 Results

### 6.3.1 Carriage

Figure 39 shows the carriage prevalence of *M. catarrhalis* by year using data from the Southampton Pneumococcal Carriage Study (all sites) and the Solent SMART Study. The trend line shows an increase in carriage over the 12 year period. $X^2$ test for trend shows that the increase in carriage over the 12 years between 2008 and 2020 was significant (P= <0.001).




| Year | Year of Study | No of *M. catarrhalis* isolated | No of participants recruited | *M. catarrhalis* carriage prevalence | Binomial exact 95% CI |
|---|---|---|---|---|---|
| 2006/07 | 1 | N/A | 324 | N/A | N/A |
| 2007/08 | 2 | N/A | 373 | N/A | N/A |
| 2008/09 | 3 | 73 | 328 | 22.3 | 17.9-27.2 |
| 2009/10 | 4 | 114 | 399 | 28.6 | 24.2-33.3 |
| 2010/11 | 5 | 89 | 287 | 31.0 | 25.7-36.7 |
| 2011/12 | 6 | 108 | 333 | 32.4 | 27.4-37.8 |
| 2012/13 | 7 | 68 | 223 | 30.5 | 24.4-37.0 |
| 2013/14 | 8 | 94 | 316 | 29.7 | 24.8-35.1 |
| 2014/15 | 9 | 112 | 351 | 31.9 | 27.1-37.1 |
| 2015/16 | 10 | 189 | 523 | 36.1 | 32.0-40.4 |
| 2016/17 | 11 | 165 | 438 | 37.7 | 33.1-42.4 |
| 2017/18 | 12 | 265 | 808 | 32.8 | 29.6-36.2 |
| 2018/19 | 13 | 386 | 996 | 38.8 | 35.7-41.9 |
| 2019/20 | 14 | 360 | 968 | 37.2 | 34.1-40.3 |

**Figure 38. Carriage of *M. catarrhalis* between 2008 and 2020.**

### 6.3.2 AMR

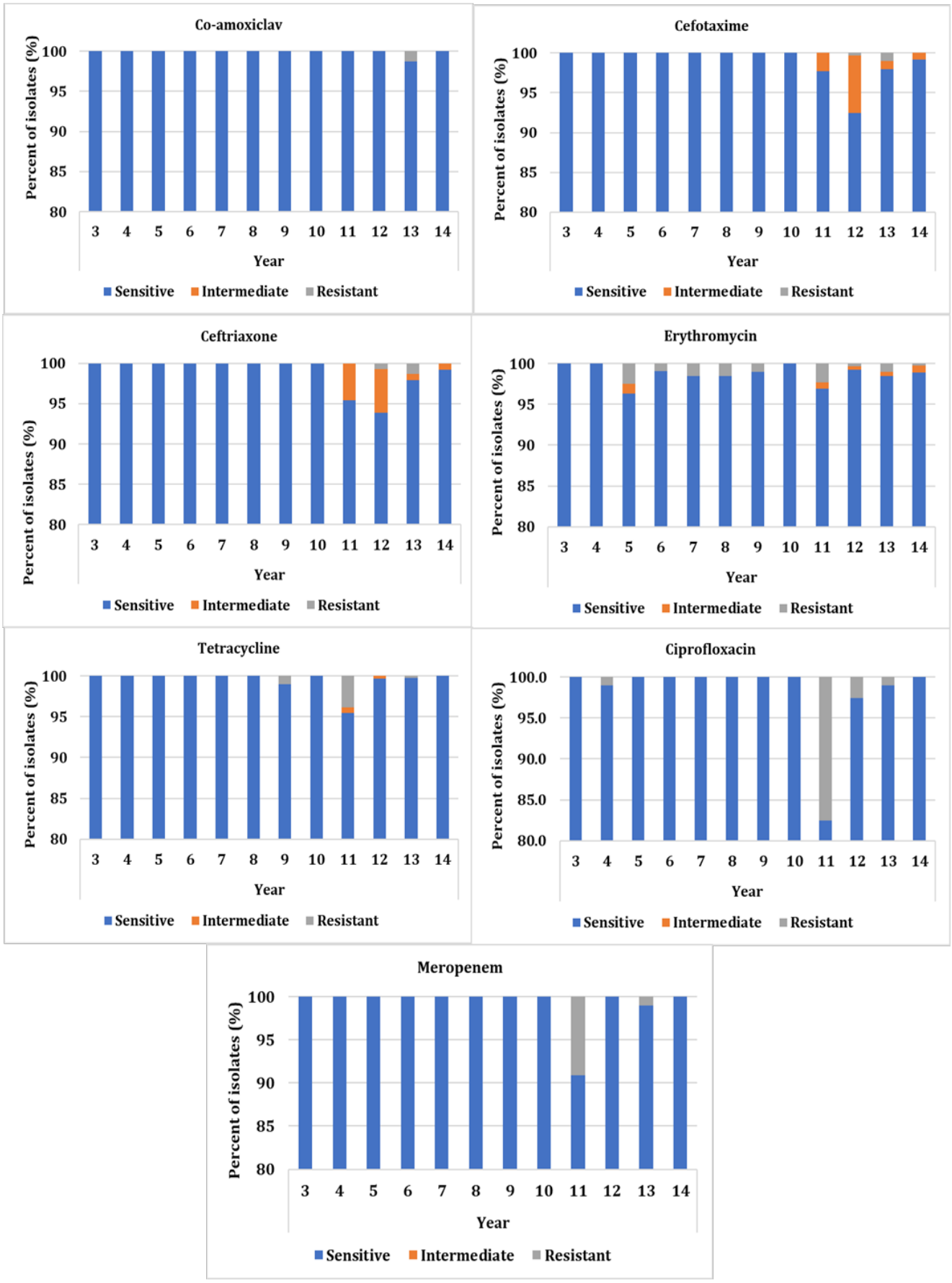



**Figure 39. Prevalence of AMR between 2008 and 2020.**

Figure 40 shows consistently low levels of resistance between 2008 and 2020. $X^2$ test for trend shows no significant change over time in resistance for the antibiotics tested.

Year 11 shows a spike in tetracycline, ciprofloxacin and meropenem resistance. To confirm whether this was a result of the inclusion of AMR data from the Solent SMART Study, the two datasets were compared. Figure 41 compares data from October 2016-March 2017 for both the Southampton Pneumococcal Carriage and Solent SMART studies, highlighting a higher prevalence of resistance in Solent SMART Study isolates. This explains the spike in AMR in year 11. Data from Solent SMART Study was also included in year 12, however there were only 12 *M. catarrhalis* isolates to include thus had less of an effect on overall percentages.

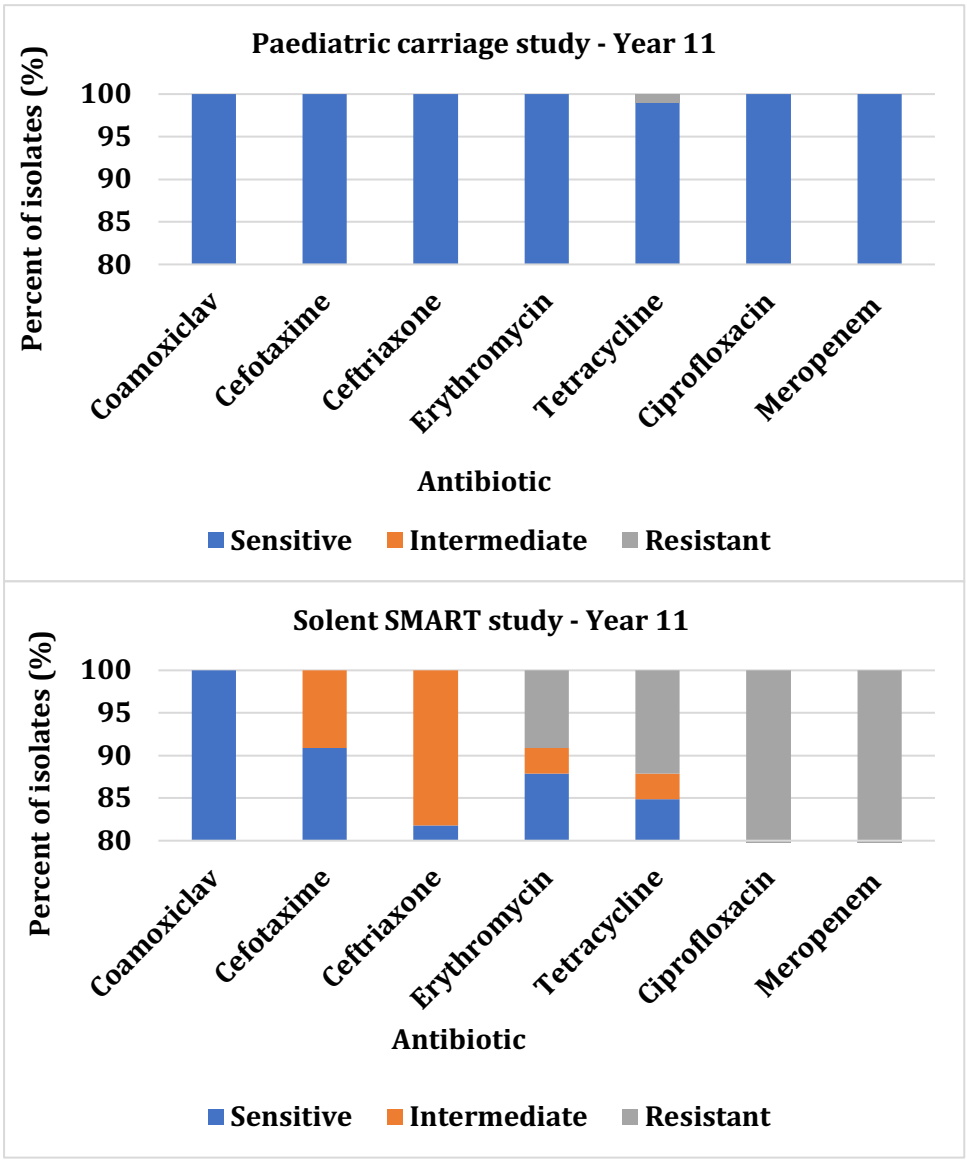



**Figure 40. Comparison of AMR data from the Southampton Pneumococcal Carriage and Solent SMART Studies**.

## 6.4 Discussion

Prevalence of *M. catarrhalis* carriage has significantly increased since 2008, however levels of resistance to co-amoxiclav, cefotaxime, ceftriaxone, erythromycin, tetracycline, ciprofloxacin and meropenem have remained low. Such data is beneficial as publication often states *M. catarrhalis* disease is an increasing problem or is increasing but it's not always clear whether *M. catarrhalis* disease is increasing in proportion as a result of a reduction in disease caused by other bacteria due to the successful vaccination schedules, or whether there is a genuine increase in the incidence and/ or prevalence of *M. catarrhalis* disease. As carriage is increasing and disease caused by VT bacteria has reduced, both are likely true (Goldstein, Murphy and Parameswaran, 2009). Furthermore, whilst various studies have shown an increase in *M. catarrhalis* infection and disease (Verduin *et al.*, 2002; Goldstein, Murphy and Parameswaran, 2009; Ramadan *et al.*, 2017) overall increases are hard to truly quantify. Unlike other pathogens such as *S. pneumoniae*, *H. influenzae* and *N. meningitidis*, infection and disease cause by *M. catarrhalis* is not surveyed or monitored. For one, many of the infections caused by *M. catarrhalis* are blindly treated with antibiotics and the causes rarely identified. Whilst some research has tested old samples suggesting an increase in *M. catarrhalis* infection, there is no regulatory data from institutions such as the PHE or WHO. Continuous research on *M. catarrhalis* as a cause of disease would be a beneficial area of future work, as would the monitoring of AMR in such isolates.

Prevalence of AMR has remained low over the 12 years between 2008 and 2020, although the emergence of intermediate and resistant isolates since 2016 (study year 11) is of interest to public health and highlights the need to monitor the AMR of *M. catarrhalis* over coming years so we are aware of the emergence of increased prevalence of AMR to inform clinical practice. The likelihood that this emergence could be a result of the inclusion of recruitment in the Solent community was considered; prior to year 11 recruitment only took place in the children's outpatients depart of UHS. It is unclear why community isolates would have more resistance / emerging intermediate sensitivity. However, looking at the raw data, this doesn't ring true. Resistant and intermediate isolates came from both UHS and Solent recruitment, suggesting genuine emergence.

# Chapter 7 Discussion

Subsequent to designing and undertaking the Solent SMART Study, this thesis set out to investigate the epidemiology of *M. catarrhalis* in community, care home and COPD cohorts. Risks factors associated with *M. catarrhalis* carriage were identified and prevalence of antibiotic resistance characterised. Furthermore, changes in the prevalence of *M. catarrhalis* carriage and AMR were investigated using supplementary data from another study.

The Solent SMART Study was a large community-based carriage study, which involved the collection of NP, OP and nasal swab samples from members of the general population. It is the only all-age community carriage study to focus on the carriage of *M. catarrhalis.* In total, 1701 participants were recruited (200 aged 0-4, 341 aged 5-16, 540 aged 17-49 and 541 aged 50+ and 79 care home residents) from 51 different sites across the Solent region, making this one of the largest community carriage studies. Swab samples were analysed using established culture-based methodology. Overall, 228 *M. catarrhalis* were isolated (202 were isolated from community-based participants whilst 26 were isolated from care/nursing home residents); 110 were isolated from NP swabs, 20 from OP swabs and 98 from nasal swabs.

## 7.1 Key findings

### 7.1.1 Carriage prevalence

True carriage prevalence of *M. catarrhalis* in community-based participants and care home residents was identified as 8.0% and 19% respectively. Such community carriage data has not previously been reported for *M. catarrhalis*, nor is there any data for *M. catarrhalis* carriage in care/nursing home residents. Those with COPD had a true carriage prevalence of 4.7%, consistent with the lower end of the range previously published which suggests 5-32% of adults with COPD carry *M. catarrhalis* (Murphy *et al.*, 2005b; Wilkinson *et al.*, 2019).

When community based carriage was broken down by age, 26% of 0-4 year olds, 7.9% of 5-16 year olds, 4.3% of 17-49 year olds and 5.2% of those aged 50+ carried *M. catarrhalis*. Published carriage prevalence in children varies drastically. Figures here are in line with the lower end of the range previously reported for young children (0-4 year olds), possibly due to use of culture rather than PCR methodology. Data for 5-16 year olds is comparable to the prevalences reported by Ejlertsen *et al* (Ejlertsen *et al.*, 1994b) *M. catarrhalis* carriage prevalence seen here in adults is higher than that commonly reported, perhaps due to an

overall increase in carriage in the community. Data here supports previous suggestions of increased carriage in older adults.

When considering site of carriage, *M. catarrhalis* was most commonly found in the NP; with swab positivity rates of 6.1% for NP samples, 1.1% for OP samples and 5.2% for nose samples from community-based participants. *M. catarrhalis* was carried in the NP, OP and nose of 15.0%, 3.8% and 13.9% of care home resident respectively, and the NP, OP and nose of 3.9%, 0% and 3.8% of those with COPD respectively. Whilst care home residents had much higher carriage prevalence of *M. catarrhalis* in the NP, OP and nose than community based participants of a similar age, this increased carriage was not significant. This lack of significance may be a result of low sample size. Again, NP and Nose carriage of *M. catarrhalis* in those with COPD was higher than in those from participants of the same age range without COPD (3.9% vs 2.5% in the NP, 3.8% vs 2.9% in the nose). However, this difference was not deemed significant possibly due to low isolation/sample size; only 4 *M. catarrhalis* were isolated from those with COPD. Few studies compare or investigate carriage of *M. catarrhalis* in multiple anatomical sites, especially from the same participant, making the data presented here novel.

Interestingly, analysis of supplementary data has shown a significant increase in the carriage of *M. catarrhalis*, feasibly a result of conjugate vaccines such as MenB and PCV. Increased carriage correlates with reports of increased *M. catarrhalis* disease (whether prevalence or proportion) (Revai *et al.*, 2006; Coker *et al.*, 2010).

### 7.1.2 Risk factors associated with *M. catarrhalis* carriage

Multivariate logistic regression analysis determined that anatomical site, age, microbial co-carriage, recent/concurrent cold and recent use of antibiotics were all significantly associated with the carriage of *M. catarrhalis.* NP and 0-4 years were the site and age range most associated with carriage. *S. pneumoniae*, *H. influenzae* and *N. meningitidis* were associated with an increased likelihood of carriage by 2.3, 1.4 and 5.4 times respectively. S. aureus was associated with a 60% reduction in the likelihood of *M. catarrhalis* carriage. Previous data on co-colonisation is mixed and, in some cases, conflicting; this data therefore adds further clarification of the association of *M. catarrhalis* carriage with common pathobionts. Notably prior research is mostly in young children (Appendix A Table 1), so this study increases data of co-carriage in all ages.

Recent/concurrent cold was associated with an increase (almost fourfold) in the likelihood of *M. catarrhalis* carriage, although other RTI (flu, ear infection and chest infection) was not.

However, this may be due to the lower proportion of participants who had recently suffered one of these other RTIs. Prior research suggests an association between acute URTI and increased *M. catarrhalis* carriage, however type of URTI was not presented and carriage was only investigated in 1–2-year-olds.

A lack of recent antibiotics use was also was associated with an increased risk of *M. catarrhalis* carriage. Conversely, antibiotic use has previously been associated with increased *M. catarrhalis* carriage, but only 109 young children were included in the study (Gisselsson-Solen *et al.*, 2014). However, this is comparable with the results of univariate analysis undertaken using only data from 0-4 years old, for which antibiotic use was linked to increased *M. catarrhalis* carriage (1.5 times), likely due to the antibiotics commonly prescribed to young children. However, it must be noted that the association here was not deemed significant.

### 7.1.3 AMR

Overall prevalence of AMR in isolates from the Solent SMART Study, the Southampton Pneumococcal Carriage Study and disease was low. This is consistent with prior publication showing limited resistance to tetracycline (Flamm *et al.*, 2012; Maraki and Papadakis, 2014), macrolides, cephalosporins, rifampicin, fluoroquinolones, tetracyclines, aminoglycoside and chloramphenicol (Gupta, Arora and Kundra, 2011; Sirwar *et al.*, 2013; Maraki and Papadakis, 2014; Zhang *et al.*, 2016; Król-Turmińska and Olender, 2018; Shi *et al.*, 2018). Carriage isolates from both carriage studies showed higher resistance for ciprofloxacin than any other antibiotic tested. Whilst low-level fluoroquinolone resistance has been seen in clinical isolates from Japan and Poland (Yamada and Saito, 2014; Król-Turmińska and Olender, 2018), levels here are more consistent with data from India showing levels (25-50% depending on the fluoroquinolone antibiotic) (Raveendran *et al.*, 2020), possibly suggesting an increase or spread of AMR in recent years.

What is notable, is the emergence of resistance to co-amoxiclav, as seen in isolates from both disease and carriage (Southampton Pneumococcal Carriage Study). Increased prevalence of co-amoxiclav resistance is of public health interest. As a common alternative to penicillins for β-lactamase producing bacteria like *M. catarrhalis*, such resistance could affect the successful treatment of infection. However, all isolates were sensitive to at least three of the antibiotics tested so at present there are plenty of treatment options for *M. catarrhalis* infection. Perhaps unexpectedly, when compared to disease, isolates from carriage had a significantly higher prevalence of AMR. It remains unclear why this may be.

Analysis of supplementary data has shown there has been no significant change in the prevalence of AMR over the 12 years between 2008 and 2020. Such data is reassuring in an age when increasing levels of AMR is one of the biggest public health challenges.

## 7.2 Limitations of the study

Despite best efforts there were several limitations of the study. Statistical analysis has highlighted the need for increased recruitment. While recruitment was sufficient to determine carriage prevalence; when broken down to assess carriage in certain cohorts or to assess the impact of certain variables on carriage of *M. catarrhalis,* in some cases recruitment and/or isolation of *M. catarrhalis* was too low. It is for this reason that whilst statistical analysis was undertaken on data stratified by sample site, age stratified data was not statistically analysed for the majority of variables. For instance, when looking at the effect of the receipt of the MenB vaccine on the carriage of *M. catarrhalis*, only 5 participants ages 5-16 that received the MenB vaccine carried *M. catarrhalis.*

Secondly, only those with capacity to consent (or whose parents had capacity to consent) were recruited to the study based on ethical considerations. However, by limiting participation to those with mental capacity to consent recruitment in care homes was limited and a selection bias was potentially introduced if for example patients with dementia are more or less likely to be carrying *M. catarrhalis*. Dementia has been linked with acute respiratory dysfunction in COPD patients, suggesting those with dementia may be more at risk of bacterial carriage and RTI (Liao *et al.*, 2015). Furthermore, only English speakers were recruited, again potentially introducing recruitment bias.

In order to limit bias, recruitment occurred in numerous locations, including several non-NHS sites. However increased recruitment at neutral places would have been beneficial i.e. (supermarkets, town centre, gyms, community centres, mosques etc.). Whilst this was not possible with Solent NHS Trust research team recruiting, due to the constraints for recruitment needing to be accountable to the Solent NHS Trust only, this is a consideration for future studies.

The ethnicity classifications used in the questionnaire caused confusion. This resulted in the need to use WHO defined regions rather than actual ethnicity groups. Future studies should have a more comprehensive ethnicity list, or researchers should be better prepared or encouraged to offer advice to ensure accurate classification of ethnicity.

The inability to access GP data to confirm vaccine status, health status, and recent medication (including antibiotics) meant that for some variable there were a lot of participants who answer don't know/gave no answer which may have resulted in statistical significance not being reached due to a lack of data. For example, 535 participants didn't know if they had received a pneumococcal vaccine (even after advice from the research team as to who and when the pneumococcal vaccines are given to). A further 91 participant didn't know whether they had had the annual flu vaccine, and 454 didn't know if they had received a MenB vaccine. The lack of such data will have undoubtedly affected the ability of significance to be determined.

## 7.3 Future work

During the Solent SMART Study, 228 *M. catarrhalis* were isolated from participants of all ages. Isolates should be sequenced, and the distribution of virulence factors investigated to inform vaccine development and implementation strategies. Further to this, a comparison of carriage and clinical isolates would be beneficial and likely to provide additional data to facilitate a better understanding of the differences between carriage and disease, and the identification of markers of pathogenic strains of *M. catarrhalis*. Comparisons of strain/ type prevalence, conservation and distribution of virulence factors, and the expression of such factors could elucidate the pathogenicity of *M. catarrhalis* and define factors that separate carriage vs disease states. Especially as not all cases of colonisation result in the development of infection and disease. Whilst higher disease burden is seen for 16S type 1, all types can and have caused disease. Furthermore, all strains have conserved genes for the majority of virulence factors. It is unknown whether virulence is associated with a particular subpopulation or yet undefined type and what causes differences in clinical manifestation. Certainly, there is no clear link between serum resistance, genetic lineage and disease. Furthermore, whilst numerous virulence factors and their functions (or at least some of their functions) are documented; for many it remains unknown how function is performed (function is only known via knockout experiment), when, what triggers expression, whether expression or function is dependent on other factors or environmental cues etc. Such research would be beneficial to vaccine development and the understanding of disease.

A further consideration is that perhaps asymptomatic carriers are less likely to develop disease but can pass on to others in the same environment. With COPD the acquisition of new strains is associated with exacerbation, because of the vulnerability of the airways/lung. Perhaps in healthy everyday people, acquisition either results in harmless carriage, immediate clearance or immediate infection and disease? If the latter is there a particular, as

yet unidentified, subset of *M. catarrhalis* responsible or is the development of disease solely dependent on the host and interaction with co-colonising pathobionts?

Another area of future work would be to look at the AMR profiles of *M. catarrhalis* found in multiple locations. As highlighted, of the 52 cases where *M. catarrhalis* is found in multiple anatomical locations in the same participant, 33 cases showed the isolates to have different AMR profiles (Appendix A Table 8). It would be of interest to determine whether these are the same or different strains. And if they are the same strains, investigation of the cause of the different AMR profiles; perhaps starting with what bacteria were found co-colonising at these sites and what AMR profiles these co-colonisers had to assess the potential of horizontal gene transfer.

As an alternative to traditional vaccines, some researchers have investigated the use of non-pathogenic bacteria to reduce the carriage of and disease caused by pathogenic bacteria. For instance the use of *Neisseria lactamica* human infection to protect against *N. meningitidis* (Dale et al., 2022). No such research has been done for *M. catarrhalis*, however this is a potential future area of investigation. The research presented here and previously published data suggests *M. catarrhalis* and *S. aureus* to have a competing relationship, with the presence of one reducing the carriage of the other (Coughtrie *et al.*, 2018; Dunne *et al.*, 2018). It is therefore likely there are non-pathogenic bacteria that could be used to prevent *M. catarrhalis* carriage and disease. However from the age of 3 months *Moraxella* dominant communities are associated with ecological stabilisation and are linked to fewer RTI. Therefore, *Moraxella* spp may be important in the development of a stable respiratory microbiome in infants, playing a role in preventing the colonisation of more pathogenic bacteria and development of infection (Bosch *et al.*, 2017). The impact of reduced *M. catarrhalis* carriage would need to be fully assessed, and the appropriate ages or cohorts for such treatment investigated.

## 7.4 Concluding remarks

This thesis has produced important data. Firstly, it has demonstrated successful study design that can be used to inform the design of future carriage studies. Such information will be also be applicable to larger multi-centre studies which could provide data on a UK or global scale. Secondly this thesis has provided key epidemiological information regarding carriage and AMR patterns and trends of *M. catarrhalis*. This may inform prevention strategies (including implementation of vaccines when they become available) as well as the effective targeting of antibiotic treatments. Finally, this thesis adds to published data on co-carriage with other bacterial species, which may impact successful treatment strategies of both *M. catarrhalis* and

the bacteria found to co-colonise. Further research, including investigation the distribution of virulence factors in these isolates will inform the development of new treatments such as a new vaccine.

# Appendix A Supplementary Tables

| Study population | Sample type | *S. pneumoniae* | *S. aureus* | *H. influenzae* | Study [ref] |
|---|---|---|---|---|---|
| 79 aboriginal and 88 non-aboriginal Western Australian infants enrolled at birth and followed until 2 years old | NP aspirates (taken on several occasion) | Positive association | Negative association (only significant in non-aboriginals) | Positive association | (Jacoby *et al.*, 2007) |
| 212 healthy children aged 6-36 months (followed up for 1 year) | NP swabs taken when participants experienced URTI/AOM symptoms | Positive association | Negative association | Negative association * | (Pettigrew *et al.*, 2008) |
| 333 healthy Belgian preschool children aged 3-6 years old | NP aspirates (collected on three occasions) | No association | No association | Positive association | (Jourdain *et al.*, 2011) |
| 1079 healthy Dutch children aged 2 years and under | Nasal swabs (taken at 1.5, 6, 14 and 24 months old) | Not investigated | Not investigated | Positive association | (Verhaegh *et al.*, 2011) |
| 1005 Dutch infants aged 2 years and under | NP swabs (taken at 6 weeks, 6, 12, 18 and 24 months old) | Positive association VT | Not investigated | Not investigated | (van Gils *et al.*, 2011) |
| 30 Gambian infants aged 12 months and under | NP swabs (taken on 16 occasions from 2 weeks to 12 months of age) | Positive association | Not investigated | Not investigated | (Kwambana *et al.*, 2011) |
| 161 Fijian infants aged 17 months participating in a phase II pneumococcal vaccine trial | NP swabs | Positive association | Not investigated | Positive association | (Dunne *et al.*, 2012) |
| Healthy South Korean children: 165 children aged 3-7 years from three kindergartens and 417 children aged 7-10 years from a school | Nasal aspirates | Positive association | Negative association | Positive association | (Bae *et al.*, 2012) |
| 320 healthy American infants aged 6-24 months (although at some time-points some children had symptoms of AOM) | NP and OP swabs (taken at 6, 9, 12, 15, 18 and 24 months old) | Positive association | No association | No association in healthy infants; negative association in those with AOM | (Xu *et al.*, 2012) |
| 302 healthy children aged 12-24 months from Indonesia (6 health centres across 3 regions) | NP swabs | Positive association | Negative association | Positive association | (Dunne *et al.*, 2018) |
| Healthy participants: 497 0-4 year olds, 264 5-17 year olds and 1306 18+ year olds. | Nose swabs | Positive association | Negative association | Positive association | (Coughtrie *et al.*, 2018) |
| Children aged 1-12 years with AOM | NP swabs | Positive association | Not investigated | No association | (Jinhage, Hermansson and |

**Appendix Table 1. Summary of studies looking at *M. catarrhalis* co-colonisation.**
NP – Nasopharyngeal, OP – Oropharyngeal, VT – vaccine serotypes.
*although when *H. influenzae* and *S. pneumoniae* colonise together they show a positive association with *M. catarrhalis*

**Appendix Table 2. Antibiotic prescription guidelines summarised by disease**.

| Body site | Disease / condition | Community guidelines | NICE guidelines |
|---|---|---|---|
| Ear/Nose/ Throat | Sore throat | Phenoxymethylpenicillin or clarithromycin | Penicillin V or clarithromycin (erythromycin during pregnancy) |
| | OM | Amoxicillin or clarithromycin | Amoxicillin or clarithromycin (erythromycin during pregnancy) Co-amoxiclav on worsening |
| | Acute rhinosinusitis | Amoxicillin, doxycycline or co–amoxiclav | Phenoxymethylpenicillin, doxycycline or clarithromycin (erythromycin during pregnancy) Co-amoxiclav if serious |
| RTI | Bronchitis | Amoxicillin, doxycycline, or clarithromycin | Amoxicillin, ampicillin, tetracycline, clarithromycin, azithromycin, or erythromycin |
| | Acute exacerbation of COPD (AECOPD) | Amoxicillin, doxycycline or co-amoxiclav | Amino penicillin, a macrolide, or a tetracycline |
| | Community acquired pneumonia (CAP) | Amoxicillin, clarithromycin, or doxycycline | Amoxicillin, tetracycline, or macrolide (Amoxicillin and a macrolide if moderate – severe) |
| | Pneumonia (hospitalised) | Benzylpenicillin, amoxicillin or cefotaxime | ceftazidime/avibactam (Telavancin for MRSA) |
| Invasive | Meningitis | Benzylpenicillin, cefotaxime or chloramphenicol | Cefotaxime and either amoxicillin or ampicillin (used for unconfirmed bacterial meningitis and confirmed cases in children aged <3 months) or Ceftriaxone (used for unconfirmed bacterial meningitis, confirmed cases in those >3 month and confirmed *H. influenzae* and *S. pneumoniae* cases) |

(National Institute for Health and Clinical Excellence, 2010;2019; National Health Service, 2020)

| Carriage rate | | For CI width = 1% | | | | |
|---|---|---|---|---|---|---|
| p | 100-p | CI width | N | Rounded up N | Inflated N (for 5%) | Rounded up & inflated N (for CI width 1%) |
| 0 | 100 | | | | | |
| 1 | 99 | 1 | 380.3 | 381 | 401.1 | 402 |
| 2 | 98 | 1 | 753.0 | 753 | 792.6 | 793 |
| 3 | 97 | 1 | 1117.9 | 1118 | 1176.8 | 1177 |
| 4 | 96 | 1 | 1475.2 | 1476 | 1553.7 | 1554 |
| 5 | 95 | 1 | 1824.8 | 1825 | 1921.1 | 1922 |
| 6 | 94 | 1 | 2166.7 | 2167 | 2281.1 | 2282 |
| 7 | 93 | 1 | 2500.9 | 2501 | 2632.6 | 2633 |
| 8 | 92 | 1 | 2827.4 | 2828 | 2976.8 | 2977 |
| 9 | 91 | 1 | 3146.3 | 3147 | 3312.6 | 3313 |
| 10 | 90 | 1 | 3457.4 | 3458 | 3640.0 | 3640 |
| 11 | 89 | 1 | 3760.9 | 3761 | 3958.9 | 3959 |
| 12 | 88 | 1 | 4056.7 | 4057 | 4270.5 | 4271 |
| 13 | 87 | 1 | 4344.8 | 4345 | 4573.7 | 4574 |
| 14 | 86 | 1 | 4625.3 | 4626 | 4869.5 | 4870 |
| 15 | 85 | 1 | 4898.0 | 4899 | 5156.8 | 5157 |
| 16 | 84 | 1 | 5163.1 | 5164 | 5435.8 | 5436 |
| 17 | 83 | 1 | 5420.5 | 5421 | 5706.3 | 5707 |
| 18 | 82 | 1 | 5670.2 | 5671 | 5969.5 | 5970 |
| 19 | 81 | 1 | 5912.2 | 5913 | 6224.2 | 6225 |
| 20 | 80 | 1 | 6146.6 | 6147 | 6470.5 | 6471 |
| 21 | 79 | 1 | 6373.2 | 6374 | 6709.5 | 6710 |
| 22 | 78 | 1 | 6592.2 | 6593 | 6940.0 | 6940 |
| 23 | 77 | 1 | 6803.5 | 6804 | 7162.1 | 7163 |
| 24 | 76 | 1 | 7007.1 | 7008 | 7376.8 | 7377 |
| 25 | 75 | 1 | 7203.0 | 7203 | 7582.1 | 7583 |
| 26 | 74 | 1 | 7391.2 | 7392 | 7781.1 | 7782 |
| 27 | 73 | 1 | 7571.8 | 7572 | 7970.5 | 7971 |
| 28 | 72 | 1 | 7744.7 | 7745 | 8152.6 | 8153 |
| 29 | 71 | 1 | 7909.9 | 7910 | 8326.3 | 8327 |
| 30 | 70 | 1 | 8067.4 | 8068 | 8492.6 | 8493 |
| 31 | 69 | 1 | 8217.2 | 8218 | 8650.5 | 8651 |
| 32 | 68 | 1 | 8359.3 | 8360 | 8800.0 | 8800 |
| 33 | 67 | 1 | 8493.8 | 8494 | 8941.1 | 8942 |
| 34 | 66 | 1 | 8620.6 | 8621 | 9074.7 | 9075 |
| 35 | 65 | 1 | 8739.6 | 8740 | 9200.0 | 9200 |
| 36 | 64 | 1 | 8851.0 | 8852 | 9317.9 | 9318 |
| 37 | 63 | 1 | 8954.8 | 8955 | 9426.3 | 9427 |
| 38 | 62 | 1 | 9050.8 | 9051 | 9527.4 | 9528 |
| 39 | 61 | 1 | 9139.2 | 9140 | 9621.1 | 9622 |
| 40 | 60 | 1 | 9219.8 | 9220 | 9705.3 | 9706 |

**Appendix Table 3. Sample size calculations for a CI with of 1%.**

| Carriage rate | | For CI width = 2% | | | | |
|---|---|---|---|---|---|---|
| p | 100-p | CI width | N | Rounded up N | Inflated N (for 5%) | Rounded up & inflated N (for CI width 2%) |
| 0 | 100 | | | | | |
| 1 | 99 | 2 | 95.1 | 96 | 101.1 | 102 |
| 2 | 98 | 2 | 188.2 | 189 | 198.9 | 199 |
| 3 | 97 | 2 | 279.5 | 280 | 294.7 | 295 |
| 4 | 96 | 2 | 368.8 | 369 | 388.4 | 389 |
| 5 | 95 | 2 | 456.2 | 457 | 481.1 | 482 |
| 6 | 94 | 2 | 541.7 | 542 | 570.5 | 571 |
| 7 | 93 | 2 | 625.2 | 626 | 658.9 | 659 |
| 8 | 92 | 2 | 706.9 | 707 | 744.2 | 745 |
| 9 | 91 | 2 | 786.6 | 787 | 828.4 | 829 |
| 10 | 90 | 2 | 864.4 | 865 | 910.5 | 911 |
| 11 | 89 | 2 | 940.2 | 941 | 990.5 | 991 |
| 12 | 88 | 2 | 1014.2 | 1015 | 1068.4 | 1069 |
| 13 | 87 | 2 | 1086.2 | 1087 | 1144.2 | 1145 |
| 14 | 86 | 2 | 1156.3 | 1157 | 1217.9 | 1218 |
| 15 | 85 | 2 | 1224.5 | 1225 | 1289.5 | 1290 |
| 16 | 84 | 2 | 1290.8 | 1291 | 1358.9 | 1359 |
| 17 | 83 | 2 | 1355.1 | 1356 | 1427.4 | 1428 |
| 18 | 82 | 2 | 1417.6 | 1418 | 1492.6 | 1493 |
| 19 | 81 | 2 | 1478.1 | 1479 | 1556.8 | 1557 |
| 20 | 80 | 2 | 1536.6 | 1537 | 1617.9 | 1618 |
| 21 | 79 | 2 | 1593.3 | 1594 | 1677.9 | 1678 |
| 22 | 78 | 2 | 1648.0 | 1649 | 1735.8 | 1736 |
| 23 | 77 | 2 | 1700.9 | 1701 | 1790.5 | 1791 |
| 24 | 76 | 2 | 1751.8 | 1752 | 1844.2 | 1845 |
| 25 | 75 | 2 | 1800.8 | 1801 | 1895.8 | 1896 |
| 26 | 74 | 2 | 1847.8 | 1848 | 1945.3 | 1946 |
| 27 | 73 | 2 | 1892.9 | 1893 | 1992.6 | 1993 |
| 28 | 72 | 2 | 1936.2 | 1937 | 2038.9 | 2039 |
| 29 | 71 | 2 | 1977.5 | 1978 | 2082.1 | 2083 |
| 30 | 70 | 2 | 2016.8 | 2017 | 2123.2 | 2124 |
| 31 | 69 | 2 | 2054.3 | 2055 | 2163.2 | 2164 |
| 32 | 68 | 2 | 2089.8 | 2090 | 2200.0 | 2200 |
| 33 | 67 | 2 | 2123.4 | 2124 | 2235.8 | 2236 |
| 34 | 66 | 2 | 2155.1 | 2156 | 2269.5 | 2270 |
| 35 | 65 | 2 | 2184.9 | 2185 | 2300.0 | 2300 |
| 36 | 64 | 2 | 2212.8 | 2213 | 2329.5 | 2330 |
| 37 | 63 | 2 | 2238.7 | 2239 | 2356.8 | 2357 |
| 38 | 62 | 2 | 2262.7 | 2263 | 2382.1 | 2383 |
| 39 | 61 | 2 | 2284.8 | 2285 | 2405.3 | 2406 |
| 40 | 60 | 2 | 2305.0 | 2305 | 2426.3 | 2427 |

**Appendix Table 4. Sample size calculations for a CI with of 2%.**

| Carriage rate | | For CI width = 3% | | | | |
|---|---|---|---|---|---|---|
| p | 100-p | CI width | N | Rounded up N | Inflated N (for 5%) | Rounded up & inflated N (for CI width 3%) |
| 0 | 100 | | | | | |
| 1 | 99 | 3 | 42.3 | 43 | 45.3 | 46 |
| 2 | 98 | 3 | 83.7 | 84 | 88.4 | 89 |
| 3 | 97 | 3 | 124.2 | 125 | 131.6 | 132 |
| 4 | 96 | 3 | 163.9 | 164 | 172.6 | 173 |
| 5 | 95 | 3 | 202.8 | 203 | 213.7 | 214 |
| 6 | 94 | 3 | 240.7 | 241 | 253.7 | 254 |
| 7 | 93 | 3 | 277.9 | 278 | 292.6 | 293 |
| 8 | 92 | 3 | 314.2 | 315 | 331.6 | 332 |
| 9 | 91 | 3 | 349.6 | 350 | 368.4 | 369 |
| 10 | 90 | 3 | 384.2 | 385 | 405.3 | 406 |
| 11 | 89 | 3 | 417.9 | 418 | 440.0 | 440 |
| 12 | 88 | 3 | 450.7 | 451 | 474.7 | 475 |
| 13 | 87 | 3 | 482.8 | 483 | 508.4 | 509 |
| 14 | 86 | 3 | 513.9 | 514 | 541.1 | 542 |
| 15 | 85 | 3 | 544.2 | 545 | 573.7 | 574 |
| 16 | 84 | 3 | 573.7 | 574 | 604.2 | 605 |
| 17 | 83 | 3 | 602.3 | 603 | 634.7 | 635 |
| 18 | 82 | 3 | 630.0 | 631 | 664.2 | 665 |
| 19 | 81 | 3 | 656.9 | 657 | 691.6 | 692 |
| 20 | 80 | 3 | 683.0 | 683 | 718.9 | 719 |
| 21 | 79 | 3 | 708.1 | 709 | 746.3 | 747 |
| 22 | 78 | 3 | 732.5 | 733 | 771.6 | 772 |
| 23 | 77 | 3 | 755.9 | 756 | 795.8 | 796 |
| 24 | 76 | 3 | 778.6 | 779 | 820.0 | 820 |
| 25 | 75 | 3 | 800.3 | 801 | 843.2 | 844 |
| 26 | 74 | 3 | 821.2 | 822 | 865.3 | 866 |
| 27 | 73 | 3 | 841.3 | 842 | 886.3 | 887 |
| 28 | 72 | 3 | 860.5 | 861 | 906.3 | 907 |
| 29 | 71 | 3 | 878.9 | 879 | 925.3 | 926 |
| 30 | 70 | 3 | 896.4 | 897 | 944.2 | 945 |
| 31 | 69 | 3 | 913.0 | 914 | 962.1 | 963 |
| 32 | 68 | 3 | 928.8 | 929 | 977.9 | 978 |
| 33 | 67 | 3 | 943.8 | 944 | 993.7 | 994 |
| 34 | 66 | 3 | 957.8 | 958 | 1008.4 | 1009 |
| 35 | 65 | 3 | 971.1 | 972 | 1023.2 | 1024 |
| 36 | 64 | 3 | 983.4 | 984 | 1035.8 | 1036 |
| 37 | 63 | 3 | 995.0 | 995 | 1047.4 | 1048 |
| 38 | 62 | 3 | 1005.6 | 1006 | 1058.9 | 1059 |
| 39 | 61 | 3 | 1015.5 | 1016 | 1069.5 | 1070 |
| 40 | 60 | 3 | 1024.4 | 1025 | 1078.9 | 1079 |

**Appendix Table 5. Sample size calculations for a CI with of 3%.**

| Carriage rate | | For CI width = 4% | | | | |
|---|---|---|---|---|---|---|
| p | 100-p | CI width | N | Rounded up N | Inflated N (for 5%) | Rounded up & inflated N (for CI width 4%) |
| 0 | 100 | | | | | |
| 1 | 99 | 4 | 23.8 | 24 | 25.3 | 26 |
| 2 | 98 | 4 | 47.1 | 48 | 50.5 | 51 |
| 3 | 97 | 4 | 69.9 | 70 | 73.7 | 74 |
| 4 | 96 | 4 | 92.2 | 93 | 97.9 | 98 |
| 5 | 95 | 4 | 114.0 | 115 | 121.1 | 122 |
| 6 | 94 | 4 | 135.4 | 136 | 143.2 | 144 |
| 7 | 93 | 4 | 156.3 | 157 | 165.3 | 166 |
| 8 | 92 | 4 | 176.7 | 177 | 186.3 | 187 |
| 9 | 91 | 4 | 196.6 | 197 | 207.4 | 208 |
| 10 | 90 | 4 | 216.1 | 217 | 228.4 | 229 |
| 11 | 89 | 4 | 235.1 | 236 | 248.4 | 249 |
| 12 | 88 | 4 | 253.5 | 254 | 267.4 | 268 |
| 13 | 87 | 4 | 271.6 | 272 | 286.3 | 287 |
| 14 | 86 | 4 | 289.1 | 290 | 305.3 | 306 |
| 15 | 85 | 4 | 306.1 | 307 | 323.2 | 324 |
| 16 | 84 | 4 | 322.7 | 323 | 340.0 | 340 |
| 17 | 83 | 4 | 338.8 | 339 | 356.8 | 357 |
| 18 | 82 | 4 | 354.4 | 355 | 373.7 | 374 |
| 19 | 81 | 4 | 369.5 | 370 | 389.5 | 390 |
| 20 | 80 | 4 | 384.2 | 385 | 405.3 | 406 |
| 21 | 79 | 4 | 398.3 | 399 | 420.0 | 420 |
| 22 | 78 | 4 | 412.0 | 413 | 434.7 | 435 |
| 23 | 77 | 4 | 425.2 | 426 | 448.4 | 449 |
| 24 | 76 | 4 | 437.9 | 438 | 461.1 | 462 |
| 25 | 75 | 4 | 450.2 | 451 | 474.7 | 475 |
| 26 | 74 | 4 | 462.0 | 462 | 486.3 | 487 |
| 27 | 73 | 4 | 473.2 | 474 | 498.9 | 499 |
| 28 | 72 | 4 | 484.0 | 485 | 510.5 | 511 |
| 29 | 71 | 4 | 494.4 | 495 | 521.1 | 522 |
| 30 | 70 | 4 | 504.2 | 505 | 531.6 | 532 |
| 31 | 69 | 4 | 513.6 | 514 | 541.1 | 542 |
| 32 | 68 | 4 | 522.5 | 523 | 550.5 | 551 |
| 33 | 67 | 4 | 530.9 | 531 | 558.9 | 559 |
| 34 | 66 | 4 | 538.8 | 539 | 567.4 | 568 |
| 35 | 65 | 4 | 546.2 | 547 | 575.8 | 576 |
| 36 | 64 | 4 | 553.2 | 554 | 583.2 | 584 |
| 37 | 63 | 4 | 559.7 | 560 | 589.5 | 590 |
| 38 | 62 | 4 | 565.7 | 566 | 595.8 | 596 |
| 39 | 61 | 4 | 571.2 | 572 | 602.1 | 603 |
| 40 | 60 | 4 | 576.2 | 577 | 607.4 | 608 |

**Appendix Table 6. Sample size calculations for a CI with of 4%.**

| Carriage rate | | For CI width = 5% | | | | |
|---|---|---|---|---|---|---|
| p | 100-p | CI width | N | Rounded up N | Inflated N (for 5%) | Rounded up & inflated N (for CI width 5%) |
| 0 | 100 | | | | | |
| 1 | 99 | 5 | 15.2 | 16 | 16.8 | 17 |
| 2 | 98 | 5 | 30.1 | 31 | 32.6 | 33 |
| 3 | 97 | 5 | 44.7 | 45 | 47.4 | 48 |
| 4 | 96 | 5 | 59.0 | 60 | 63.2 | 64 |
| 5 | 95 | 5 | 73.0 | 73 | 76.8 | 77 |
| 6 | 94 | 5 | 86.7 | 87 | 91.6 | 92 |
| 7 | 93 | 5 | 100.0 | 101 | 106.3 | 107 |
| 8 | 92 | 5 | 113.1 | 114 | 120.0 | 120 |
| 9 | 91 | 5 | 125.9 | 126 | 132.6 | 133 |
| 10 | 90 | 5 | 138.3 | 139 | 146.3 | 147 |
| 11 | 89 | 5 | 150.4 | 151 | 158.9 | 159 |
| 12 | 88 | 5 | 162.3 | 163 | 171.6 | 172 |
| 13 | 87 | 5 | 173.8 | 174 | 183.2 | 184 |
| 14 | 86 | 5 | 185.0 | 186 | 195.8 | 196 |
| 15 | 85 | 5 | 195.9 | 196 | 206.3 | 207 |
| 16 | 84 | 5 | 206.5 | 207 | 217.9 | 218 |
| 17 | 83 | 5 | 216.8 | 217 | 228.4 | 229 |
| 18 | 82 | 5 | 226.8 | 227 | 238.9 | 239 |
| 19 | 81 | 5 | 236.5 | 237 | 249.5 | 250 |
| 20 | 80 | 5 | 245.9 | 246 | 258.9 | 259 |
| 21 | 79 | 5 | 254.9 | 255 | 268.4 | 269 |
| 22 | 78 | 5 | 263.7 | 264 | 277.9 | 278 |
| 23 | 77 | 5 | 272.1 | 273 | 287.4 | 288 |
| 24 | 76 | 5 | 280.3 | 281 | 295.8 | 296 |
| 25 | 75 | 5 | 288.1 | 289 | 304.2 | 305 |
| 26 | 74 | 5 | 295.6 | 296 | 311.6 | 312 |
| 27 | 73 | 5 | 302.9 | 303 | 318.9 | 319 |
| 28 | 72 | 5 | 309.8 | 310 | 326.3 | 327 |
| 29 | 71 | 5 | 316.4 | 317 | 333.7 | 334 |
| 30 | 70 | 5 | 322.7 | 323 | 340.0 | 340 |
| 31 | 69 | 5 | 328.7 | 329 | 346.3 | 347 |
| 32 | 68 | 5 | 334.4 | 335 | 352.6 | 353 |
| 33 | 67 | 5 | 339.8 | 340 | 357.9 | 358 |
| 34 | 66 | 5 | 344.8 | 345 | 363.2 | 364 |
| 35 | 65 | 5 | 349.6 | 350 | 368.4 | 369 |
| 36 | 64 | 5 | 354.0 | 355 | 373.7 | 374 |
| 37 | 63 | 5 | 358.2 | 359 | 377.9 | 378 |
| 38 | 62 | 5 | 362.0 | 363 | 382.1 | 383 |
| 39 | 61 | 5 | 365.6 | 366 | 385.3 | 386 |
| 40 | 60 | 5 | 368.8 | 369 | 388.4 | 389 |

**Appendix Table 7. Sample size calculations for a CI with of 5%.**

Calculations for table 3-7 were performed by University of Southampton (UoS) statistician Ho Ming Yuen.

| | | Co-amoxiclav | Cefotaxime | Ceftriaxone | Erythromycin | Tetracycline | Chloramphenicol | Ciprofloxacin | Meropenem |
|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Isolate** | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R |
| OP | **11** | S | S | S | S | S | S | S | S |
| Nose | **11** | S | S | S | R | R | S | R | S |
| NP | **34** | S | S | S | S | S | S | S | S |
| Nose | **34** | S | S | S | S | S | S | S | S |
| NP | **48** | S | S | S | S | S | S | S | S |
| OP | **48** | S | S | S | S | S | S | R | S |
| NP | **85** | S | S | S | S | S | S | S | S |
| Nose | **85** | S | S | S | S | S | S | S | S |
| NP | **91** | S | S | S | S | S | S | S | S |
| Nose | **91** | S | S | S | S | S | S | S | S |
| NP | **104** | S | S | S | S | S | S | S | S |
| Nose | **104** | S | S | S | S | S | S | S | S |
| NP | **133** | S | S | S | S | S | S | R | S |
| Nose | **133** | S | S | S | S | S | S | R | S |
| NP | **134** | S | S | S | S | S | S | R | S |
| Nose | **134** | S | S | S | S | S | S | S | S |
| NP | **135** | S | S | S | S | S | S | R | S |
| Nose | **135** | S | S | S | S | S | S | S | S |
| NP | **157** | S | S | S | S | S | S | R | S |
| Nose | **157** | S | S | S | S | S | S | S | S |
| NP | **271** | S | S | S | S | R | R | R | S |
| Nose | **271** | S | S | S | S | S | S | R | S |
| NP | **309** | S | S | S | S | S | S | S | S |
| Nose | **309** | S | S | S | S | S | S | S | S |
| NP | **364** | S | S | S | S | S | S | R | S |
| Nose | **364** | S | S | S | S | S | S | S | S |
| NP | **493** | S | S | S | R | R | R | R | R |
| Nose | **493** | S | S | S | S | R | S | R | S |

| | | Co-amoxiclav | Cefotaxime | Ceftriaxone | Erythromycin | Tetracycline | Chloramphenicol | Ciprofloxacin | Meropenem |
|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Isolate** | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R |
| NP | **560** | S | S | S | S | S | R | R | R |
| Nose | **560** | S | S | S | S | S | S | S | S |
| NP | **618** | S | S | S | S | S | S | S | S |
| Nose | **618** | S | S | S | S | S | S | R | S |
| NP | **663** | S | S | S | S | S | S | S | S |
| Nose | **663** | S | S | S | S | S | S | R | S |
| NP | **683** | S | S | S | S | S | R | R | R |
| Nose | **683** | S | S | S | S | S | S | S | S |
| NP | **684** | S | S | S | S | S | S | S | S |
| Nose | **684** | S | S | S | S | S | S | S | S |
| NP | **701** | S | S | S | S | S | S | S | S |
| OP | **701** | S | S | S | S | S | S | S | S |
| Nose | **701** | S | S | S | S | S | S | S | S |
| NP | **704** | S | S | S | S | S | S | S | S |
| Nose | **704** | S | S | S | S | S | S | S | S |
| NP | **714** | S | S | S | S | S | R | R | S |
| Nose | **714** | S | S | S | S | S | S | S | S |
| NP | **740** | S | I | I | S | S | R | R | R |
| OP | **740** | S | I | S | S | S | S | S | S |
| NP | **744** | S | S | S | R | S | R | R | R |
| Nose | **744** | S | S | S | S | S | S | S | S |
| NP | **763** | S | S | S | S | S | S | R | S |
| Nose | **763** | S | S | S | S | S | S | S | S |
| NP | **773** | S | S | S | S | S | S | S | S |
| Nose | **773** | S | S | S | S | S | S | S | S |
| NP | **821** | S | S | S | S | S | R | R | S |
| Nose | **821** | S | S | S | S | S | S | S | S |
| NP | **826** | S | S | S | S | S | S | R | S |

| | | Co-amoxiclav | Cefotaxime | Ceftriaxone | Erythromycin | Tetracycline | Chloramphenicol | Ciprofloxacin | Meropenem |
|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Isolate** | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R |
| Nose | **826** | S | S | S | S | S | S | S | S |
| NP | **833** | S | S | S | S | S | S | R | S |
| Nose | **833** | S | S | S | S | S | S | S | S |
| NP | **874** | S | S | S | S | S | S | R | S |
| Nose | **874** | S | S | S | S | S | S | R | S |
| NP | **875** | S | S | S | S | S | R | R | S |
| OP | **875** | S | S | S | S | S | S | S | S |
| NP | **878** | S | S | I | S | S | R | R | R |
| Nose | **878** | S | S | S | S | S | S | S | S |
| NP | **881** | S | S | S | S | S | R | R | S |
| Nose | **881** | S | S | S | S | S | S | S | S |
| NP | **902** | S | S | S | S | S | S | S | S |
| Nose | **902** | S | S | S | S | S | S | S | S |
| NP | **909** | S | I | S | S | R | R | R | R |
| Nose | **909** | S | S | S | S | S | S | S | S |
| NP | **920** | S | S | S | S | R | R | R | R |
| Nose | **920** | S | S | S | S | S | S | S | S |
| NP | **925** | S | S | S | S | S | S | S | S |
| Nose | **925** | S | S | S | S | S | S | S | S |
| NP | **931** | S | S | S | S | R | R | R | R |
| Nose | **931** | S | S | S | S | S | S | S | S |
| NP | **953** | S | S | S | S | S | S | R | S |
| Nose | **953** | S | S | S | S | S | S | S | S |
| NP | **1000** | S | S | I | S | S | R | R | S |
| Nose | **1000** | S | S | S | S | S | S | S | S |
| NP | **1009** | S | S | S | S | S | S | S | S |
| OP | **1009** | S | S | S | S | S | S | S | S |
| Nose | **1009** | S | S | S | S | S | R | R | R |

| | | Co-amoxiclav | Cefotaxime | Ceftriaxone | Erythromycin | Tetracycline | Chloramphenicol | Ciprofloxacin | Meropenem |
|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Isolate** | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R | S/I/R |
| NP | **1023** | S | S | S | S | S | S | S | S |
| Nose | **1023** | S | S | S | S | S | S | S | S |
| NP | **1029** | S | S | S | S | S | S | S | S |
| Nose | **1029** | S | S | S | S | S | S | S | S |
| NP | **1035** | S | S | S | I | I | R | R | S |
| Nose | **1035** | S | S | S | S | S | S | S | S |
| NP | **1043** | S | S | S | S | S | R | R | R |
| Nose | **1043** | S | S | S | S | S | S | S | S |
| NP | **1045** | S | S | S | S | S | R | R | S |
| Nose | **1045** | S | S | S | S | S | S | S | S |
| NP | **1046** | S | S | S | S | S | S | S | S |
| Nose | **1046** | S | S | S | S | S | S | S | S |
| NP | **1049** | S | S | I | S | S | S | S | S |
| Nose | **1049** | S | S | S | S | S | S | S | S |
| NP | **1012** | S | S | S | S | S | S | S | S |
| Nose | **1012** | S | S | S | S | S | S | S | S |
| NP | **1014** | S | S | S | S | S | S | S | S |
| OP | **1014*** | | | | | | | | |
| Nose | **1014** | S | S | S | R | R | R | R | R |
| NP | **1019** | S | S | S | S | S | S | S | S |
| Nose | **1019** | S | S | S | S | S | S | S | S |
| OP | **1170** | S | S | S | S | S | S | S | S |
| Nose | **1170** | S | S | S | S | S | S | S | S |

**Appendix Table 8. AMR profiles of *M. catarrhalis* isolated from multiple sites from the same participant.**

# Appendix B Solent SMART Study documents

## B.1.1 PIS

University of Southampton

NHS Solent NHS Trust

### Information sheet (for participants aged 10 and under)

| | |
|---|---|
| Short title: | Analysis of bacterial carriage and antibiotic resistance rates in the upper respiratory tract (Solent SMART pilot study). |

Sponsor Code: 18525
REC No: 16/SS/0094
Version No: 1 dated 31/03/2016

**Study to look at germs in people's noses and mouths**

**Please ask your mum or dad to help you read this form.**

There are germs that live in everyone's noses and mouths. We are doing a study to find out exactly which germs people have in their nose and mouth.

**What do I have to do?**

Your mum, dad or guardian have been asked if you would mind having a swab taken from your nose and mouth. All you have to do is let the nurse put this cotton bud on the inside of your nose and mouth.

**Do I have to take part?**

No, but you must decide this with your mum, dad or guardian.

We are happy to answer any questions that you may have.

**Thank you**

REC Number: 16/SS/0094
Version 1 dated 31/03/2016

UNIVERSITY OF Southampton

NHS Solent NHS Trust

## Information sheet (for participants aged 11-16 years)

| | |
|---|---|
| **Short title:** | **Analysis of bacterial carriage and antibiotic resistance rates in the upper respiratory tract (Solent SMART pilot study).** |
| **Sponsor Code:** | **18525** |
| **REC No:** | **16/SS/0094** |
| **Version No:** | **1 dated 31/03/2016** |

**Study to look at the germs/bacteria in people's noses and mouths**

**1. We are trying to find out which germs are carried in people's mouths and noses.**

All of us carry germs in our mouths and noses. This is normal and most of the time these germs do not harm us. But sometimes these germs can make us ill. They can cause colds, flu or even serious illnesses which mean you might need to go to the hospital. It is important for us to learn about the germs in your mouths and noses and what medicine are effective at treating them.

**2. What will happen if I take part?**

If you decide to take part in our study we will take swabs from the back of your nose (nasopharyngeal) and back of your mouth/throat (oropharyngeal). We will also ask to swab the front of your nose (nasal), but you do not have to have this swab if you do not want to. Please talk to your parents/guardians or a member of the research team or clinical staff if you are confused about the different swabs types or confused about where the swab will be taken from you. When swabbing we rub cotton wool which is attached to a stick/wire on the inside of your nose and mouth. The swab will pick up any germs living there. All swabs will be taken by a member of the research team or any clinical staff who have undertaken training in this area either before or after the doctor's appointment today. Please let us know if you want us to show you the swabs before you decide whether to take part.

In the laboratory, we will find out what germs were found on your swabs as well as what medicines (i.e. antibiotics) may or may not be used to kill them. Germs can become resistant to some medicines so it is important for us to study this.

Before the swabs are taken we will ask you and your parents/guardians to sign a consent form and to answer a few questions on a ***'Questionnaire' sheet***. Your consent form will be given a special 'study number'. This number will be used to label your swabs and questionnaire so that they can be looked at anonymously (without anyone knowing who they came from).

All germs from the swabs, the consent form and the questionnaire will be stored by the research team for research reasons. If after taking part you decide that you or no longer want your bacteria or questionnaire to be used for our research you can let us know can we will destroy them if that is what you ask us to do.

**3. Do I have to take part?**

You do not have to take part if you do not want to. If you decide not to take part we will not mind at all.

**4. What will happen if germs are found in my nose or mouth?**

The germs we are looking for are found in many people's mouth and nose, and they do not normally harm you. If we do find germs it is nothing to worry about. Because your swabs will be under a study number and not your name, we won't know it is your swab so are unable to tell what germs you had. Any germs we find will be a gift to research and will be stored for research.

If you or your parents are worried about getting sick from germs in your nose or mouth please do talk to your doctor.

**5. There are no risks in taking part.**

The swabs may be a little scratchy or uncomfortable but they will not harm you.
If you have any questions about the research study we are happy to talk to you about it, and if you do not wish to take part this is your choice. It is OK if you decide not to take part

**6. What if something goes wrong?**

It is highly unlikely that something will go wrong as we only require that you donate swab samples. You may experience slight discomfort or a scratchy feeling when some of the swabs are taken.

If you have a complaint about the research study you should contact a member of the research team. This applies to complaints associated with any part of the study process, including how you have been dealt with, the consent process and the swabbing itself.

Please contact the following independent contact if you have any concerns or complaints, your parents can help guide you with this:

Isla-Kate Morris
Research Integrity and Governance Manager
Research Integrity and Governance Team
Research and Innovation Services
University of Southampton
Building 37, Room 4079,
University Road
Highfield
Southampton
SO17 1BJ

Tel: 02380 595058
Email: Rgoinfo@soton.ac.uk

**7. What happens when the research study stops?**

After taking part today you do not need to do anything else.

**8. Taking part will help doctors and nurses understand more about germs in our mouths and noses**

If you decide to take part, you will help us understand more about the different germs in people's mouths and noses and help us to understand how to treat those that get ill.

**9. Who is organising the research?**

The researchers of this study work at the University of Southampton.

**10. Contact for further Information**

If you have any questions please do not hesitate to ask a member of the research team or clinical staff who has approached you today.

Alternatively, you can get in touch with us through either of the following contacts:

Phone: 07500 551523
Email: idepi@soton.ac.uk

Dr S.C. Clarke
Associate Professor in Infectious Disease Epidemiology and Honorary Consultant in Public Health
Academic Unit of Clinical and Experimental Sciences
University of Southampton
Mailpoint 814, Level C
Sir Henry Wellcome Laboratories
South Block
University Hospital Southampton NHS Foundation Trust
Southampton
SO16 6YD

UNIVERSITY OF Southampton

NHS Solent NHS Trust

## Information sheet (for participants aged 17+)

| | |
|---|---|
| **Short title:** | **Analysis of bacterial carriage and antibiotic resistance rates in the upper respiratory tract (Solent SMART pilot study).** |

**Sponsor Code: 18525**
**REC No: 16/SS/0094**
**Version No: 1 dated 28/04/2016**

### 1. Invitation

You are being invited to take part in a research study. Before you agree to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Thank you for reading this.

### 2. What is the purpose of the study?

This study aims to improve our understanding of germs/bacteria in the upper airways and the resistance of these bacteria against certain antibiotics. The human airway is home to a wide variety of bacteria, most of which are harmless to us; however some have the potential to cause disease. Airway infections are a serious health concern and can develop into serious disease such as meningitis (inflammation of the membranes lining the skull) and sepsis (blood poisoning). Bacteria can also mutate to cause antibiotic resistance.

It is therefore important that we improve our understanding of what bacteria are carried in the upper airways to help us better recognise the causes of infections and what antibiotics/treatments these bacteria may be resistant to so we can determine the most appropriate ways of treating and preventing them.

### 3. Do I have to take part?

It is up to you to decide whether or not to take part. If you decide not to take part this will not affect the care you receive from your GP/ heath care professional in any way.

### 4. What will happen if I take part?

Nasopharyngeal (back of nose) and oropharyngeal (back of mouth/throat) swabs will be taken from every participant. We will also request to take a nasal swab, however this swab is optional and not mandatory for participation. All swabs will be taken by a member of the research team or clinical staff who have undertaken relevant training. The swabs will be taken before or after the doctor's appointment, today, and there will be no follow up.

All swabs will then be taken to the laboratory where they will be tested to see what types of bacteria are present. The results will help us develop a better picture of the bacteria being carried in the upper airways of people in the Solent community. We will also assess the resistance of these bacteria to known antibiotics.

Before any swabs can be taken, you need to fill out a ***'Consent form'*** and a ***'Questionnaire'***. The questionnaire is short and aims to collect information that can be used to see if there are any factors that may influence the carriage of bacteria i.e. age, sex, lifestyle and past/current medication.

Any bacteria found on the swabs, as well as the consent form and the questionnaire will be stored long term, at the University Hospital Southampton NHS Foundation Trust for research and research governance purposes. Access to these will restricted to the research team and requires swipe card or code access. If you decide to withdraw from the study within 7 working days of the swab being taken, the bacteria obtained from the swab, the consent from and questionnaire will be destroyed immediately. After 7 working days, the bacteria and associated material will be retained unless a specific request is made to destroy them.

**5. What will happen if bacteria are found in my samples?**

It is normal to carry bacteria in your upper airways. Bacteria are often carried by a large number of the population without leading to/causing infection. All samples will be anonymised as soon as they are taken so we are unable to provide results to individuals or their GP. If any bacteria is found it is nothing to worry about. Bacteria will be deemed a gift to the research study and stored for research purposes.

If you have any concerns about infection in and around your upper airways then you should consult your GP separately.

The overall findings of the study will be summarised in a poster displayed at all participating Solent NHS Trust sites and sites working in partnership with the Solent NHS Trust, including the one you have visited today. No participant details will be given or shown. We hope you will take some satisfaction from contributing to this important area of study.

**6. What happens when the research study stops?**

When the research study ends there will be no impact on you. Participation is a one-time event, after participating today there is no follow up.

**7. What if something goes wrong?**

It is highly unlikely that something will go wrong as we only require that you donate swab samples. You may experience slight discomfort or a scratchy feeling when some of the swabs are taken.

If you have a complaint about the research study you should contact a member of the research team. This applies to complaints associated with any part of the study process, including how you have been dealt with, the consent process and the swabbing itself.

Complaints will be dealt with in a timely manner and a formal response given. The normal National Health Service complaints mechanisms are also be available to you.

Please contact the following independent contact if you have any concerns or complaints:

Isla-Kate Morris

Research Integrity and Governance Manager
Research Integrity and Governance Team
Research and Innovation Services
University of Southampton
Building 37, Room 4079,
University Road
Highfield
Southampton
SO17 1BJ

Tel: 02380 595058
Email: Rgoinfo@soton.ac.uk

**8. Will taking part in this study be kept confidential?**

Information collected about you will only be used for the purpose of this research study. All information collected about you will be handled in strict confidence.

You will be given a unique 'study number' which will be added to the consent form. The swabs and questionnaire will only be labelled with this study number; there will be no participant identifiable information i.e. name or date of birth, to ensure that they are processed anonymously.

**9. What will happen to the results of the research study?**

The results of the study will help us to develop a detailed picture of the bacteria carried in the upper airways. The study will also help give an idea of the level of antibiotic resistance found in the community and to assess what antibiotics certain bacteria are or are not resistant to. We aim to publish the results in conference proceedings and in scientific journals. Any information that is useful for determining future policy to control infection will be publicised appropriately. The overall findings of the study will be summarised in a poster displayed at all participating Solent NHS Trust sites and sites working in partnership with the Solent NHS Trust, including the one you have visited today.

**10. Who is organising and funding the research?**

The study was designed and is being carried out by researchers at the University of Southampton. The Solent NHS Trust have provided funding for the staffing of this study (two research facilitators, the research staff and clinical staff). No staff or participants will receive any payment or personal incentives for their involvement in this research.

**11. Who has reviewed the study?**

The study proposal has been reviewed by the Research Governance Office at the University of Southampton, as well as the Health Research Authority (HRA).

**12. Contact for further Information**

If you have any questions please do not hesitate to ask a member of the research team or clinical staff who has approached you today.

Alternatively, you can get in touch with us through either of the following contacts:

Phone: 07500 551523
Email: idepi@soton.ac.uk

Dr S.C. Clarke
Associate Professor in Infectious Disease Epidemiology and Honorary Consultant in Public Health
Academic Unit of Clinical and Experimental Sciences
University of Southampton
Mailpoint 814, Level C
Sir Henry Wellcome Laboratories
South Block
University Hospital Southampton NHS Foundation Trust
Southampton
SO16 6YD

## Information sheet (for the parent or guardian of the participant)

| | |
|---|---|
| **Short title:** | **Analysis of bacterial carriage and antibiotic resistance rates in the upper respiratory tract (Solent SMART Pilot study).** |

**Sponsor Code: 18525**
**REC No: 16/SS/0094**
**Version No: 1 dated 28/04/2016**

### 1. Invitation

Your child is being invited to take part in a research study. As their parent/guardian we ask for your consent to allow them to participate in the study. Before you decide whether your child should take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Thank you for reading this.

### 2. What is the purpose of the study?

This study aims to improve our understanding of the germs/bacteria in the upper airways and the resistance of these bacteria against certain antibiotics. The human airway is home to a wide variety of bacteria and most of these bacteria are harmless to us; however some have the potential to cause disease. Airway infections are a serious health concern and can develop into serious disease such as meningitis (inflammation of the membranes lining the skull) and sepsis (blood poisoning). Bacteria can also mutate to become resistant to antibiotics.

It is therefore important that we improve our understanding of what bacteria are carried in the upper airways to help us better recognise the causes of infections and what antibiotics/treatments these bacteria may be resistant to so we can determine the most appropriate ways of treating and preventing them.

### 3. Does my child have to take part?

It is up to you and your child to decide whether or not to take part. If you decide not to take part this will not affect the care you or your child receives from your GP/heath care professional in any way.

### 4. What will happen if my child takes part?

Nasopharyngeal (back of nose) and oropharyngeal (back of throat/mouth) swabs will be taken from every participant. We will also request to take a nasal swab, however this swab is optional and not mandatory for participation. All swabs will be taken by a member of the research team or clinical staff who have undertaken relevant training. The swabs will be taken before or after the doctor's appointment, today, and there will be no follow up.

The swabs will then be taken to the laboratory where they will be tested to see what types of bacteria are present. The results will help us develop a better picture of the bacteria being carried in the upper airways of people in the Solent community. We will also assess the resistance of these bacteria to known antibiotics.

Before any swabs can be taken, you need to complete a ***'Consent form'*** and a ***'Questionnaire'*** on behalf of your child (or with your child if they are aged 11-16). The questionnaire is short and aims to collect information that can be used to see if there are any factors that may influence the carriage of bacteria i.e. age and sex.

Any bacteria found on the swabs, as well as the consent form and the questionnaire will be stored long term, at the University Hospital Southampton NHS Foundation Trust for research and research governance purposes. Access to these will restricted to the research team and requires swipe card or code access. If you decide to withdraw your child from the study within 7 working days of the swab being taken, the bacteria obtained from the swab, the consent from and questionnaire will be destroyed immediately. After 7 working days the bacteria and associated material will be retained unless a specific request is made to destroy them.

**5. What will happen if bacteria are found in the samples given by my child?**

It is normal to carry bacteria in your upper airways. Bacteria are often carried by a large number of the population without leading to/causing infection. All swabs and questionnaires will be anonymised as soon as they are taken so we are unable to provide results to individuals, their parents/guardians or their GP. If any bacteria is found it is nothing to worry about. Bacteria will be deemed a gift to the research study and stored for research purposes.

If you have any concerns about infection in and around the upper airways then you should consult your GP separately.

The overall findings of the study will be summarised in a poster displayed at all participating Solent NHS Trust sites and sites working in partnership with Solent NHS Trust, including the one you have visited today. No participant details will be given or shown. We hope you will take some satisfaction from contributing to this important area of study.

**6. What happens when the research study stops?**

When the research study ends there will be no impact on you and your child. Participation is a one-time event, after participating today there is no follow up.

**7. What if something goes wrong?**

It is highly unlikely that something will go wrong as your child is simply donating swab samples. Your child may experience slight discomfort or a scratchy feeling when some of the swabs are taken.

If you or your child has a complaint about the research study you should contact a member of the research team. This applies to complaints associated with any part of the study process, including how you have been dealt with, the consent process and the swabbing itself.

Complaints will be dealt with in a timely manner and a formal response given. The normal National Health Service complaints mechanisms are also available to you.

Please contact the following independent contact if you have any concerns or complaints:

Isla-Kate Morris
Research Integrity and Governance Manager
Research Integrity and Governance Team
Research and Innovation Services
University of Southampton
Building 37, Room 4079,
University Road
Highfield
Southampton
SO17 1BJ

Tel: 02380 595058
Email: Rgoinfo@soton.ac.uk

**8. Will taking part in this study be kept confidential?**

Information collected about your child will only be used for the purpose of this research study. All information collected about your child will be handled in strict confidence.

Your child will be given a unique 'study number' which will be added to the consent form. The swabs and questionnaire will only be labelled with this study number; there will be no participant identifiable information i.e. name or date of birth, to ensure that they are processed anonymously.

**9. What will happen to the results of the research study?**

The results of the study will help us to develop a detailed picture of the bacteria carried in the upper airways. The study will also help give an idea of the level of antibiotic resistance found in the community and to assess what antibiotics certain bacteria are or are not resistant to. We aim to publish the results in conference proceedings and in scientific journals. Any information that is useful for determining future policy to control infection will be publicised appropriately. The overall findings of the study will be summarised in a poster displayed at all participating Solent NHS Trust sites and sites working in partnership with Solent NHS Trust, including the one you have visited today.

**10. Who is organising and funding the research?**

The study was designed and is being carried out by researchers at the University of Southampton. The Solent NHS Trust have provided funding for the staffing of this study (two research facilitators, the research team and clinical staff). No staff or participants will receive any payment or personal incentives for their involvement in this research.

**11. Who has reviewed the study?**

The study proposal has been reviewed by the Research Governance Office at the University of Southampton, as well as the Health Research Authority (HRA).

**12. Contact for further Information**

If you have any questions please do not hesitate to ask any of the clinical staff or research team member who has approached you today.

Alternatively you can get in touch with us through either of the following contacts:

Phone: 07500 551523
Email: idepi@soton.ac.uk

Dr S.C. Clarke
Associate Professor in Infectious Disease Epidemiology and Honorary Consultant in Public Health
Academic Unit of Clinical and Experimental Sciences
University of Southampton
Mailpoint 814, Level C
Sir Henry Wellcome Laboratories
South Block
University Hospital Southampton NHS Foundation Trust
Southampton
SO16 6YD

## Information sheet (for participants in care homes)

| | |
|---|---|
| **Short title:** | **Analysis of bacterial carriage and antibiotic resistance rates in the upper respiratory tract (Solent SMART pilot study).** |

**Sponsor Code: 18525**
**REC No: 16/SS/0094**
**Version No: 1 dated 28/04/2016**

### 1. Invitation

You are being invited to take part in a research study. Before you agree to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Thank you for reading this.

### 2. What is the purpose of the study?

This study aims to improve our understanding of germs/bacteria in the upper airways and the resistance of these bacteria against certain antibiotics. The human airway is home to a wide variety of bacteria and most of these bacteria are harmless to us; however some have the potential to cause disease. Airway infections are a serious health concern and can develop into serious disease such as meningitis (inflammation of the membranes lining the skull) and sepsis (blood poisoning). Bacteria can also mutate to cause antibiotic resistance.

It is therefore important that we improve our understanding of what bacteria are carried in the upper airways to help us better recognise the causes of infections and what antibiotics/treatments these bacteria may be resistant to so we can determine the most appropriate ways of treating and preventing them.

### 3. Do I have to take part?

You can decide whether or not to take part. If you decide not to take part this will not affect the care you receive in any way.

### 4. What will happen if I take part?

Nasopharyngeal (back of nose) and oropharyngeal (back of mouth/throat) swabs will be taken from every participant. We will also request to take a nasal swab, however this swab is optional and not mandatory for participation. All swabs will be taken by a member of the research team or clinical staff who have undertaken relevant training, today, and there will be no follow up.

After we have taken the swabs they will then be transported to the laboratory where they will be tested to see what types of bacteria are present. The results will help us develop a better picture of the bacteria being carried in the upper airways of people in the community. We will also assess the resistance of these bacteria to known antibiotics.

Before any swabs can be taken, you need to fill out a ***'Consent form'*** and a ***'Questionnaire'***. The questionnaire is short and aims to collect information that can be used to see if there are any factors that may influence the carriage of bacteria i.e. age, sex, lifestyle and past/current medication.

Any bacteria found on the swabs, as well as the consent form and the questionnaire will be stored long term, at the University Hospital Southampton NHS Foundation Trust for research and research governance purposes. Access to these will restricted to the research team and requires swipe card or code access. If you decide to withdraw from the study within 7 working days of the swab being taken, the bacteria obtained from the swab, the consent from and questionnaire will be destroyed immediately. After 7 working days the bacteria and associated material will be retained unless a specific request is made to destroy them.

**5. What will happen if bacteria are found in my samples?**

It is normal to carry bacteria in your upper airways. Bacteria are often carried by a large number of the population without leading to/causing infection. All samples will be anonymised as soon as they are taken so we are unable to provide results to individuals. If any bacteria is found it is nothing to worry about. Bacteria will be deemed a gift to the research study and stored for research purposes.

If you have any concerns about infection in and around your upper airways then you should consult your GP separately.

The overall findings of the study will be summarised in a poster displayed at all participating Solent NHS Trust sites and sites working in partnership with Solent NHS Trust, including your care home. No participant details will be given or shown. We hope you will take some satisfaction from contributing to this important area of study.

**6. What happens when the research study stops?**

When the research study ends there will be no impact on you and there will be no follow up.

**7. What if something goes wrong?**

It is highly unlikely that something will go wrong as we only require that you donate swab samples. You may experience slight discomfort or a scratchy feeling when some of the swabs are taken.

If you have a complaint about the research study you should contact a member of the research team. This applies to complaints associated with any part of the study process, including how you have been dealt with, the consent process and the swabbing itself.

Complaints will be dealt with in a timely manner and a formal response given. The normal National Health Service complaints mechanisms are also be available to you.

Please contact the following independent contact if you have any concerns or complaints:

Isla-Kate Morris

Research Integrity and Governance Manager
Research Integrity and Governance Team
Research and Innovation Services
University of Southampton
Building 37, Room 4079,
University Road
Highfield
Southampton
SO17 1BJ

Tel: 02380 595058
Email: Rgoinfo@soton.ac.uk

**8. Will taking part in this study be kept confidential?**

Information collected about you will only be used for the purpose of this research study. All information collected about you will be handled in strict confidence.

You will be given a unique 'study number' which will be added to the consent form. The swabs and questionnaire will only be labelled with this study number; there will be no participant identifiable information i.e. name or date of birth, to ensure that they are processed anonymously.

**9. What will happen to the results of the research study?**

The results of the study will help us to develop a detailed picture of the bacteria carried in the upper airways. The study will also help give an idea of the level of antibiotic resistance found in the community and to assess what antibiotics certain bacteria are or are not resistant to. We aim to publish the results in conference proceedings and in scientific journals. Any information that is useful for determining future policy to control infection will be publicised appropriately. The overall findings of the study will be summarised in a poster displayed at all participating Solent NHS Trust sites and sites working in partnership with Solent NHS Trust, including your care home.

**10. Who is organising and funding the research?**

The study was designed and is being carried out by researchers at the University of Southampton. The Solent NHS Trust have provided funding for the staffing of this study (two research facilitators , the research team and clinical staff). No staff or participants will receive any payment or personal incentives for their involvement in this research.

**11. Who has reviewed the study?**

The study proposal has been reviewed by the Research Governance Office at the University of Southampton, as well as the Health Research Authority (HRA).

**12. Contact for further Information**

If you have any questions please do not hesitate to ask the nurse or research team member who has approached you today.

Alternatively you can get in touch with us through either of the following contacts:

Phone: 07500 551523
Email: idepi@soton.ac.uk

Dr S.C. Clarke
Associate Professor in Infectious Disease Epidemiology and Honorary Consultant in Public Health
Academic Unit of Clinical and Experimental Sciences
University of Southampton
Mailpoint 814, Level C
Sir Henry Wellcome Laboratories
South Block
University Hospital Southampton NHS Foundation Trust
Southampton
SO16 6YD

## B.1.2 Consent forms

UNIVERSITY OF Southampton

NHS Solent NHS Trust

# CONSENT FORM FOR PARTICIPANTS AGED 11-16 YEARS

**Title of Project:** Analysis of the carriage rates and antibiotic resistance of bacteria in the upper respiratory tract (Solent SMART pilot study)
**Name of Researchers:** Dr S Clarke

**Please initial box**

1. I confirm that I have read and understand the information sheet dated 31/03/2016(V1) for the above study and have had the opportunity to ask questions. ☐

2. I understand that I am providing consent to participate and that participation is voluntary. I know that non-participation will not affect my medical care or legal rights. ☐

3. I agree to allow a member of the research team or clinical staff, who have undertaken relevant training, to take a nasopharyngeal and oropharyngeal swab, the details of which have been made clear to me, and to complete a questionnaire (with the help from my parent/guardian). ☐

4. I agree to allow a member of the research team or clinical staff, who have undertaken relevant training, to take a nasal swab, the details, which have been made clear to me. ☐

5. I understand that information collected will only be used to obtain consent and to assist with the above research project. Consent will be stored for research governance purposes. The questionnaire and swabs will be anonymised and will only be used for the purposes of the study. Bacteria obtained from the swabs will be stored for future analysis at the University Hospital Southampton NHS Foundation Trust, access to which will need swipe card or code access. ☐

Name of participant.....

Date of birth.....

Date.....

Signature.....

As the parent/guardian, I agree for my child to take part in this study. ☐

.....

Name of Parent/Guardian Date Signature

.....

Researcher Date Signature

REC Number: 16/SS/0094
Version 1 dated 28/04/2016
Sponsor Number:18525

UNIVERSITY OF Southampton

NHS Solent NHS Trust

# CONSENT FORM FOR ADULT (17+) PARTICIPANTS

**Title of Project:** Analysis of the carriage rates and antibiotic resistance of bacteria in the upper respiratory tract (Solent SMART pilot study)
**Name of Researchers:** Dr S Clarke

**Please initial box**

1. I confirm that I have read and understand the information sheet dated 28/04/2016 (V1) for the above study and have had the opportunity to ask questions. ☐

2. I understand that I am providing consent to participate and that participation is voluntary. I know that non-participation will not affect my medical care or legal rights. ☐

3. I agree to allow a member of the research team or clinical staff, who have undertaken relevant training, to take a nasopharyngeal and oropharyngeal swab, the details, which have been made clear to me, and to complete a questionnaire. ☐

4. I agree to allow a member of the research team or clinical staff, who have undertaken relevant training, to take a nasal swab, the details, which have been made clear to me. ☐

5. I understand that information collected will only be used to obtain consent and to assist with the above research project. Consent will be stored for research governance purposes. The questionnaire and swabs will be anonymised and will only be used for the purposes of the study. Bacteria obtained from the swabs will be stored for future analysis at the University Hospital Southampton NHS Foundation Trust, access to which will need swipe card or code access. ☐

Name of participant.....

Date of birth.....

Date.....

Signature.....

.....

Researcher Date Signature

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Version 1 dated 28/04/2016
Sponsor Number:18525

# CONSENT FORM FOR PARTICIPANT'S PARENT/GUARDIAN

**Title of Project:** Analysis of the carriage rates and antibiotic resistance of bacteria in the upper respiratory tract (Solent SMART Pilot study)
**Name of Researchers:** Dr S Clarke

**Please initial box**

1. I confirm that I have read and understand the information sheet dated 28/04/2016 (V1) for the above study and have had the opportunity to ask questions. ☐

2. I understand that I am providing consent on behalf of my child and that participation is voluntary. I know that non-participation will not affect my child's medical care or legal rights. ☐

3. I agree to allow a member of the research team or clinical staff, who have undertaken relevant training, to take a nasopharyngeal and oropharyngeal swab from my child, the details of which have been made clear to me, and to complete a questionnaire. ☐

4. I agree to allow a member of the research team or clinical staff, who have undertaken relevant training, to take a nasal swab from my child, the details of which have been made clear to me. ☐

5. I understand that information collected will only be used to obtain consent and to assist with the above research project. Consent will be stored for research governance purposes. The questionnaire and swabs will be anonymised and will only be used for the purposes of the study. Bacteria obtained from the swabs will be stored for future analysis at the University Hospital Southampton NHS Foundation Trust, access to which will need swipe card or code access. ☐

Name of participant.....

Date of birth.....

.....

Name of Parent/Guardian Date Signature

.....

Researcher Date Signature

REC Number: 16/SS/0094
Version 1 dated 28/04/2016
Sponsor Number:18525

## B.1.3 Questionnaires

Medicine

UNIVERSITY OF Southampton

# QUESTIONNAIRE – 11-16 YEARS

Title of project: **Analysis of the carriage rates and antibiotic resistance of bacteria in the upper respiratory tract (Solent SMART pilot study)**

**PLEASE ASK YOUR PARENT/GUARDIAN'S TO HELP YOU COMPLETE THIS.**

Please tick the relevant boxes and provide extra information where necessary.

1. **Have you received all vaccines due in the line with the national schedule?**

Yes ☐ No ☐ Don't know ☐ Would rather not say ☐

2. **Have you received any pneumococcal vaccines (also called Prevenar)?**

Yes ☐ No ☐ Don't know ☐ Would rather not say ☐

If yes which one:……

3. **Have you received the meningitis group B vaccine (also called Bexsero)?**

Yes ☐ No ☐ Don't know ☐ Would rather not say ☐

If yes please state when:……/……/…….

4. **Have you received the meningitis group C vaccine?**

Yes ☐ No ☐ Don't know ☐ Would rather not say ☐

5. **Have you received the yearly influenza vaccine (also called Fluenz) in the past 12 months?**

Yes ☐ No ☐ Don't know ☐ Would rather not say ☐

If yes please state when: ……/……/…….

6. **Have you had a cold, flu, ear infection, or other respiratory infection during the past month?**

Cold ☐ Flu ☐ Ear infection ☐ Other chest infection ☐ None ☐

7. **Have you taken antibiotics in the past month?**

Yes ☐ No ☐

8. **If yes, do you remember the name**:

Amoxil ☐ Augmentin (coamoxiclav) ☐ Erythromycin ☐ Azithromycin ☐

Oxacillin ☐ Tetracycline ☐ Ciprofloxacin ☐ Ampicillin ☐

Chloramphenicol ☐ Penicillin ☐ Cefotaxim ☐ Other (unknown name) ☐

☐ Other (please state) ……

REC number: 16/SS/0094
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Sponsor number: 18525

**9. What ethnicity are you?**

**White**

| | | | |
|---|---|---|---|
| White British | ☐ | **Asian/Asian British** | |
| White Irish | ☐ | Indian | ☐ |
| White Gypsy or Irish Traveller | ☐ | Pakistani | ☐ |
| **Mixed/Multiple ethnic groups** | | Bangladeshi | ☐ |
| White and Black Caribbean | ☐ | Chinese | ☐ |
| White and Black African | ☐ | **Black/African/Caribbean/Black British** | |
| White and Asian | ☐ | African | ☐ |
| **Other ethnic group** | | Caribbean | ☐ |
| Arab | ☐ | **Would rather not say** | ☐ |

**Other not listed** (please state) .....

**10. What is your age?** ..... years

**11. What sex are you?**

Male ☐ Female ☐ Other ☐ Would rather not say ☐

**12. Do you have any long term illnesses or conditions?** e.g. asthma, COPD, diabetes, lung fibrosis, Chronic rhinosinusitis, etc.

Yes ☐ No ☐ Would rather not say ☐

If yes, please state .....

**13. Do you live with people who smoke cigarettes and/or cigars?**

Yes ☐ No ☐ Would rather not say ☐

**14. Do you live with people who smoke e-cigarettes?**

Yes ☐ No ☐ Would rather not say ☐

**15. How often are you exposed to second hand cigarettes and/or cigars smoke?**

Daily ☐ Once a week ☐ On occasion (i.e. once a month) ☐ Never ☐

REC number: 16/SS/0094
Version 1 dated 31/03/2016
Sponsor number: 18525

**16. How often are you exposed to second hand e-cigarette smoke?**

Daily ☐ Once a week ☐ On occasion (i.e. once a month) ☐ Never ☐

**17. What are the first 5 digits of your post code i.e. SO16 8**

Please write here: ..... Would rather not say ☐

**18. If you only had the mandatory swabs taken, please state why you did not want to have the nasal swab taken?**

.....

.....

.....

**19. Would you participate in a study like this one again?**

Yes ☐ No ☐ Would rather not say ☐

If no, please state why

.....

.....

.....

**Participant study number** (to be filled out by research team) .....

## Thank you for completing the questionnaire

Although we are unable to report individual's results, if you would like us to report back the overall findings of the study please give your email address:

.....

REC number: 16/SS/0094
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Sponsor number: 18525

**Swabbing Notes**

REC number: 16/SS/0094
Version 1 dated 31/03/2016
Sponsor number: 18525

Medicine

UNIVERSITY OF Southampton

# QUESTIONNAIRE – ADULT 17 - 49

Title of project: **Analysis of the carriage rates and antibiotic resistance of bacteria in the upper respiratory tract (Solent SMART pilot study)**

Please tick the relevant boxes and provide extra information where necessary.

1. **Have you received all vaccines due in the line with the national schedule?**

Yes ☐ No ☐ Don't know ☐ Would rather not say ☐

2. **Have you received the PPV23 pneumococcal vaccine (also called Prevenar)?**

Yes ☐ No ☐ Don't know ☐ Would rather not say ☐

3. **Have you received the meningitis group B vaccine (also called Bexsero)?**

Yes ☐ No ☐ Don't know ☐ Would rather not say ☐

If yes please state when: ……/……/……

4. **Have you received the meningitis group C vaccine?**

Yes ☐ No ☐ Don't know ☐ Would rather not say ☐

If yes please state when: ……/……/……

5. **Have you received the annual influenza vaccine in the past 12 months?**

Yes ☐ No ☐ Don't know ☐ Would rather not say ☐

If yes please state when: ……/……/……

6. **Have you had a cold, flu, ear infection, or other respiratory infection during the past month?**

Cold ☐ Flu ☐ Ear infection ☐ Other chest infection ☐ None ☐

7. **Have you taken antibiotics in the past month?**

Yes ☐ No ☐

If yes, do you remember the name:

Amoxil ☐ Augmentin (coamoxiclav) ☐ Erythromycin ☐ Azithromycin ☐

Oxacillin ☐ Tetracycline ☐ Ciprofloxacin ☐ Ampicillin ☐

Chloramphenicol ☐ Penicillin ☐ Cefotaxim ☐ Other (unknown name) ☐

☐ Other (please state) ……..

REC number: 16/SS/0094
Version 1 dated 24/03/2016
Sponsor number: 18525

8. **What ethnicity are you?**

**White**

White British ☐

White Irish ☐

White Gypsy or Irish Traveller ☐

**Mixed/Multiple ethnic groups**

White and Black Caribbean ☐

White and Black African ☐

White and Asian ☐

**Other ethnic group**

Arab ☐

**Asian/Asian British**

Indian ☐

Pakistani ☐

Bangladeshi ☐

Chinese ☐

**Black/African/Caribbean/Black British**

African ☐

Caribbean ☐

**Would rather not say** ☐

**Other not listed** (please state) .....

9. **What is your age?** ..... years

10. **What sex are you?**

Male ☐ Female ☐ Other ☐ Would rather not say ☐

11. **Do you have any long term illnesses or conditions?** e.g. asthma, COPD, diabetes, lung fibrosis, Chronic rhinosinusitis, etc.

Yes ☐ No ☐ Would rather not say ☐

If yes, please state .....

12. **Do you smoke cigarettes and/or cigars?**

Yes ☐ No ☐ Would rather not say ☐

13. **Do you smoke e-cigarettes?**

Yes ☐ No ☐ Would rather not say ☐

**If yes to questions 12 or 13** please state on average how many times you smoke per day

.....

**If no to questions 12 or 13** have you ever smoked cigarettes/cigars?

Yes ☐ No ☐ Would rather not say ☐

**If no to questions 12 or 13** have you ever smoked e-cigarette?

Yes ☐ No ☐ Would rather not say ☐

Please state on average how many times you used to smoke per day.....

Please state how long ago you quit smoking.....

**14. Do you live with people who smoke cigarettes and/or cigars?**

Yes ☐ No ☐ Would rather not say ☐

**15. Do you live with people who smoke e-cigarettes?**

Yes ☐ No ☐ Would rather not say ☐

**16. How often are you exposed to second hand cigarettes and/or cigars smoke?**

Daily ☐ Once a week ☐ On occasion (i.e. once a month) ☐ Never ☐

**17. How often are you exposed to second hand e-cigarette smoke?**

Daily ☐ Once a week ☐ On occasion (i.e. once a month) ☐ Never ☐

**18. What are the first 5 digits of your post code ie SO16 8**

Please write here: ..... Would rather not say ☐

**19. If you only had the mandatory swabs taken, please state why you did not want to have the nasal swab taken?**

.....

.....

.....

**20. Would you participate in a study like this one again?**

Yes ☐ No ☐ Would rather not say ☐

If no, please state why.....

.....

.....

REC number: 16/SS/0094
Version 1 dated 24/03/2016
Sponsor number: 18525

## Thank you for completing the questionnaire

**Participant study number** (to be filled out by research team) .....

**Swabbing Notes**

Although we are unable to report individual's results, if you would like us to report back the overall findings of the study please give your email address:

.....

REC number: 16/SS/0094
Version 1 dated 24/03/2016
Sponsor number: 18525

# QUESTIONNAIRE – ADULT 50+

Title of project: **Analysis of the carriage rates and antibiotic resistance of bacteria in the upper respiratory tract (Solent SMART pilot study)**

Please tick the relevant boxes and provide extra information where necessary.

1. **Have you received all vaccines that were due in the line with the national schedule?**

Yes ☐ No ☐ Don't know ☐ Would rather not say ☐

2. **Have you received the PPV23 pneumococcal vaccine (also called Prevenar)?**

Yes ☐ No ☐ Don't know ☐ Would rather not say ☐

3. **Have you received the annual influenza vaccine in the past 12 months?**

Yes ☐ No ☐ Don't know ☐ Would rather not say ☐

If yes please state when: ……/……/……..

4. **Have you received any other vaccines in the past 10 years?**

If yes please state what: ……

5. **Have you had a cold, flu, ear infection, or other respiratory infection during the past month?**

Cold ☐ Flu ☐ Ear infection ☐ Other chest infection ☐ None ☐

6. **Have you taken antibiotics in the past month?**

Yes ☐ No ☐

If yes, do you remember the name:

Amoxil ☐ Augmentin (coamoxiclav) ☐ Erythromycin ☐ Azithromycin ☐

Oxacillin ☐ Tetracycline ☐ Ciprofloxacin ☐ Ampicillin ☐

Chloramphenicol ☐ Penicillin ☐ Cefotaxim ☐ Other (unknown name) ☐

☐ Other (please state) ……..

REC number: 16/SS/0094
Version 1 dated 24/03/2016
Sponsor number: 18525

**7. What ethnicity are you?**

**White**

White British ☐

White Irish ☐

White Gypsy or Irish Traveller ☐

**Mixed/Multiple ethnic groups**

White and Black Caribbean ☐

White and Black African ☐

White and Asian ☐

**Other ethnic group**

Arab ☐

**Asian/Asian British**

Indian ☐

Pakistani ☐

Bangladeshi ☐

Chinese ☐

**Black/African/Caribbean/Black British**

African ☐

Caribbean ☐

**Would rather not say** ☐

**Other not listed** (please state) .....

**8. What is your age?** ..... years

**9. What sex are you?**

Male ☐ Female ☐ Other ☐ Would rather not say ☐

**9. Do you have any long term illnesses or conditions?** e.g. asthma, COPD, diabetes, lung fibrosis, Chronic rhinosinusitis, etc.

Yes ☐ No ☐ Would rather not say ☐

If yes, please state .....

**10. Do you smoke cigarettes and/or cigars?**

Yes ☐ No ☐ Would rather not say ☐

**11. Do you smoke e-cigarettes?**

Yes ☐ No ☐ Would rather not say ☐

**If yes to questions 10 or 11** please state on average how many times you smoke per day

.....

**If no to questions 10 or 11** have you ever smoked cigarettes/cigars?

Yes ☐ No ☐ Would rather not say ☐

**If no to questions 10 or 11** have you ever smoked e-cigarette?

Yes ☐ No ☐ Would rather not say ☐

Please state on average how many times you used to smoke per day.....

Please state how long ago you quit smoking.....

**12. Do you live with people who smoke cigarettes and/or cigars?**

Yes ☐ No ☐ Would rather not say ☐

**13. Do you live with people who smoke e-cigarettes?**

Yes ☐ No ☐ Would rather not say ☐

**14. How often are you exposed to second hand cigarettes and/or cigars smoke?**

Daily ☐ Once a week ☐ On occasion (i.e. once a month) ☐ Never ☐

**15. How often are you exposed to second hand e-cigarette smoke?**

Daily ☐ Once a week ☐ On occasion (i.e. once a month) ☐ Never ☐

**16. What are the first 5 digits of your post code ie SO16 8**

Please write here: ..... Would rather not say ☐

**17. If you only had the mandatory swabs taken, please state why you did not want to have the nasal swab taken?**

.....

.....

.....

**18. Would you participate in a study like this one again?**

Yes ☐ No ☐ Would rather not say ☐

If no, please state why

.....

.....

.....

**Participant study number** (to be filled out by research team) .....

REC number: 16/SS/0094
Version 1 dated 24/03/2016
Sponsor number: 18525

## Thank you for completing the questionnaire

**Swabbing Notes**

Although we are unable to report individual's results, if you would like us to report back the overall findings of the study please give your email address:

.....

REC number: 16/SS/0094
Version 1 dated 24/03/2016
Sponsor number: 18525

Medicine

UNIVERSITY OF Southampton

# QUESTIONNAIRE – PARENT/GUARDIAN/CAREGIVER

Title of project: **Analysis of the carriage rates and antibiotic resistance of bacteria in the upper respiratory tract (Solent SMART pilot study)**

Please tick the relevant boxes and provide extra information where necessary.

1. **Has your child received the pneumococcal vaccine (also called Prevenar) in line with the national immunisation schedule?**

Yes ☐ No ☐ Don't know ☐ Too young ☐ Would rather not say ☐

2. **Has your child received all other vaccines that are due in the line with the national immunisation schedule?**

Yes ☐ No ☐ Don't know ☐ Too young ☐ Would rather not say ☐

3. **Has your child received the meningitis group B vaccine (also called Bexsero)?**

Yes ☐ No ☐ Don't know ☐ Too young ☐ Would rather not say ☐

If yes please state when: ……/……/……

4. **Has your child received the influenza vaccine (also called Fluenz), in the last 12 months?**

Yes ☐ No ☐ Don't know ☐ Too young ☐ Would rather not say ☐

If yes please state when: ……/……/……

5. **Has your child had a cold, flu, ear infection, or other respiratory infection during the past month?**

Cold ☐ Flu ☐ Ear infection ☐ Other chest infection ☐ None ☐

6. **Has your child taken antibiotics in the past month?**

Yes ☐ No ☐

If yes, do you remember the name:

Amoxil ☐ Augmentin (coamoxiclav) ☐ Erythromycin ☐ Azithromycin ☐

Oxacillin ☐ Tetracycline ☐ Ciprofloxacin ☐ Ampicillin ☐

Chloramphenicol ☐ Penicillin ☐ Cefotaxime ☐ Other (unknown name) ☐

☐ Other (please state) ……

REC number: 16/SS/0094
Version 1 dated 24/03/2016
Sponsor number: 18525

**7. What ethnicity is your child?**

**White**

| | | | |
|---|---|---|---|
| White British | ☐ | **Asian/Asian British** | |
| White Irish | ☐ | Indian | ☐ |
| White Gypsy or Irish Traveller | ☐ | Pakistani | ☐ |
| **Mixed/Multiple ethnic groups** | | Bangladeshi | ☐ |
| White and Black Caribbean | ☐ | Chinese | ☐ |
| White and Black African | ☐ | **Black/African/Caribbean/Black British** | |
| White and Asian | ☐ | African | ☐ |
| **Other ethnic group** | | Caribbean | ☐ |
| Arab | ☐ | **Would rather not say** | ☐ |

**Other not listed** (please state) .....

**8. What is the age of your child? ..... years.....months**

**9. What sex is your child?**

Male ☐ Female ☐ Other ☐ Would rather not say ☐

**10. Does your child have any long term illnesses or conditions?** e.g. asthma, COPD, diabetes, lung fibrosis, Chronic rhinosinusitis, etc.

Yes ☐ No ☐ Would rather not say ☐

If yes, please state .....

**11. If too young for school does your child attend nursery or pre-school?**

Yes ☐ No ☐ Would rather not say ☐

**12. Does your child live with anyone who smokes cigarettes and/or cigars?**

Yes ☐ No ☐ Would rather not say ☐

**13. Does your child live with anyone who smokes e-cigarettes?**

Yes ☐ No ☐ Would rather not say ☐

**14. How often is your child exposed to second hand cigarettes and/or cigars smoke?**

Daily ☐ Once a week ☐ On occasion (i.e. once a month) ☐ Never ☐

**15. How often is your child exposed to second hand e-cigarette smoke?**

Daily ☐ Once a week ☐ On occasion (i.e. once a month) ☐ Never ☐

**16. What are the first 5 digits of your post code ie SO16 8**

Please write here: ..... Would rather not say ☐

**17. If your child only had the mandatory swabs taken, please state why you did not want them to have the nasal swab taken?**

.....

.....

.....

**18. Would you let your child participate in a study like this one again?**

Yes ☐ No ☐ Would rather not say ☐

If no, please state why

.....

.....

.....

**Participant study number** (to be filled out by research team) .....

## Thank you for completing this questionnaire

Although we are unable to report individual's results, if you would like us to report back the overall findings of the study please give your email address:

.....

REC number: 16/SS/0094
Version 1 dated 24/03/2016
Sponsor number: 18525

**Swabbing Notes**

REC number: 16/SS/0094
Version 1 dated 24/03/2016
Sponsor number: 18525

## B.1.4 Swabbing SOPs

Medicine

UNIVERSITY OF Southampton

| | |
|---|---|
| SOP Title: | SOP for performing nasal swabs on participants |
| SOP version: | 1 |
| Date | 01/03/2016 |

**SOP for obtaining nasal swabs from participants participating in the Solent SMART pilot study**

1. **PURPOSE AND SCOPE**

This SOP is designed to ensure that the nasal specimen collection is performed consistently and correctly.

This SOP describes the procedures for taking nasal swabs to obtain bacteria present in the nose of participant, whilst fulfilling ethical responsibilities for the rights, safety and welfare of the participants.

This SOP will apply to all members of the research team or clinical staff, involved in the swabbing of participants for the Solent SMART pilot study.

The PI will ensure that all staff read and follow the SOP, and that relevant training has been completed before the project has started.

2. **RESPONSIBILITIES**

It is the responsibility of all staff performing this procedure on any participant, to read and follow this SOP.

3. **EQUIPMENT**

- Gloves
- Soft tissues
- Sterile nasal swabs
- Transport media as per study protocol

4. **Procedure**

This SOP will be given prior to the study starting so that the member of the research team or clinical staff can ask any questions that they may have before swabbing potential participants.

1. Ensure participant or parent/guardian understands the procedure and then obtain written informed consent.
2. Put on a pair of gloves.
3. Carefully remove the swabs from the sterile packaging. *Try not to put the swab on to a surface or touch the cotton bud end with your fingers.*
4. Gently insert the cotton bud end of the swab about 0.5cm-1cm into one of the nostrils of the participant (it does not matter which nostril). The swab should be rubbed firmly against the inside wall of the nostril to ensure that the swab contains a sufficient sample.

5. Place the swab in the tube provided containing charcoal transport medium ensuring that the cap is put on and creates a seal.
6. Label tube with participant's study number.
7. Ensure patient is comfortable after the procedure.
8. Give swabs to the research team for processing in the laboratory.

**FILE LOCATION OF THE MASTER E-VERSION**

M:\RESEARCHGROUPS\STUART CLARKE GROUP\SOLENT STUDY\PILOT STUDY\STUDY DOCUMENTS\SOP'S

| SOP Title: | SOP for performing nasopharyngeal swabs on participants |
|---|---|
| SOP version: | 1 |
| Date | 24/03/2016 (based on SOP provided by the WTCRF – BRU SOP Number: WTCRF-BRU/GEN/V1/139) |

**SOP for obtaining nasopharyngeal swabs from participants participating in the Solent SMART pilot study**

## 1. BACKGROUND

The nasopharynx is the most common pathway for the introduction of airborne microorganism into the respiratory tract. Sampling of the nasopharyngeal epithelium using a nasopharyngeal swab is a technique widely applied for detecting potential respiratory pathogens.

## 2. PURPOSE AND SCOPE

This SOP is designed to ensure that the nasopharyngeal specimen collection is performed consistently and correctly.

This SOP describes the procedures for taking nasopharyngeal swabs. This SOP will apply to all members of the research team or clinical staff involved in the swabbing of participants for the Solent SMART pilot study.

The PI will ensure that all staff read and follow the SOP, and that relevant training has been completed before the project has started.

## 3. RESPONSIBILITIES

It is the responsibility of all staff performing this procedure on any participant, to read and follow this SOP.

## 4. EQUIPMENT

- Gloves
- Soft tissues
- Sterile nasopharyngeal swabs
- Transport media as per study protocol

## 5. PROCEDURE

This SOP will be given prior to the study starting so that members of the research team or clinical staff can ask any questions that they may have before swabbing potential participants.

1. Ensure participant or parent/guardian understands the procedure and then obtain written informed consent.
2. Put on a pair of gloves.

3. Carefully remove the swabs from the sterile packaging. *Try not to put the swab on to a surface or touch the cotton bud end with your fingers.*
4. Sit the patient up with head against a wall / lie them on an examination table as patients have a tendency to pull away during this procedure (or sat on their parent's lap if appropriate).
5. Measure the distance from the tip of the nose to the external auditory canal (Appendix 1). The wire shaft of the swab should only be inserted half this distance.
6. Insert a **sterile** dry swab into one nostril straight back (not upwards) and continue along the floor of the nasal passage until the measured distance is fully inserted or until resistance is felt (See Appendix 2 - 3). **Do not force** swab if obstruction is encountered before reaching the nasopharynx.
7. Rotate the swab gently for up to 10 seconds to loosen the epithelial cells.
8. Remove the swab and immediately place in the tube provided containing charcoal transport medium ensuring that the cap is put on and creates a seal.
9. Label tube with participant's study number.
10. Ensure patient is comfortable after the procedure.
11. Give swabs to the research team for processing in the laboratory.

**REFERENCES**

**1.** *Lieberman D, Lieberman D, Shimoni A, Keren-Naus A,Steinberg R, Shemer-Avni Y. "Identification of Respiratory Viruses in Adults: Nasopharyngeal versus Oropharyngeal Sampling" JOURNAL OF CLINICAL MICROBIOLOGY, 47 (11)2009, 3439–3443.*

**2.HTTP://WWW.WHO.INT/CSR/DISEASE/AVIAN_INFLUENZA/GUIDELINES/HUMANSPECIMENS/EN/**

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*David Lieberman,1,2* Elena Shleyfer,2 Hana Castel,2 Andrei Terry,2 Ilana Harman-Boehm,Jorge Delgado,2 Nechama Pe JOURNAL OF CLINICAL MICROBIOLOGY, 2006, 44(2). 525–528*

*4. .California Department of Public Health. Nasopharyngeal swab collection for Swine Influenza.. Published April 2009*

*5. Sung R, Chan P, Choi K, Yeung A, Li A, Tang J, Ip M, Tsen T, Nelson A. "Comparative Study of Nasopharyngeal Aspirate and Nasal Swab Specimens for Diagnosis of Acute Viral Respiratory Infection". JOURNAL OF CLINICAL MICROBIOLOGY,. 2008, 46(9) 3073–3076*

*6. Heikkinen T,Marttila J, Salmi A,Ruuskanen O." Nasal Swab versus Nasopharyngeal Aspirate for Isolation of*
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**FILE LOCATION OF THE MASTER E-VERSION**

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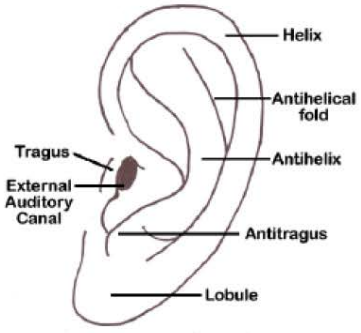
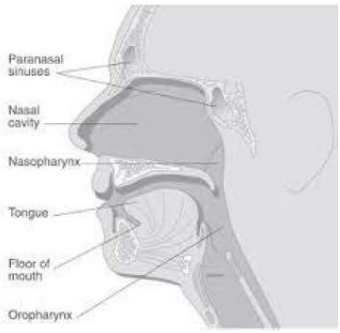
(THIS SOP IS BASED ON AN SOP PROVIDED BY THE WTCRF – BRU. THE LOCTION OF THIS SOP CAN BE FOUND - Z:\TrustHQ\Wellcome\MANAGEMENT\Governance\Policies & SOPs\SOP-Standard Operating Procedures\SOP GEN - Current\139 GEN Performing a nasopharyngeal swab_V1)

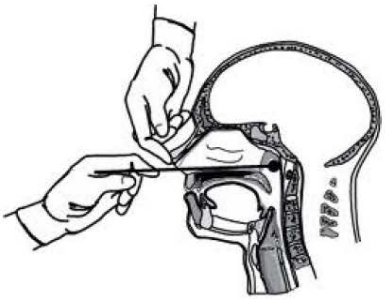
**APPENDIX 1** Anatomy of an external ear.




**APPENDIX 2** Anatomy of Nasophraynx.




**APPENDIX 3** Nasopharyngeal sampling.



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| SOP Title: | SOP for performing oropharyngeal swabs on participants |
| SOP version: | 1 |
| Date | 01/03/2016 (based on SOP provided by the WTCRF) |

**SOP for obtaining oropharyngeal swabs from participants participating in the Solent SMART pilot study**

## 1. BACKGROUND

The oropharynx is a common pathway for the introduction of airborne microorganism into the respiratory tract. Sampling of the oropharynx epithelium using an oropharyngeal swab is a technique widely applied for detecting potential respiratory pathogens.

## 2. PURPOSE AND SCOPE

This SOP is designed to ensure that the oropharyngeal specimen collection is performed consistently and correctly.

This SOP describes the procedures for taking oropharyngeal swabs. This SOP will apply to all members of the research team or clinical staff involved in the swabbing of participants for the Solent SMART pilot study.

The PI will ensure that all staff read and follow the SOP, and that relevant training has been completed before the project has started.

## 3. RESPONSIBILITIES

It is the responsibility of all staff performing this procedure on any participant, to read and follow this SOP.

## 4. EQUIPMENT

- Gloves
- Soft tissues
- Sterile oropharyngeal swabs
- Transport media as per study protocol

## 5. PROCEDURE

This SOP will be given prior to the study starting so that the members of research team or clinical staff can ask any questions that they may have before swabbing potential participants.

1. Ensure participant or parent/guardian understands the procedure and then obtain written informed consent.
2. Put on a pair of gloves.

3. Ensure the participant is comfortable, sat in a hard backed upright chair or against a wall or lying on an examination table (or sat on their parent's lap if appropriate).
4. Advise the participant that they may experience a gagging sensation whilst the swabbing is being done. This is a harmless reflex.
5. Ask the subject to extend their neck, open their mouth wide and stick out their tongue. Gently insert a tongue depressor and press lightly on the tongue, you should be able to clearly see their uvula and tonsils. If these are not obvious then put a little more pressure on the tongue depressor and/or ask the subject to open their mouth wider until you have full visualisation.
6. Carefully remove the swabs from the sterile packaging. *Try not to put the swab on to a surface or touch the cotton bud end with your fingers.*

7. Insert a **sterile** dry swab into the participant's mouth: If you are right-handed touch the tip of the swab against the outer side of the right tonsil and then sweep the swab across the back of the pharynx, behind the uvula, until it reaches the outer side of the left tonsil. If you are left-handed touch the tip of the swab against the outer side of the left tonsil and then sweep across the back of the pharynx, behind the uvula until it reaches the outer side of the right tonsil.
8. If the swab touches the tongue or teeth, discard and repeat the process, only attempt twice. If second attempt is also contaminated, retain sample and document this contamination.
9. Remove the swab and immediately place in the tube provided containing charcoal transport medium ensuring that the cap is put on and creates a seal.
10. Label tube with participant's study number.
11. Ensure patient is comfortable after the procedure.
12. Give swabs to the research team for processing in the laboratory.

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