



Vaccination as infection control: A pilot study to determine the impact of *Staphylococcus aureus* vaccination on nasal carriage

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ABSTRACT

Background: There is a critical need for an effective *Staphylococcus aureus* vaccine for the prevention of staphylococcal disease. In this study, we investigated the impact of *S. aureus* conjugate vaccine comprised of capsular polysaccharides 5 and 8 (CP5, CP8) on nasal colonization with *S. aureus*.

Methods: Healthy adults recruited from one academic medical center to participate in a lot consistency trial of StaphVAX[®] (*S. aureus* capsular polysaccharide 5 and 8 conjugate vaccine) were assessed for *S. aureus* nasal colonization at two weekly points prior to vaccination and again at six weeks post-vaccination. Serum anti-capsular antibody titers to CP5 and CP8 were obtained prior to vaccination and 42 days post-vaccination and measured by ELISA.

Results: Thirty of 88 enrolled subjects (34%) had *S. aureus* isolated from at least one of the pre-immunization cultures. Of these, 20 were termed persistent carriers due to two positive cultures one week apart; 19 of the 20 were evaluable at Day 42. Baseline anti-CP8 concentrations were higher in persistent carriers of CP8+ *S. aureus*; however, baseline anti-CP5 levels were not significantly higher in individuals persistently colonized with CP5+ *S. aureus*. Statistically significant rises in antibody concentrations were noted after vaccination. At Day 42, 14 of 19 persistent carriers remained colonized; 5 subjects did not have evidence of *S. aureus* colonization. Ten additional subjects were positive for *S. aureus* at Day 42 who were not persistently colonized at baseline. Serum antibody concentrations were not statistically different between those with persistent carriage vs. those that lost carriage or those with newly acquired carriage.

Conclusions: Immune responses to vaccine were brisk and comparable in subjects with or without persistent colonization. Despite a substantial rise in anti-CP5 and anti-CP8 antibody concentrations post-vaccination, *S. aureus* nasal colonization rates did not significantly change.

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1. Introduction

Staphylococcus aureus is a major human pathogen and results in substantial morbidity and mortality [1,2]. In recent years, the development of community-associated methicillin-resistant *S. aureus* (CA-MRSA) has complicated treatment options and previously healthy individuals are “at risk” for infection with this historically healthcare-associated pathogen [3].

S. aureus is ubiquitous, occupying the anterior nares of approximately 30% of adults [4]. Nasal colonization has been extensively studied by Nouwen et al. [5] and has been shown to resolve over time or result in a persistent carrier state. Based on repeated cultures over a 12-week period of time, Nouwen proposed a culture rule: two positive nasal cultures, performed one week apart, predict persistent carriage. Based on these data, colonization can be characterized as persistent, intermittent, or absent.

Healthcare-associated staphylococcal infections may arise from a patient's own native flora [6] or through transmission within the healthcare environment. One vector of such transmission may be healthcare workers (HCW) who may be transiently or persistently colonized with *S. aureus* themselves [7–11]. Healthcare-associated transmission of MRSA has resulted in disease outbreaks referable

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to HCW colonization [12–14]. Thus, interrupting transmission of HCW-associated *S. aureus*, either to the environment or to individual patients, is a logical goal in infection prevention.

Identifying methods to reduce transmission of staphylococci, particularly MRSA, in both the community and hospital environment has assumed greater importance as increasing disease rates have been reported and treatment options have become more limited. Vaccines are potent preventive measures for many infectious diseases, and the use of capsular polysaccharide-conjugate vaccine targeting specific serotypes of *S. aureus* has shown some efficacy in preventing staphylococcal bacteremia in hemodialysis patients in an early clinical trial, but not subsequently confirmed [15,16]. The current study was nested in a lot consistency study of StaphVAX[®] (Nabi Biopharmaceuticals, Rockville, MD) and investigated whether vaccination could eliminate carriage with *S. aureus* in vaccinated individuals recruited at one academic medical center.

2. Methods

2.1. Study design and participants

Healthy adults, age 18 years and older, enrolled in a lot consistency trial of three different lots of StaphVAX[®] were recruited to participate in an ancillary study examining *S. aureus* nasal colonization and the impact of vaccination on eradication of carriage. Exclusion criteria were serious staphylococcal infection within 3 months of study entry; any illness within two weeks prior to vaccination; a history of hypersensitivity to polysaccharide or conjugate vaccines; known HIV infection; current use of immunomodulatory agents; history of or current malignancy, with the exception of squamous cell or basal cell skin cancer, carcinoma *in situ* of the cervix, or early stage prostate cancer; pregnancy; or previous administration of StaphVAX[®]. The protocol was reviewed and approved by the Vanderbilt Medical Center Institutional Review Board, and written informed consent was obtained from all volunteers.

2.2. Study procedures

In the colonization study, subjects underwent two nasal swab cultures prior to receipt of StaphVAX[®], one at enrollment (Day –7) and one 7 days later at the time of vaccination (Day 0). This allowed for the identification of subjects who were persistent carriers of *S. aureus* (two consecutive positive cultures) as defined by Nouwen et al. [5]. Intermittent carriers were defined as those with one of two cultures positive for *S. aureus*, and those with two negative cultures were deemed non-carriers. A questionnaire was obtained at enrollment to evaluate potential risk factors for staphylococcal colonization. These included hospitalization or surgery within the past 12 months, antibiotic use in the past 6 months, incarceration within the last 12 months, chronic skin disorders (e.g., psoriasis, eczema), occupation (e.g., healthcare worker), dialysis, long-term intravenous catheter use, and previous history of staphylococcal infections.

2.3. Laboratory methods

2.3.1. Microbiology

Nasal cultures were obtained using Culturette[™] swabs moistened with Liquid Amies Medium (BD Diagnostic Systems) and inserted into each naris at each sampling period (one week prior to vaccination, on the day of vaccination, and 42 days post-vaccination). The swab was rotated along the inside of the nares for 5 s, and placed in 5 mL of tryptic soy broth supplemented with 6.5% NaCl within 12 h of collection and incubated for 18–24 h. Then a 75- μ L aliquot of the incubated sample was plated onto paired mannitol salt agar plates with and without 4 μ g/mL of oxacillin (MSA, MSA_{ox};

Dalynn Biologicals, Calgary, Alberta, Canada). All plates were incubated for 48 h at 37 °C and an additional 24 h at room temperature, and then were inspected for yellow colonies indicative of mannitol fermentation, a characteristic of *S. aureus*. Possible *S. aureus* isolates were subcultured onto tryptic soy agar with 5% sheep blood and colonies were tested for clumping factor by latex agglutination (Staphaurex[®], Remel, Lenexa, KS). Each isolate that grew on selective media (MSA_{ox}) and was clumping factor-positive by latex agglutination was considered a putative MRSA isolate. Genomic DNA was extracted and purified and the presence of the *mecA* gene was determined by polymerase chain reaction [17]. Only those isolates recovered from MSA_{ox} media and containing the *mecA* gene were classified as MRSA.

Capsular serotyping was performed in the laboratory at Nabi Biopharmaceuticals on all *S. aureus* isolates after subculturing at 37 °C for 24 h on Columbia agar plates containing 2% MgCl₂ and 0.5% CaCl₂ (Difco Sparks, MD, USA). Colonies were resuspended in 0.9% sodium chloride, and serotypes were determined by rapid slide agglutination using rabbit polyclonal antibodies specific to CP5 and CP8 (Nabi Biopharmaceuticals, Rockville, MD).

2.3.2. Vaccine

StaphVAX[®] is composed of *S. aureus* type 5 and 8 capsular polysaccharides (100 μ g per milliliter of each type) conjugated to an equal weight of recombinant exoprotein A derived from *Pseudomonas aeruginosa* in 0.01% polysorbate 80 and sodium phosphate-buffered saline (pH 7.4). These two capsular polysaccharide types include nearly 90% of all invasive *S. aureus* isolates [18]. Subjects received one of three unique lots of vaccine administered intramuscularly according to manufacturer's guidelines.

2.3.3. Serology

Serum was obtained for determination of anti-capsular polysaccharide IgG antibody concentrations (anti-CP5 and anti-CP8) by ELISA (Nabi Biopharmaceuticals) [19,20] prior to and 42 days after vaccination.

2.4. Statistical analysis

The study was primarily powered to detect significant differences in serologic response and reactogenicity following vaccination as part of the multicenter lot consistency trial. With an alpha = 0.05, beta = 0.8, and an assumed baseline colonization frequency of 33%, 90 subjects would be projected to provide the ability to detect, at a significant level, decreases in colonization of 60%. Categorical data from questionnaire responses were analyzed using Pearson's chi-squared test with logistic regression modeling. Relationships between categorical and continuous data were assessed with logistic regression or with non-parametric tests of association, where applicable. For situations in which multiple pair-wise comparisons were made, Bonferroni corrections were applied. For changes in colonization over time, a generalized estimating equation with a first-order working autocorrelation matrix was used to take into account the effect of repeated measures. All analyses were performed using Stata 8.0 and SAS.

3. Results

Of the 90 subjects enrolled in the colonization study, two consecutive pre-immunization nasal cultures were obtained from eighty-eight. Two patients voluntarily withdrew from both the lot consistency and the colonization study prior to the 2nd nares culture and were not included in the analysis. Two additional patients voluntarily withdrew from both studies prior to study completion (Day 42) and were included only in the baseline analysis of colonization. Females comprised 65% of the study population and 98%

were Caucasian. Sixty-one of the 88 subjects (69%) were healthcare workers, and 45 (74%) reported that their primary work site was hospital-based.

3.1. Colonization status pre-vaccination

Thirty of the 88 subjects (34%) were colonized with *S. aureus* at one or both of the initial pre-vaccination assessments; the remainder was not colonized. Twenty subjects (23% overall) exhibited persistent carriage based on two consecutively positive pre-vaccination cultures; nineteen of these were evaluable at Day 42. There were no significant differences in baseline staphylococcal carriage status with regards to gender, race, age, or occupation in general. Five subjects (5.6%) were colonized with MRSA in both pre-vaccination cultures. All of the MRSA carriers were healthcare workers ($p=0.046$) and all reported having cared for a patient with a staphylococcal infection within the last 6 months ($p=0.032$).

3.2. Capsular distribution in pre-vaccination nasal isolates

Fifteen of the thirty *S. aureus* isolates (50%) recovered at baseline expressed type 8 polysaccharide capsule (CP8+), 12 isolates (40%) expressed type 5 capsule (CP5+), and the remaining 3 isolates (10%) expressed neither. Of those subjects with CP5+ strains, 10/12 (83%) exhibited persistent pre-vaccination carriage; 8/15 (56%) of subjects with CP8+ strains were persistent carriers.

3.3. Humoral anti-capsular polysaccharide antibody concentrations

Overall, pre-immunization anti-capsular IgG concentrations were low; the median anti-CP5 antibody concentration was 7.5 mcg/mL (IQ range 4.4–13 mcg/mL) and the median anti-CP8 antibody concentration was 16.1 mcg/mL (IQ range 9.6–24.1 mcg/mL). There were no significant differences in median pre-immunization anti-CP5 or anti-CP8 concentrations by gender, healthcare worker status, location of primary clinical responsibilities (hospital vs. clinic), history of antibiotic use, or recent exposure to patients with staphylococcal infections. However, in subjects exhibiting persistent nasal carriage pre-immunization, median anti-CP8 concentrations were higher than in those without carriage or with intermittent carrier status (26.5 mcg/mL vs. 13.4 mcg/mL, $p=0.007$, Fig. 1). The same trend for higher pre-immunization anti-CP5 concentrations in persistent carriers was noted when

compared with intermittent or no carriage (10.5 mcg/mL vs. 7.2 mcg/mL, $p=0.47$), but was not statistically significant.

After vaccination, median anti-CP5 concentrations rose to 450.4 mcg/mL and anti-CP8 concentrations reached 236.6 mcg/mL by Day 42. Immune responses (geometric mean concentrations) were comparable among subjects of all demographic characteristics and among recipients of each of the three vaccine lots.

3.4. Relationship between serum anti-capsular polysaccharide antibody concentrations and carriage status post-vaccination

At Day 42, twenty-four of 86 subjects (28%) were colonized with *S. aureus*. The majority of these individuals (14/24, 58%) were persistently colonized prior to vaccination. Five subjects were intermittent carriers pre-vaccination, and only 5 previously non-colonized individuals became culture-positive for *S. aureus* at Day 42.

Of the 20 subjects persistently colonized prior to vaccination, 19 were evaluable at Day 42 (Table 1). Fourteen (74%) remained colonized at Day 42, a non-significant reduction after taking into account the effect of repeated measurements. Of the 5 subjects in whom colonization did not persist to Day 42, four were colonized at baseline with a CP5+ strain and one was non-typeable; 1 subject received vaccine lot 1, two received lot 2, and two received lot 3. Each of the 8 subjects who were persistent carriers of CP8+ *S. aureus* at baseline remained colonized with CP8+ *S. aureus* at Day 42, and each of the 5 subjects who were persistent carriers of CP5+ *S. aureus* at baseline remained colonized at Day 42, but two of them were colonized with strains that did not produce CP5 or CP8 (and were of different genetic lineages than the previous colonization strains by repetitive-element, sequence-based PCR). Five previously non-colonized subjects exhibited *S. aureus* colonization after vaccination: one was CP5+, two were CP8+, and two did not express either CP5 or CP8. Colonization status at Day 42 did not differ based on anti-CPS concentrations to either CP5 or CP8. Overall, vaccination had no statistically significant impact on reducing colonization from pre-vaccination rates.

4. Discussion

In this cohort of individuals receiving a *S. aureus* conjugate polysaccharide vaccine in a lot consistency trial, we have shown that vaccination with the conjugate vaccine resulted in significant increases in antibody concentrations regardless of age, occupation, or colonization status. However, despite this increase in humoral antibody concentrations, 74% of individuals with persistent colonization remained colonized and 5 new subjects acquired colonization. Thus, vaccination did not significantly reduce *S. aureus* colonization.

The capsule of *S. aureus* has been shown in animal models to promote nasal colonization [21,22], facilitate abscess formation [23,24], and to impede opsonophagocytosis [25–27]. It has also been suggested that both active and passive immunization with capsular antibodies results in improved opsonophagocytosis and protection from lethal challenge with staphylococcus [21]. Previously, it was reported that human adults have relatively high naturally occurring antibody levels to type 5 and 8 capsules [28]. This is consistent with our pre-immunization data in which nearly all subjects exhibited significant serum concentrations of anti-CP5 and anti-CP8. These levels of specific antibodies are below the calculated protective concentration of 80 mcg/mL [15], though it is unclear whether this concentration is required given the high-affinity antibodies generated by receipt of the conjugate vaccine. Initially, we hypothesized that humoral anti-capsular antibody would be higher in non-carriers or intermittent carriers since

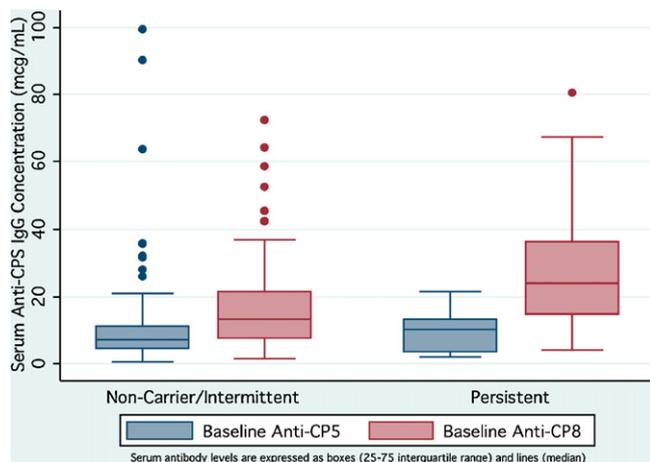


Fig. 1. Pre-immunization anti-capsular polysaccharide (CPS) concentrations stratified by pattern of colonization. Though serum anti-CP5 concentrations did not differ significantly, serum anti-CP8 concentrations were higher in persistent carriers than in intermittent carriers/non-carriers (26.5 mcg/mL vs. 13.4 mcg/mL, $p=0.007$).

Table 1

Comparison of capsular strain type, anti-CP5 concentrations, anti-CP8 concentrations, and vaccine lot at baseline and 42 days post-vaccination in subjects persistently colonized prior to vaccination and those in whom no colonization was detected prior to vaccination. Each of the subjects colonized with CP8+ strains prior to vaccination remained colonized at Day 42, though only 3/9 subjects with CP5+ strains remained colonized with CP+ strains (100% vs. 33%, $p = 0.24$). Antibody concentrations are expressed as mcg/mL. NT, non-typeable. Asterisks (*) denote subjects with MRSA carriage.

Subject	Colonization strain type (capsule)		Anti-CP5 (mcg/mL)		Anti-CP8 (mcg/mL)		Vaccine lot
	Baseline	Day 42	Baseline	Day 42	Baseline	Day 42	
1*	5	5	21.5	1164.9	4.0	698.5	1
2	5	5	13.1	2077.0	29.3	180.1	3
3*	5	5	29.0	221.0	202.1	409.7	3
4*	5	NT	13.9	567.9	18.3	1190.6	1
5	5	NT	3.5	117.3	67.6	119.5	3
6	5	–	8.0	459.1	36.1	390.7	3
7*	5	–	21.6	956.2	28.7	400.8	1
8	5	–	4.8	106.6	10.2	97.9	2
9*	5	–	20.5	626.5	14.7	246.1	2
10	8	8	10.0	490.7	16.5	104.6	3
11	8	8	11.0	254.7	60.7	228.2	3
12	8	8	3.3	122.1	24.2	138.3	2
13	8	8	6.6	65.6	34.2	120.7	1
14	8	8	13.4	740.9	33.6	118.5	3
15	8	8	4.0	69.5	15.1	129.4	2
16	8	8	2.1	406.5	80.6	517.2	1
17	8	8	2.7	282.9	40.6	688.3	2
18	NT	NT	11.5	967.2	23.0	537.9	3
19	NT	–	2.3	102.3	6.3	109.8	3
20	–	NT	19.4	1281.4	27.9	764.7	3
21	–	8	35.9	993.5	20.7	1479.2	3
22	–	NT	2.1	635.4	13.9	113.8	1
23	–	5	32.0	704.3	64.3	146.2	1
24	–	8	0.6	143.2	13.3	567.4	1

circulating antibody would bathe the mucosal surfaces and provide protection against staphylococcal colonization. However, this study clearly showed that this was not the case, since colonized individuals had higher levels of pre-immunization antibody. Others have shown similar results [29–32], thus carriage stimulates a humoral response to *S. aureus*.

The remarkable reduction in nasopharyngeal carriage of *Haemophilus influenzae* B and *S. pneumoniae* by vaccination with capsular polysaccharide protein conjugate vaccines supported the hypothesis that vaccination may prevent acquisition of nasal carriage and/or eradicate *S. aureus* nasal carriage. However, despite a substantial rise in anti-CP5 and anti-CP8 antibody concentrations after vaccination, there was no clear impact on colonization at six weeks post-immunization, indicating that even high levels of antibodies may not clear well-established colonization of the anterior nares. In fact, the majority of individuals (74%) deemed to be persistent carriers pre-vaccination remained colonized. This illustrates that the eradication of colonization may be fundamentally different than the prevention of reacquisition of *S. aureus* nasal colonization in those that have received a nasal decolonization therapy (e.g., mupirocin).

As Projan et al. have adeptly discussed [33], the example of experimental bovine mastitis vaccines ostensibly shows the potential success of active immunization against *S. aureus*. However, the role of antibody in this protection, particularly given the “camouflaging” afforded by staphylococcal protein A (by binding the Fc portion of IgG antibodies), remains entirely unclear. The argument over the role of antibody in protection from subsequent staphylococcal disease has survived for more than a half-century and will persist until more is known from both animal models and human studies of *S. aureus* disease. In addition, while capsule is likely to be an important factor in disease [21], clinicians and scientists alike are continually confronted with the virulent redundancy of the staphylococcal genome and the spectrum of pathogenic (and, ideally, antigenic) properties they possess.

There are caveats to our study. First, the study was part of a lot consistency trial and, therefore, an unimmunized control group was not included. Second, colonization was correlated only with humoral and not mucosal anti-capsular antibodies. Additionally, these antibodies were measured by ELISA and not by functional opsonophagocytic assays (OPA), raising the concern that not all anti-capsular antibodies may be created equal. Finally, the study was powered to detect differences between manufactured lots of vaccine among multiple study sites, but was underpowered to detect small changes in colonization after vaccination. The optimal design of an efficacy trial to assess vaccine impact on nasal colonization would be a randomized, placebo controlled trial with larger sample size and future studies will require such a design in order to detect any impact of a *S. aureus* vaccine [20].

In summary, the capsular polysaccharide protein conjugate *S. aureus* vaccine generated a robust immune response to both CP5 and CP8, but did not significantly reduce nasal colonization rates. New vaccine antigens or vaccine candidates using improved adjuvants or delivery methods are urgently needed in this time of increasing *S. aureus* colonization and disease.

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