Manipulating the Microbiome: Evolution of a Strategy to Prevent *S. aureus* Disease in Children

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Abstract

Hospitalized infants have the highest rates of invasive *S. aureus* disease of any population, and infection control strategies such as decolonization have been insufficient. For decades, researchers began studying the microbiome in search of new prevention strategies. The resident microbiota was found to be closely associated with susceptibility and at times, resistance to *S. aureus* colonization. The evolution of nucleic acid based techniques has enhanced our understanding of the complex relationship between the nasal microbiota and *S. aureus* colonization. We review what is known about bacterial communities in the nasal cavity of infants and discuss how future microbiome studies may help identify novel interventions to protect high-risk infants from *S. aureus* disease.

I. Introduction

Staphylococcus aureus is a leading cause of infectious morbidity and mortality in hospitalized infants and children[1]. Despite decades of research, *S. aureus* remains the second most common cause of nosocomial infections in neonates and children, including central-line associated bloodstream infection, surgical site infection, and ventilator-associated pneumonia [1, 2, 3, 4]. *S. aureus* can colonize the nasal cavity, and colonization is associated with an increased risk of subsequent infection[5, 6, 7]. Intranasal antibiotics and antiseptic agents have been used to eradicate colonization in an attempt to prevent *S. aureus* disease[8, 9]. The agents used for decolonization do not solely target *S. aureus*, but eradicate many bacterial communities with unknown long-term consequences[10]. Unfortunately, comprehensive infection control strategies including decolonization have been insufficient at preventing *S. aureus* disease in hospitalized patients, especially in neonates, who have the highest rates of invasive *S. aureus* disease of any age group [11, 12, 13, 14, 15].

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Over the last 10 years, the field of microbiome science has exploded using nucleic acid based tools to characterize organisms, including *S. aureus*, in their ecologic niche. A more comprehensive understanding of the nasal microbiota may lead to the identification of bacterial communities, interactions, and functional characteristics that are associated with *S. aureus* colonization. As clinicians and researchers, we are hopeful that these findings will uncover new strategies for the prevention of *S. aureus* disease. Below we review 1) early studies manipulating the nasal microbiome to eradicate existing *S. aureus* colonization or prevent colonization following exposure, 2) how nucleic acid based techniques have enhanced our understanding of the nasal microbiota and *S. aureus* colonization, 3) major differences between the nasal microbiome of adults and children, and 4) how future microbiome studies may identify new strategies for the prevention of *S. aureus* disease.

II. Manipulating the microbiome: evolution of a strategy to prevent *S. aureus* disease

Some bacterial communities may produce an environment in the nasal cavity that is inhospitable to *S. aureus* colonization. For decades, researchers have been investigating the role that these commensal bacterial species play on a human host's susceptibility to *S. aureus* colonization and disease. As a result, bacterial interference emerged as a strategy to use commensal bacterial species to eliminate or prevent the colonization of an anatomic niche by a pathogenic species [16].

IIa. Early trials of bacterial interference

In the 1960s, the clinical application of bacterial interference was spurred by several nursery outbreaks of highly virulent *S. aureus* strain 80/81 [9, 17, 18, 19, 20]. Light et al. found that topical decolonization with intranasal neomycin and hexachlorophene bathing failed to effectively eradicate *S. aureus* 80/81 carriage in these nurseries. They subsequently began inoculating all neonates admitted to the nursery with an attenuated *S. aureus* strain, hypothesizing that the attenuated strain 502A would occupy the nasal cavity and inhibit invasion by competing *S. aureus* strains [20, 21]. After two inoculation cycles with the attenuated *S. aureus* strain, only 1 out of 202 cultured infants tested positive for strain 80/81 [18]. This method, though successful in reducing the rates of colonization by a highly virulent strain of *S. aureus*, was discontinued after several neonates developed invasive infections with what was perceived as the non-virulent strain 502A [9, 17, 18, 19, 20]. Since the 1960s, there has been continued interest in the concept of nasal microbiome manipulation to eliminate existing *S. aureus* colonization or prevent *S. aureus* colonization following exposure.

IIb. Studies of bacterial interference to eliminate existing *S. aureus* colonization

Until recently, studies of bacterial interference to eliminate existing *S. aureus* colonization relied on culture-dependent techniques to characterize organism interactions. Researchers identified cultivable bacterial species that were associated with *S. aureus* in nasal cultures [22, 23, 24]. Species cultured from people without S. *aureus* colonization were then studied for their potential to eliminate existing *S. aureus* colonization or prevent *S. aureus* colonization following exposure. For example, Corynebacteria are some of the most

frequently identified organisms in the nares of individuals who are not colonized by *S. aureus* [25]. As common commensals of the skin and mucosal surfaces, Corynebacteria have various characteristics that confer ecological fitness, which may contribute to the prevention or elimination of colonization by competing species. Uehara et al. found that *Corynebacterium* strain Co304 had a higher affinity to nasal mucin than *S. aureus* for example, which they suggest may contribute to its ability to outcompete *S. aureus* and eliminate carriage [25]. Some species of Corynebacteria have also been shown to decrease the gene expression of factors that are important for *S. aureus* colonization and virulence like surface protein A, which allows *S. aureus* to evade detection and elimination by a host's immune system[26]. In one study, *Corynebacterium pseudodiphtheriticum* was inoculated in six MRSA colonized men and led to a decrease in abundance of *S. aureus.* However, as the abundance of *C. pseudodiphtheriticum* decreased, MRSA abundance returned to baseline level [27]. Similarly, when *Corynebacterium* strain Co304 was inoculated in the nasal cavities of over a dozen adults, *S. aureus* was eradicated in 71% of subjects and maintained in follow up periods of up to 3 years [25].

Staphylococcus epidermidis is another common commensal species found in the human nasal cavity[23]. A recent study in HIV-positive adults found that those colonized with *S. epidermidis* in the nose and throat were 80% less likely to be colonized with *S. aureus* at the same site [28]. Iwase et al. also observed that individuals who were not colonized with *S. aureus* were twice as likely to be colonized by *S. epidermidis*. This group inoculated adult *S. aureus* carriers with a serine protease-producing *S. epidermidis* strain and reported significantly reduced rates of *S. aureus* colonization[23]. These studies postulate that *S. epidermidis* may have a functional impact on the environment that makes the nasal cavity inhospitable to *S. aureus*. The exact mechanisms by which these common commensal organisms eliminate *S. aureus* colonization remain uncertain. Bacterial interference trials in humans, though few, have suggested that the bacterial species commonly found in the nasal cavity may help eliminate *S. aureus* colonization.

IIc. Studies of bacterial interference to prevent *S. aureus* colonization following exposure

The potential to prevent *S. aureus* colonization after a known exposure using certain bacterial strains, however, has only been studied in mouse models. Researchers have deliberately exposed naïve mice to *S. aureus* to assess the protective effects of other organisms. Park et al. assessed the potential of *S. epidermidis* to prevent acquisition of *S. aureus* colonization. They first treated mice with antibiotics to clear the resident microbiota, hoping that the absence of competing species would improve their success of inoculating the mice with *S. epidermidis*. After antibiotic pretreatment, mice were inoculated with *S. epidermidis.* Mice whose nasal cavities were inoculated with *S. epidermidis* prior to being exposed to MRSA showed reduced rates of *S. aureus* colonization compared to controls that were not initially inoculated with *S. epidermidis*, suggesting that *S. epidermidis* may alter its environment in the nasal cavity and make it unfavorable for *S. aureus* acquisition [29]. Similarly, Barbagelata et al. inoculated mice with an attenuated mutant strain of *S. aureus* (NK41) before exposing them to several virulent *S. aureus* strains isolated from humans[22]. Prior exposure to NK41 significantly reduced the abundance of virulent strains, suggesting that the mutant strain could exclude competing strains from the nasal cavity.

While the results of bacterial interference studies conducted to date are promising, most of the microorganisms that colonize humans cannot be cultivated, so many organisms in the nasal microbiota may be overlooked[30]. Although these prior studies to eradicate existing colonization or prevent colonization following exposure are limited by their reliance on conventional cultures, they suggest that the resident microbiota may play a significant role in regulating *S. aureus* colonization (fig 1). Nucleic acid based approaches will help to determine 1) the role of non-cultivatable organisms in resistance to *S. aureus* colonization and 2) whether a community of organisms, rather than a single species, is necessary to resist *S. aureus* colonization.

III. Nucleic acid based approaches provide insight into the nasal microbiota and *S. aureus* colonization

Until recently, researchers relied on culture-dependent techniques to identify organisms in people that did not have *S. aureus* nasal colonization, then tested those organisms for their potential to eliminate existing *S. aureus* colonization. Nucleic acid based approaches, such as 16S rRNA gene amplification and sequencing techniques, can provide a more complete and detailed characterization of the nasal microbiota and its potential for colonization resistance against *S. aureus*.

High-throughput sequencing studies in adults suggest that the nasal microbiota plays an important but complex role in *S. aureus* colonization. The abundance of certain bacterial species in the nasal microbiota is strongly associated with *S. aureus* carriage. A study conducted by Liu et al. demonstrated that the absolute abundance of *Corynebacterium* species is a predictor of *S. aureus* carriage in a threshold-dependent manner [31]. As the abundance of *Corynebacterium* species increases, the likelihood of *S. aureus* colonization decreases. In fact, multiple studies have found a higher relative abundance of *Corynebacterium* species in people not colonized with *S. aureus*[32, 33, 34, 35]. Other organisms such as the gram-positive facultative anaerobic cocci of genus *Dolosigranulum,* have been shown to negatively correlate with *S. aureus* carriage. The same group also demonstrated that there was a 16% rate of *S. aureus* colonization among individuals with a certain threshold abundance of *Dolosigranulum species. Corynebacterium* and *Dolosigranulum species* often co-colonize non-carriers, suggesting that the presence or absence of a single organism alone may not determine susceptibility or resistance to *S. aureus* colonization.

In addition to correlating *S. aureus* with other organisms in the nasal microbiota, culture-independent sequencing methods have evaluated organisms' distribution and diversity metrics to suggest that resistance to *S. aureus* colonization may be multifactorial. Frank et al. showed that the nasal microbiota of healthy adults was more than twice as diverse as those of hospitalized patients and more evenly distributed[33]. They also found that the microbiota of hospitalized adults was enriched with *S. epidermidis* or *S. aureus* but lacking in Actinobacteria, such as *Corynebacterium* and *Propionibacterium*[33]. In addition, the microbiota of *S. aureus* carriers, has a lower biodiversity and more unevenly distributed bacterial communities than that of non-carriers; however, it remains unclear whether certain commensal species foster the dynamics often found in healthy adults or whether these

dynamics favor colonization by commensal species over pathogenic bacteria [32, 36]. Microbiome studies can further inform the relative importance of biodiversity and organism composition in contributing to *S. aureus* colonization.

IV. Nucleic acid based approaches reveal important differences in the nasal microbiota of children and adults

Studies conducted in adults have revealed that a diverse nasal microbiota composed of certain beneficial bacterial communities appears to provide colonization resistance against *S. aureus* in exposed individuals[36]. However, there is a notable lack of similar data on the microbiota of neonates and children. Findings in adults cannot be extrapolated to a neonatal population for several reasons. First, the mature microbiome is more diverse than the naïve microbiome, and this diversity contributes significantly to its stability and resistance to pathogen invasion [37].

Although the mature microbiota is highly variable between individuals, it is more resistant to environmental exposures than that of infants and young children [38]. Second, the nasal microbiota of adults is composed of different organisms compared to newborns and children [39]. Both community-dwelling and hospitalized adults host a nasal microbiota dominated by organisms of the phyla Actinobacteria and Firmicutes and rarely by gram negative organisms of the phylum Proteobacteria found commonly in infants [33, 34, 35, 36, 40]. Third, studies in adults have revealed the importance of certain commensal species in the protection against colonization by pathogens like *S. aureus* [28, 31, 32, 33, 34, 36, 41, 42]. Infants, however, lack these commensals initially, and during the first years of life the microbiome is still dynamic and susceptible to extrinsic exposures [43]. Further studies are needed to characterize potentially beneficial communities and whether they can foster the same protective effects in neonates.

IVa. Emergence of a personalized microbiota in neonates

From birth, neonates are exposed to and subsequently colonized with a wide array of microbes. These organisms play a pivotal role in determining the succession of bacterial communities into mature stable configurations[44]. Neonates initially harbor a microbiota that is homogenous across different anatomic sites and significantly impacted by whether they are born by vaginal delivery or Caesarian-section[45]. Infants delivered by Caesarian-section are initially colonized by their mother's skin microbiota and therefore are more likely to possess a higher abundance of *Corynebacterium*, *Propionibacterium*, and *Staphylococcus* species[45]. These initial colonizers interact with a neonate's tolerant immune system, leading to the activation and suppression of different inflammatory responses[46]. The long-term impact of the resident microbiota on the immature immune system may also play a part in dictating when an infant will acquire important commensals and if they will acquire pathogens such as *S. aureus* [46, 47].

By 6 weeks of age, infants have developed a site-differentiated and personalized microbiota[44, 48]. At this stage, the resident microbiota varies more between different individuals than in the same individual over time[44]. Infants share many of the same

organisms in their nasal microbiomes, but in variable proportions. For this reason, the nasal microbiome is often classified by the organism with the highest relative abundance, whether *Streptococcus*, *Moraxella*, *Staphylococcus*, *Corynebacterium* and/or *Dolosigranulum* [49]. The most abundant bacterial species indicate the overall stability and diversity of other bacterial communities in the nasal cavity[50]. A high relative abundance of certain commensal organisms, such as *Corynebacterium*, is associated with more diverse communities and lower overall bacterial density [47]. These characteristics promote the stability of the microbiota over time and help maintain homeostasis in the face of external disruptions[37, 47].

As the nasal microbiome develops, commensal species may play a direct role in host protection by eliminating or excluding pathogens from their niche [16]. Children whose microbiota contains commensals *Corynebacterium* or *Dolosigranulum*, for example, are less likely to harbor potential pathogens of genera *Streptococcus*, *Staphylococcus*, and *Moraxella*[44]. Although the specific mechanisms by which commensals exclude other organisms from the nasal cavity remain unclear, studies conducted on the immature nasal microbiota suggest that early acquisition of certain commensal species may impart long-term resistance to colonization by pathogens in newborns[16, 51].

IVb. Plasticity of the neonatal microbiome

Lower biodiversity and higher bacterial burden characterize the immature microbiota and predispose it to disruption, including dysbiosis [42, 52]. Dysbiosis, or disequilibrium, has been shown to increase the risk of colonization and potential domination of a niche by pathogenic species [53]. These changes in the microbiota composition may increase an infant's risk of infection by a colonizing organism. A study by Hilty et al. explored the association between the nasal microbiota and acute otitis media (AOM) in infants. They show that the majority of infants with AOM caused by *S. pneumoniae* also had a nasal microbiota dominated by *S. pneumoniae*. These infants had a significantly higher bacterial burden overall and lower bacterial diversity in their nasal cavities than infants colonized by only commensal streptococcal species[47, 54]. In fact, the dominance of any potential respiratory pathogen, whether *Moraxella Catarrhalis*, *Haemophilus influenzae*, or *S. aureus*, in the nasal microbiome, significantly increased the likelihood of AOM with the same organism [47].

The same qualities that contribute to a vulnerability to pathogen invasion also represent an opportunity for successful colonization by protective commensal species. The nasal microbiome of infants is dynamic and receptive to new organisms [55]. A high relative abundance of certain commensal species is associated with greater biodiversity and lower bacterial burden [47]. Both are characteristics shown to promote stability and subsequently, colonization resistance.

A newborn's naïve nasal microbiome is highly sensitive to environmental exposures [55]. Breastfeeding, for example, is associated with a nasal microbiome dominated by commensal *Corynebacterium* and *Dolosigranulum* species. Formula-fed infants are significantly more likely to harbor profiles dominated by potential pathogens such as *S. aureus* [48]. Adopting habits that increase rates of colonization by potentially protective bacterial species is not always feasible, however. Antibiotics, for instance, are among the many clinical exposures related to a significant reduction in colonizing commensal species in hospitalized infants and

children [47]. The identification of species and the mechanisms by which they exclude *S. aureus* from the immature nasal microbiome could potentially make other interventions available for high-risk infants.

V. Microbiome studies may identify new strategies to prevent *S. aureus* disease

To date, scientists' understanding of the association between the nasal microbiome and *S. aureus* colonization is just the tip of the iceberg. Nucleic acid based studies are revealing that the relationship between the microbiota and *S. aureus* colonization is more complex than once thought. The microbiota seems to play an important role in colonization resistance, excluding *S. aureus* from colonizing the nasal cavity. Microbiome studies using nucleic acid based approaches extend the range of detectable organisms and facilitate genus and even species-level identification. Improving the ability to detect and distinguish bacterial species is essential for studying the association between *S. aureus* colonization and specific commensal species. The use of nucleic acid sequencing also allows for a more precise measure of organism composition and abundance. This has led to the development of new ways to measure biodiversity and population distribution that may help show the association between *S. aureus* colonization and overall dynamics of the nasal microbiota [56]. In fact, some studies have suggested that microbiota can be categorized in community types based on the organisms with the highest relative abundance[31]. These community types may potentially identify people at a higher risk for *S. aureus* colonization and infection despite vast individual variations in the nasal microbiota. In addition to community structure, sequencing enables the detection of genes encoding certain functional attributes of bacterial communities such as the ability to synthesize anti-microbial substances, possibly elucidating the mechanisms behind colonization resistance. Future functional analyses may also reveal how the interdependence of gene expression in a microbial community may be employed to attenuate *S. aureus* virulence. Burnham et al. suggest that interventions aimed at producing colonization resistance to *S. aureus* in humans may need to involve more than just one bacterial interference mechanism[57]. Introducing a community of organisms found in healthy non-carriers to the nares of high-risk infants could potentially help establish a temporally stable microbiota less susceptible to colonization by pathobionts like *S. aureus*. While hospitalized neonates and infants are especially at risk for *S. aureus* disease, there is a lack of studies on the relationship between the immature nasal microbiota and *S. aureus* colonization. The increasing accessibility of nucleic acid sequencing technologies may help to inform such studies and to make safer and more targeted prevention strategies available for high-risk infants.

VI. Conclusion

S. aureus remains a leading cause of infectious morbidity in infants and children. Prior studies suggest that the nasal microbiota is closely associated with *S. aureus* colonization, and that certain resident commensal bacteria may be capable of excluding *S. aureus* from the nasal cavity. Sequencing technologies may help identify bacterial communities and the

mechanisms by which they exclude *S. aureus* from the immature nasal microbiome and lead to novel targeted interventions to protect high-risk infants from *S. aureus* disease.

Acknowledgments

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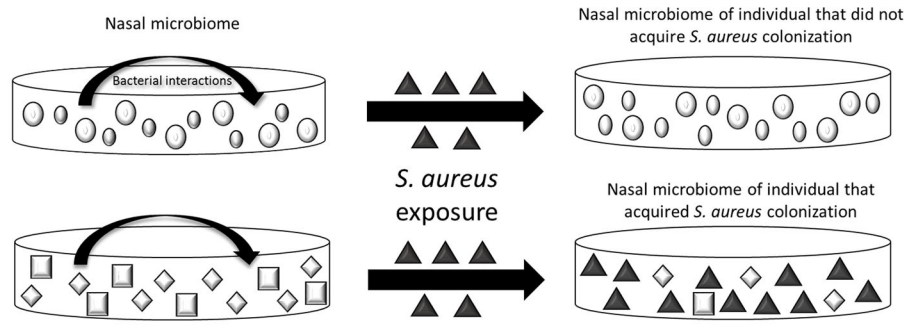
Figure 1.