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Topical Decolonization Does Not Eradicate the Skin Microbiota of Community-Dwelling or Hospitalized Adults

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Topical antimicrobials are often employed for decolonization and infection prevention and may alter the endogenous microbiota of the skin. The objective of this study was to compare the microbial communities and levels of richness and diversity in community-dwelling subjects and intensive care unit (ICU) patients before and after the use of topical decolonization protocols. We enrolled 15 adults at risk for *Staphylococcus aureus* infection. Community subjects ($n = 8$) underwent a 5-day decolonization protocol (twice daily intranasal mupirocin and daily dilute bleach-water baths), and ICU patients ($n = 7$) received daily chlorhexidine baths. Swab samples were collected from 5 anatomic sites immediately before and again after decolonization. A variety of culture media and incubation environments were used to recover bacteria and fungi; isolates were identified using matrix-assisted laser desorption ionization–time of flight mass spectrometry. Overall, 174 unique organisms were recovered. Unique communities of organisms were recovered from the community-dwelling and hospitalized cohorts. In the community-dwelling cohort, microbial richness and diversity did not differ significantly between collections across time points, although the number of body sites colonized with *S. aureus* decreased significantly over time ($P = 0.004$). Within the hospitalized cohort, richness and diversity decreased over time compared to those for the enrollment sampling (from enrollment to final sampling, $P = 0.01$ for both richness and diversity). Topical antimicrobials reduced the burden of *S. aureus* while preserving other components of the skin and nasal microbiota.

Nosocomial infections pose significant clinical and financial burdens to patients and health care systems ([1], [2]). Colonization with potential pathogens serves as an endogenous source of infection (e.g., central line-associated bloodstream infection with *Staphylococcus aureus*) ([3]). For hospitalized patients, particularly those in intensive care units (ICUs), decolonization with topical antimicrobials, including chlorhexidine and/or mupirocin, has been demonstrated to reduce the acquisition of antibiotic-resistant microorganisms and the incidence of hospital-acquired infections ([4]–[6]). While decolonization has traditionally been employed in health care settings, the emergence of methicillin-resistant *S. aureus* (MRSA) in the community and the resultant epidemic of skin and soft tissue infections (SSTIs) have led to the extrapolation of decolonization to outpatients to prevent recurrent SSTIs ([7]–[10]). As these broad-spectrum therapies are not pathogen specific (e.g., for MRSA or *Enterobacteriaceae*), these agents may suppress or eliminate other organisms on the skin and nasal mucosa, thereby potentially disrupting the balance of the microbiota, an important component of host defense against pathogenic organisms ([11], [12]). Indeed, this dysbiosis has been demonstrated for the intestinal microbiota following administration of oral antibiotics ([13], [14]).

Culture-independent molecular methods, including 16S rRNA gene sequencing and metagenomic sequence analysis, have become common approaches in studies evaluating the microbiome ([15]–[18]). While microbial culture has traditionally been regarded as insensitive for detecting a majority of the microbiota, molecular methods have informed augmented culture approaches, yielding enhanced recovery of these microorganisms ([19]–[26]). Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is a rapid and cost-effective technique that utilizes proteomic profiling for organism identification ([27]). MALDI-TOF MS facilitates the field of "cul-

turomics," which employs a variety of conditions for microbial cultivation, followed by identification by mass spectrometry ([24]). MALDI-TOF MS provides excellent species-level resolution, even for closely related Gram-positive bacteria that are major contributors to the skin microbiota. This process supplants traditional limitations of 16S rRNA gene sequencing for closely related species.

Using a culturomic approach, our objective was to discern the identity and diversity of microorganisms recovered from multiple anatomic niches in individuals at risk for infection with MRSA. As a myriad of factors influence the skin and nasal microbiota, many of which differ between outpatients and hospitalized patients, we enrolled two distinct cohorts (community-dwelling individuals and hospitalized individuals in an ICU) for this investigation. Additionally, the decolonization regimens prescribed can vary. Thus, within cohorts, we aimed to compare the richness and diversity of microorganisms within individuals prior to and following the use of topical antimicrobial decolonization measures.

MATERIALS AND METHODS

Study populations. Two participant cohorts comprised of individuals at risk for MRSA infection were enrolled. The community-dwelling cohort consisted of 8 adults living with a child (aged 6 months to 13 years) recently treated for a MRSA SSTI (these individuals represent a subset of participants in a study of MRSA transmission dynamics among households of children with MRSA infections [28]). Community-dwelling individuals completed a 5-day decolonization protocol with twice-daily application of 2% mupirocin (Teva Pharmaceuticals USA) to the anterior nares and daily bathing in dilute bleach water (1/4 cup of bleach [Clorox; The Clorox Company] to 1/4 bathtub-full of water), with soaking for ≥15 min (8, 29). The hospitalized cohort consisted of 7 adults in the 15-bed cardiac care ICU at Barnes-Jewish Hospital (BJH) in St. Louis, MO. As a component of routine clinical care, BJH ICU patients receive daily body washes from the neck down with chlorhexidine-based soap (Exidine 4%; Cardinal Health). Specifically, one 4-ounce bottle of 4% chlorhexidine-based soap is added to 4 quarts of water in a bath basin (to a final concentration of 0.125%, which has been proven to be effective at preventing MRSA transmission and infection in ICUs [30]); this solution is applied to the patient with a wash cloth and allowed to air dry (30, 31). These chlorhexidine baths are discontinued upon transfer to the general medicine unit. Although application of nasal mupirocin is not routinely prescribed in the BJH ICU, two patients received one dose each of preoperative mupirocin prophylaxis. A standardized case report form was used to collect participant demographics, health history, antibiotic use, and hygiene factors. Study procedures were approved by the Washington University Institutional Review Board, and participants provided written informed consent.

Specimen collection and processing. A standardized culturing protocol (BD Eswab; Becton Dickinson) was employed by study personnel to obtain samples from five anatomic sites, including the anterior nares and the subject's dominant-side axilla, inguinal fold, dorsal surface of the forearm, and ventral surface of the lower leg. For all sites except the anterior nares, a 2-in. by 2-in. (5.1 × 5.1 cm) area was swabbed using a collection template, with application of consistent pressure. Community-dwelling participants were sampled at three time points: day 0 (prior to initiating the 5-day decolonization protocol described above), day 5 (immediately after performing the decolonization protocol), and day 30 (1 month after completing the decolonization protocol). Hospitalized patients were sampled up to five times (in the ICU or on the general medicine unit), depending on their length of hospitalization, as follows: day 0 (upon ICU admission, prior to the first chlorhexidine bath), day 3, day 7, upon discharge from the ICU, and upon discharge from the hospital.

Microorganism identification and characterization. After vortexing, each Eswab eluate was inoculated to 9 selective and differential media and incubation environments, selected to support the growth of organisms anticipated to be recovered from the nares and skin (see Table S1 in the supplemental material) (11, 24, 32). A four-quadrant streaking method was employed. The relative abundance of each morphotype recovered in culture was approximated as follows: 0.5+, growth in broth only; 1+, rare; 2+, few; 3+, moderate; and 4+, abundant. Distinct morphotypes were selected for MALDI-TOF MS species identification as previously described (33–37). In brief, yeast and bacteria were routinely identified using the Vitek MS IVD v2.0 Knowledge Base according to the manufacturer's protocols. Any isolates not identified using this method were analyzed using the Vitek MS SARAMIS database. For a very small number of isolates, this approach was not successful and the isolate was analyzed using the Bruker Biotyper RUO database. Any isolate that remained unidentified was assigned a descriptive identity based on the Gram stain reaction and growth conditions. Filamentous fungi were identified using the Vitek MS v3.0 Knowledge Base following extraction and inactivation; any isolate that was unidentified using this method was identified using sequence-based analysis.

Disk diffusion testing was performed on all *S. aureus* isolates to detect resistance to cefoxitin (as an indicator of methicillin resistance) and mupirocin (38). Multiplex PCR was performed to detect *mupA* and *qacA/B*, conferring high-level mupirocin resistance and chlorhexidine tolerance, respectively, as described elsewhere (39).

Statistical analysis. Richness is defined as the number of different microbial species identified at a given collection site and time point (19, 32). Diversity is defined as a measure of community composition accounting for the relative abundances of species as determined by Shannon's diversity index (which considers species richness and evenness) (32, 40). Shannon's diversity index was calculated based on the relative abundance of each morphotype recovered in culture.

To evaluate trends in richness over time and across body sites, with accounting for correlation within subjects, repeated-measures GEE models assuming a Poisson distribution were used; similarly, for Shannon's diversity index, repeated-measures mixed models assuming a normal distribution were deployed. To assess the correlation between *S. aureus* and other relevant bacteria, with accounting for within-subject correlation, repeated-measures log-linear models were utilized, with adjustments for body site and time. In log-linear models, the presence of the two taxa is assessed simultaneously, with checks for the effect of the presence of one on the presence of the other. To compare skin microbial compositions at different time points, the numbers of body sites colonized with various organisms per subject over time were analyzed using related-samples Friedman's two-way analysis of variance by ranks. Analyses were performed with R statistical software, version 3.2, and SPSS 22 for Windows (IBM SPSS, Chicago, IL).

RESULTS

Participants and microbiology. The overall study population was comprised of 15 participants with a median age of 43 years (range, 26 to 66 years). Ten (67%) participants were female, seven (47%) were Caucasian, and six (40%) had experienced an SSTI in the previous year.

Overall, 174 unique organisms representing 65 genera and 158 species were identified: 137 organisms from the community-dwelling cohort and 99 from the hospitalized cohort. Sixty-two (36%) of the organisms were shared between the two cohorts; 75 (43%) were unique to the community-dwelling cohort (notably *Klebsiella pneumoniae* and *Staphylococcus pseudintermedius*), and 37 (21%) were unique to the hospitalized cohort (notably *Clostridium difficile* and *Pseudomonas aeruginosa*). In addition to the Gram-positive organisms we expected to find (e.g., *Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium* spp., and *Propionibacterium* spp.), many participants were also colonized with enteric Gram-negative bacteria and fungi (e.g., *Aspergillus* spp. and dematiaceous fungi) (Table 1). The most commonly recovered taxa were *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Propionibacterium acnes*, *Corynebacterium tuberculostearicum*, *Propionibacterium avidum*, *Enterococcus faecalis*, *Staphylococcus hominis*, *Bacillus* spp., *Finegoldia magna*, and *Staphylococcus lugdunensis*. Twenty species of *Staphylococcus*, 4 species of *Propionibacterium*, and 14 species of *Corynebacterium* were identified. Anaerobic bacteria were recovered from all 15 subjects and 168 of 225 (75%) sites sampled.

Characteristics and microbiota of community-dwelling participants. The community-dwelling cohort (*n* = 8) had a median age of 33 years (range, 26 to 43 years). Five (63%) participants were female, five (63%) were Caucasian, and five (63%) had experienced an SSTI in the previous year. Two (25%) community-dwelling participants had taken systemic antibiotics (ciprofloxacin and trimethoprim-sulfamethoxazole) in the month prior to study enrollment; two participants were taking systemic antibiotics at the time of sample collection (ciprofloxacin and trim-

TABLE 1 Comparison of skin microbial compositions at different time points[a]

| | Median no. (IQR) [range] of body sites colonized per subject | | | | | |
| | Community-dwelling cohort | | | Hospitalized cohort | | |
Organism	Enrollment (n = 8)	Day 5 (n = 8)	Day 30 (n = 8)	Enrollment (n = 7)	ICU (n = 5)	General medicine unit (final) (n = 5)
Fungi	0 (0–1.75) [0–3]	0 (0–1.5) [0–3]	0 (0–0) [0–3]	0 (0–2) [0–2]	0 (0–1) [0–2]	0 (0–1) [0–1]
Aspergilli	0 (0–0.75) [0–1]	0 (0–0.75) [0–1]	0 (0–0) [0–1]	0 (0–0) [0–2]	0 (0–0) [0–0]	0 (0–0) [0–0]
Black mold	0 (0–0) [0–3]	0 (0–0) [0–1]	0 (0–0) [0–1]	0 (0–0) [0–1]	0 (0–0) [0–0]	0 (0–0) [0–0]
Yeast	0 (0–0) [0–0]	0 (0–0) [0–0]	0 (0–0) [0–0]	0 (0–1) [0–1]	0 (0–1) [0–2]	0 (0–1) [0–1]
Bacteria	5 (5–5) [4–5]	5 (5–5) [4–5]	5 (5–5) [5–5]	5 (5–5) [4–5]	5 (4–5) [3–5]	5 (5–5) [5–5]
Gram-positive rods	5 (4–5) [3–5]	5 (3.5–5) [2–5]	4.5 (4–5) [3–5]	4 (2–5) [2–5]	3 (2.5–5) [2–5]	4 (1.5–4.5) [1–5]
Gram-positive cocci	5 (5–5) [4–5]	5 (4.25–5) [4–5]	5 (5–5) [4–5]	5 (5–5) [4–5]	5 (3.5–5) [3–5]	5 (4–5) [4–5]
Aerobes	5 (5–5) [4–5]	5 (4.25–5) [4–5]	5 (5–5) [5–5]	5 (5–5) [4–5]	5 (4–5) [3–5]	5 (4–5) [4–5]
Gram-negative aerobes	1.5 (0–2) [0–5]	1 (0–1.75) [0–5]	1 (0–1) [0–2]	1 (0–4) [0–5]	0 (0–2.5) [0–3]	1 (0.5–1.5) [0–2]
Enterobacteriaceae	0 (0–1.75) [0–3]	0.5 (0–1) [0–2]	0 (0–0.75) [0–1]	0 (0–1) [0–2]	0 (0–1.5) [0–2]	1 (0.5–1.5) [0–2]
Nonfermenting bacteria	0 (0–0) [0–0]	0 (0–0) [0–1]	0 (0–0.75) [0–2]	0 (0–1) [0–5]	0 (0–0.5) [0–1]	0 (0–0) [0–0]
Fastidious bacteria	0.5 (0–1) [0–2]	0 (0–0) [0–1]	0 (0–0) [0–1]	0 (0–1) [0–1]	0 (0–0) [0–0]	0 (0–0.5) [0–1]
Gram-positive aerobes	5 (5–5) [4–5]	5 (4.25–5) [4–5]	5 (5–5) [5–5]	5 (5–5) [4–5]	5 (4–5) [3–5]	5 (4–5) [4–5]
Cocci	5 (5–5) [4–5]	5 (4.25–5) [4–5]	5 (5–5) [4–5]	5 (5–5) [4–5]	5 (3.5–5) [3–5]	5 (4–5) [4–5]
S. aureus	3 (2.25–4) [1–4]	1.5 (1–3.5) [1–5]	0.5 (0–1.75)[b] [0–3]	0 (0–2) [0–2]	2 (0–4.5) [0–5]	2 (0–3) [0–3]
Other *Staphylococcus* spp.	5 (4.25–5) [4–5]	5 (4.25–5) [4–5]	5 (4.25–5) [4–5]	5 (5–5) [4–5]	5 (3.5–5) [3–5]	5 (4–5) [4–5]
Enterococcus spp.	0 (0–0.75) [0–3]	0 (0–2.5) [0–3]	1.5 (0–2) [0–4]	2 (0–3) [0–4]	4 (0–4) [0–4]	3 (0–4.5) [0–5]
Streptococcus-like bacteria	1 (1–1.75) [0–3]	1 (0.25–2.75) [0–4]	2 (1–2) [0–3]	1 (0–3) [0–3]	1 (0–2) [0–3]	1 (0–1.5) [0–2]
Rods	2.5 (2–4) [1–5]	3.5 (2–4.75) [1–5]	3.5 (2.25–4.75) [2–5]	3 (2–4) [0–5]	2 (2–4) [2–5]	2 (0.5–2) [0–2]
Corynebacterium spp.	1.5 (0.25–4) [0–5]	2 (1–4) [0–4]	2.5 (1.25–4) [1–4]	3 (2–4) [0–5]	1 (0.5–3) [0–4]	1 (0.5–1.5) [0–2]
Spore-forming bacteria	1 (1–2) [0–5]	2 (0.25–3.75) [0–5]	2.5 (1–4.5) [0–5]	0 (0–1) [0–1]	1 (0.5–2) [0–2]	0 (0–1) [0–1]
Bacillus spp.	1 (1–2) [0–5]	2 (0.25–3.75) [0–5]	2.5 (1–4.5) [0–5]	0 (0–0) [0–1]	1 (0.5–2) [0–2]	0 (0–1) [0–1]
Other spore-forming bacteria	0 (0–0) [0–1]	0 (0–0) [0–1]	0 (0–0) [0–0]	0 (0–1) [0–1]	0 (0–0.5) [0–1]	0 (0–0) [0–0]
Anaerobes	4 (3.25–4.75) [2–5]	5 (3.25–5) [2–5]	3.5 (3–5) [1–5]	4 (3–4) [2–5]	4 (2–4.5) [1–5]	4 (2.5–4.5) [1–5]
Clostridium/spore-forming bacteria	0 (0–0) [0–0]	0 (0–0) [0–1]	0 (0–0) [0–2]	0 (0–1) [0–1]	0 (0–0) [0–1]	0 (0–0.5) [0–1]
Propionibacterium spp.	4 (2.25–4) [2–5]	3.5 (2–4.75) [1–5]	3 (1.5–3.75) [1–4]	2 (1–4) [0–5]	1 (1–3.5) [1–4]	3 (0.5–4.5) [0–5]
Other non-spore-forming bacteria	2 (0.25–2.75) [0–3]	2 (0.25–4.75) [0–5]	2 (0.5–3) [0–3]	1 (0–4) [0–4]	3 (0.5–4) [0–5]	1 (0–2.5) [0–3]

[a] Abbreviations: IQR, interquartile range (25th to 75th percentiles); ICU, intensive care unit.
[b] $P = 0.004$ across samplings within community-dwelling participants by related-samples Friedman's two-way analysis of variance by ranks; for the remaining organisms, there was not a statistically significant change in the number of body sites colonized over time.

ethoprim-sulfamethoxazole) (indicated by a capsule graphic in the figures).

For community-dwelling participants, the median number of distinct taxa (i.e., richness) across all body sites combined was 21 (interquartile range [IQR], 13.75 to 26.5) at enrollment, 17.5 (IQR, 15.25 to 26.75) at day 5, and 20 (IQR, 14.25 to 26) at day 30 (Table 2). Richness did not differ significantly between

sample collections across time points ($P = 0.48$) (see Fig. S1 in the supplemental material). Richness did differ across body sites ($P < 0.01$), with the largest number of taxa recovered from the inguinal fold and the lowest from the axilla. Specifically, the inguinal fold richness was significantly greater than that of the axilla, anterior nares, and leg ($P < 0.01$ for each comparison).

TABLE 2 Sample richness at each body site over time[a]

| | Median no. of species (IQR) [range] | | | | | |
| | Community-dwelling cohort | | | Hospitalized cohort | | |
Site	Enrollment (n = 8)	Day 5 (n = 8)	Day 30 (n = 8)	Enrollment (n = 7)	ICU (n = 5)	General medicine unit (final) (n = 5)
Axilla	5 (3.25–5) [1–11]	4 (2–5.75) [0–8]	3.5 (1.25–5.75) [1–7]	5 (3–7) [2–10]	5 (3–6.5) [3–7]	4 (2–6.5) [1–8]
Forearm	8 (6.25–9) [6–10]	7.5 (6–10.25) [4–13]	6.5 (5.25–9.75) [4–15]	6 (5–7) [4–10]	5 (1–6.5) [0–7]	4 (3–6.5) [2–7]
Inguinal fold	10 (4.75–13) [4–15]	8 (5.25–11) [4–13]	7 (6.25–10.75) [6–14]	5 (4–13) [0–15]	6 (2–9) [2–10]	7 (2–8) [1–9]
Nares	5.5 (4.25–8) [1–10]	7 (3.75–9.25) [3–10]	7 (4.5–9.5) [4–10]	6 (5–7) [1–7]	7 (4.5–7) [4–7]	4 (2.5–7) [1–8]
Shin	7 (5.25–8) [3–10]	6.5 (2.25–10.25) [2–11]	6 (4.25–6.75) [1–9]	6 (4–6) [2–9]	3 (1–6.5) [0–8]	3 (1.5–3) [1–3]
All sites	21 (13.75–26.5) [11–27]	17.5 (15.25–26.75) [14–34]	20 (14.25–26) [11–28]	16 (14–24) [10–29]	12 (10–17.5) [9–20]	15 (7.5–15.5) [5–16]

[a] Abbreviations: IQR, interquartile range (25th to 75th percentiles); ICU, intensive care unit. Note that within the community-dwelling cohort, richness did not differ significantly between collections across time points in a repeated-measures model ($P = 0.48$). Within the hospitalized cohort, richness decreased over time compared to that for the enrollment sampling, with a trend for lower richness at the second ICU sampling (after initiation of chlorhexidine bathing; $P = 0.08$) and significantly lower richness at the third sampling (after discharge from the ICU to the general medicine unit; $P = 0.01$).

TABLE 3 Sample diversity at each body site over time[a]

	Mean Shannon's diversity index ± SD					
	Community-dwelling cohort			Hospitalized cohort		
Site	Enrollment ($n = 8$)	Day 5 ($n = 8$)	Day 30 ($n = 8$)	Enrollment ($n = 7$)	ICU ($n = 5$)	General medicine unit (final) ($n = 5$)
Axilla	1.31 ± 0.63	1.26 ± 0.46	0.98 ± 0.70	1.43 ± 0.46	1.45 ± 0.43	1.17 ± 0.74
Forearm	1.99 ± 0.20	1.90 ± 0.38	1.88 ± 0.41	1.74 ± 0.26	1.43 ± 0.54	1.33 ± 0.42
Inguinal fold	2.03 ± 0.49	1.87 ± 0.39	2.00 ± 0.33	1.92 ± 0.60	1.34 ± 0.69	1.34 ± 0.84
Nares	1.44 ± 0.63	1.62 ± 0.45	1.69 ± 0.38	1.45 ± 0.64	1.60 ± 0.31	1.21 ± 0.75
Shin	1.79 ± 0.37	1.56 ± 0.69	1.50 ± 0.64	1.58 ± 0.45	1.31 ± 0.57	0.76 ± 0.46
All sites	1.71 ± 0.55	1.65 ± 0.52	1.61 ± 0.61	1.61 ± 0.50	1.43 ± 0.48	1.16 ± 0.64

[a] Abbreviations: SD, standard deviation; ICU, intensive care unit. Note that within the community-dwelling cohort, diversity did not differ significantly between samplings across time points ($P = 0.71$) in a repeated-measures model. Within the hospitalized cohort, diversity decreased over time, with a trend for less diversity at the second ICU collection (after initiation of chlorhexidine bathing; $P = 0.09$) and significantly less diversity at the third collection (after ICU discharge to the general medicine unit; $P = 0.01$).

For community-dwelling participants, the mean (± standard deviation) Shannon's diversity index across all body sites combined was 1.71 (±0.55) at enrollment, 1.65 (±0.52) at day 5, and 1.61 (±0.61) at day 30 (Table 3). Diversity did not differ significantly between samplings across time points ($P = 0.71$) (see Fig. S2 in the supplemental material). The inguinal fold had the most diversity, which was significantly greater than that of the axilla and anterior nares ($P < 0.01$ and $P = 0.05$, respectively); there was a trend for greater diversity in the inguinal folds than on the shin ($P = 0.08$). The axilla had the least diversity.

The composition of skin flora for each body site over time is displayed in Fig. 1A for one representative community-dwelling participant. The core Gram-positive flora (e.g., coagulase-negative staphylococci and *Corynebacterium* spp.) were relatively consistent over time and across body sites, while the accessory bacteria (e.g., *Enterobacteriaceae*, enterococci, and anaerobes) were more variable. The composition of nasal flora for all community-dwelling participants over time is displayed in Fig. 1B. While the core Gram-positive organisms within the nares were well represented across all individuals, the accessory bacteria were more similar within individuals over time than between individuals.

Characteristics and microbiota of hospitalized participants. The hospitalized cohort ($n = 7$) had a median age of 56 years (range, 47 to 66 years). Five (71%) patients were female, two (29%) were Caucasian, and one (14%) had experienced an SSTI in the previous year. Four (57%) hospitalized patients had taken systemic antibiotics in the month prior to study enrollment; five patients were receiving at least one systemic antibiotic (up to four antibiotics per sampling) at ≥1 sampling time points (indicated by a capsule graphic in the figures).

For the hospitalized cohort, the median richness over all body sites combined was 16 (IQR, 14 to 24) at enrollment, 12 (IQR, 10 to 17.5) at the second ICU sampling, and 15 (IQR, 7.5 to 15.5) after discharge to the general medicine unit (Table 2). Within the hospitalized cohort, richness decreased over time compared to that of the enrollment sampling, with a trend for lower richness at the second ICU sampling (after initiation of chlorhexidine bathing; $P = 0.08$) and significantly lower richness at the third sampling (after discharge from the ICU to the general medicine unit; $P = 0.01$) (see Fig. S3 in the supplemental material). There was a trend for differing richness levels across body sites overall ($P = 0.06$); the inguinal fold again had the highest richness, and the shin had the lowest richness.

For the hospitalized cohort, the mean (± standard deviation) Shannon's diversity index over all body sites combined was 1.61 (±0.50) at enrollment, 1.43 (±0.48) at the second ICU sampling, and 1.16 (±0.64) after discharge to the general medicine unit (Table 3). Diversity decreased over time, with a trend for less diversity at the second ICU collection (after initiation of chlorhexidine bathing; $P = 0.09$) and significantly less diversity at the third collection (after ICU discharge to the general medicine unit; $P = 0.01$) (see Fig. S4 in the supplemental material). The inguinal fold had the most diversity and the shin the least diversity, though these differences were not statistically significant.

The composition of skin flora for each body site over time is displayed in Fig. 1C for one representative hospitalized patient; while the overall *Staphylococcus* spp. were maintained across anatomic sites and time, the accessory microbiota were less variable over time than those in the community-dwelling participant (Fig. 1A). Note that *Propionibacterium* spp. comprised a larger portion of the microbiota in the hospitalized patient than in the community participant (Fig. 1C and A, respectively). The composition of inguinal fold flora for all hospitalized participants over time is displayed in Fig. 1D.

Relationships between *S. aureus* and other microbes. *S. aureus* was recovered from 13 participants (all eight community-dwelling and five hospitalized subjects) at ≥1 time points over the study period. Of the 71 *S. aureus* isolates available for susceptibility testing, 25 (35%) isolates (from seven participants) were MRSA. None of the isolates exhibited mupirocin resistance; two isolates, both of which were methicillin-susceptible *S. aureus* (MSSA) (one from a community-dwelling and one from a hospitalized participant), possessed genes conferring chlorhexidine tolerance (*qacA/B*) (Fig. 2). Of six community-dwelling participants colonized with *S. aureus* in the nares at enrollment, two (colonized with MRSA) remained colonized at all three samplings despite application of mupirocin, while four participants (one with MRSA and three with MSSA) were decolonized (Fig. 2A).

Microbial richness and diversity within each cohort over time and across body sites (detailed above) were also evaluated in the context of *S. aureus*; adjusting for the presence of *S. aureus* did not affect the richness or diversity models. The relationships between *S. aureus* and other relevant organisms (i.e., inhibition of or coexistence with *S. aureus* by other components of the microbiota) were evaluated, with adjustments for multiple body sites within an individual across all time points. In both community-dwelling
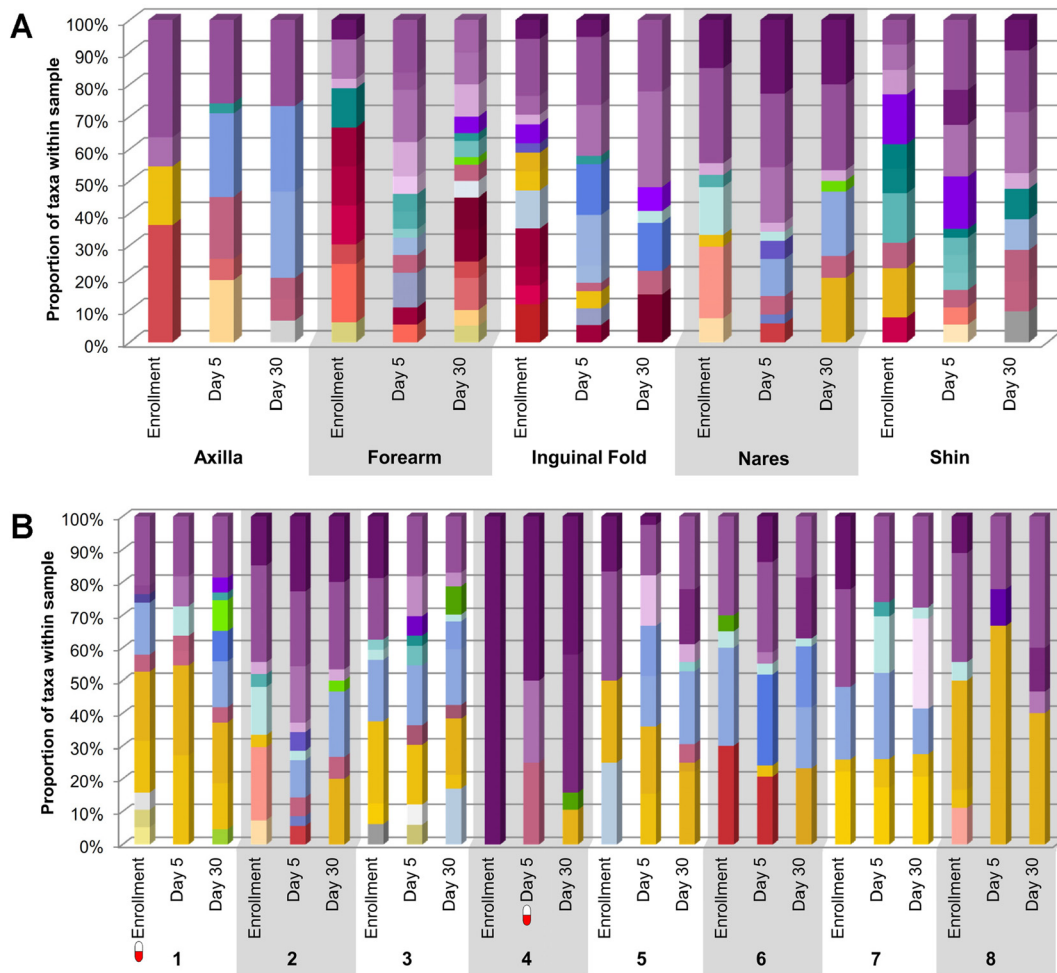
FIG 1 Skin microbiota. The height of each color within each bar represents the relative abundance of the corresponding taxon within each sample. A red and white "capsule" symbol represents a participant who was taking systemic antibiotics at the time of sampling. No growth, no organisms were cultivated from the sample. D/C, discharge. (A) Community-dwelling participant (ID 2). The data show the skin microbiota at each body site and time point for one community-dwelling participant. (B) Community-dwelling cohort anterior nares. The data show the nasal microbiota for all community-dwelling participants at each time point. (C) Hospitalized patient (ID 13). The data show the skin microbiota at each body site and time point for one hospitalized patient. (D) Hospitalized cohort inguinal folds. The data show the inguinal fold microbiota for all hospitalized patients at each time point.

and hospitalized populations, the presence of *S. aureus* was independent of colonization with *S. epidermidis*, *Propionibacterium* spp., *Enterococcus* spp., or *Enterobacteriaceae*. The presence of these organisms for each participant at each body site over time is depicted in Fig. 2. In both cohorts, colonization with these organisms was relatively consistent over time, despite decolonization efforts and, for some participants, administration of systemic antibiotics. Note that in the community-dwelling cohort, the number of body sites colonized with *S. aureus* significantly decreased over time (*P* = 0.004) (Table 1 and Fig. 2A).

DISCUSSION

Given the effectiveness of decolonization in infection prevention across broad study populations, the practice of topical antimicrobial application has become common in health care and community settings (4, 5, 8, 9). While the outcomes of these decolonization studies have focused on reducing the colonization burden and infection incidence with a specific organism, the overall community composition of the skin microbiota has not been evalu-

ated. These topical antimicrobials may result in a modulation of the normal skin microbiota, accommodating both the presence and prosperity of potential pathogens. Furthermore, a reduction in microbiome diversity has been associated with inflammatory skin disorders (41).

Although many studies evaluating microbial communities have applied a genomic approach, culturomics is emerging as a robust technique for detailed microbial identification to the species level (19–26). As demonstrated herein, this level of detail is especially important for the skin and nasal flora, where closely related Gram-positive species predominate. Sequencing reference databases are less comprehensive for taxa common to these body niches than for taxa comprising the gut microbiota. Furthermore, depending on the specific sequencing technique used and/or the portion of the 16S rRNA gene evaluated, important taxonomic groups, such as *Propionibacterium* spp., may be underestimated (16). Using our culturomic approach, we were able to recover and identify a wide variety of bacteria with high species-level resolution, including 20 staphylococcal species and 14 species of *Coryne-*
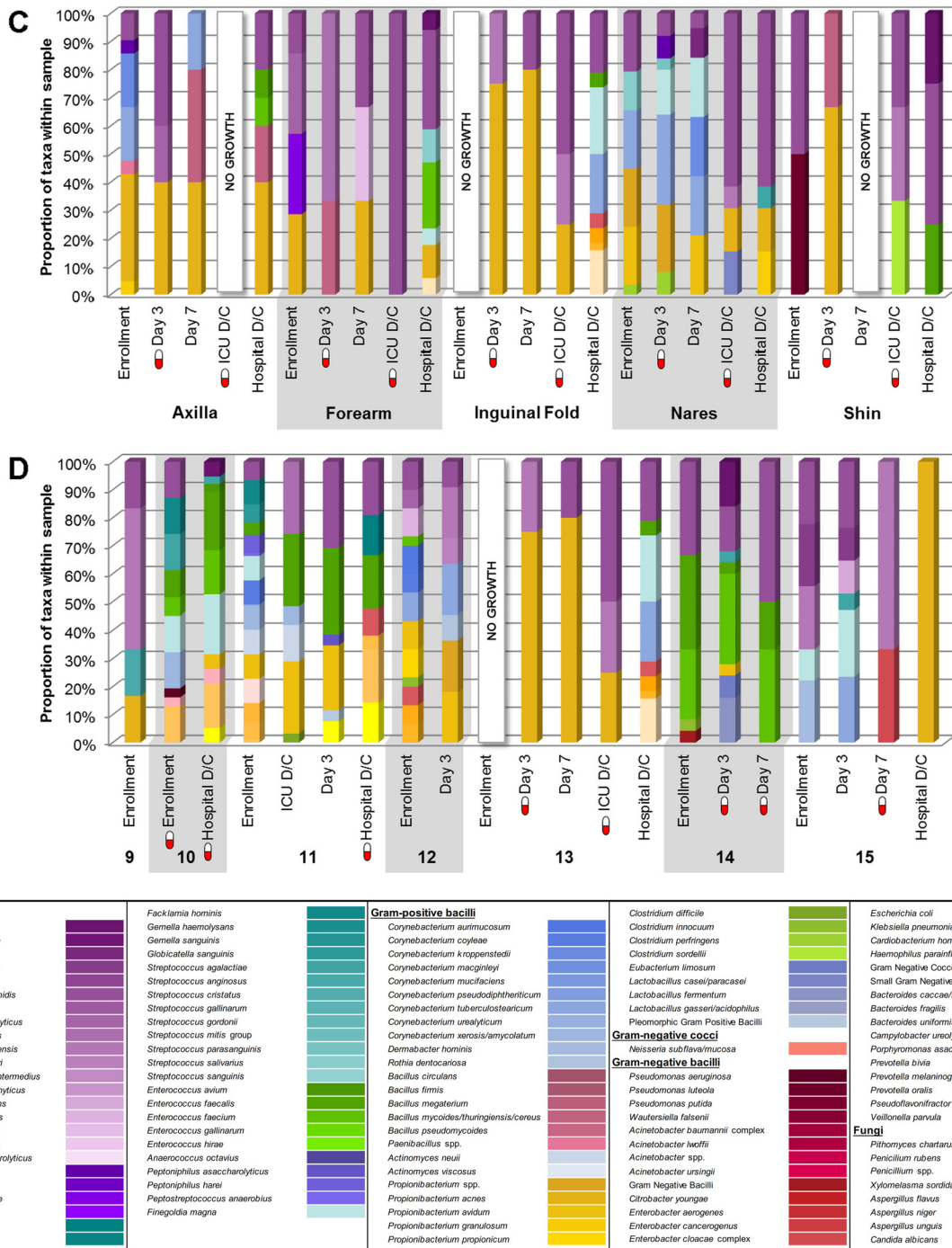
FIG 1 continued

In the present study, unique communities of organisms were recovered from the hospitalized and community participants, which is an important consideration in interpreting similar inves-

tigations, as findings from one population may not be generaliz- able. In our cohort of hospitalized patients who were bathed daily with chlorhexidine, the microbial richness and diversity decreased over time, a finding which may be affected by use of broad-spec- trum systemic antibiotics and medical and surgical interventions occurring concomitantly with topical antisepsis. Similarly, in a study of adult ICU patients in France, microbial diversity was lower among patients receiving chlorhexidine body washes than among those bathed with soap and water (19). Among the mem-
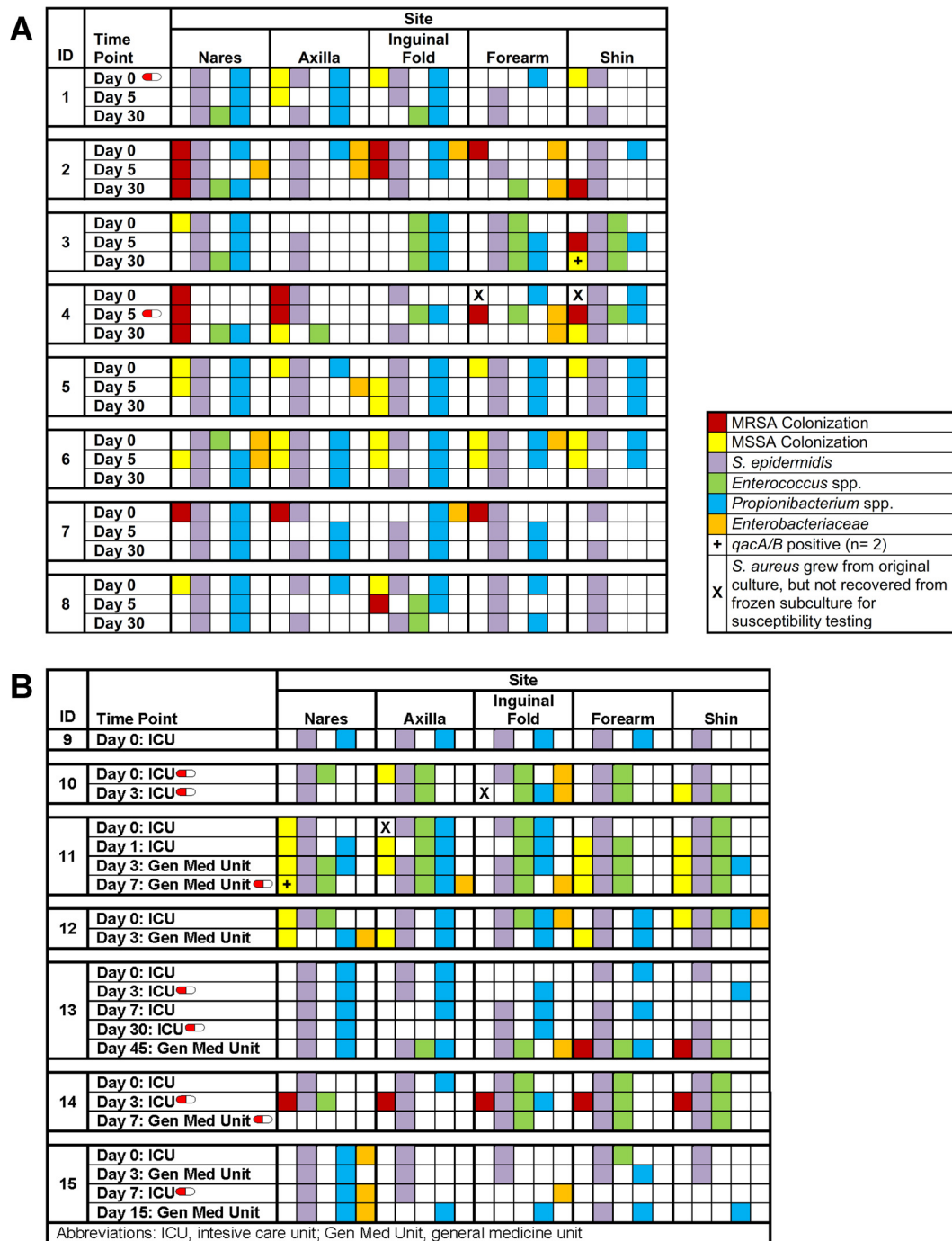
FIG 2 Presence of *S. aureus*, *S. epidermidis*, *Enterococcus* spp., *Propionibacterium* spp., and *Enterobacteriaceae* at each body site over time. A red and white "capsule" symbol represents a participant who was taking systemic antibiotics at the time of sampling. (A) Community-dwelling cohort. (B) Hospitalized cohort.

bers of our community-dwelling cohort, although adherence to the prescribed decolonization protocol was high (data not shown), the richness and diversity of the microbiota did not change significantly upon performance of the 5-day decolonization regimen with intranasal mupirocin application and bleach-water baths, although it is important that these indices do not discern the specific organisms present. Additionally, while the burden of *S. aureus* (i.e., number of body sites colonized) was

reduced following decolonization, topical antimicrobials did not eradicate or significantly reduce the burden of other components of the microbiota.

In both the community-dwelling and hospitalized cohorts, microbial richness and diversity differed significantly between anatomic niches. In the community-dwelling population, the axilla yielded the lowest richness and diversity (which may perhaps be attributed to additional exogenous factors, such as deodorant

use), while the shin was the site with the lowest richness and diversity in the hospitalized cohort. Among both cohorts, the inguinal fold consistently maintained the highest richness and diversity, which may be reflective of its proximity to the perirectal area as well as the temperature and humidity in this region (32). Note that enteric Gram-negative rods were found not only in the inguinal fold but also at all body sites. Overall, our data suggest that even following decolonization, an individual's skin microbiota is a "personal trait" and is temporally stable, similar to recent findings by Oh and colleagues (17). Thus, over time, the microbiota of the skin of an individual is more similar to "self" than to "others."

This study has numerous strengths, such as the inclusion of both hospitalized and community-dwelling individuals, with multiple longitudinal samplings, and the robust number of taxa identified to the species level, a degree of granularity that is imperative for assessing the skin microbiota. However, this study is not without limitations, including the limited sample size, the application of different decolonization regimens between the cohorts (which may have differential activity on the microbiota and thus precludes comparisons between cohorts), and the variable number of samplings obtained from hospitalized patients.

As endogenous microbial communities exist in a delicate balance within a niche, many microorganisms produce toxins and bacteriocins or augment host immune responses to ward off potentially pathogenic organisms. We were thus encouraged by the findings of the present study demonstrating that topical antimicrobial therapies do not completely eradicate the commensal microbes, or so-called "good bacteria," on the skin. Although prior decolonization investigations in hospital and community settings have demonstrated reduced burdens of *S. aureus* colonization and infection with the administration of topical antimicrobials (4, 5, 8), important considerations of these therapies include the longevity of effectiveness as well as microbial development of resistance to these agents (39, 42–44). Thus, development of novel preventive strategies is essential, and one promising approach is that of "bacterial interference," or the application of nonpathogenic organisms to impede or outcompete colonization or infection by potential pathogens. Indeed, this practice was implemented in the 1960s to abrogate nursery outbreaks caused by hypervirulent *S. aureus* strains (phage type 80/81) (45–48).

In conducting the present study, we postulated that the concept of bacterial interference might have clinical utility in the contemporary era as well. While we anticipated identification of one or two key organisms sufficient and necessary to outcompete *S. aureus* within a niche, this hypothesis was not substantiated. In prior investigations of *S. aureus* in the context of other constituents of the skin and nasal microbiota, an inverse relationship was observed between the presence of *S. aureus* and that of a variety of other flora, including *S. epidermidis*, *Corynebacterium* spp., *Streptococcus* spp., and *Propionibacterium* spp., suggesting competition or, again, bacterial interference between these organisms within the niche (49–53). In the present study, however, *S. aureus* often coexisted with *Propionibacterium* spp. and *S. epidermidis*, and in contrast to studies suggesting that *S. aureus* predominates in the niche, the presence of *S. aureus* did not affect microbial richness or diversity (52). While the concept of a single-organism probiotic is an attractive "natural" method for *S. aureus* eradication and infection prevention, it is likely that a community of organisms is required, similar to the approach of normal flora restoration therapy for *Clostridium difficile* infection. Restoration of the normal skin and nasal flora as an infection prevention approach is an exciting avenue for future investigation.

In conclusion, we demonstrated that topical antimicrobials reduced the burden of *S. aureus* on the skin of community-dwelling and hospitalized individuals while preserving other components of the skin and nasal microbiota. Specific taxa permitting or precluding *S. aureus* colonization were not identified.

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