

## Review Article

# Nasopharyngeal Bacterial Carriage in the Conjugate Vaccine Era with a Focus on Pneumococci

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Seven-valent pneumococcal conjugate vaccine (PCV7) was included in the UK national immunisation program in 2006, and this was replaced by thirteen-valent PCV in 2010. During this time, the carriage of vaccine-type *Streptococcus pneumoniae* decreased but pneumococcal carriage remained stable due to increases in non-vaccine-type *S. pneumoniae*. Carriage studies have been undertaken in various countries to monitor vaccine-type replacement and to help predict the serotypes, which may cause invasive disease. There has been less focus on how conjugate vaccines indirectly affect colonization of other nasopharyngeal bacteria. If the nasopharynx is treated as a niche, then bacterial dynamics are accepted to occur. Alterations in these dynamics have been shown due to seasonal changes, antibiotic use, and sibling/day care interaction. It has been shown that, following PCV7 introduction, an eradication of pneumococcal vaccine types has resulted in increases in the abundance of other respiratory pathogens including *Haemophilus influenzae* and *Staphylococcus aureus*. These changes are difficult to attribute to PCV7 introduction alone and these studies do not account for further changes due to PCV13 implementation. This review aims to describe nasopharyngeal cocarriage of respiratory pathogens in the PCV era.

## 1. Introduction

Invasive pneumococcal disease (IPD) is a cause of substantial morbidity and mortality worldwide, with over 5,000 cases reported in the UK per year [1]. For patients with IPD in the United States, around 10% will die from the illness [2]. In 2008, globally there were an estimated 476,000 deaths attributed to pneumococcal infection among children less than five years of age. As of 2014, globally 59% of infants still live in countries where a PCV has yet to be added to the national immunisation program [3]. The seven-valent pneumococcal conjugate vaccine (PCV7) (Prevenar, Pfizer, previously Wyeth) was added to the US immunisation schedule in 2000 and to the UK immunisation schedule in 2006. The effect of PCV7 on pneumococcal carriage in children

has been investigated, with vaccine serotypes decreasing since PCV introduction [4–6]. In the UK and elsewhere, the incidence of IPD has decreased after the implementation of PCVs [7–10]. As PCV13 was introduced, the rates of vaccine-type carriage and IPD have similarly declined [11–13], and as additional higher valency PCVs are introduced it is expected that IPD incidence will continue to decrease [14].

The effect of pneumococcal vaccination on other bacterial species known to occupy the same niche as *S. pneumoniae* has not been fully investigated, in particular determination of how changes in the human microbiome can be attributed to external pressures or vaccine introductions [15, 16]. This paper reviews what is known about cocarriage of nasopharyngeal bacteria.

## 2. Pneumococcal Disease

*S. pneumoniae* are Gram-positive diplococci often found to occupy the nasopharynx. Pneumococci are typed according to the serological response to their external polysaccharide capsule. Strains of *S. pneumoniae* that do not react with type-specific antisera are deemed nontypeable (NT) *S. pneumoniae*. Currently 94 pneumococcal serotypes have been characterised [17–22]. Individuals become colonised with *S. pneumoniae* and other nasopharyngeal flora during their first few months of life [23], although the age of pneumococcal colonisation varies and may be attributed to environmental factors such as having siblings, attending day care, or geographical location [23, 24]. Colonisation with a pneumococcal isolate is a prerequisite for pneumococcal infection; the capsule type of *S. pneumoniae* rather than genotype is thought to modulate the degree of infection [25]. The capsular type of the pneumococcus dictates the duration of colonization in children with common serotypes retained longer in carriage. As the age of a child increases, so does their immune mediated clearance of pneumococcal serotypes [26]. Disease caused by pneumococcal infections can be divided into two groups: invasive pneumococcal disease (IPD) and noninvasive disease.

## 3. Immunisation to Reduce Disease Burden

The 23-valent pneumococcal polysaccharide vaccine (PPV23) (Pneumovax II, Aventis Pasteur) is a plain polysaccharide vaccine that induces an immune response to the polysaccharide capsule of an infectious organism to induce short-term memory B-cells and antibody production. As the immune response produced is classed as “slow, no immune memory,” this type of vaccine is not effective in young children and infants (IPD risk groups). T-cell independent vaccines also appear not to prevent carriage of the bacterial species [27] after the short-lived immune response has finished.

Conjugate vaccines contain bacterial polysaccharides from the outer capsule of an organism, for example, PCV7 (seven-valent pneumococcal conjugate vaccine, Prevenar, Pfizer); 10-valent PCV (Synflorix, GSK) and 13-valent PCV (Prevenar 13, Pfizer) are currently licensed pneumococcal conjugate vaccines. The polysaccharide is converted into a T-cell dependent antigen through the presence of the carrier protein. Long-term memory B-cells mature so that the immune system has both a short-term and long-term response invoked when those polysaccharides are encountered again. This reduces colonisation of the serotypes included within the vaccine, helping to prevent infection even in the very young. PCVs are given to children rather than a PPV to produce a stronger and more long-lasting immune response [28] and PCVs have also been found to be more effective against vaccine serotypes than PPV in older adults [29]. The 10-valent pneumococcal vaccine, PHiD-CV10 (GSK), also includes conjugation of nontypeable *Haemophilus influenzae* protein D. PCV10 is comparable to PCV7 at preventing invasive pneumococcal disease [30] and has been found to have a higher immunologic coverage for acute otitis media than PCV13 [31].

## 4. Pneumococcal Conjugate Vaccination

Prior to implementation of 7-valent pneumococcal conjugate vaccination, the majority of invasive disease globally was caused by seven of the pneumococcal serotypes [32]. PCV13 introduction has further addressed IPD caused by the 6 serotypes included in the new vaccine in Europe and North America [33]. PCV effectiveness is subject to strains undergoing capsular changes including (a) serotype replacement/shifting [34], where prevalence of a nonvaccine serotype increases as prevalence of a vaccine serotype decreases and the non-vaccine-type bacteria overcome vaccine challenges in a community [35], and (b) capsular switching, where an individual bacterium can undergo changes in the capsular genes, causing the bacteria to change serotype [36]. Through alteration of capsular expression and the increase in prevalence of serotypes not included in vaccine formulations, serotypes in carriage may be replaced with more virulent serotypes [37]. However it has been reported that capsular switching resulting from vaccine pressures will only contribute to an increase of a maximum of three extra cases of IPD per 100,000 vaccinated children cumulated over a ten-year period [38]. The additional maximum of three cases per year was deduced using a mathematical model of pneumococcal transmission based on IPD data presented in previous European publications [38]. However until there is evidence from more studies of capsular switching after PCV, IPD from non-vaccine-type pneumococci may be a more pressing issue [39]. Antibiotic resistance in pneumococcal isolates has also been shown to be present globally in both carriage [40–42] and disease [43–45] cases. Reasons for pneumococcal antibiotic resistance include vaccine pressures as well as overprescribing and overuse of antibiotics acting as a selective pressure for current strains to undergo clonal expansion [46, 47].

## 5. Interactions of Nasopharyngeal Microbiota

The microbiota of the human nasopharynx contains both commensal and potentially pathogenic species with external environmental factors and the presence of antibiotic resistant species contributing to disease states [48].

Bacteria found to reside in the nasopharynx other than *S. pneumoniae* include *H. influenzae*, *Moraxella catarrhalis*, alpha-haemolytic streptococci ( $\alpha$ -HS), *Staphylococcus aureus*, and *Neisseria meningitidis* which are included in this review as respiratory bacteria capable of causing significant infections. *M. catarrhalis* is a nonmotile Gram-negative human commensal and opportunistic pathogen responsible for a range of infections, including causing an estimated 10% of adult chronic obstructive airways disease (COPD) exacerbations [49]. It has been shown that even the colonisation of *M. catarrhalis* in a COPD patient can contribute to the progression of airway disease [50]. *S. pneumoniae*, *Streptococcus mutans*, and *Streptococcus sanguis* are all species of  $\alpha$ -haemolytic streptococci ( $\alpha$ -HS) that are both commensal and pathogenic in people; *Streptococcus viridans* are a group of streptococcal organisms including *Streptococcus mitis*, *Streptococcus salivarius*, and *S. mutans*. *S. viridans* have

been shown to be involved in causing empyema thoracis and lung abscesses [51]. *S. aureus* is a commensally carried Gram-positive bacterium that can act as an opportunistic respiratory pathogen in susceptible individuals. Nasal carriage of *S. aureus* is positively associated with noninvasive and invasive infections, compared to those who do not carry *S. aureus* [52]. It is important to assess the carriage of *S. aureus* and to monitor if carriage of methicillin-resistant (MRSA) bacteria is increasing in the community. Carriage of the Gram-negative diplococcus *N. meningitidis* is higher in young children under 1 year of age and then, after 15 years of age [53], *N. meningitidis* can result in a wide range of infections and bacterial load is associated with mortality, particularly for serogroup C that produces a higher bacterial load in patients [54]. *H. influenzae* are Gram-negative coccobacilli, serologically typed a–f, as well as a large, distinct population [55] that are unencapsulated, termed nontypeable *H. influenzae* (NTHi). Certain bacterial genes of NTHi have been found to be associated to aid bacterial persistence within the lower airways of patients with COPD [56] as well as now being known to cause invasive disease in risk groups [57].

## 6. Monitoring Bacterial Carriage

Bacterial colonization is thought to be a prerequisite for an individual to become infected, but bacterial colonisation does not normally result in infection [58]. There are many relevant studies, both completed and ongoing, that monitor bacterial carriage in individuals [59–63]. Bacterial carriage may be monitored to detect before and after changes following the implementation of a preventative vaccination strategy [4, 59, 64–68]. Carriage can be monitored for changes attributed to age, health status, geographical location, ethnicity, and many other environmental factors [69, 70]. Carriage of a number of bacteria or carriage of a single bacterial species can be monitored. Respiratory bacteria can be detected using relatively noninvasive means such as a nasopharyngeal [71, 72] or nose swab, meaning that larger numbers of patients can be recruited to strengthen the results gained from the study.

Pneumococcal carriage studies are highly informative and beneficial for a number of reasons. Through surveillance of invasive disease studies, we have seen how PCVs are effective against invasive vaccine-type (VT) pneumococcal disease [73], and through carriage studies we can see the reduction of VT pneumococcal colonisation and VT pneumococcal transmission [74]. It is also possible to monitor indirect effects of PCVs (Table 1), such as changes in dynamics of the nasopharynx to detect microbial shifts [75], where the bacterial species change in content or numbers due to an external pressure.

## 7. A Niche of Carriage and Infection

Bacterial cocolonisation of the nasopharynx niche can be classed as dynamic as it comprises both synergistic and competitive associations. These associations can change depending on whether or not the niche is in a healthy or a disease state [76]. A lower diversity of nasopharyngeal

flora has been positively associated with higher carriage rates of nasopharyngeal pathogens including *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* [77]. Individuals with spontaneous otorrhea that have multiple pneumococcal serotypes colonising at any one time were more likely to present with other species cocolonising [78]. Viral infection has been to leave the middle ear vulnerable to infection by bacteria that normally reside in the nasopharynx [79].

External factors such as sibling interaction and interaction with other children can play a part in polymicrobial carriage, and such relationships are associated with more frequent nasopharyngeal carriage of potential pathogens [80]. Adult associations with more frequent carriage of potential pathogenic species include, but are not limited to, the presence of children either at home or at work, preexisting allergic conditions, and respiratory conditions including COPD and asthma [80]. Such data imply that children are reservoirs for bacterial pathogens. With increased contact between children and other children or children and adults, there is a greater chance for bacterial transmission between the individuals.

*S. pneumoniae* and *H. influenzae* are frequently found to cocolonise the nasopharynx, and competition may exist between the two organisms for nutritional resources and for dominance of the niche. It has been shown that *H. influenzae* when colonising with *S. pneumoniae* may outcompete them for survival through signaling of nucleotide-binding oligomerisation domain-1 (Nod1) to facilitate clearance of *S. pneumoniae* [81], but virulent *S. pneumoniae* serotypes show resistance to host cell-mediated clearance as a mechanism to overcome these attacks [82]. Both organisms cause immune responses in colonised individuals and cocolonisation by these two pathogens can result in exaggerated immune responses with prolonged hospitalization particularly for young asthmatics experiencing their first count of wheezes [83].

Before the inclusion of the *H. influenzae* type b (Hib) conjugate vaccine in the UK routine paediatric immunisation schedule in 1992 [84], around 95% of invasive *H. influenzae* disease was attributed to serotype b alone [85]. After Hib vaccination there was a dramatic 98% decrease in invasive disease by 1998 [86]. However a small amount of Hib disease has still been reported in some vaccinated populations, ranging from invasive disease due to vaccine failure in the UK and elsewhere [86, 87]. Increased disease incidence has been reported for non-Hib serotypes [88–90]. NTHi has also been reported as a cause for invasive disease [90, 91]. The surveillance following Hib vaccination indicates that vaccine-type replacement is seen with other strains not included in vaccine formulation; however studies have not yet fully elucidated the effects on cocolonising niche species.

Polymicrobial cocarriage can consist of more than one bacterial species; this can also include viruses and fungi [92]. A polymicrobial infection combining both bacteria and fungi can mount a greater immune response within a host than infection by either bacteria or fungi [93]. Preinfection with a virus can destroy epithelial cells and allow better adhesion for bacteria, thus priming the middle ear for further infection that can result in otitis media [94]. Bacterial

TABLE 1: Examples of studies identifying respiratory cocarriage of bacterial species in the PCV era.

Study	Study detail: vaccination status, species selected, and origin of samples	Methodology
Wiertsema et al. [104]	Post-PCV7, Spn, and Hflu (Australia)	Nasopharyngeal swab (NP) and conventional culture
Xu et al. [101]	Post-PCV7, Spn, Hflu, and Mcat (USA)	Nasal swab (NS), oropharyngeal sample (OP), and conventional culture
Spijkerman et al. [105]	Post-PCV7, Spn, SA, Hflu, and Mcat (Netherlands)	NP swab and conventional culture
Principi et al. [24]	Pre-PCV7, Spn, Hflu, and Mcat (Italy)	NP swab and conventional culture
Biesbroek et al. [75]	Post-PCV7 and pre-PCV7, Spn, Mcat, SA, and Spn (Netherlands)	NP swab and 454 pyrosequencing
Xu et al. [76]	Post-PCV7, Spn, Hflu, Mcat, and SA (USA)	NP, OP, and conventional culture
Pettigrew et al. [77]	Post-PCV7, Spn, Hflu, and Mcat (USA)	NP and 454 pyrosequencing
Laufer et al. [102]	Post-PCV7, Spn (USA)	NS swab and 454 pyrosequencing
Bogaert et al. [103]	Post-PCV7 and reduced dose PCV7, Spn, Hflu, Mcat, and SA (Netherlands)	NP swab and 454 pyrosequencing

This table includes vaccination status, species chosen for monitoring, and the origin of the samples as well as the methodology used for a comparison. Spn: *S. pneumoniae*; Hflu: *H. influenzae*; SA: *S. aureus*; Mcat: *M. catarrhalis*. 454 pyrosequencing is nonculture based identification.

communities that aggregate together on a surface are known as biofilms. Biofilms have a number of mechanisms to increase persistence and survive, which protect from both therapeutic attack and host immune responses [95, 96]. Bacterial biofilms contribute to a range of chronic respiratory and otolaryngeal diseases. Bacterial biofilms are commonly detected and implicated in pathogenicity in children with recurrent acute otitis media [97, 98], chronic middle ear effusion [99], and other chronic respiratory conditions.

## 8. Indirect Effects of PCV Implementation

Reports are emerging of the effect of PCV implementation on cocarriage and disease caused by bacteria other than pneumococci. Where vaccine serotypes of *S. pneumoniae* have been eradicated, there has been an increase in nontypeable *H. influenzae* isolated in cases of otitis media [100]. A large study of healthy children and children with recurrent otitis media, all less than 36 months of age in Western Australia, has shown that with a decrease in *S. pneumoniae* and *S. pneumoniae* PCV7 VT serotypes there is a corresponding increase in *H. influenzae*, particularly NTHi. Another study has shown that colonisation with of *S. pneumoniae* invasive serotype 19A is associated with a decrease in colonisation of *H. influenzae* [101]. To study the indirect effects of PCV implementation without introducing bias through standard microbiology culture, 16S-sequencing was used to sequence nasopharyngeal swabs of children with or without otitis media, which demonstrated that an infection is associated with increased *S. pneumoniae* and *H. influenzae* being present with a lack of protective flora present [102]. Another study set out to characterize the nasopharyngeal niche to deduce which, if any, external factors (such as viral carriage and day care level) have an effect on the microbiota of the nasopharynx of young children. Results showed that seasonal changes

were occurring but that these were unrelated to viral or antibiotic causes and that seasonal variations corresponded to “healthy” probiotic species being more abundant in summer rather than autumn [103].

## 9. Summary

Pneumococcal conjugate vaccines (PCVs) affect the carriage of *S. pneumoniae* and the carriage of vaccine-type (VT) serotypes. With a decrease in *S. pneumoniae* PCV7 VT serotypes, there was a corresponding increase in *H. influenzae*, particularly NTHi [100], exemplifying the dynamic, potentially competitive relationship between these two organisms [106–108]. The eradication of PCV7 VT in the nasopharynx has also been associated with higher rates of *H. influenzae* and *S. aureus* carriage in young children and infants, highlighting those virulent serotypes of *S. pneumoniae* also having a competitive relationship with *S. aureus* as well as *H. influenzae* [75, 105]. Incidences of bacterial cocarriage are important to report to inform future vaccine developments. This is important as the effect of vaccines targeting nasopharyngeal pathogens may produce indirect effects, as the ultimate balance between cocolonising organisms is unknown. The future of vaccination is under scrutiny with each vaccination implemented against specific serotypes or serogroups, as vaccine-type replacement is detected in the years following vaccination. When it is so difficult to predict the effects after vaccination of the target species, it is even more difficult to account for the indirect effects on cocolonising species.

- (i) IPD remains an important disease both in the UK and worldwide responsible for morbidity and mortality.
- (ii) PCVs are currently the most effective pneumococcal vaccinations available at both reducing colonization of invasive serotypes and invasive disease.

- (iii) The role of conjugate vaccines in the nasopharyngeal niche remains unclear as studies initially focused on serotype/serogroup replacement of the target species.
- (iv) Serotype replacement occurs after PCV implementation, driving the need to develop new vaccination strategies independent of pneumococcal serotype inclusion.
- (v) Bacterial carriage studies in the conjugate vaccine era have primarily focused on carriage of the target species; there have not been as many studies looking at nasopharyngeal cocarriage.
- (vi) Studies that have looked at nasopharyngeal cocarriage in the PCV era have shown changes in carriage of *H. influenzae* and *S. aureus* following PCV implementation, implying that a reduction in vaccine-type pneumococci will result in an increase of carried *H. influenzae* and *S. aureus*.
- (vii) It is currently difficult to define all potential indirect effects of PCV on the carriage of nonpneumococcal organisms.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

## Authors' Contribution

S. C. Clarke and S. N. Faust have contributed equally to this work.

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