Journal of Infection 79 (2019) 582–592

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

S. aureus colonization in healthy Australian adults receiving an investigational *S. aureus* 3-antigen vaccine

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ARTICLE INFO

Article history:
Accepted 27 September 2019
Available online 1 October 2019

Keywords:
Staphylococcus aureus vaccine
Methicillin-resistant *Staphylococcus aureus*
Colonization
Staphylococcus aureus acquisition
Spa typing

SUMMARY

Objectives: Assess *Staphylococcus aureus* (*S. aureus*) colonization in healthy Australian adults receiving an investigational *S. aureus* 3-antigen vaccine (SA3Ag).
Methods: In this phase 1, double-blind, sponsor-unblinded study, participants were randomized to receive a single dose (1 of 3 dose levels) of SA3Ag or placebo and a booster dose or placebo at 6 months. *S. aureus* isolates from nasal, perineal, and oropharyngeal swabs before and through 12 months post-vaccination were identified.
Results: Baseline *S. aureus* colonization prevalence was 30.6% (any site), with nasal carriage (27.0%) more common than oropharyngeal/perineal (3.2% each). Following initial vaccination (low-dose: 102; mid-dose: 101; high-dose: 101; placebo: 102) and booster (low-dose: 45; mid-dose: 44; high-dose: 27; placebo: 181), placebo and SA3Ag groups showed similar *S. aureus* carriage through 12 months. Most colonized participants (74.0%) were colonized by single *spa* types. Placebo and SA3Ag groups had similar persistence of colonization, with 19.6–30.7% due to single *spa* types. Acquisition was observed in mid- and high-dose recipients (~20%) and low-dose and placebo recipients (~12%). Vaccination resulted in substantial increases in antibodies to all 3 antigens, irrespective of carriage status.
Conclusions: Based on descriptive analyses of this small study, SA3Ag vaccination did not impact *S. aureus* acquisition or carriage. Carriage status did not impact antibody responses to SA3Ag.

Introduction

Staphylococcus aureus (*S. aureus*) is a leading cause of infection-related morbidity and mortality in all age groups.[1] *S. aureus* can cause invasive skin, respiratory tract, and bloodstream infections in previously healthy children and adults, with sequelae including endocarditis, pericarditis, osteomyelitis, and septic arthritis.[2] Globally, *S. aureus* is the most frequent cause of surgical site infections (SSI), and is associated with greater morbidity and mortality than most other infectious organisms.[3] Despite efforts to reduce infections, invasive *S. aureus* disease remains a significant burden on healthcare systems,[4,5] requiring the development of novel preventative measures such as vaccines.

Asymptomatic carriage of *S. aureus* occurs soon after birth, with one study reporting >40% of infants were *S. aureus* carriers within 8 weeks after birth.[6] Carriage may involve multiple sites of the

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https://doi.org/10.1016/j.jinf.2019.09.018

body, including the nose, oropharynx, and perineum, with the anterior nares being the most frequent site of *S. aureus* colonization.[7] Based on patterns of *S. aureus* carriage observed in longitudinal studies, healthy adults can be classified into 2–3 groups. In some studies, individuals have been categorized as carriers and non-carriers,[8–10] while other studies categorize them as persistent carriers, intermittent carriers, and non-carriers.[11–14] This difference in categorization may be due to sampling techniques. Indeed the definition of persistent carriage can vary according to study criteria; persistent carriers can generally be described as individuals who are consistently positive for *S. aureus* carriage during serial sampling.[15] Intermittent carriers are not consistently positive for *S. aureus* carriage on serial sampling, while non-carriers are individuals with no positive sampling results.[15] It is estimated that approximately 20% of individuals are persistent carriers, 30% are intermittent carriers, and 50% are non-carriers.[7]

While *S. aureus* colonization generally does not result in symptomatic infection, there is a well-established association between *S. aureus* nasal carriage and potential subsequent infection (including SSI).[16–19] Patients colonized with methicillin-resistant *S. aureus* (MRSA) are more likely to develop an SSI, with the colonizing strain usually identified as the infecting organism.[18,20] Decolonization strategies[21] including *S. aureus* screening, followed by nasal mupirocin ointment and chlorhexidine gluconate application, have been associated with significant reductions in *S. aureus* infections in patients undergoing orthopaedic surgery including hip and knee arthroplasty, spinal surgery, and cardiac surgery.[22–25] World Health Organization (WHO) guidelines therefore strongly recommend decolonization of nasal carriers of *S. aureus* in these surgical populations.[26] Given the importance of colonization as a risk factor for *S. aureus* infection, a comprehensive understanding of *S. aureus* carriage is important in the development of novel preventative strategies such as prophylactic vaccines. Previous studies of meningococcal, and pneumococcal capsular polysaccharide conjugate vaccines have shown that vaccination can affect bacterial carriage by preventing acquisition.[27]

Although *S. aureus* vaccines have been developed to provide protection against invasive infection, to date, no vaccine has been found to be efficacious.[28,29] While some studies of investigational *S. aureus* vaccines have assessed carriage prior to vaccination,[30] there is limited data on the impact of investigational *S. aureus* vaccines on colonization. A study of an *S. aureus* vaccine candidate that is no longer in development (StaphVAX, Nabi Biopharmaceutical, Rockville, MD, USA) showed no apparent changes in *S. aureus* nasal colonization rates after vaccination;[30] however, this study was not powered to demonstrate reduction in colonization. It is clear that to power a study correctly, a comprehensive understanding of baseline carriage dynamics is required.

A *S. aureus* 4-antigen vaccine (SA4Ag) was being developed to prevent invasive *S. aureus* disease in at-risk adults;[31,32] the further development of SA4Ag is now under evaluation due to recent declaration of futility (low probability that the study will meet its primary efficacy objective) in the Phase 2b efficacy study.[33] A Phase 1 study to evaluate the safety and immunogenicity of a first-generation 3-antigen vaccine formulation (SA3Ag) was previously conducted in healthy Australian adults 18–24 and 50–85 years of age (ClinicalTrials.gov identifier: NCT01018641).[34,35] The results of this study showed that a single dose of SA3Ag elicited robust and sustained functional immune responses with no notable safety or tolerability concerns. In this context, immune responses are considered functional due to their ability to facilitate the killing of *S. aureus* or neutralization of associated virulence mechanisms, as assessed using different antigen-specific assays. Participants were also assessed for *S. aureus* colonization at the nasal, oropharyngeal, and perineal anatomical sites at multiple time points in this 12-month study, thus providing important baseline data on *S. aureus* carriage dynamics in Australian adults.

Methods

Study population

This first-in-human, Phase 1, participant- and investigator-blinded, sponsor-unblinded, ascending dose level, randomized, placebo-controlled study was conducted at five study centers in Australia. Healthy adults 18–24 years and 50–85 years of age were enrolled into this study.[34,35] This study was approved by the Human Research Ethics Committee of each participating institution. All participants provided written informed consent prior to undergoing any study-related procedures. SA3Ag was administered as a non-adjuvanted, lyophilized vaccine containing capsular polysaccharide serotypes 5 and 8 (CP5 and CP8) conjugated to the non-toxic mutant form of diphtheria toxin (cross-reactive material 197 [CRM197]) and a recombinant mutated form of surface protein clumping factor A (rClfA*m*) at low, medium, and high dose levels, reconstituted with 60 mM NaCl, as reported elsewhere.[34] Randomization within each SA3Ag dose level cohort (low, mid, and high) was 3:1 active to placebo, such that overall, randomization was approximately 1:1:1:1 (low:mid:high:placebo). At 6 months after the initial injection in Stage 1, participants who had received the active vaccine and continued into Stage 2 were randomized to receive a booster vaccination of SA3Ag at the same dose level or placebo at a 1:1 ratio. Those who received placebo in Stage 1 and continued into Stage 2 received placebo again. Administration of active vaccine or placebo in both Stage 1 and Stage 2 was carried out in a blinded manner by study site staff.

Laboratory assessment

Swab samples were obtained from the nares, oropharynx, and perineum of all participants on the day of vaccination (prior to vaccine administration) and at multiple time points (baseline, days 8, 15, 29 and months 2, 3, 6, 7, 9, and 12) for *S. aureus* culture. Participants were permitted to self-collect perineal swabs, with instructions provided on how to obtain a satisfactory sample.

S. aureus isolates were identified using standard methodology.[36] Briefly, *Staphylococcus* species were identified on selective (CNA; colistin, nalidixic acid agar; Hardy Diagnostics, Santa Maria, CA) and non-selective (horse blood agar) plates followed by Gram-staining, catalase testing, and latex agglutination.[36,37] DNAse plate testing[38] was performed to confirm *S. aureus* and a single isolate per subject/visit/body site was selected for further characterization. Cefoxitin disk testing was used to establish MRSA as previously described.[39]

S. aureus isolates were genotyped using *S. aureus* protein A gene (*spa*) typing.[40] *Spa* typing is a single locus strain typing method for *S. aureus* that is proven to provide discriminatory power for evaluation of local hospital outbreaks as well as global surveillance. DNA sequence analysis of a polymorphic 21 to 30 base pair variable number tandem repeat region in the 3′ coding region of the *spa* gene is the basis for the technique. Each new base/repeat composition of the polymorphic repeat region is assigned a unique repeat code and the aggregate of the repeat codes for a given strain determines its *spa* type assignment.

Comparative studies of multiple genotyping methods revealed that *spa* typing is more discriminating than pulsed-field gel electrophoresis and multi-locus sequence typing.[40] S*pa* clusters align well with multi-locus sequence typing, which permits assignment to established clonal clusters.[41] The laboratories that performed the microbiology and *spa* typing included Sonic, New South Wales,

Australia (*S. aureus* identification and methicillin susceptibility), and the Public Health Research Institute, Newark, USA (*spa* typing of *S. aureus* isolates). Molecular characterization of the isolates by *spa* typing facilitated an understanding of the overall diversity of isolates in the collection, enabling determination of participants who were colonized with multiple isolates (genetically different) at the same anatomical site (over multiple visits) and at different anatomical sites. Molecular characterization was used to define persistent carriage as well as acquisition of isolates through the term of the study.

For isolates that failed to generate valid *spa* type sequences or in cases where the *spa* type sequence could not be unambiguously assigned to a clonal complex, further characterization using whole genome sequencing (WGS) analysis was performed as previously described.[42] Multi-locus sequence typing (MLST) was performed as previously described[43] and assigned MLST types were used to cluster isolates to clonal complexes.[44] Following this procedure, only 7 isolates remained untyped.

Persistence of *S. aureus* colonization was defined as ≥3 consecutive visits that were positive for *S. aureus* with the same *spa* type. *S. aureus* isolates were assumed to be the same strain if the *spa* type was identical over different visits. Acquisition of *S. aureus* colonization was defined as negative at baseline and positive for any *spa* type at ≥1 subsequent visit. Clearance of *S. aureus* colonization was defined as a positive swab at baseline and a negative swab at ≥1 subsequent visit.

Immunogenicity

The impact of *S aureus* colonization and acquisition on the immune response of the participant to the initial dose of SA3Ag was also evaluated. Immunogenicity was assessed using a triplex competitive Luminex® immunoassay (cLIA) which measures competition between serum immunoglobulins and antigen-specific monoclonal antibodies for binding to the respective antigen-coated microspheres.[45]

Statistical analysis

No formal statistical analysis was conducted and results are descriptive. This exploratory analysis was not powered to assess the impact of SA3Ag on colonization (including acquisition). The Stages 1 and 2 modified intent-to-treat colonization populations included all randomized participants who had at least one valid and determinate assay result from blood draws at Stage 1 or Stage 2.

Geometric mean titers (GMT) were calculated at each time point for the following sets of participants: (a) negative for *S. aureus* at each visit, (b) first positive for *S. aureus* at day 15, (c) first positive at day 29, (d) first positive at month 3, and (e) positive at all visits. Geometric means were obtained by taking log transformations of the titers, calculating arithmetic averages, then exponentiating the result. The intention was to assess the effect, if any, of acquisition of *S. aureus* on subsequent titers.

Results

A total of 408 consenting participants (including those who received placebo) were included in the Stage 1 colonization and immunogenicity component. All participants were randomized into four groups (102 participants per vaccination group, each comprising 78 participants 50–85 years of age and 24 participants 18–24 years of age). Generally, demographic characteristics were comparable among vaccine groups in the 50–85-year age stratum.[34] All participants received study vaccination, except for one participant in the mid-dose SA3Ag group who was assessed as ineligible after randomization but prior to vaccination (due to receipt of a prohibited medication) and one participant in the high-dose SA3Ag group who withdrew consent prior to vaccination.

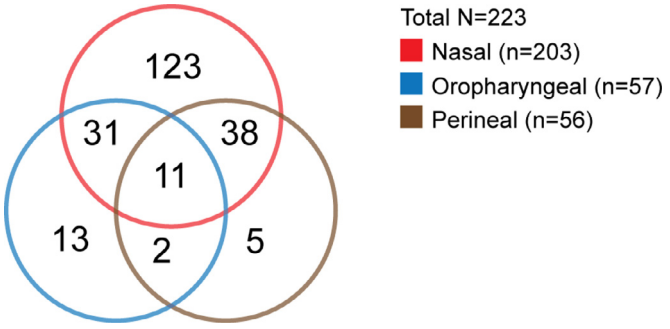

Fig. 1. Prevalence of *S. aureus* colonization at the nasal, oropharyngeal, and perineal sites at any visit.
The number of nasal (105) and perineal (12) isolates at baseline differs from the numbers presented in Table 1 (nasal: 110; perineal: 13) due to the fact that 5 baseline nasal isolates and one baseline perineal isolate were not viable and, therefore, could not be *spa* typed.

In all, 406 participants were vaccinated (low-dose: 102; mid-dose: 101; high-dose: 101; and placebo: 102). One additional participant withdrew before day 29 due to refusal to undergo additional blood draws. A total of 392 participants completed the month 6 visit. Of the 345 participants who entered Stage 2, 297 received a booster dose of SA3Ag or placebo (low-dose: 45; mid-dose: 44; high-dose: 27; and placebo: 181), and 328 participants completed the study, as previously described.[35] During the 12-month study, a total of 1248 *S. aureus* isolates were obtained from 223 participants for *spa* typing.

S. aureus colonization prevalence

Prevalence (at baseline)

Overall, the prevalence of *S. aureus* colonization at any anatomical site at baseline was 30.6% (range across vaccine groups: 24.0% to 45.6%) (Table 1). Nasal carriage was most frequently observed, with 27.0% colonized with *S. aureus* at baseline (range across vaccine groups 20–38.6%) (Table 1). Carriage occurred less frequently at non-nasal sites, with only 3.2% of participants colonized with *S. aureus* at the oropharyngeal anatomical site and 3.2% at the perineal anatomical sites at baseline. Participants may have been positive for *S. aureus* carriage at multiple anatomical sites. MRSA carriage rates at baseline were low overall, with only 0.7% of participants identified as positive at the nasal anatomical site.

Overall prevalence (at any visit)

Within each anatomical site, the prevalence of *S. aureus* remained generally consistent across vaccine groups and visits. There were no substantial differences in colonization of participants aged 18–24 and 50–85 years due to either methicillin-susceptible *S. aureus* or MRSA (data not shown). Following the initial vaccination, there were no clear differences in the rates of *S. aureus* carriage between placebo recipients and SA3Ag recipients. Administration of the booster dose did not appear to have a noticeable effect on the prevalence of *S. aureus* carriage.

Prevalence at different anatomical sites (at any visit)

Of the colonized participants ($N = 223$), 203 (91%) had nasal colonization at any visit and 123/203 (61%) were colonized only in the nose (Fig. 1). For participants with oropharyngeal ($N = 57$, 26%) and perineal colonization ($N = 56$, 25%) a minority of participants were carriage-positive only in the oropharynx (13/57, 23%) or perineum (5/56, 9%) (Fig. 1).

Table 1
S. aureus carriage in the mITT colonization study population.

Anatomic Site	Visit	Stage 1 vaccination/Stage 2 vaccination (as randomized)							
		Placebo/None and placebo/Placebo [*n*/*N* (%)][a]	Low-dose SA3Ag/None and low-dose SA3Ag/Placebo [*n*/*N* (%)][a]	Low-dose SA3Ag/Low-dose SA3Ag [*n*/*N* (%)][a]	Mid-dose SA3Ag/None and Mid-dose SA3Ag/Placebo [*n*/*N* (%)][a]	Mid-dose SA3Ag/Mid-dose SA3Ag [*n*/*N* (%)][a]	High-dose SA3Ag/None and high-dose SA3Ag/Placebo [*n*/*N* (%)][a]	High-dose SA3Ag/High-dose SA3Ag [*n*/*N* (%)][a]	Total [*n*/*N* (%)][a]
Nasal	Stage 1								
	Day 1	27/102 (26.5)	22/57 (38.6)	12/45 (26.7)	15/58 (25.9)	10/44 (22.7)	15/75 (20.0)	9/27 (33.3)	110/408 (27.0)
	Day 8	24/102 (23.5)	20/56 (35.7)	12/44 (27.3)	15/57 (26.3)	12/44 (27.3)	17/73 (23.3)	8/27 (29.6)	108/403 (26.8)
	Day 15	23/102 (22.5)	21/56 (37.5)	13/45 (28.9)	13/56 (23.2)	15/44 (34.1)	21/74 (28.4)	10/27 (37.0)	116/404 (28.7)
	Month 1	26/102 (25.5)	22/57 (38.6)	14/45 (31.1)	14/56 (25.0)	15/44 (34.1)	23/74 (31.1)	8/27 (29.6)	122/405 (30.1)
	Month 2	21/101 (20.8)	20/55 (36.4)	13/45 (28.9)	13/55 (23.6)	11/44 (25.0)	18/73 (24.7)	9/27 (33.3)	105/400 (26.3)
	Month 3	20/100 (20.0)	20/55 (36.4)	11/45 (24.4)	16/53 (30.2)	8/44 (18.2)	22/72 (30.6)	6/27 (22.2)	103/396 (26.0)
	Stage 2								
	Month 6	19/80 (23.8)	14/44 (31.8)	11/45 (24.4)	10/43 (23.3)	12/44 (27.3)	17/62 (27.4)	5/27 (18.5)	88/345 (25.5)
	Month 7	24/79 (30.4)	14/44 (31.8)	13/44 (29.5)	13/42 (31.0)	9/44 (20.5)	20/61 (32.8)	7/27 (25.9)	100/341 (29.3)
	Month 9	23/78 (29.5)	16/43 (37.2)	11/43 (25.6)	10/41 (24.4)	15/44 (34.1)	19/58 (32.8)	9/27 (33.3)	103/334 (30.8)
	Month 12	19/76 (25.0)	15/43 (34.9)	10/42 (23.8)	11/40 (27.5)	14/44 (31.8)	16/56 (28.6)	8/27 (29.6)	93/328 (28.4)
Oropharyngeal	Stage 1								
	Day 1	2/102 (2.0)	3/57 (5.3)	2/45 (4.4)	0/58 (0.0)	3/44 (6.8)	3/75 (4.0)	0/27 (0.0)	13/408 (3.2)
	Day 8	2/102 (2.0)	1/56 (1.8)	1/44 (2.3)	0/57 (0.0)	2/44 (4.5)	2/73 (2.7)	0/27 (0.0)	8/403 (2.0)
	Day 15	1/102 (1.0)	1/56 (1.8)	1/45 (2.2)	1/56 (1.8)	1/44 (2.3)	3/74 (4.1)	0/27 (0.0)	8/404 (2.0)
	Month 1	4/102 (3.9)	4/57 (7.0)	2/45 (4.4)	1/56 (1.8)	0/44 (0.0)	3/74 (4.1)	0/27 (0.0)	14/405 (3.5)
	Month 2	1/101 (1.0)	1/55 (1.8)	1/45 (2.2)	1/55 (1.8)	2/44 (4.5)	2/73 (2.7)	0/27 (0.0)	8/400 (2.0)
	Month 3	1/100 (1.0)	1/55 (1.8)	2/45 (4.4)	2/53 (3.8)	3/44 (6.8)	1/72 (1.4)	0/27 (0.0)	10/396 (2.5)
	Stage 2								
	Month 6	3/80 (3.8)	1/44 (2.3)	1/45 (2.2)	3/43 (7.0)	0/44 (0.0)	2/62 (3.2)	0/27 (0.0)	10/345 (2.9)
	Month 7	0/79 (0.0)	2/44 (4.5)	3/44 (6.8)	1/43 (2.3)	0/44 (0.0)	2/61 (3.3)	1/27 (3.7)	9/342 (2.6)
	Month 9	3/78 (3.8)	1/43 (2.3)	0/43 (0.0)	3/41 (7.3)	5/44 (11.4)	2/58 (3.4)	0/27 (0.0)	14/334 (4.2)
	Month 12	0/76 (0.0)	0/43 (0.0)	0/42 (0.0)	1/40 (2.5)	1/44 (2.3)	1/56 (1.8)	1/27 (3.7)	4/328 (1.2)
Perineal	Stage 1								
	Day 1	4/102 (3.9)	3/57 (5.3)	1/45 (2.2)	1/58 (1.7)	4/44 (9.1)	0/75 (0.0)	0/27 (0.0)	13/408 (3.2)
	Day 8	3/102 (2.9)	0/56 (0.0)	1/44 (2.3)	3/57 (5.3)	1/44 (2.3)	0/73 (0.0)	0/27 (0.0)	8/403 (2.0)
	Day 15	2/102 (2.0)	0/56 (0.0)	2/45 (4.4)	1/56 (1.8)	1/44 (2.3)	2/74 (2.7)	0/27 (0.0)	8/404 (2.0)
	Month 1	2/102 (2.0)	2/57 (3.5)	2/45 (4.4)	1/56 (1.8)	2/44 (4.5)	0/74 (0.0)	2/27 (7.4)	11/405 (2.7)
	Month 2	5/101 (5.0)	0/55 (0.0)	1/45 (2.2)	1/55 (1.8)	2/44 (4.5)	0/73 (0.0)	1/27 (3.7)	10/400 (2.5)
	Month 3	3/100 (3.0)	0/55 (0.0)	2/45 (4.4)	2/53 (3.8)	1/44 (2.3)	1/72 (1.4)	2/27 (7.4)	11/396 (2.8)
	Stage 2								
	Month 6	4/80 (5.0)	0/44 (0.0)	1/45 (2.2)	1/43 (2.3)	2/44 (4.5)	0/62 (0.0)	0/27 (0.0)	8/345 (2.3)
	Month 7	2/79 (2.5)	1/44 (2.3)	3/44 (6.8)	2/43 (4.7)	1/44 (2.3)	2/61 (3.3)	1/27 (3.7)	12/342 (3.5)
	Month 9	3/78 (3.8)	1/43 (2.3)	1/43 (2.3)	0/41 (0.0)	6/44 (13.6)	2/58 (3.4)	1/27 (3.7)	14/334 (4.2)
	Month 12	0/76 (0.0)	2/43 (4.7)	2/42 (4.8)	0/40 (0.0)	3/44 (6.8)	0/56 (0.0)	3/27 (11.1)	10/328 (3.0)
Any anatomic site[b]	Stage 1								
	Day 1	28/102 (27.5)	26/57 (45.6)	15/45 (33.3)	15/58 (25.9)	14/44 (31.8)	18/75 (24.0)	9/27 (33.3)	125/408 (30.6)
	Day 8	28/102 (27.5)	20/56 (35.7)	13/44 (29.5)	16/57 (28.1)	14/44 (31.8)	18/73 (24.7)	8/27 (29.6)	117/403 (29.0)
	Day 15	24/102 (23.5)	22/56 (39.3)	15/45 (33.3)	13/56 (23.2)	16/44 (36.4)	23/74 (31.1)	10/27 (37.0)	123/404 (30.4)
	Month 1	29/102 (28.4)	25/57 (43.9)	15/45 (33.3)	14/56 (25.0)	16/44 (36.4)	24/74 (32.4)	10/27 (37.0)	133/405 (32.8)
	Month 2	24/101 (23.8)	20/55 (36.4)	14/45 (31.1)	13/55 (23.6)	13/44 (29.5)	18/73 (24.7)	9/27 (33.3)	111/400 (27.8)
	Month 3	20/100 (20.0)	20/55 (36.4)	14/45 (31.1)	17/53 (32.1)	10/44 (22.7)	23/72 (31.9)	8/27 (29.6)	112/396 (28.3)
	Stage 2								
	Month 6	20/80 (25.0)	14/44 (31.8)	12/45 (26.7)	11/43 (25.6)	14/44 (31.8)	18/62 (29.0)	5/27 (18.5)	94/345 (27.2)
	Month 7	24/79 (30.4)	15/44 (34.1)	15/44 (34.1)	14/43 (32.6)	10/44 (22.7)	22/61 (36.1)	8/27 (29.6)	108/342 (31.6)
	Month 9	25/78 (32.1)	16/43 (37.2)	11/43 (25.6)	10/41 (24.4)	20/44 (45.5)	21/58 (36.2)	9/27 (33.3)	112/334 (33.5)
	Month 12	19/76 (25.0)	16/43 (37.2)	10/42 (23.8)	11/40 (27.5)	16/44 (36.4)	17/56 (30.4)	10/27 (37.0)	99/328 (30.2)

S. aureus carriage was positive if the colony count was ≥1.
[a] *n* = Number of participants with positive *S. aureus* at that visit; *N* = number of participants with swab at that visit.
[b] *n* = Number of participants with positive *S. aureus* at any anatomic site at that visit; *N* = number of participants with at least one swab at that visit.
Abbreviations: mITT, modified intent-to-treat.

Spa *typing (at any visit)*

Of the colonized participants (*N* = 223), 165 (74.0%) were colonized with single strains (i.e. all strains carried in all colonized sites had the same *spa* type) rather than multiple strains. A total of 26.0% (*N* = 58) of participants were colonized by multiple strains, with only one strain identified at each anatomical site at each visit (no more than one isolate from each site was selected for identification). Participants were colonized by multiple strains at the same anatomical site (55.2%) at different visits or at different anatomical sites (44.8%); most instances of multiple strain colonization at the same anatomical site were observed in the nasal site (96.8%), followed by the oropharyngeal site (3.1%). A total of 26 (11.7%) participants had co-colonization with multiple strain types (2–3) in different anatomical sites, which included the nasal site in all cases.

A significant diversity of *spa* types was found in this study population (Table 2). The majority (57%) of colonized participants were positive for *spa* types found in <3 participants only, or for novel *spa* types that had not previously been identified (Table 2).

S. aureus isolates from several different clonal complexes (CC; based on *spa* typing and WGS analysis) were identified at each of the study sites (Fig. 2). Isolates belonging to CC5, 22, 34, 45, and 88 were found in participants at all 5 study sites, with each site having isolates from approximately 8–20 CCs (Fig. 2). The Adelaide 2 study site had the lowest diversity of isolates, with only isolates belonging to CC20, 22, 30, 34, 45, 5, 8, and 88 identified (Fig. 2). However, compared with the other study sites, the Adelaide 2 study site had the largest proportion of isolates belonging to CC45 (17.4%). Examining the isolates from all the study sites together, the most commonly observed CCs overall were CC5 (14.6%), CC15 (13.3%), CC30 (8.8%), and CC45 (7.7%).

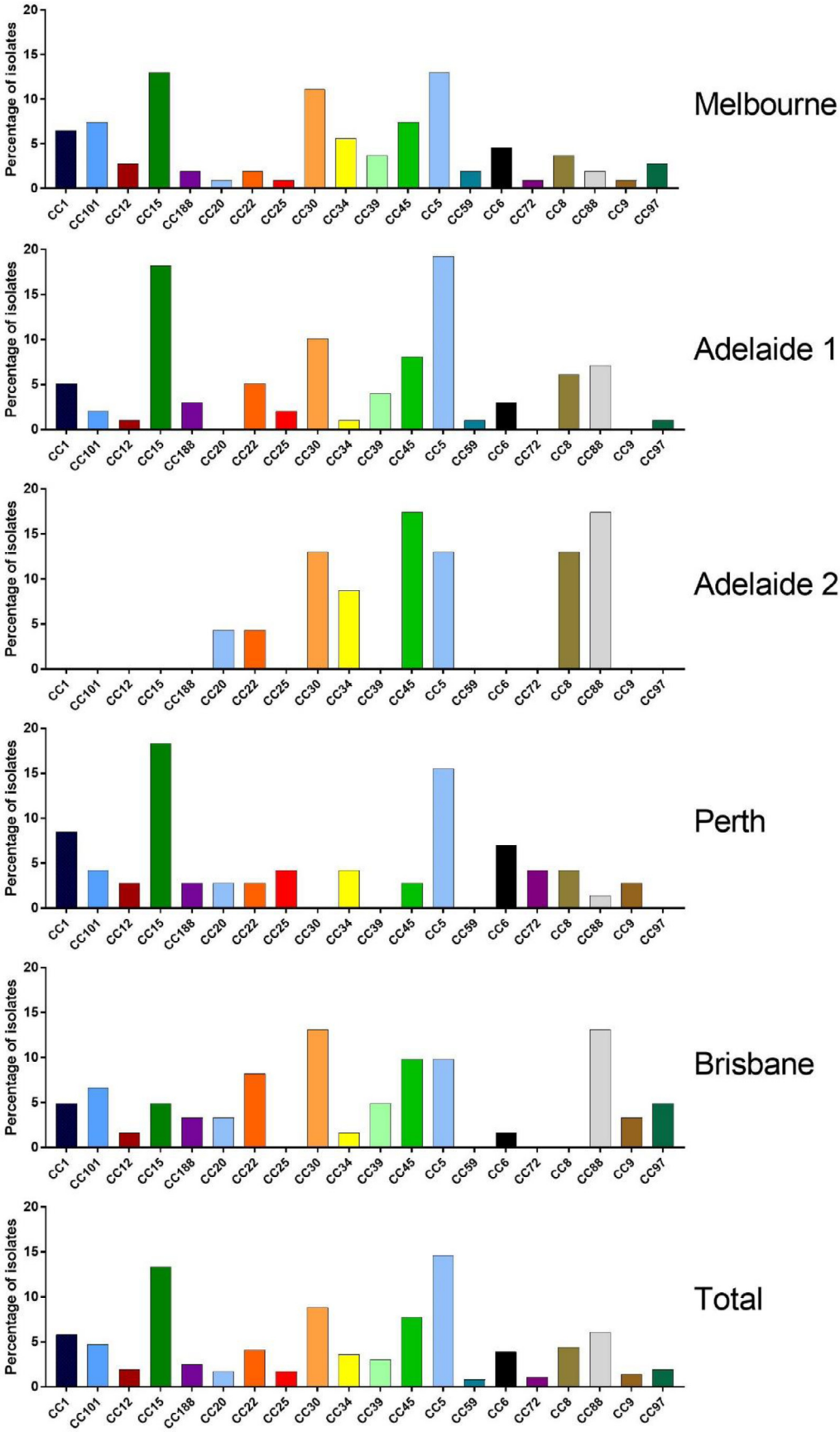

Fig. 2. Clonal complex diversity of *S. aureus* isolates by investigational site.
Clonal complex was inferred from *spa* type. A total of 7 isolates remained untyped following *spa* typing and WGS analysis. CC, clonal complex.

Table 2
Distribution of *spa* types at any visit, mITT colonization population.

Spa type	$N^a = 289 n^b$ (%)	Inferred clonal complex
t035	15 (5.1)	CC1
t021	13 (4.4)	CC15
t033	12 (4.1)	CC30
t002	11 (3.8)	CC5
t122	7 (2.4)	CC188
t073	6 (2.0)	CC45
t001	5 (1.7)	CC8
t029	5 (1.7)	CC5
t154	5 (1.7)	CC6
t155	5 (1.7)	CC15
t565	5 (1.7)	CC34
t105	4 (1.4)	CC97
t141	4 (1.4)	CC12
t156	4 (1.4)	CC15
t176	4 (1.4)	CC5
t370	4 (1.4)	CC5
t414	4 (1.4)	CC20
t203	3 (1.0)	CC5
t532	3 (1.0)	CC30
t960	3 (1.0)	CC15
Other/new *spa* type[c]	167 (57.0)	N/A

Spa types are counted per participant after combining all visits, but no *spa* type is counted more than once for any participant.
[a] N = total number of participants with at least one positive swab; in some cases subjects carried multiple strains with different *spa* types; therefore the N is greater than 223.
[b] n = number of participants with indicated *spa* type in at least one visit.
[c] Includes *spa* types found in less than 3 participants and novel *spa* types not previously identified.
Abbreviations: mITT, modified intent-to-treat.

Persistence of S. aureus *colonization*

S. aureus persistence after the initial vaccination (positive for *S. aureus* at ≥3 consecutive visits in Stage 1, including visits conducted at baseline, days 8, 15, 29 and months 2 and 3) at the nasal anatomical site due to colonization by a single *spa* type was observed in 19.6% of placebo recipients, and 30.7%, 25.0%, and 20.8% of low-, mid-, and high-dose SA3Ag recipients, respectively. Only 2 participants had persistent colonization with the same *S. aureus spa* type at the oropharyngeal site, and 3 participants at the perineal site.

The dynamics of *S. aureus* carriage at the nasal anatomical site across selected visits through 12 months is displayed in Fig. 3(A) and (B). A total of 22/77 (28.6%) of SA3Ag recipients and 7/27 (25.9%) of placebo recipients were positive at baseline and at each of the selected visits through month 12.

Acquisition of S. aureus *colonization*

A higher proportion of participants in the mid- and high-dose SA3Ag groups (20.0% and 20.8%, respectively) had acquired *S. aureus* colonization in at least one anatomical site after the initial vaccination (negative at baseline and positive for any *spa* type at any visit through month 3), compared with participants receiving placebo or low-dose SA3Ag (12.7% and 12.9%, respectively) (Table 3). However this acquisition was generally transient, with only a small proportion of these participants becoming persistent carriers (placebo: 2.9%, low-dose SA3Ag: 5.9%, mid-dose SA3Ag: 8.0%, and high-dose SA3Ag: 3.0%). There were no notable differences identified between age groups, with the exception of the high-dose group at the nasal anatomical site, where the older age group acquired more *S. aureus* colonization (Stage 1: 23.4% in older participants compared to 8.3% in younger participants). Nasal acquisition of *S. aureus* is shown at selected visits through month 12 (Fig. 3(A) and (B)), and was observed in 34.5% of SA3Ag recipients compared with 20% of placebo recipients.

The acquisition rates were also used to estimate the size of a clinical study that would be required to determine whether SA3Ag could have an effect on *S. aureus* acquisition with a power of over 80% (Table 4). Power was obtained with 2-sided alpha=0.05. Given the acquisition proportions for placebo recipients (Table 3), sample sizes needed to detect a 50% reduction in acquisition in the SA3Ag group were calculated, although the results of this exploratory analysis did not show lower acquisition among SA3Ag recipients compared with placebo recipients. Study sizes varied depending on the anatomical site investigated, from approximately 359 participants per group (any anatomical site) to >1200 (perineal) participants per group (Table 4).

Clearance of S. aureus *colonization*

There were no clear differences between placebo and SA3Ag recipients in the proportions positive for *S. aureus* at any time point. Within each anatomical site, similar percentages of participants demonstrated clearance of colonization across vaccine groups, defined as positive for *S. aureus* colonization at baseline and negative for *S. aureus* colonization at ≥1 subsequent visit.

Immunogenicity following acquisition of S. aureus *colonization*

Overall, there were no discernible differences in the immune responses to vaccination among participants grouped by timing of *S. aureus* acquisition, as measured by cLIA GMT for each antigen following nasal acquisition of *S. aureus* at any time point (Fig. 4). Participants who were positive for *S. aureus* colonization (regardless of acquisition time) still exhibited substantially elevated cLIA GMTs; these GMTs were similar in magnitude to those of participants that were negative for *S. aureus* colonization at all visits.

Discussion

This study describes *S. aureus* colonization in Australian adults who participated in a Phase 1 investigational *S. aureus* 3-antigen vaccine (SA3Ag) clinical trial.[34,35] A single dose of SA3Ag had an acceptable safety profile and elicited robust functional immune responses in adults 18–24 and 50–85 years of age. cLIA titers were up to approximately 80-fold (CP5), 30-fold (CP8), and 40-fold (ClfA) higher than baseline at day 29 after vaccination in adults 50–85 years of age.[34] Antibody titers remained substantially above baseline through 12 months after vaccination, with little additional booster response seen in participants administered a second dose of SA3Ag at 6 months.[35] Colonization results from this analysis showed that the majority of *S. aureus* isolates were methicillin-susceptible and that the nasal anatomical site was more frequently colonized compared with the perineum and oropharynx. Baseline *S. aureus* carriage rates at the nasal and perineal anatomical sites (20.0–38.6% and 0.0–9.1%, respectively) were similar to rates in a carriage study conducted in the USA (25.1% and 4.9%, respectively).[46] However, baseline *S. aureus* carriage rates in the oropharynx were higher in the participants in the USA (18.7%) compared with this Australian study (0.0–6.8%). Reasons for this difference are unknown. Despite low MRSA carriage rates observed in the community in this study, MRSA-associated clinical disease is seen in up to 32% of SSIs[47] and 19% of cases of *S. aureus* bacteremia[48] in Australia. SA3Ag and SA4Ag were designed to be effective against both methicillin-sensitive *S. aureus* (MSSA) and MRSA.

Due to the substantial burden of invasive *S. aureus* disease, there remains an unmet medical need to develop a safe and effective *S. aureus* vaccine, especially given that previous attempts have failed.[49–51] While one previous *S. aureus* vaccine study assessed *S. aureus* carriage prior to vaccination,[52] there are few data on the

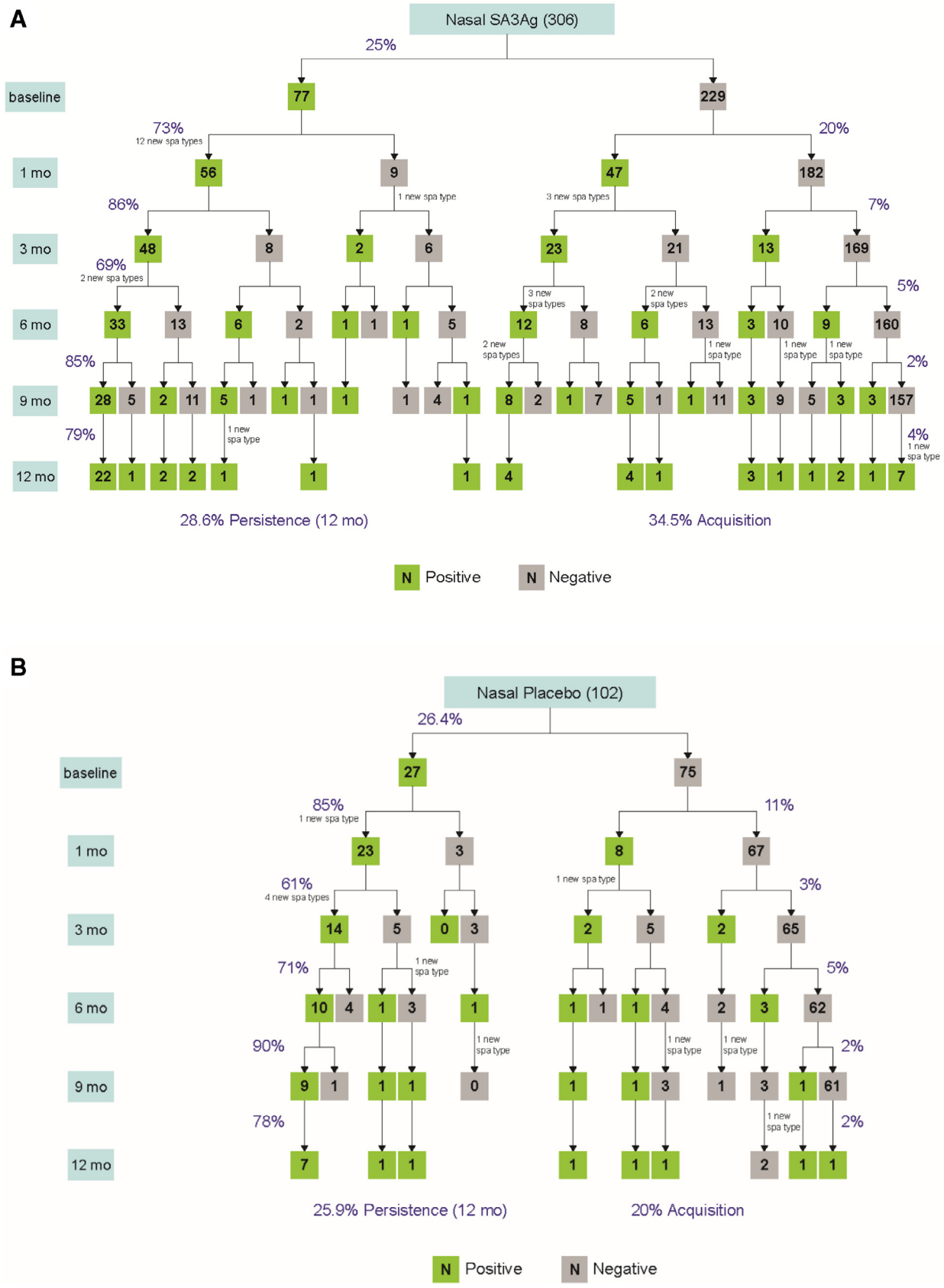

Fig. 3. Tree diagrams showing persistence and acquisition of nasal *S. aureus* colonization in the SA3Ag (A) and placebo (B) groups over 12 months. The number of patients who were positive or negative for *S. aureus* colonization at each stage are shown in green and gray boxes, respectively.
mo, months.

Table 3
Acquisition of *S. aureus* colonization (Stage 1 mITT colonization population).

Anatomic site	Vaccine group (as randomized)							
	Placebo		Low-dose SA3Ag		Mid-dose SA3Ag		High-dose SA3Ag	
	N^a	n^b (%)	N^a	n^b (%)	N^a	n^b (%)	N^a	n^b (%)
Nasal	102	10 (9.8)	101	14 (13.9)	100	21 (21.0)	101	20 (19.8)
Oropharyngeal	102	8 (7.8)	101	8 (7.9)	100	8 (8.0)	101	2 (2.0)
Perineal	102	4 (3.9)	101	5 (5.0)	100	5 (5.0)	101	8 (7.9)
Any site	102	13 (12.7)	101	13 (12.9)	100	20 (20.0)	101	21 (20.8)

Acquisition is defined as negative at baseline and positive for any *spa* type in at least one subsequent visit.
[a] N = number of participants with 3 or more consecutive visits.
[b] n = number of participants with *S. aureus* colonization acquisition.
Abbreviations: mITT, modified intent-to-treat.

Table 4
Sample sizes required to detect 50% lower *S. aureus* acquisition rates in SA3Ag recipients compared with placebo recipients.

Anatomical site	Proportion of placebo recipients (%)	Proposed proportion of SA3Ag recipients (%)	Actual power	*N* (per group)	Total sample size (assuming 2 groups)
Nasal	9.8	4.9	0.801	475	950
Oropharyngeal	7.8	3.9	0.800	605	1210
Perineal	3.9	2.0	0.800	1243	2486
Any site	12.7	6.4	0.801	359	718

Acquisition is defined as negative at baseline and positive for any *spa* type in at least one subsequent visit.

impact of investigational *S aureus* vaccines on carriage pre- and post-vaccination.[30]

In our study, the prevalence of *S. aureus* remained generally consistent across vaccine groups and visits and there was no clear difference between placebo and active vaccine recipients in rates of *S. aureus* carriage after vaccination. Prevalence was different between the nasal, oropharyngeal, and perineal sites. In addition, the booster dose vaccine administered at 6 months after the initial dose had no discernible impact on the prevalence of *S. aureus* carriage. Although a higher proportion of participants in the mid- and high-dose groups acquired *S. aureus* at the nasal site compared with the placebo and low-dose groups, only a small proportion of these individuals became persistent carriers. Immune responses to SA3Ag as measured using the specified assays were not substantially impacted by acquisition of colonization; no assessment was made as to whether there were changes in cellular or other immune functions. Creech and coworkers assessed whether vaccination induced cellular responses and did not observe changes for the factors that they investigated.[31] It is important to note that the study was not powered to evaluate whether the vaccine could impact carriage rates or acquisition of *S. aureus*, and all analyses of colonization were descriptive; therefore conclusions about SA3Ag impact on colonization are limited. However now that the acquisition rates have been established, it is possible to estimate the size of a study that would be required to establish whether an *S. aureus* vaccine has an effect on carriage. Another limitation of the study is the fact that baseline colonization was defined by a single sample taken just prior to vaccine administration, rather than by samples collected over a defined period prior to study entry. The latter approach may have resulted in a more accurate determination of the baseline colonization status of the participants.

Large-scale studies of other bacterial capsular polysaccharide-conjugate vaccines have demonstrated that vaccination can reduce acquisition of bacterial colonization. Following vaccination of infants and toddlers with pneumococcal conjugate vaccines, high IgG concentrations were associated with a reduction in nasopharyngeal acquisition of *Streptococcus pneumoniae* serotypes, with potential impact on transmission and herd protection.[53] Similarly, a vaccination program with the meningococcal serogroup C conjugate vaccine in those under 19 years of age in the United Kingdom led to a reduction in serogroup C meningococcal carriage.[27] This reduction was noted in both vaccinated and unvaccinated individuals, without replacement by other meningococcal serotypes. In another example, the initiation of a widespread vaccination program with the 7-valent pneumococcal conjugate vaccine in the Netherlands led to changes in bacterial carriage of other pathogens, including an increase in *Streptococcus pneumoniae* nasopharyngeal colonization by the non-vaccine serotype 19A, along with changes in *S. aureus* carriage rates.[54] These findings demonstrate the importance of understanding the impact of vaccines on colonization.

Another important finding of our study is the diverse range of *spa* types identified, many of these being novel in comparison to studies carried out in North America and Europe. Previous studies in healthy populations in Australia and New Zealand have shown similarly diverse *spa* types: however, there is still little understanding of how the distribution of novel *spa* types isolated from carriers in this study relates to disease-causing *spa* types in the Australian context.[55] Data from the US show that diverse *spa* types are associated with both clinical disease and colonization.[56] In our study, CC5 (14.6%), CC15 (13.3%), CC30 (8.8%), and CC45 (7.7%) were the most commonly observed CCs overall. Similarly, a study of community-associated (CA) and community-onset *S. aureus* isolates belonging to CA epidemic clones in European countries also found that CC15 (17%) and CC30 (14%) were among the most commonly observed CCs.[57] However, CC8 (27%) was the most commonly observed CC in the European study, in contrast to our findings. A study of clinical *S. aureus* isolates from an integrated healthcare provider in California in the US found that the most commonly observed CCs were CC8 (40%) and CC5 (20%), with 46% of the isolates tested being MRSA.[58]

Spa typing analyses also indicated that the prevalence of distinct *S. aureus* strains was likely due to persistent carriage of the same strain, as evidenced by a single *spa* type per participant. Carriage of multiple strains of *S. aureus* was observed in >25% of participants in the study. Persistence of colonization with the same *S. aureus spa* type was common at the nasal anatomical site.

Over the last decade, epidemiological, disease, and transmission studies have benefited immensely from the development of WGS analysis.[59] We employed WGS analysis in this study to identify isolates that were not able to be assigned using conventional *spa* typing. The use of WGS in this and other studies has increased the resolution of strain comparisons, and the size and quality

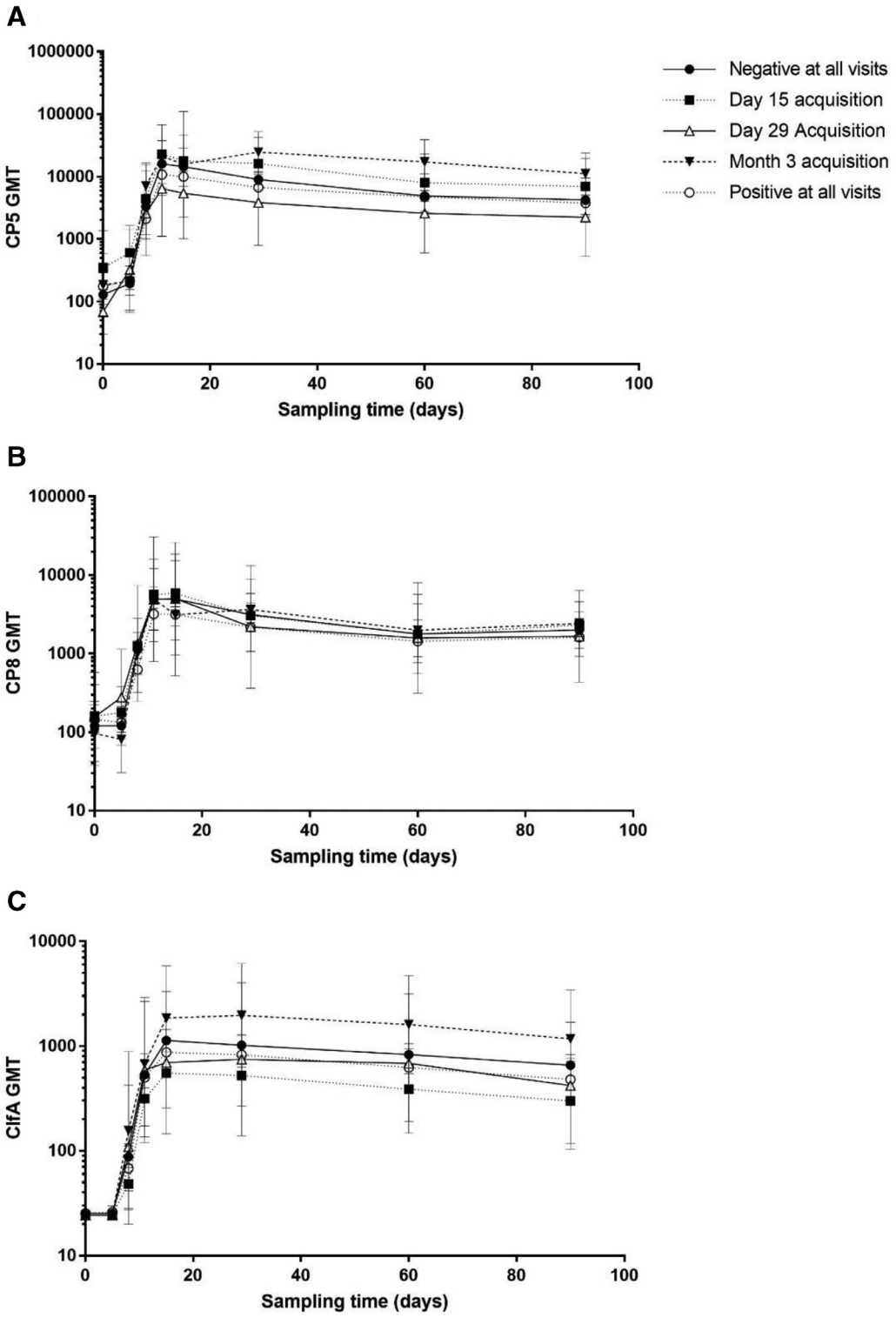

Fig. 4. GMTs for CP5 (A), CP8 (B), and ClfA (C) through month 3 (at the nasal site for Stage 1 mITT colonization and immunogenicity populations [SA3Ag recipients]). Error bars represent 95% confidence intervals.
ClfA, clumping factor A; CP5, capsular polysaccharide serotype 5; CP8, capsular polysaccharide serotype 8; GMT, geometric mean titer; mITT, modified intent-to-treat.

of the genome databases provide a rich resource to correlate genotypic and phenotypic data and identify markers to predict resistance, relative virulence, and outcome. Employing this single technique, clinicians now have access to a large amount of data on a pathogen that can be used to follow a specific isolate through the course of disease. WGS can also be used to explore within-host diversity, as well as to inform treatment decisions.[59] Beyond antibiotic resistance and the presence of specific virulence factors, clonal associations of organisms have been shown to be useful in predicting outcomes.[60]

In conclusion, these results for *S. aureus* colonization show that the isolates likely came from a single strain, as determined

by *spa* typing. The prevalence of *S. aureus* colonization was relatively consistent in both SA3Ag and placebo recipients throughout the 12-month study period. There was no evidence of a SA3Ag vaccine-related effect on *S. aureus* carriage, however, the study colonization analyses were observational and descriptive, and the study was not designed to assess the impact of the vaccine on carriage. Further development of SA4Ag is under evaluation pending analysis of an efficacy study of patients undergoing elective spinal surgery (Study B3451002: *STaphylococcus auReus* Inpatient Vaccine Efficacy [STRIVE]; NCT02388165). Data from the current study may inform the design of future studies to assess the impact of *S. aureus* vaccines on colonization.

Data availability

Upon request, and subject to certain criteria, conditions and exceptions (see https://www.pfizer.com/science/clinical-trials/trial-data-and-results for more information), Pfizer will provide access to individual de-identified participant data from Pfizer-sponsored global interventional clinical studies conducted for medicines, vaccines and medical devices (1) for indications that have been approved in the US and/or EU or (2) in programs that have been terminated (i.e., development for all indications has been discontinued). Pfizer will also consider requests for the protocol, data dictionary, and statistical analysis plan. Data may be requested from Pfizer trials 24 months after study completion. The de-identified participant data will be made available to researchers whose proposals meet the research criteria and other conditions, and for which an exception does not apply, via a secure portal. To gain access, data requestors must enter into a data access agreement with Pfizer.

Funding

This study was sponsored by Pfizer. Pfizer was involved in the study design, data collection, data analysis, preparation of the manuscript and the decision to submit the article for publication. Editorial and medical writing support was provided by Paul Hassan, Ph.D. and Chu Kong Liew, Ph.D. of Engage Scientific Solutions and was funded by Pfizer.

Declaration of Competing Interest

Helen Marshall has been a principal investigator on vaccine clinical trials sponsored by GSK, Pfizer, and Novartis, with her institution receiving funding to conduct these trials. She has received travel support to present scientific data at international meetings from GSK. Her institution has received grant funding for investigator–led studies from Pfizer, GSK, and Seqirus, none being relevant to this study. Michael Nissen receives personal fees from GSK Vaccines as a full-time employee since October 2014 and he has been principal investigator of clinical trials sponsored by Baxter, bioCSL, GSK, Merck, Sanofi, and Pfizer and has received travel support to present these data at scientific conferences. Michael Nissen has also received an independent study grant from Abbott Australia which is not relevant to the submitted work. Peter Richmond has been principal investigator of clinical trials sponsored by GSK, Merck, Sanofi, and Pfizer and has received travel support to present these data at scientific conferences. Peter Richmond has also received institutional funding from GSK for investigator-led studies and participated in scientific advisory boards for Pfizer and GSK. Sepehr Shakib was principal investigator as a contractor on this study at the CMAX clinical trial unit. He has no conflict of interest to declare. Barry Kreiswirth receives consultancy fees from Pfizer and Shionogi. James Baber, William Gruber, Kathrin U. Jansen, Annaliesa S. Anderson, and C. Hal Jones are employees of Pfizer. Joseph Severs, Edward T. Zito, and Joseph Eiden are former employees of Pfizer.

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