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EXPERT REVIEWS

The fundamental link between pneumococcal carriage and disease

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Streptococcus pneumoniae (pneumococcus) is a major cause of worldwide mortality and morbidity, and to a large extent is vaccine-preventable. Nasopharyngeal carriage of pneumococcus precedes disease and is the source of pneumococcal spread between people. The use of vaccine effect on carriage as part of the vaccine licensure and post-vaccine introduction evaluation could facilitate and expand the licensure of new, life-saving pneumococcal vaccines and enable a comprehensive estimate of population effects after vaccine introduction. The authors provide a review of the evidence supporting pneumococcal carriage at the individual level as an immediate and necessary precursor to pneumococcal disease. Based on such a causal link between carriage and disease, the authors emphasize the role of information on pneumococcal carriage in vaccine trials and in public health decision-making.

Keywords: herd immunity • invasive pneumococcal disease • pneumococcal carriage • pneumococcal colonization • pneumococcal vaccine licensure • pneumococcal vaccines • pneumococcus • vaccine effect on colonization

Streptococcus pneumoniae (pneumococcus) is a pathogen associated with high worldwide morbidity and mortality, particularly among children and in countries with the least access to diagnosis and treatment. The nasopharynx (NP) in humans is readily colonized by pneumococci, and hence acts as the reservoir and source of pneumococcal transmission between individuals. Occasionally, in an individual the colonizing pneumococci spread from the NP to the surrounding tissue or invade the bloodstream, causing disease that can range from a mild upper respiratory tract infection (acute otitis media, sinusitis) to a severe and potentially life-threatening condition (pneumonia, bacteremia, meningitis). Pneumococcal mortality and morbidity are, to a large extent, vaccine-preventable. Pneumococcal conjugate vaccines (PCVs) effectively prevent the most serious forms of pneumococcal disease caused by the serotypes included in the vaccine (VTs) and also reduce the risk of NP carriage by those serotypes. To date, 7-, 10- and 13-valent formulations of PCV have been licensed (PCV7, PCV10 and PCV13) and additional PCV products are expected to be licensed in the coming years [1]. Use of these highly efficacious pneumococcal vaccines is expected to lead to dramatic reductions in deaths among children throughout the world due to pneumonia, sepsis and meningitis caused by the VTs.

Strengthening the existing pneumococcal vaccine licensure pathway for PCVs and facilitating a licensure pathway for novel pneumococcal vaccine formulations not based on capsular polysaccharide antigens would accelerate the worldwide access to new, life-saving pneumococcal vaccines. Evaluating the efficacy of new PCV products against disease end points has become extremely difficult, if not largely unfeasible for some end points, because of ethical considerations as well as sample size requirements. The current evaluation of new PCV products for licensure is therefore predominantly based on demonstrating safety and non-inferiority of the immunogenicity of a new PCV with the currently licensed products. However, the need to rely on immunogenicity measures poses limitations. First, comparative immunogenicity studies alone, with licensed vaccines, may fail to demonstrate the value of a new candidate vaccine, since the currently approved immunological criteria are applicable for invasive pneumococcal disease (IPD), but not for any of the non-IPD end points, including colonization. Second, addressing the direct vaccine effects alone among the immunized population ignores the importance of the profound indirect effects (herd immunity and serotype replacement) on disease among the

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unimmunized population, which influence the overall impact of the vaccine use [Davis S, Deloria-Knoll M, O'Brien KL; PneumoCarr Consortium. Impact of pneumococcal conjugate vaccines on nasopharyngeal carriage and invasive disease among unvaccinated people: review of evidence on indirect effects (2012), Submitted]. Third, the current pathway does not allow for the evaluation of protein and other novel-mechanism vaccines, several of which are currently in development [2].

The time when a pneumococcal strain establishes itself within the host is referred to as acquisition, whereas ongoing NP colonization is defined as carriage. Measurement of the vaccine effect on acquisition, duration of carriage, colonization density and/or clearance could be used to support decision-making for both existing and new pneumococcal vaccines. More precisely, inclusion of the vaccine effect on NP carriage in the vaccine licensure process would facilitate and expand the licensure of new pneumococcal vaccines and enable the estimation of population effects after vaccine introduction. To achieve this, the link between pneumococcal carriage and pneumococcal disease needs to be adequately understood. In this article, the authors provide a review of the evidence supporting the fact that pneumococcal carriage at the individual level is the first essential step in all pneumococcal disease. Based on the causal link between the carriage and disease, the authors outline the role of pneumococcal carriage in pneumococcal vaccine trials as a marker of protection and a candidate surrogate for pneumococcal disease end points.

Colonization as the precursor to disease

This section reviews epidemiological evidence of the direct link between pneumococcal carriage and disease at the individual level. In most cases, data concern common and mild mucosal infections in children (such as acute otitis media [AOM]), whereas data about the direct link between pneumococcal carriage and pneumonia or IPD are more scarce, but do exist. Nevertheless, in the context of any pneumococcal disease manifestation, NP carriage emerges as a predisposing factor and a first step in the causal chain of pneumococcal pathogenesis. Elementary evidence for this comes from experimental challenge studies in animal models, which shows that the nasal inoculation of pneumococci leads to otitis media [3–5] or invasive disease [6,7]. The focus of this section, however, is in observational data from humans, in particular from young children aged less than 5 years.

There are only a few studies that have prospectively investigated the temporal relationship of pneumococcal carriage with a pneumococcal disease manifestation in the same study subjects. The predominant disease manifestation in these studies has been AOM, the most common infection following pneumococcal colonization. Gray *et al.* [8] investigated carriage and disease in 82 infants followed from birth up to 2 years of age. In this study they routinely sampled the NP of children monthly for the first 6 months of life and at 2–3-month intervals thereafter until 2 years of age and collected information about any disease episodes experienced by the children. By correlating acquisition episodes and carriage periods of pneumococcus with culture-confirmed pneumococcal disease, they concluded that pneumococcal infection, most of which (28/31) was AOM, is mainly associated with a newly acquired serotype. In particular, although prolonged duration of carriage was common, 74% of infections were caused by serotypes found less than a month before the illness. Carriage itself was not found to pose risk for disease and the authors hypothesized the role of prolonged carriage as one of the protective factors against pneumococcal disease.

In another study (FinOM cohort), 329 children were followed from 2 months until 2 years of age with ten scheduled NP samples and comprehensive information of respiratory infection and etiologically confirmed AOM episodes was recorded [9]. During the weeks preceding respiratory infection, children with pneumococcal AOM had a significantly lower frequency of carriage specific to the serotype/group causing the disease compared with children of the same age who also carried pneumococci during a respiratory infection, but did not have pneumococcal AOM, thus confirming the role of recent acquisition of the disease-causing serotype. Notably, in this study the serotype causing AOM was found in 99% of the simultaneously obtained NP samples from the children affected by AOM [10].

In a third study, NP samples from 213 infants were obtained biweekly from 2 weeks to 3 months of age and then monthly until 6 months of age. In this study, pneumococcal acquisition was significantly associated with office visits to general practitioners for nonspecific respiratory infections [11]. By contrast, there was no evidence for an association between the presence of carriage as such and physician medical visits. The three longitudinal studies summarized above emphasize carriage as a necessary predisposing condition for mucosal pneumococcal disease, the main predisposing event for disease being the pneumococcal acquisition event rather than the prolonged carrier state.

Several studies show that at the time of pneumococcal AOM or other pneumococcal disease syndromes there is a disproportionately high prevalence of pneumococcal carriage in the affected children [7,11–14]. Although such simultaneous detection of pneumococci in the NP and in a normally sterile site (middle ear, lung or bloodstream) documents only a temporal association between carriage and disease, it is congruent with the argument that pneumococcal carriage is a precondition for disease. The pneumococcal strain detected in the middle ear fluid during AOM is in the vast majority (>90%, and sometimes almost 100%) of cases and also found in the simultaneous NP sample from the same study subject [10,12,16]. Similar findings apply to IPD. In the Pakistani study 94% (101/108) of children with IPD simultaneously carried pneumococci, compared with 52% (69/133) in healthy controls [17]. Similarly, in The Gambia, the proportions were 90% (73/81) in children with IPD and 76% (86/113) in healthy controls [18]. In the Pakistani study, the proportion of the typed NP isolates that were concordant with the serotype causing the disease was 99% (69/70). In the Gambian study, a lower proportion (~60%) of concordant serotypes may be affected by the insensitivity in detecting colonization with multiple serotypes.

Direct observations of a temporal occurrence of pneumococcal acquisition and subsequent development of IPD are difficult to make, because pneumococcal serotypes and strains differ both in their duration of carriage [19] and their invasiveness (i.e., ability to

progress from carriage to IPD) [20,21]. Disease-causing serotypes that are carried for a longer time are more readily identified in concurrent NP colonization studies, whereas certain serotypes/serogroups are only rarely found in carriage (e.g., serotypes 1, 5, 7, 12), presumably due to very short episodes of carriage or because they are carried as a sub-dominant type mixed in with a more dominant serotype. Consequently, these so called 'invasive types' are invariably found more often as causes of IPD than predicted by colonization [18,22,23]. However, early studies from 1915 to 1937 show that serotype 1 can be isolated from NP of children with pneumonia or from close contacts of those with serotype 1 pneumococcal disease [24]. A more recent Gambian study [18] found higher prevalence of serotypes 1, 5 and 14 in the family members of children with IPD caused by these serotypes compared with the control families. The serotypes that are particularly rare in carriage (i.e., 1 and 5) often appear as causes of IPD in outbreaks. When carriage is studied during outbreaks, carriage of these invasive serotypes, especially in closed communities, is common [24,25]. Furthermore, adult pneumonia patients have more dense pneumococcal colonization than asymptomatic colonized controls, suggesting that the transition from asymptomatic carriage to disease may happen at a critical NP colonization density [26]. Although those with pneumococcal disease may more easily transmit pneumococci carried in their NP than asymptomatic carriers, carriage studies in close contacts of cases clearly support the understanding that exposure to and colonization with pneumococci is a predisposing event to disease. Analogous evidence has been gathered from outbreaks of two similar bacterial pathogens also having a carriage state, *Haemophilus influenzae* type b in families [27] and *Neisseria meningitidis* in garrisons [28,29].

Ecological associations between pneumococcal carriage & disease

In this section, the authors review associations between pneumococcal carriage and disease measured at the population level. Particularly, the authors compare patterns of carriage and disease by age strata and geography. A clear age-related association between the incidence of pneumococcal carriage and disease is detected, as is the geographical association between high carriage and disease rates.

Pneumococcal carriage & acute otitis media

The incidence of pneumococcal carriage is highest in young children, peaking during the first 2 years of life and starting to decrease gradually after the age of 3–5 years in most developed country populations [30–33]. However, in developing country settings, or in settings of high disease burden (e.g., American Indians), high carriage rates are observed to persist further into childhood [34,35]. Among children less than 3 years of age, the prevalence of colonization generally ranges from 20 to 50% [30,32], while in certain indigenous populations practically all children are colonized with pneumococci by the age of 3 months [36]. The peak incidence of bacterial AOM occurs between the ages of 6 and 18 months [37], the age with the highest rate of NP colonization of *S. pneumoniae* and other AOM pathogens. The four serogroups 6, 14, 19 and 23, sometimes referred to as 'pediatric serotypes', are among the most commonly carried ones in children and at the same time cause the most cases of pneumococcal AOM [9,18,38–41]. Thus, the authors have learned that by contrast with IPD (see below), there are only small differences in the ability of pneumococcal serotypes and clones to cause AOM, most serotypes causing AOM at a frequency that is proportional to their prevalence in NP carriage [42]. Some serotypes (i.e., serotypes 3, 5, 1, 12F, 19F, 19A) seem to have a higher AOM disease potential once carriage is established [43]. By geography, the serotypes causing AOM do not vary markedly: the most common pneumococcal serotypes globally causing AOM are 3, 6A, 6B, 9V, 14, 19A, 19F and 23F [44].

Carriage & IPD

A limited number of serotypes cause most IPD worldwide, the seven most common serotypes in the era prior to PCV introduction include 1, 5, 6A, 6B, 14, 19F and 23F [45]. In contrast to the clear-cut association between pneumococcal carriage and AOM, the direct link between carriage and IPD is more nuanced as the serotype distribution in IPD is not concordant with that found in NP colonization. The lack of NP and IPD concordance at the serotype level is potentially related to failure to detect minority clones in the NP. New technologies (e.g., microarray) providing greater attention to co-colonization will likely strengthen the case for carriage as a predictor of IPD seroepidemiology [46]. For instance, serotypes 1 and 5 are rarely detected in colonization, but are found disproportionately in disease (i.e., have high invasiveness) [43,47,48]. In general, the invasiveness of a given serotype summarizes the serotype's relative ability to cause invasive disease per a given episode of colonization. The invasiveness measure for a given serotype has been found to be relatively stable across different populations [21].

The incidence of IPD is greatest at the extremes of age, among the very young and the elderly. The highest incidence of IPD generally occurs among children aged 6–11 months [49], at the very same age when the incidence of pneumococcal acquisition is high. In the pre-PCV7 era, the commonly carried pediatric serogroups 6, 14, 19 and 23 are frequent causes of IPD in children [50–53]. Apart from the high incidence of pneumococcal carriage, the high risk of IPD among young children is also related to their immature immunological response, especially to the pneumococcal polysaccharide capsule [49]. Among adults and particularly in the elderly, the link between IPD incidence and carriage is more difficult to quantify, since the risk of IPD is more closely related to the underlying medical and/or socio-demographic conditions than to frequency of carriage. Also, with regard to the overall susceptibility to pneumococcal disease, elderly individuals are a more heterogeneous population compared with young children, who have a relatively homogenous immunological immaturity.

Among certain indigenous populations (e.g., Alaska Native, American Indian and Australian aboriginal populations) and in the developing countries, the prevalence of pneumococcal carriage is much higher and the first acquisition happens at a much younger age compared with the industrialized settings. At the same time, the frequency of IPD and pneumococcal pneumonia among these populations is high, underlining the ecological link between the frequencies of pneumococcal carriage and disease [54,55].

Pneumococcal carriage & pneumonia

Due to the lack of reliable diagnostic methods and the difficulty in obtaining adequate sample for culture from the infection site, identification of the etiologic agents of pneumonia has remained a challenge [56]. This, in turn, may hamper understanding the relationship between pneumococcal carriage and pneumonia. In spite of these diagnostic challenges, in children aged less than 5 years pneumococcus is recognized as the most important cause of bacterial pneumonia [57]. In a study by Greenberg *et al.* in the era prior to PCV introduction [21], serotypes 1, 5, 7F, 9V, 14, 19A and 22F were shown to have a higher potential to cause childhood pneumonia – that is, these serotypes had higher odds of being carried during radiologically diagnosed community-acquired alveolar pneumonia compared with healthy controls.

Biologic determinants of pneumococcal carriage & disease pathogenesis

Pneumococcal infection is a complex interplay between various pathogen- and host-specific factors. In this section, the authors describe the pneumococcal virulence factors playing a role in the pathogenesis of pneumococcal diseases. Many of these factors have dual functions in disease pathogenesis, as well as in pneumococcal carriage and transmission into new hosts. Since the vast reservoir of pneumococci in the population is found in the commensal state of NP carriage, the selective pressures driving its behavior must reflect the requirements of colonization (i.e., the attributes allowing for its virulence must also somehow promote its commensal lifestyle) [58]. The host defense mechanisms constraining pneumococcal multiplication in the human body are summarized in the end of this section.

Pathogen-specific factors

Capsule

The main pneumococcal virulence factor is the polysaccharide capsule attached to the bacterial surface. Based on the chemical structure of the capsule, pneumococci are divided into over 90 different serotypes [59,60]. Pneumococcal serotypes differ substantially in their duration of carriage and capacity to cause disease, but exactly how serotype confers these differences is not known. Along with the capsule, there are also several other virulence factors contributing to the ability of a particular strain to cause disease [60]. In early colonization, the negatively charged capsule is suggested to have a physiochemical effector mechanism by limiting mucus-mediated clearance of pneumococci [61]. Later on, to neutralize the inhibitory effect of negatively charged capsule on adhesive interaction with epithelial host cells, the bacteria are able to bind positively charged host molecules on their surface and thereby enhance pneumococcal adherence [62,63]. During disease pathogenesis, the capsule confers resistance to complement-mediated opsonophagocytosis (i.e., ingestion of bacteria by the host phagocytic cells) [64,65]. The capsule forms a physical barrier that limits access of antibodies and complement to the pneumococcal surface [66,67]. By reducing binding of IgG and C-reactive protein, the capsule further inhibits activation of the classical complement pathway.

Factors contributing to pneumococcal adherence & invasion

Upon establishment of pneumococcal colonization, multiple receptor-ligand interactions are required for the attachment of bacteria to the host respiratory epithelial cells [68–72]. Pneumococci express several surface-attached exoglycosidases that are able to modify the host molecules by deglycosylation and thereby expose novel receptors for pneumococcal adherence, inhibit mechanisms of clearance and provide carbon source for bacterial growth [73–75]. The first pneumococcal surface adhesin to be described was the choline binding protein A [76]. Besides adherence, several other virulence properties have been attributed to this protein [76–80]. Choline binding protein A belongs to a family of pneumococcal surface proteins non-covalently anchored to the cell wall structure phosphorylcholine (ChoP) [76–78]. ChoP is another pneumococcal surface molecule shown to function as an adhesin. ChoP is necessary for pneumococcal binding to the receptor for plateletactivating factor, which besides enhancing pneumococcal adherence also serves as a gateway for pneumococcal invasion [81,82]. Some pneumococcal isolates are able to express a pilus-like structure, which extends beyond the polysaccharide capsule and mediates adherence to the respiratory epithelium [83–85]. Once access to basement membranes is reached, an important feature of pneumococcal disease pathogenesis is the ability to adhere to the components of host extracellular matrix by several specific pneumococcal surface adhesins [86–88]. Further, greater access to the tissue is achieved via function of the surface-attached pneumococcal hyaluronidase that degrades hyaluronic acid, a major polysaccharide component of the host connective tissues [89].

Phase variation

The efficiency of adherence and virulence of a given pneumococcal strain is greatly affected by a spontaneous, high-frequency phenotypic variation (also known as phase variation), which results in different display of various bacterial features in different conditions [90]. The varying expression of certain cell surface properties correlates with the ability of the pneumococci on one hand to asymptomatically colonize the NP mucosa and on the other hand to cause an overt disease in other compartments of the body. Phase variation can be recognized by colony morphology changing between two phenotypes: opaque and transparent [90]. The capabilities of these two phenotypes for adhesion and invasion are different. The transparent colony variants with a thinner capsule layer and other characteristics promoting the binding to host tissues have a selective advantage for adherence and establishing NP colonization in the infant rat model [90,91], whereas the opaque variants with thicker capsule and resistance to opsonophagocytic killing survive better in the bloodstream [92].

Biofilm formation

There is evidence for pneumococcal biofilm formation occurring naturally during nasopharyngeal colonization and AOM [93,94]. The clinical significance of this biological mechanism is largely unknown, but it has been suggested that pneumococcal biofilms may confer a quiescent mode of growth during colonization rather than directly contributing to the development of IPD [95].

Interaction with the resident microflora

An additional factor contributing to the success of pneumococcal colonization is the composition of the resident co-colonizing microflora in the host upper respiratory tract. Pneumococci have to compete for nutrients and attachment sites with these co-colonizing bacteria and depending on the relative degrees of interspecies competition or symbiosis, interaction with other bacteria may either facilitate or impede pneumococcal colonization. Besides interspecies competition, pneumococci also have intraspecies competition mediated by bacteriocins (pneumocins) targeting other members of the same species that do not express a certain cognate immunity factor [96]. In addition to competition, pneumococci may also collaborate with other members of resident nasopharyngeal microflora, as suggested by a recent finding of interaction between pneumococci and nontypeable *H. influenzae* in chronic rhinosinusitis [97]. On the other hand, an inverse relationship between nasopharyngeal colonization with VT pneumococci and *Staphylococcus aureus* has been indicated, suggesting natural competition [98,99].

Host-specific factors

As a result of concerted action by host innate and acquired immune mechanisms, pneumococcal colonization does not proceed into a syndrome of a disease in the majority of individuals. The intact mucosal surface of the upper respiratory tract provides an initial, nonspecific barrier against pneumococci and other pathogens invading the body. It is not only the integrity of epithelium, but also the simultaneous action of mucus, ciliated cells and antibacterial peptides that efficiently prevent the bacteria from spreading into the surrounding tissue, lower respiratory tract and blood. If invasion, however, does occur, additional nonspecific molecules and cells are recruited to eliminate the bacteria from a non-immune host [67,100,101].

The acquired immune system is specifically directed against a certain microbial target. The two arms of the acquired immune system, antibody- and cell-mediated, act in concert to defend the host against pneumococcal infection. The antibody-dependent immunity is essential in defense against IPD, whereas its role in the development of resistance to pneumococcal colonization has remained obscure. Natural serum derived anti-capsular antibodies may mediate protection at the mucosal surfaces and can certainly be detected in the circulation following NP carriage of pneumococci and are associated with protection against carriage [102]. However, local B-cell-derived antibodies, perhaps of the IgA class, may be more critical molecules associated with mediating protection against acquisition or invasion than IgG, but data available to support this hypothesis are at the moment scarce. In animal models, the acquired immunity to pneumococcal carriage has been proposed to derive from the development of pneumococci-specific $CD4^+$ T cells that reduce the duration of carriage and may also impact mucosal disease [103], as has been suggested by the colonization studies [104,105] and intranasal immunization studies [106,107].

Direct effect of pneumococcal vaccines on carriage & disease

In this section, the authors review the evidence for the direct effects of pneumococcal vaccination. The indirect effects of PCV vaccination will be described in a separate review by Davis *et al.* [Davis S, Deloria-Knoll M, O'Brien KL; PneumoCarr Consortium. Impact of pneumococcal conjugate vaccines on nasopharyngeal carriage and invasive disease among unvaccinated people: review of evidence on indirect effects (2012), Submitted]. The fact that vaccination protects the individual concurrently against carriage and disease is essential in drafting the role of vaccine effect on colonization (VE-col) in the evaluation and licensure of new pneumococcal vaccines. Specifically, this observation supports considering carriage as a marker of protection against disease (see section 'Carriage as a marker of direct protection'). Also, understanding the magnitude of the direct vaccine effect on carriage and its relation to the concurrent vaccine effect on disease helps to formulate the role of VE-col in the public health impact evaluation of vaccination and include this as part of the vaccine licensure process.

Pneumococcal polysaccharide vaccines are generally understood to have little or no effect on pneumococcal carriage. However, the pneumococcal polysaccharide vaccine trials among mine workers in 1970s did demonstrate a reduction in pneumococcal carriage [108]. Specifically, vaccination had a significant effect on pneumonia and, at the same time, pneumococcal colonization decreased by up to 70%. It has thereby been concluded that carriage and pneumonia were prevented concurrently and that the effect on pneumonia was mediated via protection from carriage [108]. However, other studies with pneumococcal polysaccharide vaccine have not been able to show such an effect [109–111].

Trials among children and infants have convincingly shown that vaccination with PCVs confers concurrent direct and indirect effects on both pneumococcal carriage and disease. The direct effect of vaccination is understood as a vaccine-induced change in the risk of carriage or disease in the vaccinated individual. In general, direct effects of pneumococcal vaccines include reduction of VT carriage and disease as well as the secondary, simultaneous increase in occurrence of non-vaccine serotypes (NVTs). The increase in NVT carriage has been reported to occur in vaccinated individuals even during a short follow-up period after vaccination with PCV (**Table 1**). The indirect (herd) effect is understood as the protection against the VTs in the unvaccinated population due to reduced circulation of pneumococcal VT strains and it usually appears after the vaccine has been in widespread use in the community for some time. In settings with closed contacts, herd protection may emerge promptly [112]. Because the vaccinated individual is also affected by the herd effect, the actual direct vaccine effect can be shown only during pre-licensure efficacy trials with short follow-up periods or fairly soon after implementation of pneumococcal vaccine in a routine vaccination program. After the PCV has been used widely and it is in the national immunization program, the authors can measure only the combined direct and indirect effects (i.e., the total effect).

Besides a profound direct vaccine-induced reduction in the risk of VT disease, many studies show concurrent marked reduction in the risk of VT carriage within the same population. Specifically, evidence of a direct link between carriage and disease has accumulated from several PCV efficacy trials with relatively short follow-up periods, so that any confounding indirect effects can be

Table 1. Pneumococcal conjugate vaccine trials with data on the concurrent direct vaccine effect on colonization and disease.

Trial site/ population/ vaccine (carriers)†	Vaccine schedule	Disease end points and vaccine efficacy	Impact of vaccination on carriage†	Notes	Ref.
South Africa (HIV infected and uninfected) PCV9 (CRM)	6–10–14 weeks	HIV-uninfected: IPD: 83 (39–97)%; Pneumonia (radiol): 20 (2–35)%	At 9 months: VT: vaccinated 18%, unvaccinated 36% ($p < 0.001$) NVT: 36 versus 25% ($p = 0.007$)	The carriage study was done prior to the efficacy trial and was among mainly HIV-uninfected children	[113,114]
The Gambia PCV9 (CRM)	3 doses starting at 6–51 weeks	VT IPD: 77 (51–90)%; Pneumonia: 37 (27–45)%	RR at 9–15 months: VT: 0.56 (0.49–0.65); NVT: 1.59 (1.41–1.79)	Large sample size in the carriage study; serotype-specific data available	[115,116]
USA/American Indians PCV7 (CRM)	3 doses starting at 6 weeks to 7 months, a booster at 12–15 months catch-up cluster randomized	VT IPD: 76.8 (9.4–95.1)%; VT AOM: 64 (-34–90)%	OR at 7 months: VT: 0.40 (0.23–0,67), NVT: 0.72 (0.43–1.23) OR at 12 months: VT: 0.51 (0.34–0.78), NVT: 1.02 (0.61–1.72) OR at 18 months: VT: 0.81 (0.51–1.31), NVT: 1.67 (1.02–2.78)		[117–119]
Czech Republic and Slovakia PCV11 (Hi protein D)	2–4–6–12 months	VT AOM: 57.6 (41.4–69.3)% NVT AOM: 8.5 (-64.2–49.0)%	After booster: VT: 42.8 (16.7–71.9)%; NVT: 15.3 (-78.9–59.7)%	Efficacy against carriage detected later than efficacy against AOM	[126,127]
Finland two PCV7s (CRM and OMPC)	2–4–6–12 months	PncOMP: VT AOM: 56 (44–68)%; NVT AOM: -27 (-70–6)% PncCRM: VT AOM: 57 (44–67)%; NVT AOM: -33 (-80–1)%	PncCRM: at 12 months 17% (VT, NS) At 18 months: VT: $p < 0.001$, NVT increase: $p = 0.02$	No published data on carriage available. Efficacy against carriage detected later than efficacy against AOM	[124,125]
The Philippines PCV11 (D/T)	6–10–14 weeks	Radiol. Pneumonia: 22.9 (-1.1–41.2)%	At 24 months: VT: 35 (8–54)%; NVT: no effect	No published data on carriage available	[120,121]
Argentina, Panama, Colombia/PCV10 (Hi PD/D/T) 23738/2000	2, 4, 6 and 15–18 months	Likely bacterial community-acquired pneumonia: 18 (6–29)% WHO pneumonia: 23 (9–36)%	At 7 months: VT: 18 (-7–37)%; NVT: -15.2 (-55–14) At 12 months: VT: 28 (5–48)%; NVT: 3 (-32–29) 3 months post-booster: VT: 28 (2–57)%; NVT: -28 (-78–7) 9 months post-booster: VT: 29 (-0.2–49)%; NVT: -22 (-74–15) Any visit: VT: 26 (13–37)%; NVT: -6 (-25–10)	Carriage trial in Panama only; no published data available	[122,123]
Israel/day care attendees PCV9 (CRM)	Toddlers 2 doses for those <1 year, 1 dose for older	RR: URI 0.85 (0.75–0.97) LR: 0.84 (0.72–0.98) AOM: 0.83 (0.67–1.02) Use of antibiotics: 0.85 (0.75–0.97)	OR 1 month post-PCV9: VT: 0.50 (0.3–0.66); NVT: 1.59 (1.28–1.96)	Disease end points are not pneumococcus specific serotype-specific carriage data for some VT serotypes	[128,129]

†The trial-specific data are given in several ways: either % protection, RR or OR, with 95% CI.
AOM: Acute oitis media; CRM: Nontoxic mutant of diphtheria toxin; D: Diphtheria toxoid; Hi protein D: Protein D of *H. influenzae*; IPD: Invasive pneumococcal disease; NVT: Nonvaccine serotype; OMPC: Outer membrane protein complex of *N. meningitidis*; OR: Odds ratio; PCV: Pneumococcal conjugate vaccine; RR: Risk ratio; T: Tetanus toxoid; VT: Vaccine serotype.

presumed to be minimal. In addition, there are studies reporting the impact of PCV on carriage and disease after wide adoption of the vaccine. However, those data represent either the total (direct and indirect) effects in vaccine recipients or even the overall effects detected in the entire population of vaccinated and unvaccinated individuals, and therefore are excluded from this review.

Most placebo-controlled PCV trials have shown that vaccination has concurrent effects on carriage and disease (Table 1). In a South African study [113,114], the carriage and disease trials were not nested and the carriage trial was completed prior to the vaccine efficacy trial with disease end points. In this study, the vaccine efficacy in HIV-uninfected populations on IPD was 83% (95% CI: 39–97) and the data from mainly HIV-uninfected children showed a reduced VT carriage at 9 months of age but not earlier. At the same time, NVT carriage increased significantly [114].

The PCV efficacy trial in The Gambia [115] with a very high colonization rate showed 77% (95% CI: 51–90) efficacy against IPD and 37% (95% CI: 27–45) efficacy against radiologically confirmed pneumonia. The efficacy against IPD was significant for the individual serotypes 14-, 5- and 23F. The nested carriage study had a large sample size (~1000 children per group) and showed effects on carriage of several individual serotypes [116]. As above, the protective efficacy against VT carriage was significant (RR: 0.56; 95% CI: 0.49–0.65), while the NVT carriage increased significantly (RR: 1.59; 95% CI: 1.41–1.79).

The cluster-randomized trial among American Indians [117] showed 77% (-9–95) efficacy against IPD. The study also showed 64% (-34–90) efficacy against spontaneously draining VT-pneumococcal AOM [118]. The nested carriage study [119] showed a decrease in VT carriage after the primary series at 7 and 12 months (odds ratio [OR]: 0.40 and 0.51, respectively), but after the booster the difference between the PCV and control groups was not significant (Table 1), possibly due to a small sample size. An increase in NVT carriage was observed after the fourth PCV dose (OR: 1.67; 1.02–2.78), but not earlier. It has to be noted, however, that this cluster-randomized study was not designed to measure direct effects but rather the total effect [119].

A study conducted among Filipino infants showed 23% (-1–41) efficacy against radiologically confirmed pneumonia [120], whereas the nested carriage study showed a decrease in VT carriage not earlier than at 24 months [121]. No increase in NVT carriage was detected. The COMPAS study [122], with likely bacterial community-acquired pneumonia as the primary end point, showed 18% (6–29) efficacy against bacterial community-acquired pneumonia and 23% (9–36) against WHO-defined pneumonia. The efficacy against VT carriage [123] was lower than in most of the other trials, showing an efficacy of around 25–30% after the booster dose (Table 1).

Two trials with AOM as the primary end point also reports the effect on carriage in nested studies. In the Finnish FinOM vaccine trial using two PCV7s in parallel, the efficacy on VT AOM and the increase in NVT AOM was seen already after the primary series [124], whereas the effect on colonization was detected only at 18 months – that is, 6 months after the booster dose [125]. The POET study conducted in the Czech Republic and Slovakia [126] showed an efficacy of 58% (41– 69) against VT AOM, with no increase in NVT AOM during the follow-up period. Similarly to the FinOM vaccine trial, the impact on carriage was detected only after the booster dose [126], whereas the effect on AOM was seen already after the primary series. The increase in NVT carriage was detected only at the age of 24–27 months, which is concordant with the NVT AOM data showing no replacement during the 18-month follow-up.

The Israeli efficacy study of a 9-valent PCV among toddlers attending day care centers showed an effect on carriage acquisition of some VT pneumococci [128] concomitant with an effect on respiratory infections and antibiotic use [129]. Although this study did not show any pneumococcal species or type-specific effects on disease, it clearly speaks for a concurrent direct vaccine effect on both colonization and disease.

The magnitude of direct PCV effect on VT-pneumococcal colonization seems to be, with few exceptions (Table 1), quite constantly around 50% despite various potential confounding factors, such as infection pressure, sampling time, vaccine product and different schedules of vaccination. One possible reason for not finding an effect on carriage after the primary series in some settings may be a low carriage rate with low rate of acquisitions before the booster, since some although not all carriage studies conducted in countries with higher acquisition rates in the infants show an effect already after the primary series (Table 1). It is also possible that vaccination affects the density of carriage in addition to the rate of carriage. This was shown to be the case in the American Indian trial in which those receiving PCV7 and nevertheless were colonized with VT strains had lower density of colonization than those who received the control vaccine and were colonized with VT pneumococci [119]. The same was found in the FinOM vaccine trial [130]. While in most trials the risk of NVT colonization increased among PCV- vaccinated subjects, no significant increase was found in NVT IPD. The changes in the NVT IPD are difficult to show during the short follow-up period, mainly due to the small sample size, whereas changes in the NVT AOM can already be seen rather soon after vaccination (Table 1).

As a whole, the above data on direct concurrent vaccine effects on pneumococcal colonization and disease clearly show that there exists a link between colonization and disease. The vaccine-induced reduced risk of pneumococcal carriage in an individual is reflected in a subsequent reduced risk of pneumococcal disease. For estimation of the overall effectiveness of a vaccination program, it is of importance to know both the direct and indirect vaccine effects. The data on pneumococcal NP acquisition after vaccination can be generated from Phase II and III trials with relatively short follow-up periods, and it can inform the effects of vaccination on replacement and on transmission to unvaccinated population [131]. The same is also true for future pneumococcal protein vaccines, if they affect colonization.

Carriage as a marker of direct protection

Based on the causal link between pneumococcal carriage and disease, this section characterizes pneumococcal carriage as a marker of the direct protection against pneumococcal disease in the individual. In particular, the authors view carriage as a

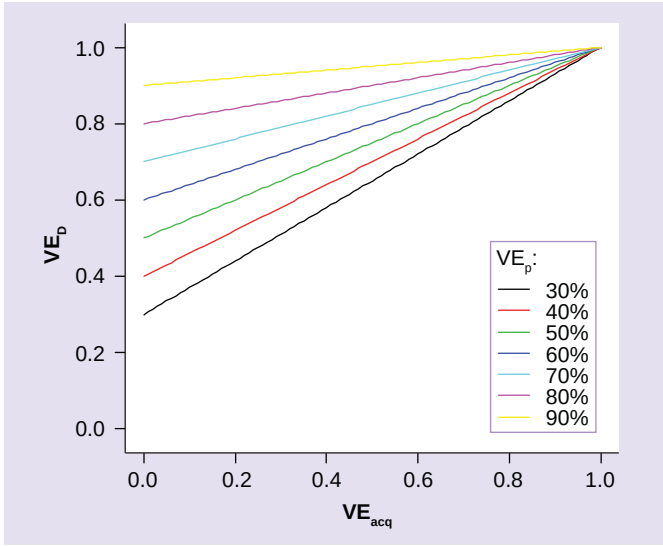

Figure 1. Dependence of vaccine efficacy against disease on vaccine efficacy against acquisition. The dependence is plotted with different levels of vaccine efficacy against carriage progression to disease (VE_p = 90, 80, 70, 60, 50, 40, 30 and 0% from top to bottom). For smaller values of VE_p, the lines are steeper, indicating a larger predictive value of carriage as marker of the direct protection against disease in the individual (see text). VE_{acq}: Vaccine efficacy against acquisition; VE_D: Vaccine efficacy against disease; VE_p: Vaccine efficacy against carriage progression to disease.

candidate surrogate for protection through assessing its value in predicting the risk of disease at the individual level. This is based on the observation that vaccination may afford protection separately against the two steps in the causal pathway from exposure to pneumococci to disease; namely pneumococcal carriage (including its acquisition, duration and density) and subsequently progression of pneumococcal carriage to a disease. The authors argue that carriage can be viewed as a marker of protection for most pneumococcal disease outcomes.

The direct protective effect of a vaccine on the clinical outcome is generally quantified as vaccine efficacy against disease (VE_D). This causal effect of vaccination is the relative reduction in the incidence of disease: $VE_D = 1 - RR$, where RR the ratio of the incidence rates of the disease manifestation in the individual if vaccinated and if the (same) individual was not vaccinated. VE_D is the primary parameter of interest in Phase III trials with disease end points. For PCVs, VE_D has been found to be very high (>85%) against VT-specific IPD but considerably lower for VT otitis media (Table 1), and not known but likely considerably less than 100% for VT pneumonia.

Corresponding to the two steps in the pathway from exposure to disease, the incidence rate of the disease outcome is a product of the acquisition rate (λ) and the case-to-carrier ratio (π). This means that the vaccine efficacy against disease can be expressed as follows (V for vaccinated, C for control):

$$VE_D = 1 - \frac{\pi_V \lambda_V}{\pi_C \lambda_C} = 1 - (1 - VE_p)(1 - VE_{acq}) \qquad (1)$$

This shows how VE_D depends on vaccine efficacies against VT acquisition of ($VE_{acq} = 1 - \lambda_V/\lambda_C$) and on progression of carriage to disease ($VE_p = 1 - \pi_V/\pi_C$). Specifically, at the first step, VE_{acq} quantifies the causal effect of being vaccinated on the rate of VT acquisition: the overall VE_{acq} against all VT colonization is approximately 50% (Table 1), with some variability depending on the epidemiological setting [132]. At the second step, even if a vaccinated individual becomes a carrier of any of the serotypes included in the vaccine formulation, the vaccine may protect against carriage progressing to disease. This protective effect of the vaccine is quantified by VE_p (the relative reduction in the conditional risk π of disease, given acquisition of carriage).

Figure 1 shows VE_D as function of VE_{acq} for different levels of vaccine efficacy against progression (VE_p). VE_D is always at least as large as vaccine efficacy against acquisition (VE_{acq}). The value of carriage as a marker of protection is the stronger, the smaller the difference between the two vaccine efficacies is (i.e., the smaller VE_p; cf.; Figure 1). For example, if VE_p is only 30% (the black line), more than half of protection against disease is due to reduced acquisition whenever VE_{acq} exceeds 43%. This may well be the case with pneumococcal pneumonia. Specifically, VE_{acq} of 50% and VE_p of 30% produce VE_D (efficacy against disease) as 65%. In such a case, protection against disease (pneumonia) in the individual would be mainly mediated by reduction in VT acquisition (cf. Klugman *et al.* [108]).

According to expression (1), VE_D is the percent reduction in the incidence of disease for a vaccinated subpopulation with a given rate of carriage acquisition, compared with if the population was not vaccinated. This interpretation corresponds to a general framework for identifying correlates of protection [133]. Specifically, the change in VE_D as a function of VE_{acq} directly quantifies the level of causal protection that is expected, given the vaccine-induced reduction in acquisition of carriage. In other words, the relationship between VE_D and VE_{acq} describes the predictive capacity of carriage as a candidate surrogate to project the disease incidence in a vaccinated subpopulation compared with if it was not vaccinated. The relevant surrogate measurement is the rate of acquisition or, equivalently, the expected number of at-risk events (acquisition) for disease.

In summary, the authors argue that the rate of carriage acquisition serves as a predictor of vaccine-induced protection against disease in the individual. Its predictive value is best for disease manifestations with less than 100% efficacy (VE_D) against the disease end point. In practice, the reduction in the risk of disease can be predicted in terms of the reduction in rate of acquisition (i.e., through VE_{acq}). This prediction can be calculated specifically for most of the serotypes included in the vaccine, since it is possible to estimate VE_{acq} for much larger number of serotypes than VE_D for IPD. Finally, if the vaccine's principal mechanism of protection is not directed against acquisition, as may occur with some of the non-polysaccharide based pneumococcal vaccine antigens, vaccine efficacy against colonization (the first step in the causal chain) can also be taken to incorporate the effects on duration and density.

Expert commentary

As has been shown above, there is considerable epidemiological and ecological evidence for pneumococcal acquisition being a precursor for pneumococcal disease, and pneumococcal carriage, mainly in young children, being the main source of pneumococcal transmission between persons. The concurrent direct and indirect effects of PCVs on pneumococcal colonization and disease further strengthen the argument for such a link between pneumococcal carriage and disease. As a result of these associations, pneumococcal carriage can be used as a marker of vaccine-induced protection especially in children, but also in adults (including the elderly) when the carriage rate in this age group is high.

The current PCV licensure process, anchored on immunological criteria, has proved to be an effective pathway for licensing new PCV products, but it can be improved. It does not include vaccine effect on carriage, does not address the overall impact of vaccination, does not apply to non-invasive disease manifestations and is not applicable to novel-mechanism vaccines. Assessment of VE-col would provide a straightforward means to project the expected minimum level of direct protection against all VT disease manifestations in vaccinated individuals. Carriage information would also make it easier to evaluate and predict the indirect effects of vaccination and thus take these larger population-level effects into consideration for licensure. In comparison to the current pathway, the use of carriage data in the vaccine licensure pathway would lead to a more informed and accurate decision-making regarding the likely effects of a new pneumococcal vaccine at the individual and population level.

To meaningfully include VE-col as part of the vaccine assessment licensure approach, standardization of both the measurement (including sampling and culturing of NP samples) and estimation procedures of VE-col is important. This would allow for results that can be compared across different vaccine products and epidemiological settings (e.g., differences in the case-carrier ratios; i.e., invasiveness of pneumococcal strains) so that any observed differences in VE-col can be clearly understood to derive from product, setting or other inherent biological difference rather than confounded by differences in the study design.

Five-year view

With over a decade of PCV7 experience in infant routine use, the vaccine has resulted in near elimination of VT disease and colonization among people of all ages. Nevertheless, with full replacement of NVT colonization, and a smaller degree of replacement disease, PCV products can be improved upon. The licensure of PCV products with extended valency, specifically the 10- and 13-valent products, and their rapid uptake into routine immunization schedules in both developed and developing world countries offers the promise of significantly accelerating disease prevention further.

Information from the PCV routine-use settings on both disease and colonization effects will allow a full understanding of serotype-specific performance, variability in serotype distribution, prediction of serotype emergence and knowledge of perturbations to strain invasiveness characteristics (i.e., case-to-carrier ratios). Next on the horizon are new PCV products with additional or different serotypes, and various carrier proteins that are in clinical trials and moving toward licensure. Similarly non-polysaccharide vaccines (whole-cell and pneumococcal protein vaccine) are entering or are in clinical trials. Further, new information will also gather on mucosal administration of the novel vaccines. For all of these products, the indirect impact of the vaccine is a key element to the total impact of the product and will be an essential component of policy decision-making. In middle-income and developing countries that have not introduced pneumococcal vaccines, the determination of VE-col (including acquisition, density and duration) will likely become an important part of evaluating new pneumococcal vaccines, both PCV products and protein/whole-cell vaccines. Furthermore, determination of VE-col could be used in surveillance of pneumococcal epidemiology after introduction of pneumococcal vaccines. Particularly for pneumococcal vaccines that are species-specific rather than serotype-specific, the role of colonization studies to delineate the pneumococcal colonization impact also includes microbiota studies to detail any cross-species interactions that are occurring.

Pneumococcal vaccines are not the only ones that will benefit from the incorporation of colonization as part of the evaluation and licensure pathway. Determining VE-col will aid in the evaluation of vaccines against other diseases that develop via mucosal colonization, like those caused by *Neisseria meningitidis* and group B streptococci. New epidemiological data on vaccine impact and better understanding of pneumococcal immunology, disease and colonization pathogenesis will help to develop improved models for prediction of overall vaccine effects of various vaccines. Even with more sophisticated colonization assessments of vaccine impact, there will be unanswered questions especially related to serotypes that are infrequently carried, populations in whom carriage is less frequent (e.g., adult populations) and for these situations VE-col may still be insufficient. The way forward will need to include new immunologic markers of vaccine effect that go beyond the production of antibody.

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Key issues

- In summary, pneumococcal disease episodes are preceded by acquisition of a pneumococcal strain.
- Pneumococcal conjugate vaccines are known to reduce carriage acquisition and density, resulting in a reduced risk of pneumococcal disease. Accordingly, pneumococcal conjugate vaccines have been shown to produce a concurrent direct and indirect effect on both pneumococcal carriage and disease.
- Many pneumococcal virulence factors (e.g., capsule and pneumococcal surface molecules such as choline binding protein A and phosphorylcholine) have dual functions both in colonization and virulence, and thus vaccines directed against these targets are expected to have an impact on both colonization and disease.
- At the population level, vaccine-induced protection against carriage translates into prominent indirect (herd) effects. Based on the present knowledge, it is important that pneumococcal vaccines reduce pneumococcal colonization acquisition, density or duration, and thereby reduce transmission and, through that mechanism, induce the herd effect, protecting all individuals.
- The effects of vaccine against a disease end point can be broken into two components: vaccine efficacy against acquisition of carriage, and vaccine efficacy against carriage progressing to disease. These determine the vaccine's efficacy against disease. The relationship between vaccine efficacy against disease and vaccine efficacy against acquisition of carriage directly describes the predictive capacity of carriage as a candidate surrogate to project the disease incidence in a vaccinated subpopulation.
- Standardization of the study designs (e.g., ways of sampling, sampling time points, microbiological methodology, analyses of the data) would allow for results that are comparable across different study settings and different vaccine products.
- Future colonization studies to delineate pneumococcal colonization impact should also include microbiota studies to analyze any cross-species interactions.

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