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Staphylococcus aureus and *Streptococcus pneumoniae* interaction and response to pneumococcal vaccination: Myth or reality?

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S. aureus and *S. pneumoniae* are both common pathogens that are also carried by a large proportion of healthy individuals in the nasal and nasopharyngeal spaces. A negative association between carriage of *S. aureus* and *S. pneumoniae* has been reported in children in various epidemiologic studies from different geographical regions. Most studies found that the negative association between *S. pneumoniae* and *S. aureus* was significant only for carriage of vaccine-type *S. pneumoniae* strains. In this review, we summarize the various suggested mechanisms of this suggested bacterial interference, and the clinical implications reported following PCV introduction to date in various geographical regions.

Introduction

S. aureus and *S. pneumoniae* are both common pathogens that are also carried by a large proportion of healthy individuals in the nasal and nasopharyngeal spaces. *S. aureus* is a major source of morbidity and mortality worldwide, with infections ranging from minor skin infections to invasive infections such as endocarditis and toxic shock syndrome. It is persistently carried by approximately 25% of the healthy population.[1] As with *S. pneumoniae*, carriage of *S. aureus* serves as the first step to infection as well as the frequent source of transmission between one individual to another.

Over a decade ago, two studies independently reported a negative association between carriage of *S. aureus* and *S. pneumoniae*.[2,3] During the following years multiple epidemiological studies in different geographical regions observed similar findings of a negative association between carriage of *S. pneumoniae* and *S. aureus* in young children.[4-8] The carriage of both species was associated with age, with the peak *S. pneumoniae* carriage and lowest *S. aureus* carriage at 6 months to 3 years[4-10] and peak *S. aureus* colonization at age <6 months and 5–7 y[11] The negative association was significant even after adjusting for age, but this interference was not observed in older children and adults.[8,12] Interestingly, most studies[2,3,5,9] found that the inverse correlation between *S. pneumoniae* and *S. aureus* was significant only for carriage of vaccine-type *S. pneumoniae* strains, which were carried more commonly before the introduction of the pneumococcal vaccine.

This finding, together with an earlier clinical trial that reported increased *S. aureus* otitis media following PCV vaccination,[13] raised much concern[3,7,14,15]; if *S. pneumoniae* carriage protects from *S. aureus* carriage and the introduction of the pneumococcal conjugate vaccines (PCV) results in decreased *S. pneumoniae* carriage, this could potentially lead to an increase in *S. aureus* carriage, and infection. In this review, we summarize the various suggested mechanisms of this inverse correlation, and the clinical implications reported following PCV introduction to date in various geographical regions.

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Suggested Mechanisms of Interaction between *S. pneumoniae* and *S. aureus*

As with any bacterial interaction, the association between *S. pneumoniae* and *S. aureus* can theoretically be caused by either direct or indirect interactions. Direct interactions, such as direct competition for adhesion sites, resources and receptor-mediated interactions are unlikely in this case due to the fact that the 2 bacteria reside in closely located, yet different niches. Interactions through secreted factors are more likely, as well as indirect interactions mediated through other bacteria, or through the immune system.

Suppression of *S. aureus* by H_20_2 production by *S. pneumoniae*

Hydrogen peroxide produced by *S. pneumoniae* was first postulated to have a role in the inhibition of *S. aureus* and other respiratory pathogens by Mcleod and Gordon in 1922.[16] Nearly a century later Pericone et al. observed that H_20_2 in *S. pneumoniae* culture supernatant was bactericidal against *H. influenza* and *N. meningitidis*, and to a lesser extent against *M. catarrhalis*.[17] Regev-Yochay et al found that the in vitro bactericidal activity of *S. pneumoniae* toward *S. aureus* is indeed mediated through hydrogen peroxide; The bactericidal effect was reversible with

catalase and *S. pneumoniae spxB* mutants that do not produce H_20_2 were not bactericidal.[18] Following this observation, Selva et al. suggested that the mechanism of interference is activation of *S. aureus* resident prophages by low levels of hydrogen peroxide produced by *S. pneumoniae*,[19] which then lyse *S. aureus* cells.

In vivo murine studies that assessed this issue are conflicting. In line with the theory of H_20_2 interference, Park et al demonstrated that *S. aureus* catalase expression contributes to its ability to colonize and survive in the presence of *S. pneumoniae* in an in vivo mouse model of nasal co-colonization.[20] However, in a neonatal rat model, Margolis observed that *S. aureus* density did not differ whether co-colonized with hydrogen peroxide producing or non-producing *S. pneumoniae*, or whether catalase or non-catalase producing *S. aureus* strains were tested.[21]

To assess the role of hydrogen peroxide in the patterns of human co-colonization, Regev-Yochay et al. assessed the variation of bactericidal activity in *S. pneumoniae* strains isolated from children co-colonized with *S. pneumoniae* and *S. aureus* compared to those colonized only with *S. pneumoniae*. They showed only a trend toward a negative correlation between co-colonizing *S. pneumoniae* strains and bactericidal activity and concluded that the variation in hydrogen-peroxide production alone does not fully explain the pattern of co-colonization.[22]

Genetic bacterial determinants of interference

Melles et al. assessed the possibility of a genotype-specific association between *S. aureus* and *S. pneumoniae* carriage and did not find such a correlation. They suggested that only more subtle genetic variations may possibly play a role in the interference between the two.[23] In line with this, a study by Nouwen et al. showed that *S. aureus* carriage of a strain is not dependent on bacterial genotype, suggesting that it is instead related to host factors.[24] Margolis et al. determined that the colonizing strain of *S. aureus* in a neonatal rat model is determined solely by which strain is first to colonize and not by the characteristics of that strain.[25]

Interactions with other residents of the upper respiratory tract microbiome

S. aureus and *S. pneumoniae* are not the only species present in the nasopharyngeal region. Over the years, many studies have observed interactions between various bacterial species and viruses carried in the upper respiratory tract.[26-30] These bacteria and viruses compete for space and resources[25,30,31] and in some cases, such as influenza virus and *S. pneumoniae*, the virus and bacteria act synergistically to cause increased *S. pneumoniae* adhesion to host cells.[32]

The most clinically relevant and commonly studied interactions and competition are those between the upper respiratory tract pathogens, namely *S. pneumoniae, S. aureus, H influenzae, and M. catarrhalis.* The prevalence of these pathogens varies between populations, but most children are colonized by at least one of these species in the first year of life.[5,8,33-37] Positive correlations between *S. pneumoniae, M. catarrhalis* and *H. influenza*, and negative correlations between *H. influenza* and *S. aureus* have been reported in epidemiological studies[5,7,8,38,39] as well as infection models.[40,25,41] Pettigrew et al. showed that colonization with *M. catarrhalis* and *H. influenza* together doubled the likelihood of co-colonization with *S. pneumoniae*. [7]

Recent advances in sequencing technology have allowed for the detection of the entire nasal microbiome, not only of culturable strains.[42] Metagenomic analyses showed the presence of a highly diverse nasopharyngeal microbiome including up to 2042 observed operational taxonomic units and as many as 1,219,310 observed unique sequences.[4,43,44] Considering the vast diversity in the nasal microbiome with which the two pathogens interact, the relationship between the two pathogens should take into consideration the possible roles of other species in the nasal microbiome, possibly by inhibiting or promoting the growth of *S. aureus* or *S. pneumoniae.*

In a study by Cremers et al., in which nasal microbiomes of *S. pneumoniae* carriers and non-carriers were assessed before and after artificial inoculation of non-carriers with *S. pneumoniae*, they observed that colonization was less likely to be successful if the individual's microbiome was rich in Staphylococcal species.[45] In line with this, Laufer et al. observed that *S. pneumoniae* colonization was more frequently detected when the microbiome had lower population diversity.[4]

S. aureus has also been shown to affect and be affected by other species in the upper respiratory niche. Analysis of the nasal microbiome by Lina et al. found a negative association between *Corynebacterium* species and *S. aureus* carriage,[28] and similarly, Yan et al. found that various species of *Corynebacterium* were either positively (*C. accolens)* or negatively (*C.* pseudodiphtheriticum) associated with the presence of *S. aureus*. Evidence for these interactions was seen in vitro[44] and in vivo, as artificial inoculation of *S. aureus* carriers with a species of *Corynebacterium* was shown to eradicate a resident *S. aureus* species.[46]

Another bacteria that was also suggested to interact with *S. aureus* in the nose is *Staphylococcus epidermidis*. Iwase et al. showed that a serine protease produced by some *S. epidermidis* strains inhibited *S. aureus* biolfilm formation. These inhibitory strains were more likely to be isolated from individuals who did not carry *S. aureus*, while non- inhibitory strains were isolated from *S. aureus* carriers.[47]

The role of the immune system in the bacterial interference

While some studies focused on potential direct mechanisms of interference, i.e. competition for space and resources[30,31,46] or direct inhibition through bactericidal factors,[18,21,41,48] the fact that *S. aureus* and *S. pneumoniae* colonize closely related, yet not precisely the same region in the upper respiratory niche, suggests that indirect inhibition, such as immune mediated inhibition is more plausible. The concept of interspecies immune-mediated cross-reactivity is not new, with some examples being cowpox and small pox, as discovered by Edward Jenner,[49] or certain enteric commensal *Escherichia coli*.[50]

Repeated *S. pneumoniae* carriage episodes in early childhood eventually induces an immune response that leads to shorter and less dense colonization of *S. pneumoniae* in older children and adults.[51] In contrast, *S. aureus* appears to elicit a futile antibody response, allowing the bacteria to escape immune responses and

recurrent colonization and infections with *S. aureus* are common.[52-54] Moreover, ~20% of healthy adult individuals are persistent carriers of a single strain for many years,[55,56] while young healthy adults are typically carriers of *S pneumoniae* only for very short durations, even following experimental exposures.[57] Shak et al. showed that when *S. aureus* carriers were artificially inoculated with *S. pneumoniae*, successful colonization resulted in a decrease in *S. aureus* carriage, but only 14 d later. The authors suggested that this delayed response may point to an immune mediated interaction.[41]

Initial suggestive data on the role of immune-mediated interference arose from studies that showed that *S. pneumoniae* – *S. aureus* interference was observed only in HIV uninfected children, but did not exist in HIV-infected individuals.[58,59] Colonization rates with *S pneumoniae* alone have been shown to be the same[60,61] or higher[62] in HIV infected compared to uninfected children, and HIV-positive patients have been shown to carry a wider range of *S. pneumoniae* serotypes.[63] In HIV treated children, the interference was once again observed as in uninfected children.[64] Indeed, pneumococcal-specific $CD4^+$ T cells have been found to return to normal levels following anti-retroviral therapy.[65] This suggests that the interaction between the *S. aureus* and *S. pneumoniae* may be $CD4^+$ T cell-mediated.

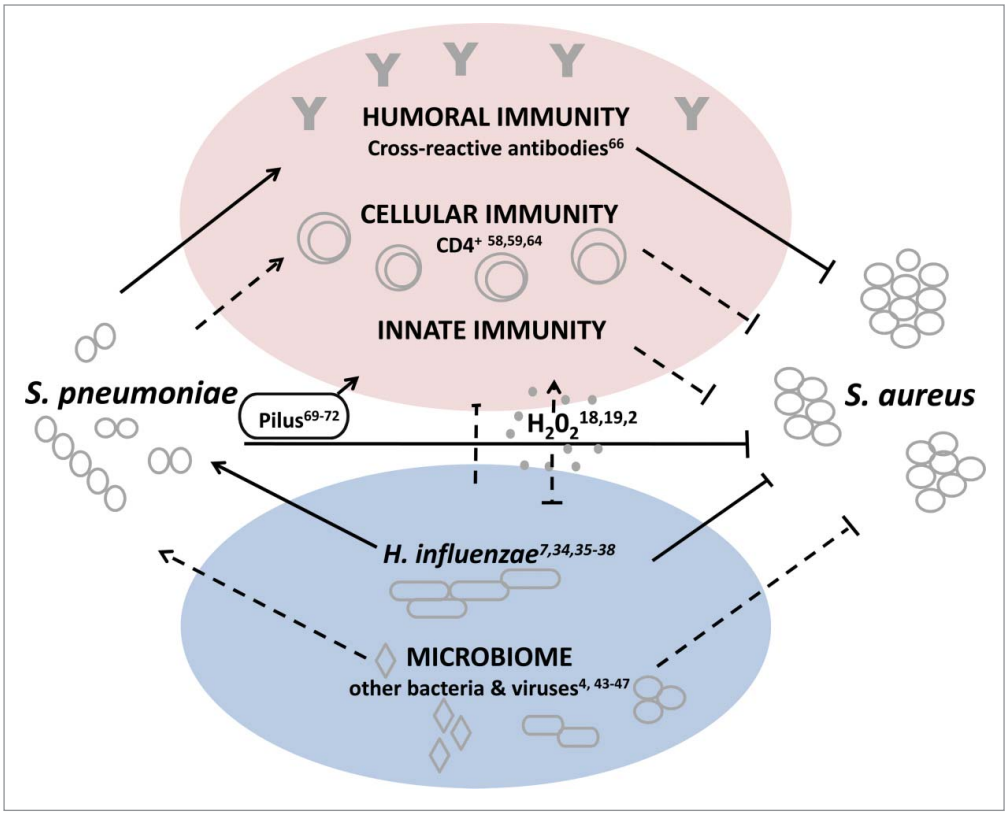

Figure 1. Model of possible mechanisms for *S. aureus* and *S. pneumoniae* interference. Straight lines indicate interactions that have been reported between *S. aureus* and *S. pneumoniae*. Dotted lines indicate interactions and/or directionality that have been suggested, but have not yet been observed experimentally.

While the HIV studies may suggest that T-cell immune response plays a role in the interference, Lijek et al. demonstrated in an in vivo murine model that *S. pneumoniae* colonization inhibits subsequent *S. aureus* acquisition in an antibody-dependent manner, via cross-reactive antibodies targeting conserved dehydrogenases, 1-pyroline-5-carboxylate dehydrogenase (P5CDH) of *S. aureus* and a putative *S. pneumoniae* dehydrogenase: SP_1119.[66] Yet, these antibodies have not been found in humans. In addition, other studies did not find correlations between levels of particular *S. aureus* or *S. pneumoniae* antibodies and rates of *S. aureus* colonization.[67,68]

S. pneumoniae pilus as a potential determinant in the interference mechanism

Another possible mechanism of immune-mediated interaction that has been suggested is through an immune response elicited toward the pneumococcal pilus. The pneumococcal pilus is a long filamentous structure that plays a role in host cell adhesion and pathogenesis and has also been shown to elicit an inflammatory immune response from the host.[69,70] Vaccine-type strains were more likely to carry a pilus[71] and indeed, following vaccine implementation, the rates of carriage of vaccine-type strains, including piliated ones, declined. However other piliated strains emerged, either by acquiring the pilus, or due to expansion and unmasking.[72] Piliated pneumococcal strains have been found to be negatively associated with *S. aureus* carriage regardless of whether those strains were included in the PCV7 vaccination, but carriage of non-piliated strains had no significant correlation with carriage of *S. aureus*.[72] It is therefore possible that the negative association between *S. aureus* and vaccine-type *S. pneumoniae* is due to the presence of the pilus, or a pilus-associated virulence factor. Since many of these new strains were then included in PCV13,[73] we have yet to see whether new piliated strains will again emerge.

While different mechanisms for a potential interference have been suggested, indirect mechanisms seem more plausible. The current reported data suggests that immune mediated mechanisms elicited by *S. pneumoniae* either specifically or non-specifically, directly or through other members of the microbiome interfere with *S. aureus* carriage (**Fig. 1**). Further studies are required to determine whether cross-reactive

antibodies, non-specific immune response elicited by a particular pneumococcal structure or other mechanisms can fully explain this bacterial interference.

Clinical Implications of the PCV effect on the Interference and on *S. aureus* Carriage

The discovery of the inverse correlation between *S. aureus* and vaccine-type strains of *S. pneumoniae* came at a time when PCV7 was just being introduced to the pediatric national immunization plan in many countries. Since vaccine-type strains were those found to be correlated with *S. aureus* carriage, this raised much concern that the introduction of the vaccine would indirectly cause a rise in *S. aureus* carriage and infection.[7,11,14,15] The idea that external interventions in ecosystems could damage the natural equilibrium by eliminating a less virulent 'predator' and result in undesirable emergence of a possibly more virulent 'prey' is not new and was shown decades ago for pests and pesticides.[74] The clinical trial that first suggested interference between *S. pneumoniae* and *S. aureus* found a rise in *S. aureus* otitis media cases following vaccination with PCV7 compared to a non-vaccinated group.[13] Others alerted that the emergence in CA-MRSA in the USA may be related to the introduction of PCV7 which took place at the same time.[75]

Several studies have examined the dynamics of *S. aureus* carriage post-PCV introduction. Lee at al observed no changes in *S. aureus* carriage rates in children from Massachusetts 3 to 7 y following PCV7 implementation, but they did not compare their results to pre-vaccination *S. aureus* carriage rates. Interestingly, they also did not observe an inverse correlation between *S. aureus* and *S. pneumoniae* in their sample.[11] The lack of observed interference is possibly due to the low rates of vaccine-type *S. pneumoniae* in their samples which were collected following PCV7 vaccination.[11,76] The correlation could also be hidden in the sample due to the large age range of children in their study ranging from 3 months to 7 y Other studies that looked at older children, or looked at a wide range of children without differentiating between older and younger children did not see the individual or population level interference either,[12,77] whereas those that only looked at children under the age of 2 y saw the interference more clearly.[10] It is therefore difficult to interpret the results of this study regarding the effect of PCV7 on *S. aureus* carriage, particularly since they did not observe an inverse correlation throughout their study periods.

Two studies in the Netherlands observed temporary increases in *S. aureus* carriage in children aged 11–12 months following vaccination with PCV7.[76,78] One of these was a randomized control vaccine trial done before the introduction of PCV7 in the Netherlands health program; the other was a cross-sectional surveillance study on nasopharyngeal bacterial carriage in the years following PCV7 introduction. The observed increase in *S. aureus* carriage coincides with a decrease in overall *S. pneumoniae* carriage, seen at age 11 months following vaccination with PCV7.[76] In the same study population a higher bacterial diversity of the microbiome was observed among PCV7 vaccinated children aged 12 months compared to unvaccinated children.[79] The relationship to age could be due to the fact that all 3 doses were needed in order to decrease carriage of *S. pneumoniae* strains, or it could be due to the immune maturation that occurs around age 12 months.[80]

Interestingly, the cross-sectional study reported increased parental *S. aureus* carriage concomitant to the rise of *S. aureus* in the child (at the age of 11 months).[76] However, the randomized study did not observe an increase in parental carriage and surprisingly they observed decreased parental carriage when the child was 24 months old.[78] A surveillance study from the pre-PCV era in South Africa reported constant maternal *S. aureus* colonization rates during times of dynamic *S. aureus* and *S. pneumoniae* carriage in the child.[8] The seemingly conflicting results of these 3 studies may be explained by changes in carried serotypes in the population due to herd effects following widespread vaccination which are not observed in clinical trials.

PCV7 implementation did not result in long-term increases in *S. aureus* carriage and infection, including *S. aureus* otitis media and MRSA levels, as reported in studies done in countries around the world, including the USA, Netherlands, Israel, and China.[7,11,76-78,81-84] Studies that observed increased *S. aureus* carriage in children age 11 months no longer saw this increase by age 24 months.[76]

Myth or Reality

The relationship between *S. aureus* and *S. pneumoniae* is very complex. The inverse correlation between carriage of *S. aureus* and carriage of vaccine-type *S. pneumoniae* in young children has been seen throughout many geographical areas, and through the years, including post-PCV7 implementation. However, the mechanism behind it and the clinical implications have yet to be fully determined.

Fears that vaccinating against *S. pneumoniae* would cause an increase in *S. aureus*, and more specifically MRSA carriage and infection seem, as of now, to be unsubstantiated. Changes following PCV7 introduction in overall *S. aureus* carriage rates were only short-term.

Carriage rates of both *S. pneumoniae* and *S. aureus* are dynamic. These two strains do not reside alone in the nose; their presence or absence can be affected by other species and other competitive factors, or external interventions such as vaccination or antibiotic use. It is still unclear whether the balance between the 2 species will continue to exist following widespread vaccination, and whether further vaccination with pneumococcal vaccines will cause a rise in *S. aureus* carriage and infection. It appears that the interaction between the two bacteria may be reduced due to vaccination, but the emergence of new strains and the evolution of existing strains makes it difficult to predict the implications. Many of the strains that replaced the vaccine-type strains following PCV7

implementation are now included in PCV13 and their prevalence rates are expected to decline as well. Complicating further, if piliated strains play a major role in the interference, determining the impact of PCVs on the prevalence of piliated strains will define the effect on *S. aureus* carriage.

Since the mechanism of interaction between the two species is not yet fully understood, it is impossible to predict whether the implementation of newer vaccines will result in further serotype replacement, a rise in *S. aureus* carriage, or a rise in a different species altogether. Clearly, further studies are required, both on the epidemiological effects of PCV vaccination, and on the mechanisms of this interaction.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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