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Prevalence of *Staphylococcus aureus* Colonization and Risk Factors for Infection Among Military Personnel in a Shipboard Setting

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ABSTRACT Staphylococcal skin and soft tissue infections (SSTIs), especially those due to methicillin-resistant *Staphylococcus aureus* (MRSA) are an important public health issue for the military. Limited data exist regarding the prevalence of *S. aureus* colonization in the shipboard setting. We conducted a cross-sectional, observational study to determine the point prevalence of *S. aureus* colonization among military personnel onboard a naval vessel. Asymptomatic active duty personnel completed a survey for risk factors associated with colonization and SSTIs. Culture specimens were obtained from the anterior nares, pharynx, groin, and perirectal regions. MRSA isolates underwent testing for antimicrobial resistance, virulence factors, and pulsed-field type. 400 individuals were enrolled, 198 (49.5%) of whom were colonized with *S. aureus*, with MRSA identified in 14 participants (3.5%). No significant risk factors were associated with MRSA colonization. USA800 was the most common colonizing MRSA strain in the cohort and was detected in 10 participants (71%). Two participants (14%) were colonized with USA300 MRSA. In this first report of *S. aureus* epidemiology in a shipboard setting, we observed high rates of *S. aureus* and MRSA colonization. Longitudinal studies are needed to document the incident rates of *S. aureus* colonization during shipboard deployment and its impact on SSTI risk.

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INTRODUCTION

Staphylococcal skin and soft tissue infections (SSTIs), especially those caused by methicillin-resistant *Staphylococcus aureus* (MRSA), are common among military service members.[1] Prior epidemiologic studies in high-risk groups, such as military recruits, have reported MRSA colonization rates between 2% and 6%,[2–5] and have noted a higher risk of subsequent SSTI in MRSA colonized recruits as compared to noncolonized recruits or those colonized with methicillin-sensitive *S. aureus* (MSSA). Moreover, with regard to MRSA SSTI, USA300 has emerged as the predominant pulsed-field type (PFT), causing the bulk of disease in military personnel.[2,5,6]

Although SSTI has been studied in land-based military settings, shipboard deployments represent a similarly high-risk environment for the spread of virulent *S. aureus* strains due to crowded conditions, shared equipment, and limited opportunities for personal hygiene that facilitate colonization.[7] However, there is insufficient data on the epidemiology of *S. aureus* colonization among active duty personnel on shipboard deployments. This is in large part due to the logistical challenges posed by limited laboratory facilities onboard ship, which would be required for culture and storage of colonization swabs.

We conducted a cross-sectional pilot study onboard a naval vessel to evaluate *S. aureus* (MRSA and MSSA) colonization rates among shipboard active duty personnel. We also evaluated the utility of freezing colonization swabs during deployment with subsequent plating after returning to the home port. Finally, we evaluated the genotypic and phenotypic characteristics of MRSA isolates including PFT, antimicrobial resistance, and purported virulence factors.

METHODS

The study was performed on an amphibious assault ship in September 2011 during a 3-week training exercise in the Atlantic Ocean. The ship consisted of 1,000 Navy crewmembers and a contingent of 1,200 Marines. Study recruitment was performed through an introductory e-mail, and approaching crewmember groups while underway. Research staff started enrollment on the second day after leaving the port and completed enrollment in 2 weeks. Adult active duty military members who were willing to comply with study procedures were included in the study. All participants provided written informed consent.

All enrollees completed a survey detailing their demographics, history of SSTI or relevant dermatologic conditions, living quarters, daily routines during deployment, recent history of antibiotic use, and health care exposures. Culture specimens were obtained at enrollment from the anterior nares, pharynx, groin, and perirectal regions. Study coordinators obtained the nares and pharyngeal swabs. Participants were instructed on the collection method of the groin and perirectal swabs, after which the specimens were self-collected. Because of constraints in available laboratory space for processing and storing of samples, culture swabs for the nares and pharynx were streaked onto a single Mannitol Salt Agar (Becton Dickinson, Heidelberg, Germany) plate (termed "nasopharyngeal culture"), as were the culture swabs for the groin and perirectal regions (termed groin/perirectal culture). Mannitol-fermenting colonies were streaked for isolation on Trypicase Soy Agar (Becton Dickinson) with 5% sheep blood (sheep blood agar). Presumed *S. aureus* isolates were inoculated into Microbank vials (Pro-Lab diagnostics, Richmond Hill, Ontario, Canada), after confirming catalase and Staphaurex Plus (Murex diagnostics, Dartford, England) positivity. All presumed *S. aureus* isolates and corresponding culture swabs were stored at −70°C pending return to port (approximately 4 weeks). Upon return, presumed *S. aureus* culture swabs were confirmed using the same microbiologic workup described above, and tested for methicillin resistance using the MicroScan Walkaway-automated system (Beckman Coulter, Brea, California). Colonization was defined as a positive culture for *S. aureus* at either (i.e., nasopharyngeal or groin/perirectal) site.

Additional antibiotic susceptibility testing was carried out on all MRSA isolates using the Kirby–Bauer disc diffusion method according to Clinical and Laboratory Standards Institute guidelines[8] and confirmed using the MicroScan Walkaway-automated system. The inducible MLS_{β} phenotype was detected using the double erythromycin–clindamycin disc test (D-test).

Molecular Testing

Confirmed MRSA isolates underwent pulsed field gel electrophoresis (PFGE) and polymerase chain reaction (PCR) testing. PFGE was carried out according to a published protocol[9]; gels were analyzed using the BioNumerics (Applied Maths Inc., Austin, TX) software and clonality assessed using established criteria.[10] Isolates were screened by PCR for the presence of the staphylococcal chromosomal cassette (*SCC*) *mec* element type using previously published methods.[11] PCR detection was also performed for virulence genes (Panton-Valentine leukocidin [*pvl*], phenol-soluble modulin [*psm-α*], toxic shock syndrome toxin [*tst*], enterotoxins [*seC, seK*]), and factors involved in the establishment of colonization and infection (clumping factor B [*clfB*], sortase enzyme [*srtA*]). Genomic DNA was extracted from isolates using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. Real-time PCR was performed for all virulence and colonization/infection factors with Quantifast SybrGreen (see Table I for primers). Each reaction mixture contained 12.5 μL of SybrGreen (Qiagen, Valencia, CA), 5 μL each of the forward and reverse primers, and 2.5 μL of template DNA. An initial denaturation step of 95°C for 5 minutes was followed by 40 cycles of amplification, 95°C for 30s and 60°C for 10 seconds. All PCR products were run on a 1.5% gel in 1× Tris/Ethylenediaminetetraacetic acid buffer, stained with ethidium bromide and visualized under ultraviolet light.

Statistical Analysis

An enrollment target of 400 individuals was set to detect a point prevalence between 3% and 6% with 95% confidence interval (CI). The point prevalence of MSSA and MRSA colonization was calculated as the total number of study participants with a positive isolate (nasopharyngeal, groin/perirectal, or both) divided by the total number of participants. Odds ratios (ORs) to determine risk factors for MSSA and MRSA colonization were simultaneously estimated using a generalized logit model. Evaluation of time since departure from port was analyzed by a data-driven smooth of log odds of MSSA colonization. All statistical analyses were conducted using SAS software (version 9.1.3; SAS Institute, Cary, NC).

RESULTS

400 active duty personnel were enrolled during a 2-week period, approximately 18% of the ship's total complement. 192 enrollees (48%) were part of the Navy crew, of whom 139 (72%) worked onboard ship before the training exercise for a median period of 13 months (interquartile range [IQR]: 8–23). The remaining participants included 208 individuals (52%) who were part of the Marine augmentation that occurred during and shortly after departure from the home port. The median interval between departure and enrollment was 8 days (IQR: 5–12 days). The median age of enrollees was 22 years (IQR: 21–27 years) and was predominantly male (91%). The naval contingent was significantly older and included more women (mean age: Navy: 28 years vs.

TABLE I. Primers Used for Detection of Virulence Genes

Target Gene	Primer Direction	Primer Sequence (5′–3′)	Amplicon Size (bp)	Positive Control Strain
pvl	Forward	5′-TGAAATGTTTTTAGGCTCAAGAC-3′	82	BAA-1556
	Reverse	5′-CCTTTTTTGGCTGCTTTAAGATT-3′		
srtA	Forward	5′-CCTTTTTTGGCTGCTTTAAGATT-3′	70	700699
	Reverse	5′-CAATTGCAGGACACACTTTCA-3′		
clfB	Forward	5′-GCGTTTTCTAAACAATAGTAATGATCCTA-3′	86	BAA-1556
	Reverse	5′-CAGGAGATAAGAGCGAAAACACA-3′		
tst	Forward	5′-TCCAATAACCACCCGTTTT-3′	68	700699
	Reverse	5′-AATTCGTCATCAGCTAACTCAAA-3′		
psm-α	Forward	5′-TCAAACATAAAACACGCCACAA-3′	76	BAA-1556
	Reverse	5′-CTTGGTGGGACGTACGGAAT-3′		
seA	Forward	5′-GGAAACGGTTAAAACGAATAAGAA-3′	77	700699
	Reverse	5′-TTTCCTGTAAATAACGTCTTGCTT-3′		
seB	Forward	5′-GTATGGTGGTGTAACTGAGC-3′	164	BAA-1556
	Reverse	5′-CCAAATAGTGACGAGTTAGG-3′		
vseC	Forward	5′-CGTTTTAGCAGAGAGTCAACCA-3′	76	700699
	Reverse	5′-ACCCATCGTACCAGTAAACTCA-3′		
seD	Forward	5′-CGTACAAGAATTAGATGCACAAGCA-3′	79	BAA-1556
	Reverse	5′-TCCTCCGAGAGTATCATTATTATACAATTT-3′		
seE	Forward	5′-AGGTTTTTTCACAGGTCATCC-3′	209	27664
	Reverse	5′-CTTTTTTTTCTTCGGTCAATC-3′		
seH	Forward	5′-AATGTCTATATGGAGGTACAACACTAAA-3′	75	MW2
	Reverse	5′-CCCAAACATTAGCACCAATC-3′		
seK	Forward	5′-ATGGCGGAGTCACAGCTACT-3′	197	BAA-1556
	Reverse	5′-TGCCGTTATGTCCATAAATGTT-3′		
seQ	Forward	5′-GGAAATCACAAAACTATTTCTACCA-3′	97	BAA-1556
	Reverse	5′-CTTGAAGGTACTTTCTTAATTTGACA-3′		

Marines 22 years; proportion of females: Navy 18% vs. Marine 1%; $p < 0.05$ for both comparisons).

Staphylococcus aureus Colonization

Preliminary cultures obtained at enrollment from 400 participants identified 239 presumed *S. aureus* isolates. *S. aureus* was recovered from 236 of the 239 (98.7%) frozen swabs recultured 4 weeks later. Three swabs grew coagulase-negative staphylococci on reculture. *S. aureus* colonization was detected in 198 participants (prevalence: 49.5%; 95% CI: 44.6%–54.4%), of whom 14 (prevalence: 7%; 95% CI: 4.5%–9.5%) were colonized with MRSA and 184 (prevalence: 46%; 95% CI: 41.1%–50.9%) were colonized with MSSA. Table II shows the distribution and overlap of colonization sites. Inclusion of the groin/perirectal culture yielded an additional 6 MSSA- and 2 MRSA-positive participants over nasopharyngeal culture alone, representing approximately 5% and 18% of all MSSA and MRSA isolates, respectively. Colonization rates did not differ significantly between participants enrolled in the first week and second week of deployment (first week: MSSA: 44.6% (83/186), MRSA: 4.8% (9/186); second week: MSSA: 54% (116/214), MRSA: 2.34% (5/214); $p > 0.05$).

TABLE II. Distribution of *S. aureus* Colonization Among 400 Active Duty Personnel Enrolled During a Ship-Board Deployment

	Number of Participants Colonized With *S. aureus* (Total Number of Participants = 400)	
Colonization Site	MSSA	MRSA
Nasopharynx Only[a]	140 (35.0)	10 (2.5)
Groin/Perirectal Only[a]	35 (8.8)	2 (0.5)
Nasopharynx and Groin/Perirectal	9 (2.3)	2 (0.5)

MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*.
[a]Culture swabs for the nares and pharynx were streaked onto a single Mannitol Salt Agar plate, termed nasopharyngeal culture; the culture swabs for the groin and perirectal regions were similarly combined and termed groin/perirectal culture.

Participants were surveyed about potential risk factors associated with colonization: 292 (73%) reported gymnasium use several times per week, 188 (47%) reported depilating groin or body hair routinely, and 8 (2%) reported sharing personal items (towels, razors, etc.) with other crewmembers. 15% ($n = 60$) reported a history of SSTI in the prior year, though only 10 of 32 individuals who reported "staph infection" indicated that the diagnosis was culture based.

Univariate risk factors for MSSA colonization included male gender (RR 3.5, 95% CI 1.6–8.0), working onboard ship for >6 months before the training exercise (OR 1.9, 95% CI 1.2–2.9), and self-reported MRSA contact history (OR 2.2; 95% CI 1.0–5.0) (Table III). Navy service members were at significantly lower risk for MSSA colonization than Marines (RR 0.5, 95% CI 0.3–0.7). Age, rank, prior/current history of SSTI, and medical occupation were not significant risk

TABLE III. Factors Associated With MSSA and MRSA Colonization

Demographic		Total (%) (n = 400)	Noncolonized (%) (n = 202)	MSSA Colonized (%) (n = 184)	MSSA Colonization OR (95% CI)	MRSA Colonized (%) (n = 14)	MRSA Colonization OR (95% CI)
Service	Marines	208 (52)	86 (43)	113 (61)	(Ref)	9 (6)	(Ref)
	Navy	192 (48)	116 (57)	71 (39)	**0.5 (0.3–0.7)**	5 (36)	0.4 (0.1–1.3)
Gender	Female	37 (9)	28 (14)	8 (4)	(Ref)	1 (7)	(Ref)
	Male	363 (91)	174 (86)	176 (96)	**3.5 (1.6–8.0)**	13 (93)	2.1 (0.3–16.6)
Age (Years)	≥35	32 (8)	16 (8)	14 (8)	(Ref)	2 (14)	(Ref)
	26–35	91 (23)	55 (27)	33 (18)	0.7 (0.3–1.6)	3 (21)	0.4 (0.1–2.8)
	18–25	277 (69)	131 (65)	137 (7)	1.2 (0.6–2.5)	9 (64)	0.6 (0.1–2.8)
Rank	Officer	29 (7)	16 (8)	11 (6)	(Ref)	2 (14)	(Ref)
	Enlisted	371 (93)	186 (92)	171 (9)	1.1 (0.5–2.4)	12 (86)	0.5 (0.1–2.5)
Stationed Onboard for > 6m[a]		288 (72)	133 (66)	144 (78)	**1.9 (1.2–2.9)**	11 (79)	1.9 (0.5–7)
Prior Medical Occupation		40 (10)	17 (8)	21 (11)	1.4 (0.7–2.7)	2 (14)	1.8 (0.4–9)
History of Repeated SSTI[b]		29 (7)	21 (20)	8 (4)	0.4 (0.2–0.9)	0	
Current Pimples		96 (24)	43 (21)	51 (28)	1.4 (0.9–2.3)	2 (14)	0.6 (0.1–3)
Current SSTI[b]		83 (21)	39 (19)	42 (23)	1.1 (0.7–1.7)	2 (14)	0.7 (0.2–2)
MRSA Contact History[c]		31 (8)	9 (4)	20 (11)	2.2 (1.0–5)	2 (14)	3.2 (0.7–16)

MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*. Odds Ratios associated with p-value < 0.05 are shown in bold. [a]Some members of the Navy crew worked onboard ship before the training exercise for a median period of 13 months (IQR 8–23). [b]SSTI, skin and soft-tissue infection; History of repeated SSTI, history of repeated skin infections over past 5 years. [c]Report of family, friends, or roommate with MRSA.

factors. Among Marines, duration of time aboard the ship was a significant risk factor for MSSA colonization (increased risk of colonization per day [OR 1.07; CI 95% 1.02, 1.13]); no trend was noted for Navy service members. Other factors explored (number of personnel sharing living quarters, shaving practices, and frequency of gymnasium use, shower use, or uniform/towel laundry) were not statistically significant. In addition, no demographic or risk factors were significantly associated with MRSA colonization.

Molecular Characteristics and Antibiotic Susceptibilities of MRSA Isolates

Of the 14 MRSA colonized participants, two were co-colonized with the same strain of MRSA in both groin/perirectal and nasopharyngeal regions. The USA800 strain was the most common colonizing strain in the cohort and was detected in 10 participants (Table IV). Two participants (14%) were colonized with USA300 MRSA. *SCCmec* typing demonstrated that all USA800 and USA 300 isolates contained *SCC mec* IV, and the 1 USA100 isolate contained *SCCmec* II. All MRSA isolates were sensitive to trimethoprim–sulfamethoxazole and rifampin while a substantial proportion of USA800 isolates were resistant to clindamycin, erythromycin, and tetracycline; two USA300 isolates were resistant to erythromycin and levofloxacin (Table IV). Of the isolates tested, *pvl* was present !in approximately half of the USA300 and USA800 strains, while *psm-α* and *tst* were present in 11/14 MRSA isolates. The gene for sortase A (*srtA*) associated with the evasion of phagocytosis and persistence in host tissues was absent in all USA300 isolates.

DISCUSSION

To our knowledge, this is the first study evaluating the prevalence of *S. aureus* colonization in the shipboard setting. We observed high rates of colonization for both MRSA (3.5%) and MSSA (46%). Table V summarizes prior studies evaluating rates of *S. aureus* colonization in military service members. The MRSA colonization rate in our study was comparable to the rate observed in recruits during military training (2%–4%) and we noted a higher frequency of MSSA colonization. This may be in part due to the sampling of several colonization sites in our study, as compared to other studies that largely utilized nares cultures alone. Vento et al[3] observed a similar increase in colonization rates by increasing the number of sampling sites. By comparison, rates of MRSA colonization reported in the National Health and Nutrition Examination Survey, a nationally representative sample of the civilian and noninstitutionalized U.S. population, is approximately 1.5%.[12]

The high prevalence of colonization that we observed likely reflects unique shipboard factors, including enclosed and confined spaces, in addition to limited contact with external environments. We did not observe associations with MRSA colonization, such as prior antibiotic use or history of SSTI, which may be due to our limited sample size.[2,6] We observed that Marines had a higher rate of MSSA colonization, potentially resulting from tighter living quarters.

USA800 was the most prevalent PFT among MRSA isolates (10/14), with relatively few USA300 (2/14). This is in contrast to previous studies in military cohorts where USA300 was the most common MRSA strain, accounting for more than half of all MRSA isolates.[2,5,6] USA800 strains, previously associated with nosocomial acquisition, are being increasingly recognized as colonizing strains in military cohorts. 23% of MRSA isolates were USA800 in a study reported by Millar et al.[5] Although USA800 isolates have some features in common with USA300 strains, such as the presence of the

TABLE IV. Characteristics of Methicillin- resistant *S. aureus* Isolates by Pulse-field Type

Characteristic	Pulse-field (USA) Type			
	USA300 (n = 2)	USA800 (n = 10)	USA100 (n = 1)	No PFT type (n = 1)
Colonization Site				
Nasopharynx Only[a]	2 (100)	6 (60)	1 (100)	1 (100)
Nasopharynx and Groin/Perirectal	0	2 (20)	0	0
Groin/Perirectal Only[a]	0	2 (20)	0	0
SCCmec Type				
II	0	0	1 (100)	0
IV	2 (100)	10 (100)	0	1 (100)
Virulence and Colonization Factors				
pvl	1 (50)	4 (40)	0	0
srtA	0	6 (60)	0	0
psm-α	2 (100)	8 (80)	0	1 (100)
clfB	2 (100)	7 (70)	1 (100)	0
tst	1 (50)	9 (90)	1 (100)	0
seC	2 (100)	6 (60)	0	1 (100)
seK	1 (50)	7 (70)	0	0
Antibiotic Susceptibility				
Clindamycin	2 (100)	6 (60)	0	1 (100)
Erythromycin	0	1 (10)	0	1 (100)
Levofloxacin	0	9 (90)	0	1 (100)
Moxifloxacin	2 (100)	10 (100)	0	1 (100)
Rifampin	2 (100)	10 (100)	1 (100)	1 (100)
Tetracycline	2 (100)	7 (70)	1 (100)	1 (100)
Trimethoprim–Sulfamethoxazole	2 (100)	10 (100)	1 (100)	1 (100)

[a]Culture swabs for the nares and pharynx were streaked onto a single Mannitol Salt Agar plate, termed nasopharyngeal culture; the culture swabs for the groin and perirectal regions were similarly combined and termed groin/perirectal culture.

TABLE V. Summary of Reported *S. aureus* Colonization Rates Among Military Service Members

Reference (Sample Size)	Year of Study	Setting	Colonization Swab Sample	Rates of *S. aureus* Colonization		
				MRSA	MSSA	No *S. aureus*
Present Study (n = 400)	2011	Amphibious Assault Ship	Nares, Oropharynx, Groin, Perirectal	3.5%	46%	50.5%
Ellis et al[2] (n = 812)	2003	U.S. Army Soldiers Training at Fort Sam Houston, San Antonio, TX	Nares	3%	28%	69%
Vento et al[3] (n = 100)	2011	U.S. Army Soldiers Deployed in Afghanistan	Nares, Oropharynx, Axilla, Groin, Hand, Foot, Perirectal	6	65	29
Vento et al[3] (n = 100)	2011	Nondeployed Troops in the Clinic Setting, San Antonio, TX	Nares, Oropharynx, Axilla, Groin, Hand, Foot, Perirectal	4	71	25
Whitman et al[4] (n = 1,562)	2007	Military Recruits Attending Training Classes, Quantico, VA	Nares and Axilla	2	42.9	55.1
Millar et al[5] (n = 1,706)	2010	Army Soldiers Undergoing Infantry Training, Fort Benning, GA	Nares	4.2	56	39.8
Ellis et al[6] (n = 3,447)	2005	Army Soldiers Undergoing Training at Fort Sam Houston, San Antonio, TX	Nares	3.9	38	58.1

MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin resistant *S. aureus*.

SCC*mec*IV element (observed in all USA800 isolates in our cohort) and the *pvl* gene (4 of 10 isolates in our cohort), they have not been associated with increased risk of SSTIs.[6]

Several findings may inform future guidelines regarding the treatment, infection control, and outbreak investigation of community-acquired MRSA in the U.S. Navy and Marine Corps.[13] The viability of *S. aureus* after frozen storage in the BBL CultureSwab system (99% recovery) was higher than expected, providing a reasonable alternative for *S. aureus* isolation in resource-limited environments. We observed minimal colonization via groin/perirectal culture, though inclusion of this culture yielded an additional six MSSA and two MRSA-positive subjects over nasopharyngeal culture alone. These lower rates of isolation in the groin/perirectum may have been due to technique, with subject-obtained swabs used for this anatomic site. Alternatively, it could be postulated the increase in *S. aureus* colonization seen in the shipboard setting occurs primarily in the nares due to mechanisms beyond the recognized risk factors of hygiene or crowding. Despite the few additional cases identified, given the relative ease of the procedure[14] it is reasonable to include a groin/perirectal swab in MRSA screening algorithms in high-risk environments such as deploying ships. Finally, based on our antibiotic susceptibilities, development of operationally based (rather than hospital-based) antibiograms may help inform treatment decisions among active duty personnel.

Our study had several limitations. This was a pilot effort to determine the point prevalence of *S. aureus* colonization and as a result, clinical data on SSTI during deployment was not obtained, and we did not follow participants to determine changes in colonization. Selected data pertaining to risk factors were self-reported, a common problem in MRSA surveillance studies, and our small sample size prevented us from determining factors associated with MRSA colonization. Finally, combined plating of colonization swabs and self-collection of groin/perirectal culture swabs, limited our ability to identify colonizing sites and ensure appropriate collection of samples, respectively. Nonetheless, as one of the first studies of its kind, our findings provide direction for future epidemiologic studies involving fleets and ships of different sizes.

In conclusion, this pilot study has shown the feasibility of large-scale study enrollments and processing of cultures in the deployed shipboard environment. Future surveillance studies involving large, prospective cohorts of deployed Naval personnel are needed to document the changing rates of *S. aureus* colonization and their impact on SSTI risk in this population. Ultimately, these efforts would help better understand the bacterial, host, and environmental risk factors that may be amenable to intervention in the shipboard setting.

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