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Diversity of *Staphylococcus aureus* Strains Colonizing Various Niches of the Human Body

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Summary

Objectives—As individuals may be colonized with multiple strains of *Staphylococcus aureus* at different body sites, the objectives of this study were to determine whether *S. aureus* polyclonal colonization exists within one body niche and the optimal sampling sites and culture methodology to capture the diversity of *S. aureus* strains in community-dwelling individuals.

Methods—Swabs were collected from the nares, axillae, and inguinal folds of 3 children with community-associated *S. aureus* infections and 11 household contacts, all with known *S. aureus* colonization. *S. aureus* isolates were recovered from each body niche using 4 culture methods and evaluated for polyclonality using phenotypic and genotypic strain characterization methodologies.

Results—Within individuals, the mean (range) number of phenotypes and genotypes was 2.4 (1–4) and 3.1 (1–6), respectively. Six (43%) and 10 (71%) participants exhibited phenotypic and genotypic polyclonality within one body niche, respectively. Broth enrichment yielded the highest analytical sensitivity for *S. aureus* recovery, while direct plating to blood agar yielded the highest genotypic strain diversity.

Conclusions—This study revealed *S. aureus* polyclonality within a single body niche. Culture methodology and sampling sites influenced the analytical sensitivity of *S. aureus* colonization detection and the robustness of phenotypic and genotypic strain recovery.

Keywords

Staphylococcus aureus; polyclonal colonization; strain diversity; broth enrichment

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Conflicts of interest

None.

Introduction

Staphylococcus aureus is a commensal bacterium with the potential to cause community-associated and nosocomial infections.[1,2] *S. aureus* often resides in the anterior nares, oropharynx, inguinal folds, axillae, and rectum of otherwise healthy people.[3–6] Colonization with *S. aureus* is a known risk factor for skin and soft tissue infections; in extreme cases, *S. aureus* may cause invasive life-threatening infections such as osteomyelitis, bacteremia, and pneumonia.[7,8]

Within an individual, multiple colonizing strain types of *S. aureus* have been recovered from different anatomical niches.[9] This finding raises the inquiry of polyclonality within an anatomic niche. *S. aureus* polyclonality is an important consideration from a biological and clinical standpoint. The simultaneous carriage of more than one subtype of *S. aureus* at one body site opens the opportunity for horizontal gene transfer; the transfer of antibiotic resistance and virulence factors between co-colonizing strains could contribute to the pathogenicity of the particular isolate.[10]

When studying the carriage and transmission of *S. aureus*, it is important to not only consider the prevalence of polyclonal colonization within one niche, but also the culture methods resulting in the recovery of the highest strain diversity. The majority of studies rely on the selection and analysis of a single colony from a positive culture plate, not accounting for polyclonality despite its potential importance to molecular epidemiology investigations.[11] Polyclonality has primarily been studied in healthcare settings or immunocompromised patients. These studies have demonstrated that 7–30% of individuals colonized with *S. aureus* possess multiple strain types within one body niche.[10,12] While important, these findings within populations with healthcare exposure may be limited in their application to individuals in community settings.

In the current study, we sought to address the issue of intra-individual *S. aureus* strain diversity in pediatric index patients diagnosed with community-associated methicillin-resistant *S. aureus* (CA-MRSA) infections and their household contacts. Using genotypic and phenotypic analysis, the objective of this study was to determine whether multiple strain types of *S. aureus* coexist in one body niche within an individual. Additionally, we sought to determine the optimal sampling sites and culture methodology to capture the greatest diversity of *S. aureus* strains within a population.

Methods

Participant Selection

This study population was derived from a longitudinal study and consisted of 14 participants, all of whom were colonized with *S. aureus*: 3 pediatric index patients with cutaneous or invasive CA-MRSA infections and 11 healthy household contacts of these patients. The cohort was recruited from Saint Louis Children's Hospital and community pediatric practices as described previously.[13] At enrollment, swabs (Eswab, Becton Dickinson [BD], Franklin Lakes, NJ) were collected for culture from the anterior nares, axillae, and inguinal folds of each participant to determine *S. aureus* colonization status. In

the present study, *S. aureus* isolates recovered from the same niche (i.e. the axillae, inguinal folds, or anterior nares) of 14 participants (designated A-N) were evaluated for polyclonality as described below. This study was approved by the Washington University School of Medicine Human Research Protection Office. Written informed consent was obtained from all participants.

Laboratory Procedures

Culture methods—From each culture swab, 100 μL of Eswab eluate was inoculated onto each of 1) trypticase soy agar with 5% sheep blood agar plate (BAP) (BBL, BD), 2) Spectra MRSA (Remel, Lenexa, KS) (a chromogenic agar selective and differential for MRSA), and 3) tryptic soy broth with 6.5% NaCl (BBL, BD), and then incubated overnight at 35°C. From the broth cultures, 100 μL aliquots were plated to BAP and Spectra MRSA and incubated at 35°C overnight. For each BAP, up to 10 colonies consistent with *S. aureus* (large, cream to golden colored and β-hemolytic) were selected by an experienced microbiologist; for each Spectra MRSA, up to 5 colonies consistent with *S. aureus* (denim blue in color) were selected. Each colony selected from BAP and Spectra MRSA were subsequently subcultured to individual BAPs and incubated overnight at 35°C (Figure 1).

In all, 4 separate culture methods were compared: 1) direct plating to BAP, 2) direct plating to Spectra MRSA, 3) broth enrichment with subculture to BAP, and 4) broth enrichment with subculture to Spectra MRSA (Figure 1). Results from a rapid latex agglutination test for *S. aureus* identification (Staphaurex, Remel), catalase activity, and Gram staining confirmed the identity of *S. aureus* isolates. Variations exist in the number of isolates recovered from each individual in the study due to instances where fewer colonies consistent with *S. aureus* were present or isolates selected were subsequently identified to be an organism other than *S. aureus*.

Phenotypic analysis—Antibiotic susceptibility testing for each clone was performed on Mueller-Hinton agar (BBL, BD) using the Kirby-Bauer disk-diffusion method and interpreted in accordance with Clinical and Laboratory Standards Institute 2012 Guidelines.[14] Susceptibility to cefoxitin (as a surrogate for methicillin resistance), clindamycin (including inducible clindamycin resistance as determined by the double disk diffusion D-test), erythromycin, trimethoprim-sulfamethoxazole (TMP-SXT), rifampin, tetracycline, ciprofloxacin, linezolid, and ceftaroline were recorded; for the purpose of analysis, isolates with a positive D-test were classified as clindamycin resistant.[15] Unique antibiotic susceptibility patterns were assigned a designation (AA, BB, etc.), followed by an –R or –S annotation, reflecting resistance or susceptibility to methicillin, respectively (Table 1).

Genotypic analysis—DNA extraction for each clone was performed using the BiOstic Bacteremia DNA kit (MoBio Laboratories, Carlsbad, CA). Strain typing by repetitive-sequence PCR (repPCR) was performed as described previously.[13,16] For the purpose of our analyses, distinct repPCR strains were assigned a designation (RS1, RS2, etc).

Statistical Analysis

Data were analyzed with descriptive statistics using SPSS 22 for Windows (IBM SPSS, Chicago, IL). The numbers of colonies needed to be selected in order to detect all strain types within each body niche, as well as the strain diversity recovered by sampling multiple body sites, were calculated. T-tests were used to compare mean colonization burden across levels of genotypic strain diversity. The analytical sensitivity of each method was calculated using the following formula:

$$\frac{\#\ of\ individuals\ with\ S.\ aureus\ recovery\ at\ body\ niche\ using\ a\ specific\ culture\ method}{Total\ \#\ of\ individuals\ with\ S.\ aureus\ recovery\ at\ body\ niche\ using\ ALL\ culture\ methods}$$

Methods of characterization were compared by the discriminatory index (*D*), calculated using the standard formula for this metric:[17]

$$D=1-\frac{1}{N(N-1)}\sum_{j=1}^{S}n_j(n_j-1)$$

where N is the total number of strains in the sample population, S is the total number of subtypes described, and n_j is the number of strains belonging to each of the subtypes. A value of 1 is considered to be highly discriminatory and a value of 0 is not discriminatory.[17]

Results

Of 14 participants, 8 (57%) were male, 10 (71%) were African-American, and the median age was 8.5 years (range 0.5–37 years). Colonization cultures collected from the anterior nares, axillae, and inguinal folds by all culture methods yielded 336 *S. aureus* isolates. By niche, 178 (53%) isolates were from the anterior nares, 74 (22%) were from the axillae and 84 (25%) were from the inguinal folds. Six subjects were colonized at 1 body site (most commonly the nares) and 8 were colonized at multiple body sites. Fifteen repPCR strain types (genotypes) were recovered; 1 strain type (designated RS7) comprised 39% of all isolates (Table 2). Based on patterns of antibiotic resistance (phenotypes), a total of 2 methicillin-susceptible *S. aureus* (MSSA) phenotypes and 4 MRSA phenotypes were recovered (Table 1). All isolates recovered in this investigation were susceptible to TMP-SXT, rifampin, tetracycline, linezolid, and ceftaroline.

A discriminatory index was calculated for each method of characterization: genotypic, phenotypic, and a combination of the genotypic and phenotypic strain characterization methods (aggregate). RepPCR was more discriminatory than antibiotic susceptibility patterns, but the aggregate resulted in the highest discriminatory power (Table 3).

Of note, some *S. aureus* repPCR strain types appeared to be commonly detected together, i.e. they were only recovered when a certain other strain type was present in the same body niche of the same participant. This pattern was observed for repPCR strain type RS8

(correlated with RS7), RS10 (correlated with RS2) and RS4 (correlated with RS7). Polyclonality with these strains was not specific to a particular niche.

Strain Diversity by Culture Method

Within each body niche, the analytical sensitivity of each culture method to recover *S. aureus* was determined. Broth enrichment to BAP was the most sensitive of the four culture methods with 100% recovery of *S. aureus* and 75% recovery of MRSA from both the anterior nares and axillae and 100% recovery of both *S. aureus* and MRSA from the inguinal folds. The direct plating to BAP culture method was the second most sensitive recovering 42%, 57%, and 63% of *S. aureus* isolates from the anterior nares, axillae, and inguinal folds, respectively (Table 4). In all circumstances in which direct plating techniques yielded S*. aureus* isolates, broth enrichment techniques also recovered S*. aureus* isolates.

The broth enrichment to BAP culture method recovered 201 isolates (60% of all recovered isolates), yielding 9 distinct genotypes. Of note, 4 genotypes were uniquely recovered by this method (RS1, RS4, RS9, RS13). The direct plating to BAP culture method recovered 88 isolates (26%), yielding 11 distinct genotypes; 5 genotypes were only recovered by this method (RS5, RS6, RS12, RS14, RS15). The broth enrichment to Spectra MRSA culture method recovered 28 isolates (8%), yielding 1 distinct genotype. The direct plating to Spectra MRSA method recovered 19 isolates (6%), yielding 2 distinct genotypes; genotype RS11 was solely recovered by this method. Overall, broth enrichment culture methods recovered more phenotypes (6) than direct plating methods (3). The mean genotypic strain diversity recovered by the direct plating to BAP culture method was higher than the broth enrichment to BAP culture methods (Table 5).

Strain Diversity within Individuals

The mean number of isolates recovered per participant (by all culture methods) was 24 (range 5–53). Five participants were colonized exclusively with MSSA (36%), 3 exclusively with MRSA (21%), and 6 with both MRSA and MSSA (43%). Both MRSA and MSSA phenotypes were recovered within a single body niche from all 6 individuals in the latter group. The mean number of phenotypes detected within an individual was 2.4 (range 1–4). The mean number of genotypes recovered from an individual was 3.1 (range 1–6).

Using the number of isolates recovered per participant as a surrogate for burden of *S. aureus* colonization, patients more heavily colonized with *S. aureus* had greater genotypic strain diversity. On average, 12 isolates were recovered from participants with 1 *S. aureus* genotype, while 29 isolates were recovered from participants with multiple genotypes ($p=0.049$). Employing the median number of genotypes to generate groups for comparison, 36 isolates were recovered from participants with more than 2 genotypes, compared to 12 isolates in participants with 2 or less genotypes ($p<0.001$).

Strain Diversity by Body Niche

The mean number of isolates recovered per body niche (by all culture methods) was 12 (range 3–28). Participants were colonized most frequently in the anterior nares (12, 86%), followed by the inguinal folds (8, 57%), and axillae (7, 50%). MRSA constituted 46% (82 of

178) of the isolates recovered from the anterior nares, 24% (18 of 74) of the axillae isolates, and 33% (28 of 84) of the inguinal folds isolates. Eight of 14 individuals were colonized with *S. aureus* in multiple body niches: 5 were colonized in all three body niches, 2 were colonized in the nares and inguinal folds, and 1 was colonized in the nares and axillae. The remaining 6 individuals were colonized exclusively in one body site: 4 in the nares, 1 in the axillae, and 1 in the inguinal folds.

Six (43%) participants exhibited phenotypic polyclonality within a single body niche. Two of these participants (33%) exhibited polyclonality at all body niches in which *S. aureus* was recovered. The body niches most frequently colonized with multiple phenotypes were the anterior nares and axillae (3 of 6 participants each, 50%), while 2 participants (33%) had different phenotypes recovered from the inguinal folds. The most predominant phenotypes were AA-R and EE-S; both were recovered from all three body sites. Phenotype FF-S was only recovered from the inguinal folds while BB-R, CC-R, and DD-R were only recovered from the anterior nares. The median number of colonies needed to be selected per plate to detect all the phenotypic strain diversity within a body niche (by all 4 culture methodologies) was 1.

Ten (71%) participants exhibited genotypic polyclonality within one body niche. Of these, 8 (80%) exhibited polyclonality in each body niche in which *S. aureus* was recovered. The body niche most frequently colonized with multiple *S. aureus* genotypes was the axillae (5 of 7 participants, 71%) followed by the anterior nares (8 of 12 participants, 67%) and inguinal folds (5 of 8 participants, 63%). Five repPCR strain types were recovered from all three body sites (RS2, RS3, RS7, RS8, RS10), with RS7 being the predominant strain type in all three sites. Strain types RS5, RS6, RS11, RS12 and RS13 were unique to the anterior nares, RS1 and RS15 were unique to the axillae, and RS9 was unique to the inguinal folds. The median number of colonies needed to be selected to detect all the genotypic strain diversity within a body niche was 3 (by direct to BAP culture methodology), 2 (by broth enrichment to BAP), and 1 (by both direct and broth enrichment to Spectra).

Selecting one colony from a sample derived from each of the three body niches within a participant (anterior nares, axillae, and inguinal folds) recovered a majority of the genotypic and phenotypic strain diversity. Nine of the 15 genotypes and 4 of the 6 phenotypes were recovered. The remaining 6 genotypes and 2 phenotypes not recovered by selecting just one colony from each body niche were each recovered only once in the study population.

Discussion

While *S. aureus* colonization is a symbiotic state for many individuals, for some, this colonization poses risk for subsequent infection or transmission of the organism to others.[4,5] Given this risk, active surveillance is frequently conducted in healthcare settings to identify carriers of MRSA and subsequently implement precautions to prevent transmission to other patients. Given the varying burden of organism at various body niches, the sensitivity of the culture method to detect colonized individuals is an important consideration. Several investigators have demonstrated the advantage of broth enrichment in the recovery of *S. aureus.* Mernelius et al, determined that the use of broth enrichment increased recovery of *S.*

aureus isolates by 46%.[18,19] In the present study, participants were swabbed at 3 body sites (anterior nares, axillae, and inguinal folds) and 4 culture methods were employed, including broth enrichment, to detect *S. aureus* colonization. This study confirmed that inclusion of a broth enrichment step increases the recovery of *S. aureus* isolates. The omission of broth enrichment would have led to falsely negative cultures in 6 of 14 participants (43%), while the omission of the direct plating culture method would not have led to any falsely negative cultures for *S. aureus* recovery. An important consideration is the finding that, in the present study, broth enrichment resulted in slightly decreased genotypic strain diversity than direct plating to BAP, likely due to the propagation of a predominant clone which out-competes other strains. Investigators should consider overall study goals when selecting optimization of *S. aureus* strain type diversity vs. recovery. As expected, Spectra MRSA culture methods yielded fewer colonies given the lower number of colonies chosen (5, rather than 10) and the MRSA selectivity of Spectra MRSA.

Consistent with prior investigations, sampling multiple body niches in the present cohort also increased the recovery of *S. aureus* isolates and strain diversity.[4–6,20,21] In a community-based study of children presenting with *S. aureus* skin infections and their household contacts, participants were swabbed in the anterior nares, axillae, and inguinal folds to detect *S. aureus* colonization. Obtaining extra-nasal cultures resulted in identification of 32% of *S. aureus*- colonized individuals, and specifically 41% of MRSA-colonized individuals, who would not have been detected if only the anterior nares had been sampled.[5] In the present study, had the anterior nares been the only body site sampled, *S. aureus* colonization would not have been detected in 14% of individuals; 47% of isolates and 3 genotypes would not have been recovered.

The present study of community-dwelling participants also aimed to determine whether multiple strain types of *S. aureus* colonize an individual and whether polyclonal colonization exists within one body niche within an individual. Several prior studies have addressed this question in populations either associated with a healthcare setting or considered immunocompromised. Mongkolrattanothai and colleagues analyzed nasal and perianal samples of children undergoing elective surgery for polyclonal colonization and found that 30% of positive *S. aureus* swabs contained multiple genotypic strains as determined by multiple-locus variable-number tandem-repeat fingerprinting (MLVF).[12] In a study of nasal colonization in current and past intravenous drug users, Cespedes et al. employed pulsed-field gel electrophoresis (PFGE) as well as multilocus sequence (MLST), *S. aureus* protein A (*spa*), and accessory gene regulator (*agr*) typing to develop a mathematical prediction model of polyclonality among colonized individuals. This model predicted 7% of *S. aureus*-colonized individuals would carry more than one strain.[10] Using genotypic and phenotypic analysis, we determined that 71% of our study population was colonized with more than one strain of *S. aureus* overall, and more specifically, within the same body niche. Of note, polyclonality was consistent across body sites.

This study has several limitations. First, although our study population was limited, we sampled multiple body sites and interrogated a large number of isolates with multiple culture methods and strain characterization methods. Additionally, due to low colonization

abundance for some subjects, the inability to consistently collect 10 colonies for analysis from BAP and 5 colonies from Spectra MRSA precluded normalization of the results.

This study revealed the presence of *S. aureus* polyclonality within a single body niche. Further, selection of culture method and sampling sites influenced the analytical sensitivity of *S. aureus* colonization detection, as well as the robustness of genotypic and phenotypic strain recovery. The analysis of co-colonizing strains may contribute to a better understanding of horizontal gene transfer. Additionally, these analyses may inform the mechanisms by which co-colonizing strains interact and influence the progression to or protection from infection and disease states. Prospective studies following individuals with polyclonal colonization will address these pertinent questions.

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Highlights

- *S. aureus* polyclonality was assessed by phenotypic and genotypic methods.
- Polyclonality existed within a single body niche in community-dwelling individuals.
- Inclusion of broth enrichment increased recovery of *S. aureus* isolates.
- Sampling multiple body niches increased recovery and diversity of *S. aureus* isolates.

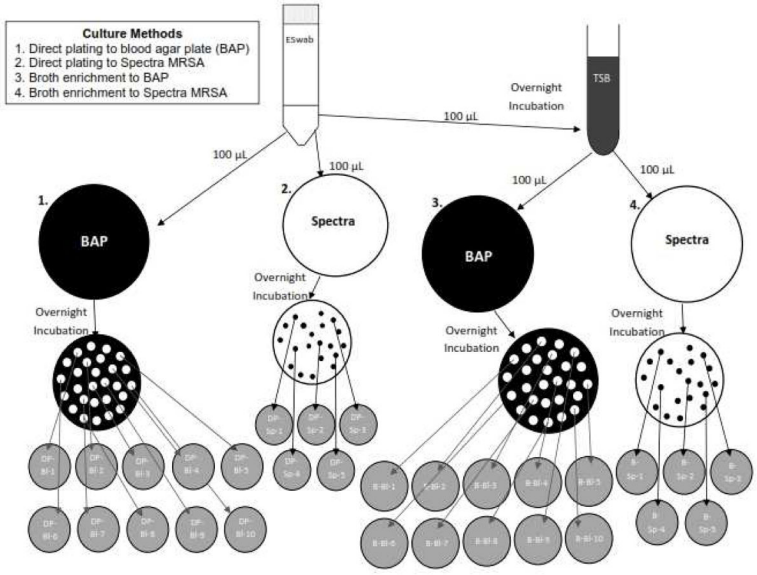

Figure 1.

Four separate culture methods were compared: 1) direct plating to blood agar plate (BAP), 2) direct plating to Spectra MRSA, 3) broth enrichment with subculture to BAP, and 4) broth enrichment with subculture to Spectra MRSA.

Table 1

Phenotypic *Staphylococcus aureus* strain characterization by antibiotic susceptibility patterns

Antibiotic susceptibility pattern[a]	Methicillin[b]	Clindamycin[c]	Erythromycin	Ciprofloxacin
AA-R	Res	Susc	Res	Susc
BB-R	Res	Susc	Susc	Susc
CC-R	Res	Susc	Susc	Inter
DD-R	Res	Susc	Res	Res
EE-S	Susc	Susc	Susc	Susc
FF-S	Susc	Res	Res	Susc

Abbreviations: Res, resistant; Susc, susceptible; Inter, intermediate

Note: All isolates were susceptible to trimethoprim-sulfamethoxazole, rifampin, tetracycline, linezolid, and ceftaroline.

[a]The –R or –S designation to the strain classification reflects resistance (-R) or susceptibility (-S) to methicillin, respectively.

[b]As predicted by cefoxitin testing.

[c]One isolate was both clindamycin susceptible and D-test positive and thus considered clindamycin resistant.

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Table 2

Genotypic and phenotypic *Staphylococcus aureus* strain characterization by body site and culture method

		Number of isolates collected by body niche and culture method					
		Anterior Nares		Axillae		Inguinal folds	
repPCR strain type	Phenotype	Broth enrichment	Direct plating	Broth enrichment	Direct plating	Broth enrichment	Direct plating
RS1, n=1 (%)	EE-S	33	33	1 (100)		33	
RS2, n=61 (%)	EE-S	19 (31)	12 (20)	17 (28)	1 (2)	4 (6)	8 (13)
RS3, n=28 (%)	AA-R	1 (3.5)					
	EE-S	6 (21)	5 (18)	3 (11)	3 (11)	9 (32)	1 (3.5)
RS4, n=29 (%)	AA-R	2 (7)					
	EE-S	17 (59)		10 (34)		3	
RS5, n=1 (%)	EE-S	33	1 (100)				
RS6, n=3 (%)	EE-S		3 (100)				
RS7, n=130 (%)	AA-R	36 (28) 3	15 (12)	16 (12)	3 (2)	11 (8) 3	16 (12)
	BB-R	6 (4)	2 (2)			33	
	CC-R	1 (1)				33	
	DD-R	15 (12)				33	
	EE-S		2 (2)	7 (5)		33	
RS8, n=38 (%)	AA-R	2 (5)				1 (3)	
	EE-S	3 (8)	1 (3)	7 (18) 3	6 (16)	13 (34)	4 (10)
	FF-S					1 (3)	
RS9, n=1 (%)	EE-S	33				33	1 (100)
RS10, n=35 (%)	EE-S	10 (28)	16 (46)		1 (3)	8 (23)	
RS11, n=1 (%)	AA-R	33	1 (100)	3		33	
RS12, n=1 (%)	AA-R		1 (100)				

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		Number of isolates collected by body niche and culture method					
		Anterior Nares		Axillae		Inguinal folds	
repPCR strain type	Phenotype	Broth enrichment	Direct plating	Broth enrichment	Direct plating	Broth enrichment	Direct plating
RS13, n=1 (%)	EE-S	1 (100) 3					
RS14, n=5 (%)	EE-S		2 (40)		3 (60)		
RS15, n=1 (%)	EE-S	33	33		1 (100)	33	

Abbreviations: repPCR, repetitive-sequence PCR

Note: Phenotype descriptions are listed in Table 1. Percentages represent proportion of isolates within each repPCR strain type.

Table 3

Discriminatory indices of strain characterization methods within participants

	Strain characterization method					
	Genotypic		Phenotypic		Aggregate[a]	
Participant ID	# Distinct strains	Discriminatory index	# Distinct strains	Discriminatory index	# Distinct strains	Discriminatory index
A	6	0.60	1	n/a	6	0.60
B	5	0.40	4	0.51	9	0.61
C	6	0.78	3	0.43	7	0.81
D	1	n/a	1	n/a	1	n/a
E	6	0.71	2	0.47	8	0.83
F	3	0.29	1	n/a	3	0.29
G	2	0.60	2	0.60	2	0.60
H	2	0.34	1	n/a	2	0.34
I	5	0.76	2	0.29	5	0.76
J	3	0.59	3	0.33	5	0.64
K	1	n/a	1	n/a	1	n/a
L	2	0.54	1	n/a	2	0.54
M	1	n/a	1	n/a	1	n/a
N	1	n/a	1	n/a	1	n/a

Abbreviations: n/a, not applicable

[a]Aggregate represents the combination of genotypic and phenotypic strain characterization methods.

Table 4

Analytical sensitivity for recovering *Staphylococcus aureus* by different culture methods within a body niche

	Anterior Nares		Axillae		Inguinal Folds	
	All *S. aureus* n=12 (%)	**MRSA only n=8 (%)**	**All *S. aureus* n=7 (%)**	**MRSA only n=4 (%)**	**All *S. aureus* n=8 (%)**	**MRSA only n=3 (%)**
Direct plating to BAP	5 (42%)	1 (13%)	4 (57%)	1 (25%)	5 (63%)	3 (100%)
Direct plating to Spectra MRSA[a]	3 (25%)	3 (38%)	n/a	n/a	2 (25%)	2 (67%)
Broth enrichment to BAP	12 (100%)	6 (75%)	7 (100%)	3 (75%)	8 (100%)	3 (100%)
Broth enrichment to Spectra MRSA[a]	4 (33%)	4 (50%)	1 (14%)	1 (25%)	1 (13%)	1 (33%)

Abbreviations: MRSA, methicillin-resistant *S. aureus*; BAP, blood agar plate

[a]Spectra MRSA is selective for the growth of MRSA.

Table 5

Strain diversity by culture methods and body niche

	Overall				METHODS															
					Direct plating to BAP				Broth enrichment to BAP				Direct plating to Spectra MRSA				Broth enrichment to Spectra MRSA			
	Genotypic		Phenotypic		Genotypic		Phenotypic		Genotypic		Phenotypic		Genotypic		Phenotypic		Genotypic		Phenotypic	
Sites	Mean	Median (Range)	Mean	Median (Range)	Mean	Median (Range)	Mean	Median (Range)	Mean	Median (Range)	Mean	Median (Range)	Mean	Median (Range)	Mean	Median (Range)	Mean	Median (Range)	Mean	Median (Range)
All sites	3.1	2.5 (1–6)	1.7	1.0 (1–4)	2.1	2.0 (1–4)	1.3	1.0 (1–3)	2.3	2.0 (1–4)	1.5	1.0 (1–4)	1.3	1.0 (1–2)	1.0	1.0 (1–1)	1.0	1.0 (1–1)	1.3	1.0 (1–2)
Anterior Nares	2.8	2.0 (1–6)	1.6	1.0 (1–4)	2.6	2.0 (2–4)	1.4	1.0 (1–3)	1.8	1.5 (1–3)	1.3	1.0 (1–3)	1.3	1.0 (1–2)	1.0	1.0 (1–1)	1.0	1.0 (1–1)	1.3	1.0 (1–2)
Axillae	2.0	2.0 (1–4)	1.4	1.0 (1–2)	2.3	2.0 (2–3)	1.3	1.0 (1–2)	1.6	1.0 (1–3)	1.7	2.0 (1–2)	n/a	n/a	n/a	n/a	1.0	1.0 (1–1)	1.0	1.0 (1–1)
Inguinal Folds	1.9	2.0 (1–3)	1.3	1.0 (1–2)	1.4	1.0 (1–2)	1.2	1.0 (1–2)	1.9	2.0 (1–3)	1.3	1.0 (1–2)	1.0	1.0 (1–1)	1.0	1.0 (1–1)	1.0	1.0 (1–1)	1.0	1.0 (1–1)

Abbreviations: BAP, blood agar plate; MRSA, methicillin-resistant *S. aureus*

Note: n/a – no isolates recovered using direct plating to Spectra MRSA from axillae cultures. Data represented as the mean and median (range) number of genotypic and phenotypic strain types by culture method at each body site. Range represents the minimum and maximum number of strain types recovered.