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Disturbance of the Nasopharyngeal Microbiome in Children After Introduction of PCV

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B.S.
College of William and Mary
2016

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An abstract of
A thesis submitted to the Faculty of the
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Abstract

Disturbance of the Nasopharyngeal Microbiome in Children After Introduction of PCV

By Sarah Stallard

The nasopharynx is a complex ecological niche, home to thousands of microbes. Some of the most common bacteria found there, *Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus,* and *Moraxella catarrhalis,* are also the leading causes worldwide for respiratory illness and other disease. While the introduction of the pneumococcal conjugate vaccine led to a decrease in pneumococcal disease, it completely changed the environment within the nasopharynx, leading to a shift in *S. pneumoniae* serotypes and possible changes in the carriage rates of *H. influenzae, S. aureus,* and *M. catarrhalis.* This has led to fears of increases in illness causes by these microbes. This review focuses on the effects of the pneumococcal conjugate vaccine on the nasopharyngeal microbiome in various populations. With the use of PCV, *S. pneumoniae* serotypes have completed shifted from vaccine-type to non-vaccine type. The three most common NVT serotypes after PCV were 19F, 19A, and 6C. *H. influenzae* experienced a net increase in carriage after PCV, while *S. aureus* and *M. catarrhalis* showed no change overall. The increase in *H. influenzae* carriage has been shown in correlation with an increase in non-typeable *H. influenzae* infection in populations vaccinated by PCV. While PCV has been successful at lowering pneumococcal disease rates, its effects on the nasopharyngeal microbiota demonstrate that the use of vaccines on bacteria that co-exist within diverse microcosms may lead to unintended consequences.

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Introduction

Streptococcus pneumoniae is the leading cause worldwide for meningitis, pneumonia, and sepsis. In 2015, it was estimated that *S. pneumoniae* caused approximately 335,000 (240,000-460,000) deaths globally in children under the age of five (1). *S. pneumoniae*, along with *Haemophilus influenzae* and *Moraxella catarrhalis*, are also the predominant causes for otitis media, which can cause hearing loss, and impair learning development. Otitis media is the most frequently reported childhood bacterial infection (2).

Colonization with *S. pneumoniae* is a prerequisite for pneumococcal infection, with serotypes modulating the intensity of infection. Carriage of *S. pneumoniae* is most common in children and elderly, with the highest carriage rates found in infants under 2 years of age, though this can vary due to a multitude of environmental and genetic factors (3, 4). Carriage rates can vary from <15% to up to >90% depending on the country and population. Age, location, attending day care, having siblings, and the presence of a smoking parent in the household are all environmental risk factors for carriage. Nasopharyngeal carriage of *S. pneumoniae* is a major reservoir that allows continued circulation of the bacteria within populations (3, 5).

The nasopharyngeal microbiota

The human body is host to many different microbiota, each home to trillions of microbes. The existence of these microbiomes is thought to be beneficial to the host, as they have been shown to simulate immune system development, maturation, and function. While the majority of microbes are commensal, disturbance of nasopharyngeal

microbiota, and colonization of respiratory tract outside of the nasopharynx, may to lead to illness. Children are the most susceptible to these infections (6, 7).

The most prominent genera in healthy children under the age of 2 are *Moraxella, Haemophilus, Streptococcus, Flavobacteria, Dolosigranulum, Corynebacterium,* and *Neisseria*. In children aged 2 years to 16 years, the twelve most common species are *Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis, Moraxella lacunata, Moraxella lincolnii, Bordetella hinzii, Neisseria flavescens, Veillonella dispar, Dolosigranulum pigrum, Fusobacterium nucleatum, Lactococcus lactis,* and *Corynebacterium propinquum.* Out of these bacteria, *Haemophilus influenzae, Moraxella lacunata,* and *Moraxella lincolnii* have the highest abundance (6, 7).

The composition of the nasopharyngeal microbiota is highly variable: studies show that there is a greater diversity of bacteria within the nasopharynx of healthy children than in sick children, and that the overall composition of bacteria shifts depending on season and location within the nasal cavity. In children ill with pneumonia, 92% of the overall colonization composition of the nasopharynx is made up of the genera *Moraxella, Haemophilus,* and *Streptococcus,* while in healthy children it was only 76%. While there has been no evidence of a protective commensal species within nasopharynx, high nasopharyngeal diversity is associated with lower rates of infection (6, 7).

The pneumococcal conjugate vaccine

Introduced in 2000, the seven-valent pneumococcal conjugate vaccine (PCV7) was the first vaccine engineered to target younger age groups for *S. pneumoniae* prevention. Pneumococci are classified by serotype, which are determined by the

serological response to their external polysaccharide capsule. Some strains do not react with type-specific antisera, and are therefore labeled nontypeable (NT). PCV7 contained 7 different pneumococci serotypes: 4, 6B, 9V, 14, 18C, 19F, 23F. These serotypes accounted for about 80% of disease caused by *S. pneumoniae* before introduction of PCV. Later, vaccine coverage was expanded upon with PCV10 (containing PCV7-serotypes and serotypes 1, 5, 7F) and PCV13 (containing PCV10-serotypes and serotypes 3, 6A, and 19A) (8, 9).

Pneumococcal conjugate vaccines are currently in use in 142 countries out of 194 WHO member states, with high-income countries adopting the vaccine recommendations first. However, it is estimated that 53% of the global infant population are not receiving PCV (10). With the introduction of PCV, carriage of vaccine-type (VT) *S. pneumoniae* virtually disappeared in immunized countries, and mortality rate of *S. pneumoniae* disease outcomes dropped, with an estimated 190,000 pneumococcal deaths averted due to the vaccine from the years 2000-2015 (11).

The Centers For Disease Control recommends a four-dose regimen of PCV for all children under the age of two. The dosages are administered at 2 months, 4 months, 6 months, and 12 through 15 months. Children who miss their shots during this timetable should still get the vaccine. For adults over the age of 65, one dose of PCV is administered followed by one dose of PPSv23 (12). PPSv23 (also known as 23vPPV) is recommended for ages two through 64 who have long-term health problems, asthma, or weakened immune systems. While PPSv23 has been tested and used in some countries on children under two, it has had no significant effect on pneumococcal disease in this age group, and is therefore recommended only for individuals older than two (13).

Review purpose and criteria

Despite the effectiveness of the PCV vaccine at preventing mortality, *S. pneumoniae* carriage rates have not changed, as non-vaccine type (NVT) serotypes replaced VT serotypes in the nasopharyngeal niche in a process coined as serotype replacement (1-5, 11, 14-16). As explained earlier, the nasopharynx holds a complex microbiome, with other prominent bacteria species being *Haemophilus influenzae, Staphylococcus aureus,* and *Moraxella catarrhalis*. The sudden shift in *S. pneumoniae* serotypes has led to unexpected interactions within this niche, with changes in density and carriage being reported for *H. influenzae, S. aureus, and M. catarrhalis* (2, 8, 11, 16-26). This review analyses the studies that were conducted after the introduction of PCV to explore the potential consequences of the shifts in *S. pneumoniae* serotypes, to the interactions between *S. pneumoniae* and other bacteria listed above, and the possible consequences of shifting established microbiome environments on nasopharyngeal colonization.

Studies were selected by searching PubMed and Google Scholar for relevant articles in English on the four bacteria featured in this paper using the terms "nasopharynx", "carriage", "children", "PCV", "pneumococcal conjugate vaccine", "interactions", "vaccines", "colonization", "nasopharyngeal microbiome", "nasopharynx microbiome", "nasal microbiome", "oropharyngeal microbiome", "nasal microbiota", "nasopharyngeal microbiota", "oropharyngeal microbiota" and also by searching for publications that cited the studies looking at the interactions between all relevant bacteria after PCV implementation (2, 19). All publications were sorted into primary and

supplementary sources. Primary sources focused on *S. pneumoniae* carriage in the nasopharynx and its interactions with other bacteria after PCV implementation in children. Supplementary sources touched upon PCV effects, *S. pneumoniae* carriage, *S. pneumoniae* serotype shifts, adult populations, and bacterial interactions without matching the primary criteria. Papers surveying solely adult populations were excluded.

Effects of PCV on *Streptococcus pneumoniae* carriage and serotype replacement

PCV immunizations were introduced in 2000 with the purpose of lowering overall *S. pneumoniae* carriage, and preventing invasive pneumococcal disease (IPD). While 142 countries have since adopted pneumococcal conjugate vaccines, overall carriage rates for *S. pneumoniae* have remained stable (1-5, 10, 11, 14-16). Carriage of VT serotypes has dropped significantly in almost all countries, and carriage of NVT serotypes has risen as NVT serotypes replaced VT serotypes within the nasopharynx (Table 1)(1-5, 11, 14-16, 27-30).

Table 1: Shift between vaccine type and non-vaccine type carriage after PCV implementation

Study	Region	Age	Serotyping Method	Carriage prevalence of *S. pneumoniae* strains							
				Pre-PCV		Post-PCV7		Post-PCV10		Post-PCV13	
				VT (%)	NVT (%)	VT (%)	NVT (%)	VT (%)	NVT (%)	VT (%)	NVT (%)
Bosch et al., 2015 (3)	The Netherlands	11 months 24 months	Culture	38.0 36.0	29.0 30.0	8.0*** 4.0***	39.0* 45.0**	2.0*** 1.0***	57.0*** 55.0***	- -	- -
Brandileone et al., 2016 (17)	São Paulo, Brazil	12-23 months	Culture, PCR	19.8	8.2	-	-	1.8***	23.5***	-	-
Hammitt et al., 2014 (18)	Kilifi, Kenya	<5 years	Culture, PCR	34.0	41.0	-	-	13.0***	57.0***	-	-
von Mollendorf et al., 2019 (11)	Ulaanbaatar, Mongolia	5-8 weeks 12-23 months	DNA microarray	12.9 42.2	16.2 26.4	- -	- -	- -	- -	6.3** 19.7***	17.4 40.2***
Satzke et al., 2018 (29)	Lao PDR	5-8 weeks 12-23 months	PCR	45.0 54.5	- -	- -	- -	- -	- -	30.0* 38.5**	- -
Sharma et	Atlanta,	6-59	Culture,	60.0	-	0.7***	-	-	-	-	-

al., 2012 (5)	Georgia, USA	months	PCR								
Flasche et al., 2010 (14)	England	<5 years old	Culture	31.9	15.3	3.6**	45.3**	-	-	-	-

*: p ≤ .05
**: p ≤.001
***: p ≤ .0001

Children vaccinated with PCV are less likely to carry *S. pneumoniae*, and therefore are less likely to transmit PCV serotypes to unvaccinated individuals leading to herd immunity against VT-mediated disease. The change in nasopharyngeal environment in children has also been reflected in unvaccinated populations (3, 5, 8, 14, 18, 20, 22). This, combined with an increase in disease caused by NVT serotypes, has stoked fear among researchers that type replacement may offset the benefits provided by PCV.

After the introduction of PCV7 and PCV10, carriage of serotypes 19A and 6A rose (Table 2)(3-5, 8, 11, 14-16, 22, 28-32). Serotype 19A became the greatest contributor to IPD in vaccinated populations. Later, PCV13 immunization included serotypes 19A and 6A, but they still remain prominent in countries that do not use PCV13. In England, four years after the introduction of PCV13, NVT IPD suddenly increased. The study found that in those four years, the evolving competition of serotypes within the nasopharynx resulted in a serotype mix with a higher case carrier ratio, and that 19A has stabilized and remains prominent despite use of PCV13 in the population (32).

Table 2: Shift in carriage of most prominent serotypes before and after PCV

Study	**Population**	**Serotype prevalence (%)**			
		Pre-PCV	**Post-PCV7**	**Post-PCV10**	**Post-PCV13**
Bosch et al. (3)	The Netherlands, 11-months	19F (11.0) 23F (11.0) 6B (7.0)	19A (12.0) 6C (4.5) 11A (4.0)	19A (8.5) 6C (6.0) 23B (6.0)	- - -
Brandileone et al. (17)	Italy, 12-23 months	6B (14.5) 19F (13.0) 6A (10.1)	- - -	6C (22.7) 6A (8.1) 11A (7.1)	- - -

Hammitt et al. (18)	Kilifi, Kenya, <5 years	19F (12.0) 6B (10.0) 6A (7.0)	- - -	6A (9.0) 19F (5.0) 11A (4.0)	- - -
Southern et al. (32)	England, <5 years	6B (5.5) 19F (3.2) 23F (3.1)	19A (2.6) 7F (1.5) 3 (1.4)	- - -	3 (0.4) 19A (0.3) 19F (0.3)
Kaur et al. (15)	Rochester, New York, 6-30 months	- - -	19A (25.0) 23B (9.0) 15B (6.0)	- - -	35B (13.0) 23B (11.0) 21 (8.0)
von Mollendorf et al. (11)	Ulaanbaatar, Mongolia, 5-8 week, 12-23 months	6A (12.5) 19F (9.8) 23F (6.0)	- - -	- - -	19F (8.2) 15A (7.5) 34 (7.0)
Satzke et al. (29)	Lao PDR, 12-23 months	6B (7.7) 23F (6.7) 19F (6.4)	- - -	- - -	15B/C (4.9) 19F (4.9) 23F (4.1)
Sharma et al. (5)	Atlanta, GA, 6-59 months	19F (18.0) 6B (18.0) 6A (16.0)	19A (21.0) 6C (17.0) 35B (10.0)	- - -	- - -

Notes: Only included top three serotypes from each study. Prevalence of serotypes is in percentages.

The nasopharyngeal microbiome in healthy children after PCV

There are few studies that have examined the effects of PCV on the entirety of the nasopharyngeal microbiome. While *S. pneumoniae, H. influenzae, S. aureus, and M. catarrhalis* have been the primary focus of research, the shift in these microbes likely caused shifts in the overall microbiome as well. Higher diversity and higher stability within the nasopharyngeal tract has been shown to be correlated with lower rates of disease, while the presence of common pathogens in the *Streptococcus, Haemophilus,* and *Staphylococcus* genus lower diversity and stability (33-37).

Mika et al. analyzed the nasopharyngeal microbiome of infants in their first year of life after introduction of PCV7 and PCV13 using *16s rRNA* sequencing (33). They found that microbial diversity was significantly higher, and that there was greater richness based on the Shannon Diversity Index (SDI), in children who had received PCV13 compared to children who had received PCV7. This was hypothesized to be due to a lower rate of *S. pneumoniae* carriage in PCV13 children than PCV7 children.

Utilizing oligotyping (OT) for more precise taxonomic grouping, Mika et al. profiled five bacterial families *Moraxellaceae, Streptococcaceae, Staphylococcaceae, Pasteurellaceae,* and *Corynebacteriaceae*. They found that *H. influenzae* abundance increased significantly while *Corynebacterium accolens* decreased from PCV7-era to PCV13-era, and that PCV13 children had far greater microbiota stability than PCV7 children (33). Another study conducted in Switzerland had similar results, with increased density and diversity in children vaccinated with PCV in the first year of life, and increased abundance of *Staphylococcaceae* and *Corynebacteriaceae* within the first three months of life (34).

The nasopharyngeal microbiome changes over time, often developing into distinct profiles for each individual by six weeks after birth. These profiles are associated with microbiota stability and changes in microbiome for the next two years of life. Biesbroek et al. found that certain bacteria carriage early on was associated with differences in stability (35). For example, infants with early colonization with *Moraxella* and *Dolosigranulum* combined with *Corynebacterium* had more stable profiles in the first two years of life compared to infants with *Streptococcus* or *Haemophilus*-dominated profiles. The composition also varies due to weather and breast-feeding (35, 36). These results supplement the earlier findings that infants with PCV had more stable and diverse microbiomes compared to those without.

Streptococcus pneumoniae* and *Haemophilus influenzae

S. pneumoniae and *H. influenzae* are commensal bacteria that commonly inhabit the nasopharynx of healthy humans, though they colonize children and the elderly at

much higher rates. Both bacteria are capable of causing disease by migrating into different anatomical niches: lungs for pneumonia, blood for septicemia, ears for otitis media, and to the brain for meningitis (2, 38). *Haemophilus influenzae* type B vaccine (Hib) was introduced in the 1980s, and has nearly eliminated *H. influenzae* type b. Severe *H. influenzae* infection has decreased by over 80% in countries using it (39). Non-typeable *H. influenzae* (NTHi) is now found more commonly in the nasopharynx.

Bacteria within these biological niches have the capability to interact synergistically to promote colonization (positive association), or to interact competitively (negative association). These interactions can deeply affect the ecological niche, and in doing so also affect incidence and severity of disease (2, 39). *S. pneumoniae* and *H. influenzae* have been shown to have a positive association. Individuals infected with one species are more likely to carry the other species as well (39). This complex relationship has become a source of study after the introduction of PCV, as studies have published conflicting reports on the rate of *H. influenzae* carriage and disease (Table 3).

Out of the studies assessed that looked at changes in *H. influenzae* carriage after the introduction of PCV in this review, five of them showed a statistically significant increase in *H. influenzae* after PCV immunization (Table 3). In the Netherlands study, *H. influenzae* carriage increased from 46% to 63% from 2005-2013 after PCV immunization (3). In Brazil, there was increase in NTHi of 62.1% from a prevalence of 26.0% pre-PCV to 43.6% post- PCV (17). In Italy, *H. influenzae* carriage rose and occurred preferentially with NVT serotypes 23, 15B, and 6C (19). No other studies determined associations between serotype and infection. Two of the studies assessed showed a decrease in *H. influenzae*, one of which was in Kilifi, Kenya (18). The study notes,

however, that *H. influenzae* carriage prevalence rebounded in year 2 of the vaccine period, so PCV as the causative agent of the decrease is suspect. *H. influenzae* carriage prevalence among children age 3-12 years in Mpumalanga, South Africa decreased significantly, but remained stable in children under 2 (20). In Fiji, there was no significant change. However, the Fiji study was a randomized control trial testing the effects of 23vPPV on children under two years of age. 23vPPV had no significant impact on this population, therefore it is likely that this is why there was no change in carriage (21).

Most cases of NTHi are found in children under 20 weeks of age and elderly over 65, where they can develop into bacteremia or pneumonia. Invasive cases of NTHi have increased 6-fold in the Netherlands from 1992 to 2013. Fatality rates for NTHi in these populations is 10-20% (40). This increase has coincided closely with PCV vaccination, leading to the possibility that this could be due to new interactions between NTHi and NVT *S. pneumoniae* (41).

Streptococcus pneumoniae **and** ***Staphylococcus aureus***

Studies have shown that interactions between *S. pneumoniae* and *S. aureus* are competitive in healthy children (26, 41, 42). In a study by Bogaert et al., there was negative correlation for co-colonization between VT *S. pneumoniae* and *S. aureus*, but not for NVT serotypes and *S. aureus* (24). This has increased fears that with the use of PCV, *S. aureus* carriage rates and *S. aureus*-mediated disease will rise in vaccinated populations.

In two studies conducted among children in the Netherlands, and one study from Fiji, *S. aureus* carriage rates rose statistically significantly after the introduction of PCV (3, 21, 22). In Fiji, the change was contributed more to ethnic differences rather than the vaccine, 23vPPC, which, while recommended solely for adults, was being tested on children for efficacy (21). A longitudinal study, also in the Netherlands, showed no overall significance during the first 2 years of life. However, at 12 months carriage was found to be doubled, before dropping again (24). In Kenya, South Africa, and Taiwan, there was no significant change, although Taiwan's results are possible skewed due to low rates of vaccination among the population (Table 3) (18, 20, 25). Therefore, the only increase in *S. aureus* carriage found was short-term, at 11-12 months, before stabilizing long-term (3, 22).

S. aureus have been more frequently isolated from children with acute otitis media after PCV-7 immunization (23). However, in studies from the US, Israel, China, and the Netherlands there was no change in carriage, and deaths due to *S. aureus* have not risen (43).

Table 3: Change in nasopharyngeal carriage of prominent bacteria after PCV implementation

Study	**Region**	*Haemophilus influenzae*	*Staphylococcus aureus*	*Moraxella catarrhalis*
Bosch et al. (3)	The Netherlands	Increase	No change	No change
Brandileone et al. (17)	São Paulo, Brazil	Increase	ND	ND
Hammitt et al. (18)	Kilifi, Kenya	Decrease	No change	ND
Camilli et al. (19)	Northern Italy	Increase	ND	ND
Nzenze et al. (20)	Mpumalanga, South Africa	Decrease	No change	ND
Boelsen et al. (21)	Fiji	No change	Increase	Increase
Spijkerman et al. (22)	The Netherlands	Increase	Increase	No change
Oikawa et al.(16)	Japan	Increase	ND	Increase

Notes: ND is Not Done; the study listed did not have results for the bacteria.

Streptococcus pneumoniae* and *Moraxella catarrhalis

Colonization between *S. pneumoniae* and *M. catarrhalis* are positively associated (2, 41). In Tanzania, the carriage rates and density of *S. pneumoniae* and *M. catarrhalis* (along with *H. influenzae* and *S. aureus)* were assessed comparing healthy children and children with acute respiratory illness (ARI) and pneumonia. Children with concurrent infection by *S. pneumoniae* and *M. catarrhalis* were must more likely to have respiratory infections or pneumonia. The study found that *M. catarrhalis* had the highest association with respiratory illness, with 90.8% of children with disease carrying it (44). In Fiji and Japan, there was an increase in *M. catarrhalis* carriage after pneumococcal immunization (16, 21). However, in all other studies conducted between the two there was no change in *M. catarrhalis* carriage rates after PCV introduction to the population (Table 3).

Discussion

While there has been a shift in *S. pneumoniae* serotypes, there has been no change in the overall carriage rate after the implementation of PCV immunization. Overall pneumococcal illness rates have fallen, but the risk of NVT-mediated disease needs to be continuously monitored. A majority of the *H. influenzae* studies point towards an increase in *H. influenzae* carriage, while others have noted a significant increase in NTHi illnesses in countries with the vaccine (3, 16, 17, 19, 22). *S. aureus* illness has not increased, and any detected raise in carriage rates eventually returned to pre-vaccine levels after some time (3, 18, 20-22, 39). This should still be monitored in the future, as these relationships are complex, and can change due to a multitude of factors. *M. catarrhalis* carriage rates have mostly remained unaffected (3, 16, 21, 22).

Vaccines are currently being developed to target NTHi in the wake of PCV. One such vaccine is the 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D-conjugate vaccine (PHiD-CV10), which is supposed to target both *S. pneumoniae* and NTHi. Early trials have shown a decrease in NTHi-mediated illness, but no change in carriage or density of NTHi within the nasopharynx (45-47).

Due to the specific nature of this review, which was focusing on the after effects of PCV, there were a limited number of appropriate studies. Many of the studies assessed for this review were cross-sectional or longitudinal, which could affect the strength of the reported results. Some studies did not use molecular methods to evaluate carriage rates when serotyping, which can lead to mistyping or misidentifying the appropriate bacteria. Molecular methods are more effective for such studies, as culturing is not as sensitive as.

The implementation of PCV has been a success, with IPD and mortality rates falling significantly. However, carriage rates have not changed, and the side effects of PCV on other bacteria species within the nasopharynx have demonstrated that the long-term effects of vaccines that target bacteria within complex biological niches needs to be monitored more carefully in the future. By targeting a specific group, we may create a domino effect leading to unintended consequences.

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