Molecular Characterization of Methicillin-Susceptible *Staphylococcus aureus* Clinical Isolates in the United States, 2004 to 2010

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While much is known about the geographic distribution of different clonal types of methicillin-resistant *Staphylococcus aureus* (MRSA), few studies have assessed the molecular epidemiology of methicillin-susceptible *S. aureus* (MSSA), despite its continued clinical importance. In each U.S. Census region, reference laboratories collected successive MSSA isolates from patients with invasive or superficial staphylococcal infections for use in the Tigecycline Evaluation and Surveillance Trial. All isolates from the periods of 2004 to 2005 and 2009 to 2010 underwent antimicrobial susceptibility testing and characterization of their staphylococcal protein A (*spa*) type. Of the 708 isolates analyzed, 274 *spa* types were identified and divided into 15 genetic clusters. The most common clones were *spa* t002 ($n = 63$, 8.9%) and t008 ($n = 56$, 7.9%). While the distribution of the predominant *spa* types did not differ by U.S. Census region or time period, *spa* t008 was nearly twice as common in community skin and soft tissue infections than in nosocomial bloodstream infections (11.1% versus 5.6%, respectively; $P = 0.008$). Despite such differences, both community and nosocomial settings had diverse staphylococcal clonal types representing all major *spa* clusters. In contrast to those of MRSA, MSSA infectious isolates show wide genetic diversity without clear geographical or temporal clustering. Notably, the prevalent MSSA strains (*spa* t002 and *spa* t008) are analogous to the predominant MRSA clones, further demonstrating the importance of both lineages.

The epidemiology of *Staphylococcus aureus* infection has changed dramatically over the past 15 years. Methicillin-resistant *S. aureus* (MRSA) infections, once confined to individuals in the hospital setting, are now routinely seen in the community (4, 9, 11). Coordinated research efforts have successfully characterized the distribution of MRSA clonal types across different geographic regions and care settings. Although methicillin-susceptible *S. aureus* (MSSA) accounts for a large proportion of staphylococcal infections, comparatively little is known about its molecular epidemiology.

A 2008 investigation by Goering et al. comprehensively described the molecular characteristics of 292 MSSA and MRSA infectious isolates obtained through global clinical trials (7). Utilizing pulsed-field gel electrophoresis (PFGE), multilocus sequence typing, staphylococcal cassette chromosome *mec* (SCC*mec*) typing, and testing for the presence of the Panton-Valentine leukocidin genes, the study found significant strain diversity among staphylococcal clinical isolates, with considerable overlap between the genetic backgrounds of MRSA and MSSA. In 2010, Grundmann et al. characterized the geographic distribution of 2,890 invasive MRSA and MSSA isolates across Europe and noted widespread diversity of the methicillin-susceptible strains without a predominant geographic pattern (8). This was in contrast to the clonal clustering found with MRSA isolates, which suggested the selection and spread of a limited number of methicillin-resistant strains. In the United States, several smaller studies have addressed the relationship between MRSA and MSSA in asymptomatic colonization, superficial skin and soft tissue infection, and invasive infection (12, 14, 16–18, 20, 21, 24, 25). A recent report by David et al. examined the epidemiology of staphylococcal infections at a large academic hospital and noted MSSA to be more frequently associated with bacteremia, endocarditis, and sepsis than MRSA,

suggesting a possible “reversal of roles” between the organisms with the two resistance patterns (6).

Despite the large burden of disease caused by MSSA in both nosocomial and community-associated infections, the distribution of specific strains across the United States remains poorly understood. This study aims to characterize systematically the molecular epidemiology and antimicrobial susceptibility of U.S. MSSA clones by geographic region, type of infection, and time period.

MATERIALS AND METHODS

This study utilized MSSA isolates collected through the Tigecycline Evaluation and Surveillance Trial (TEST Program) between 2004 and 2010 (2). The larger TEST collection included multiple Gram-negative and Gram-positive bacterial pathogens obtained from 358 study centers in 60 countries, with organism identification and data management coordinated by a single reference laboratory (Laboratories International for Microbiology Studies, International Health Management Associates [IHMA], Schaumburg, IL). All isolates included in TEST were cultured from patients with community-onset or healthcare-associated infections from blood, urine, respiratory tract, skin, wounds, or other defined sources (2). Isolates defined as “community onset” were cultured in the outpatient setting; those classified as “hospital associated” were obtained after ≥ 72 h of hospitalization. No more than one isolate was obtained per

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TABLE 1 Clinical and temporal demographics of *spa* isolates analyzed by U.S. Census region

U.S. Census region	No. of isolates (% of region)				Total no. (per region)
	2004-2005	2009-2010	CA-SSTI ^a	HA-BSI ^b	
East North Central	53 (49.1)	55 (50.9)	40 (37)	68 (63)	108
East South Central	7 (50)	7 (50)	9 (64.3)	5 (35.7)	14
Mid-Atlantic	142 (63.7)	81 (36.3)	72 (32.3)	151 (67.7)	223
Mountain	29 (85.3)	5 (14.7)	26 (76.5)	8 (23.5)	34
New England	28 (90.3)	3 (9.7)	17 (54.8)	14 (45.2)	31
Pacific	41 (93.2)	3 (6.8)	27 (61.4)	17 (38.6)	44
South Atlantic	101 (86.3)	16 (13.7)	46 (39.3)	71 (60.7)	117
West North Central	66 (79.5)	17 (20.5)	44 (53)	39 (47)	83
West South Central	53 (98.1)	1 (1.9)	17 (31.5)	37 (68.5)	54
Total (all regions)	520 (73.4)	188 (26.6)	298 (42.1)	410 (57.9)	708

^a CA-SSTI, community-acquired skin and soft tissue infection.^b HA-BSI, hospital-acquired bloodstream infection.

patient for inclusion in the study. Subcultures of each isolate were frozen in a 1:1 ratio of glycerol to Trypticase soy broth (TSB) and stored at -80°C .

For the purposes of our study, all U.S. bloodstream infection (BSI) and skin and soft tissue infection (SSTI) MSSA isolates from 2004 to 2005 and 2009 to 2010 were obtained from the TEST archives for strain typing and antimicrobial susceptibility testing. The 712 clinical isolates initially included in our study represented invasive BSIs from the hospital setting and superficial SSTIs from the community setting in each U.S. Census region over the two time periods (2004 to 2005 and 2009 to 2010). A total of 142 hospital laboratories were included in this sample, divided by region: 23 from East North Central; 6 from East South Central; 37 from Mid-Atlantic; 7 from Mountain; 8 from New England; 10 from Pacific; 29 from South Atlantic; 12 from West North Central; and 10 from West South Central. One hundred twenty-eight laboratories were represented in the earlier time period, and 34 were represented in the later time period; 20 laboratories (14%) had samples included from both time periods.

All samples underwent staphylococcal protein A (*spa*) typing by PCR sequencing to determine their clonal type. Species-specific SA442 PCR was used to confirm organism identification on those isolates not typed by *spa* PCR (13). In order to type organisms with mutations at the *spa* primer binding sites, nontypeable *S. aureus*-confirmed isolates (SA442 positive) underwent repeat *spa* typing with primers that were upstream and downstream of the standard primer binding sites (1). Isolates that we were unable to type using this modified protocol were considered nontypeable and excluded from the genetic analysis. Sequenced *spa* genes were analyzed and assigned a specific genotype by Ridom StaphType software (10, 22). Using the integrated based-upon-repeat-pattern (BURP) algorithm, *spa* types were clustered into *spa* clonal complexes if the cost differences (the minimum number of “steps” in the evolution from one *spa* type to another) were <4 . Repeats of <5 were excluded, as they are too short to cluster reliably (15). A subset of isolates identified as *spa* t002 and *spa* t008 underwent pulsed-field gel electrophoresis (PFGE) for comparison with PFGE reference strains (USA100, USA300, and USA800). SmaI digestion and electrophoresis were conducted as described previously (3). Construction of dendrograms was performed using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium).

The MICs for select Gram-positive antimicrobials were determined using broth microdilution panels purchased from MicroScan (Dade Behring, West Sacramento, CA). Positive combination type 34 panels were set up per the manufacturer’s instructions, and the appropriate quality-control organisms (*Enterococcus faecalis* ATCC 29212 and *S. aureus* ATCC 29213 for MIC testing; and *S. aureus* ATCC 29213, ATCC 43300, and BAA-977 for the cefoxitin screen and inducible clindamycin test) were tested weekly. MICs were interpreted in accordance with the most recent guidelines published by the Clinical and Laboratory Standards Institute

(5). The antimicrobial agents tested included clindamycin, cefazolin, daptomycin, erythromycin, levofloxacin, linezolid, penicillin, rifampin, trimethoprim-sulfamethoxazole, and vancomycin. Expression of inducible clindamycin resistance due to the *erm* gene was determined by the MicroScan inducible clindamycin (ICd) test, as described previously (23). A subset of samples was tested for mupirocin susceptibility using Etest strips (bioMérieux, Marcy l’Etoile, France).

Data were analyzed with SPSS software (IBM, Armonk, New York). A two-tailed Pearson chi-square test was utilized for categorical data. Statistical significance was set to an α value of ≤ 0.05 .

RESULTS

Although 712 isolates were initially included in this study, four were found to be SA442 negative by PCR and were subsequently excluded. Of the 708 specimens that we characterized, 57% were isolated from men and 43% from women. The mean age of the infected individuals was 47.6 years, with a range of 1 month to 100 years. The geographic, clinical, and temporal distributions of this collection are noted in Table 1. Nosocomial BSI isolates were more frequently included than were community SSTI isolates. More samples were included from the earlier time period (2004 to 2005) than from the later period (2009 to 2010).

FIG 1 Overall frequency of *spa* types. Types with $<0.5\%$ overall prevalence (≤ 3 isolates per type) are categorized as “others” ($n = 244$ of 277 total *spa* types).

TABLE 2 Frequencies of prevalent *spa* clusters

Cluster (prevalent types)	No. of <i>spa</i> types	2004-2005 ^a	2009-2010 ^a	<i>P</i> ^b	CA-SSTI ^{a,c}	HA-BSI ^{a,d}	<i>P</i> ^e	Total ^a	Cumulative total ^a
t002 (t002, t179, t242, t548, t688)	31	85 (16.4)	35 (18.6)	0.49	45 (15.1)	75 (18.4)	0.25	120 (17)	120 (17)
t012 (t012, t018, t019, t021, t122, t338)	37	89 (17.2)	26 (13.8)	0.29	47 (15.8)	68 (16.7)	0.75	115 (16.3)	235 (33.3)
t008 (t008, t334, t701)	17	62 (12)	25 (13.3)	0.64	48 (16.1)	39 (9.6)	0.01	87 (12.3)	322 (45.6)
t084 (t084, t346)	27	59 (11.4)	12 (6.4)	0.05	25 (8.4)	46 (11.3)	0.208	71 (10.1)	393 (55.7)
t004 (t004, t015, t065)	33	37 (7.1)	15 (8)	0.7	15 (5)	37 (9.1)	0.04	52 (7.4)	445 (63)
t127 (t127, t267, t359, t922)	18	37 (7.1)	15 (8)	0.7	26 (8.7)	26 (6.4)	0.24	52 (7.4)	497 (70.4)
t189 (t189, t209)	4	24 (4.6)	10 (5.3)	0.7	19 (6.4)	15 (3.7)	0.1	34 (4.8)	531 (75.2)
Others		125 (24.1)	50 (26.6)	0.5	73 (24.5)	102 (25)	0.88	175 (24.8)	706 (100)

^a Data are number (%) of clusters.^b *P* values compare the data from the earlier time period (2004 to 2005) to those of the later time period (2009 to 2010).^c CA-SSTI, community-acquired skin and soft tissue infection.^d HA-BSI, hospital-acquired bloodstream infection.^e *P* values compare CA-SSTI data to HA-BSI data.

Among the 708 MSSA isolates examined, 274 *spa* types were identified, 58 of which were newly assigned during this study. The frequency of each *spa* type was variable, with 1 to 63 isolates per clonal type. The most commonly encountered *spa* types were t002

(*n* = 63, 8.9%), t008 (*n* = 56, 7.9%), t012 (*n* = 31, 4.4%), and t084 (*n* = 30, 4.2%). Of note, two of the most prevalent clones identified in our study, *spa* t002 and *spa* t008, are also the predominant MRSA strains circulating in hospitals and the community, respec-

FIG 2 Frequency of *spa* clusters by clinical condition (community SSTI and hospital BSI) and time period (2004 to 2005 and 2009 to 2010). SSTI, skin and soft tissue infection; BSI, bloodstream infection.

FIG 3 Prevalent MSSA strains (t002, t008, and t012) by U.S. Census region. Data are expressed as numbers (percentages).

tively (19, 25, 26). Two of the 708 MSSA isolates (0.3%) were not able to be assigned a *spa* type through the protocol described above and were consequently excluded from clustering analyses. Figure 1 illustrates the prevalences of the *spa* types within our sample. Using the integrated BURP algorithm, the 274 *spa* types clustered into 15 groups, with 59 *spa* types not falling into any group. The prevalence of each *spa* clonal complex (cluster) is noted in Table 2.

Both individual *spa* types and *spa* clusters showed heterogeneity over the earlier and later time periods. Similar heterogeneity was noted among *spa* types and clusters between nosocomial BSI and community SSTI isolates. Of note, *spa* t008 was nearly twice as prevalent in community SSTIs than in invasive healthcare-associated BSIs (11.1% versus 5.6%, respectively; $P = 0.008$). Despite this, strains of both types (*spa* t002 and *spa* t008) remain common in each setting. Table 2 and Fig. 2 reflect the prevalence of *spa* clusters over the two time periods and the two clinical settings. Figure 3 illustrates the prevalences of strains of types *spa* t002, *spa* t008, and *spa* t012 by region in the U.S. Census.

PFGE was performed on 21 of the 63 isolates (33.3%) identified as *spa* t002 and on 22 of the 56 isolates (39.3%) identified as *spa* t008. Among the 21 *spa* t002 isolates analyzed by PFGE, 20 isolates (95.2%) demonstrated >80.0% similarity with each other and the USA100 and USA800 reference strains. All *spa* t008 isolates compared with the USA300 reference strain showed >80% similarity with each other. Banding patterns and dendrograms are shown in Fig. S1 in the supplemental material.

There was no clinically significant variation based on time period, geographic location, or clinical setting in the susceptibilities to common staphylococcal antibiotics (Table 3). However, statistically significant differences in antimicrobial susceptibility were identified based on strain type. Two hundred one samples underwent testing for mupirocin susceptibility. Four isolates (2%) had intermediate-level resistance (MIC, 8 to 256) and two isolates (1%) had high-level resistance (MIC ≥ 512). Resistant isolates

were present in the 2004–2005 and 2009–2010 time periods, inpatient and outpatient settings, and comprised several strain types: *spa* t002 ($n = 2$) and t021, t065, t067, and t084 ($n = 1$ each).

DISCUSSION

Significant research has characterized the evolution and spread of MRSA in hospital and community settings. Despite this, comparatively little is known about the evolution and spread of MSSA. Several reports have examined the relationship between MRSA and MSSA in both colonization and infection and have noted high rates of MSSA invasive disease (6). The predominant strains have shown variability between studies, possibly due to the temporal or geographic heterogeneity of both endemic and epidemic clones. This study addresses these gaps by systematically characterizing the genotypes and antimicrobial susceptibilities of infectious MSSA clones across the United States by geographic region, type of infection, and time period.

In contrast to the geographic clustering noted with MRSA strains, MSSA infectious isolates appear to have preserved genetic diversity across all U.S. Census regions. This finding is consistent with the previous report of Grundmann et al. which showed similar MSSA heterogeneity across Europe (8). Differences in molecular epidemiology between MRSA and MSSA might be related to several processes that affect staphylococcal genetic evolution. Importantly, two of the more prevalent MSSA clones found in our study (*spa* t002 and *spa* t008) are analogous to commonly circulating MRSA strains, further demonstrating the importance of these lineages. Our PFGE analysis of a subset of *spa* t002 and t008 isolates suggests that the majority would be characterized as USA100 and USA300 (the reference strains), respectively. These data support the previous findings of Orscheln et al. and suggest that the spread of *S. aureus* through hospitals and the community is related more to the characteristics of particular clones than to methicillin resistance (20).

While antimicrobial susceptibility testing did yield some statis-

TABLE 3 Isolates not susceptible to specific antistaphylococcal agents^a

Isolates	n	No. (%) of isolates not susceptible to indicated antibiotic ^b									
		CLI	CFZ	DAP	ERY	LVX	LZD	PEN	RIF	SXT	VAN
Overall	708	41 (5.8)	3 (0.4)	1 (0.1)	220 (31.1)	82 (11.6)	0	560 (79.1)	0	4 (0.6)	0
Clinical setting											
Outpatient	298	10 (3.4)	0	0	98 (32.9)	30 (10.1)	0	243 (81.5)	0	1 (0.3)	0
Inpatient	410	31 (7.6)	3 (0.7)	1 (0.2)	122 (29.8)	52 (12.7)	0	317 (77.3)	0	3 (0.7)	0
P ^c		0.02	0.14	0.4	0.37	0.28	NA	0.17	NA	0.49	NA
Time period											
2004–2005	520	31 (6)	0	1 (0.2)	156 (30)	61 (11.7)	0	421 (81)	0	4 (0.8)	0
2009–2010	188	10 (5.3)	3 (1.6)	0	64 (34)	21 (11.2)	0	139 (73.9)	0	0	0
P ^d		0.75	0.004	0.55	0.31	0.84	NA	0.04	NA	0.23	NA
Genotype											
t002	63	18 (28.6)	3 (4.8)	0	35 (55.6)	25 (39.7)	0	41 (65.1)	0	0	0
t008	56	1 (1.8)	0	0	35 (62.5)	8 (14.3)	0	48 (85.7)	0	0	0
t012	31	1 (3.2)	0	0	8 (25.8)	3 (9.7)	0	30 (96.8)	0	0	0
All others	556	19 (3.4)	0	1 (0.2)	140 (25.2)	44 (7.9)	0	439 (79)	0	4 (0.7)	0
P ^e		<0.001	<0.001	0.97	<0.001	<0.001	NA	0.002	NA	0.781	NA
U.S. Census region											
East North Central	108	7 (6.5)	0	0	25 (23.1)	16 (14.8)	0	81 (75)	0	2 (1.9)	0
East South Central	14	0	0	0	7 (50)	1 (7.1)	0	12 (85.7)	0	0	0
Mid-Atlantic	223	12 (5.4)	0	0	73 (32.7)	29 (13)	0	173 (77.6)	0	1 (0.4)	0
Mountain	34	1 (2.9)	0	0	8 (23.5)	1 (2.9)	0	28 (82.4)	0	0	0
New England	31	1 (3.2)	0	0	15 (48.4)	5 (16.1)	0	25 (80.6)	0	0	0
Pacific	44	3 (6.8)	0	0	11 (25)	9 (20.5)	0	34 (77.3)	0	0	0
South Atlantic	117	7 (6)	3 (2.6)	0	42 (35.9)	14 (12)	0	95 (81.2)	0	1 (0.9)	0
West North Central	83	10 (12)	0	0	26 (31.3)	6 (7.2)	0	70 (84.3)	0	0	0
West South Central	54	0	0	1 (1.9)	13 (24.1)	1 (1.9)	0	42 (77.8)	0	0	0
P ^f		0.19	0.06	0.146	0.07	0.06	NA	0.87	NA	0.77	NA

^a Not susceptible includes resistant and intermediate isolates.^b CLI, clindamycin; CFZ, cefazolin; DAP, daptomycin; ERY, erythromycin; LVX, levofloxacin; LZD, linezolid; PEN, penicillin; RIF, rifampin; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin; NA, not applicable.^c P values compare statistical differences in antimicrobial susceptibility between clinical settings.^d P values compare statistical differences in antimicrobial susceptibility between time periods.^e P values compare statistical differences in antimicrobial susceptibility between genotypes.^f P values compare statistical differences in antimicrobial susceptibility between geographic regions.

tically significant variations based on time period, clinical setting, and geographic region, the majority of these differences were not clinically significant (Table 3). An exception was the difference seen in antibiotic susceptibility based on genetic strain, with *spa* t008 isolates having a higher prevalence of susceptibility to clindamycin and levofloxacin than *spa* t002 isolates.

The present study contributes to our understanding of MSSA molecular epidemiology in several important ways. First, the systematic nature of isolate selection has resulted in data with significant clinical, geographic, and longitudinal breadth that are likely to have high external validity. Second, the analysis provides a contemporaneous snapshot of U.S. MSSA genetics that can serve as a baseline for future time points or more focused epidemiologic investigations. Third, the study employs a standardized reproducible method of genotyping that can easily be compared with data from other present or future research groups. Finally, the results might be applicable to public health practitioners for use in the control of staphylococcal transmission and infection. While both nosocomial and community MRSA strains often show clonal spread, MSSA isolates are comparatively diverse. As such, targeted interventions aimed at interrupting the transmission of a limited

number of clones will likely be ineffective for the methicillin-susceptible strains.

This study is not without limitations. Given the survey nature of the investigation, our results are largely descriptive. As we lack detailed clinical data that correspond to staphylococcal isolates, we cannot infer the importance of certain microbiological data (e.g., strain type, antimicrobial MICs) to disease severity or treatment outcome. Furthermore, the comprehensive nature of this survey lacks sufficient detail to characterize predominant clones in individual geographic locations. For example, our laboratory has found a high prevalence of the MSSA clone ST398 (previously associated with zoonotic infections in the Netherlands) in our local community of northern Manhattan, NY (27). This study failed to illustrate the high prevalence of this predominant clone in our location, likely because the U.S. Census regions themselves are geographically large and heterogeneous in nature. This problem is compounded by the variable numbers of hospitals and laboratories included from different clinical settings, time periods, and U.S. Census regions. While larger numbers of participating laboratories would provide a better estimation of population-level diversity, we believe that all regions have sufficient laboratory par-

ticipation to prevent one hospital's molecular epidemiology from dominating that of the entire geographic region.

In conclusion, our survey of MSSA molecular epidemiology in the United States revealed a preserved genetic diversity across different clinical conditions, time periods, and geographic regions. While previous studies on MRSA have shown clonal clustering around geographic locations, healthcare facilities, and patient populations, our survey failed to illustrate such findings among the methicillin-susceptible strains. Despite this difference, predominant MSSA strains appear to be analogous to major MRSA lineages. Additional studies examining larger numbers of clinical isolates and other MSSA genetic elements would be helpful in defining further the molecular epidemiology of this important pathogen.

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