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Genotypic Characteristics of *Staphylococcus aureus* Isolates from a Multinational Trial of Complicated Skin and Skin Structure Infections▽†

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The impact of bacterial genetic characteristics on the outcome of patients with *Staphylococcus aureus* infections is uncertain. This investigation evaluated potential associations between bacterial genotype and clinical outcome using isolates collected as part of an international phase 2 clinical trial (FAST II) evaluating telavancin for the treatment of complicated skin and skin structure infections (cSSSI). Ninety *S. aureus* isolates from microbiologically evaluable patients with cSSSI enrolled in the FAST II trial from 11 sites in the United States (56 isolates, or 62%) and 7 sites in South Africa (34 isolates, or 38%) were examined for staphylococcal cassette chromosome *mec*, *agr*, and the presence of 31 virulence genes and subjected to pulsed-field gel electrophoresis (PFGE). South African methicillin-susceptible *S. aureus* (MSSA) isolates were more likely to carry certain virulence genes, including *sdrD* ($P = 0.01$), *sea* ($P < 0.01$), and *pvl* ($P = 0.01$). All 44 (49%) methicillin-resistant *S. aureus* (MRSA) isolates were from the United States; 37 (84%) were strain USA 300 by PFGE. In the United States, MRSA isolates were more likely than MSSA isolates to carry genes for *sdrC* ($P = 0.03$), *map/eap* ($P = 0.05$), *fnbB* ($P = 0.11$), *tst* ($P = 0.02$), *sea* ($P = 0.04$), *sed* ($P = 0.04$), *seg* ($P = 0.11$), *sej* ($P = 0.11$), *agr* ($P = 0.09$), *V8* ($P = 0.06$), *sdrD*, *sdrE*, *eta*, *etb*, and *see* ($P < 0.01$ for all). MRSA isolates were more often clonal than MSSA isolates by PFGE. Isolates from patients who were cured were significantly more likely to contain the *pvl* gene than isolates from patients that failed or had indeterminate outcomes (79/84 [94%] versus 3/6 [50%]; $P = 0.01$). *S. aureus* strains from different geographic regions have different distributions of virulence genes.

Staphylococcus aureus causes a diverse spectrum of infections in humans, ranging from superficial skin infections to endocarditis, bone and joint infections, and septic shock (8). A growing body of evidence suggests that the presence of specific bacterial genetic characteristics can contribute to the severity of infection (1, 2, 11). However, despite significant advances in our understanding of the pathogenesis of *S. aureus* infections, the full impact of bacterial strain characteristics on the outcome of patients with *S. aureus* infections is unknown.

In the current study, we genotyped a collection of *S. aureus* isolates collected as part of an international clinical trial (FAST II) evaluating telavancin for the treatment of complicated skin and skin structure infections (cSSSI). Using these results, we compared the presence of distinct genotypic characteristics to the outcome and examined the geographic distribution of strains.

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MATERIALS AND METHODS

Patients and settings. Methods for FAST II were previously described (30). In brief, FAST II was a randomized, double-blind, active-control, parallel-group international phase II clinical trial which compared intravenous telavancin (10 mg/kg q 24 h) to intravenous standard therapy (vancomycin, 1g administered every 12 h [q 12 h]; nafcillin or oxacillin, 2g q 6 h; or cloxacillin, 0.5 to 1g q 6 h) for the treatment of cSSSI. For the current study eligible patients were males or nonpregnant females who were ≥18 years of age and who (i) had a diagnosis of cSSSI (defined as the presence of a major abscess requiring surgical drainage; deep, extensive cellulitis; an infected wound or ulcer; or an infected burn accompanied by purulent discharge and at least three other signs or symptoms of infection); (ii) had a pure culture of *S. aureus* isolated from the infected soft tissue site; and (iii) were evaluated by study investigators at a test-of-cure visit, conducted 7 to 14 days after administration of the last dose of the study medication. The investigation was approved by Duke University Medical Center Institutional Review Board.

PFGE. Pulsed-field gel electrophoresis (PFGE) with SmaI was performed on all isolates, and the gels were analyzed using the BioNumerics software (Applied Maths, Kortrijk, Belgium) as described by McDougal et al. (19). Dice coefficients (pairwise similarity) were calculated for each pair of isolates, and a dendrogram was constructed using an optimization value of 0.50% and a position tolerance ranging from 1.25% to 1.35% (end of the fingerprint).

PCR assays for genotyping. *S. aureus* strains were grown on trypticase soy agar overnight (37°C), harvested by gently scraping the cells off each plate, and resuspended in (300 μl) MicroBead solution containing 20 μl of lysostaphin (1 mg/ml; Sigma Aldrich, St. Louis, MO). The genomic DNA was extracted using an Ultraclean Microbial DNA Kit (MolBio Laboratories, Carlsbad, CA) according to manufacturer's instructions, and DNA concentration was determined by an ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The DNA samples were stored at −20°C until used for subsequent analysis. Thirty-three bacterial determinants were examined using PCR assays; these included

TABLE 1. PCR primers and conditions used in PCR assays

PCR product[a]	Primer description	Primer sequence	Product length (bp)	Positive control (alias strain[s])
Adhesins				
bbp	Forward	5′-TCAAAAGAAAAGCCAATGGCAAACG-3′	500	NRS71 (Sanger 252)
	Reverse	5′-ACCGTTGGCGTGTAACCTGCTG-3′		
clfA	Forward	5′-ATTGGCGTGGCTTCAGTGCTTG-3′	357	ATCC 25904 Newman
	Reverse	5′-GCTTGATTGAGTTGTTGCCGGTGT-3′		
clfB	Forward	5′-TGGCGGCAAATTTTACAGTGACAGA-3′	404	ATCC 25904 Newman
	Reverse	5′-AGAAATGTTCGCGCCATTTGGTTT-3′		
cna	Forward	5′-TTCACAAGCTTGGTATCAAGAGCATGG-3′	452	ATCC 25923
	Reverse	5′-GAGTGCCTTCCCAAACCTTTTGAGC-3′		
ebpS	Forward	5′-GCAAGTAATAGTGCTTCTGCCGCTTCA-3′	550	NRS71 (Sanger 252)
	Reverse	5′-CATTTTCCGGTGAACCTGAACCGTAGT-3′		
fnbA	Forward	5′-GCGGCCAAAATGAAGGTCAACA-3′	205	NRS133 (RN0025, NCTC8325)
	Reverse	5′-TCTGGTGTTGGCGGTGTTGGAG-3′		
fnbB	Forward	5′-CAGAAGTACCAAGCGAGCCGGAAA-3′	258	NRS133 (RN0025, NCTC8325)
	Reverse	5′-CGAACAACATGCCGTTGTTTGTTGA-3′		
map/eap	Forward	5′-GCATGATAGAGGTATCGGGGAACGTG-3′	655	ATCC 25904 Newman
	Reverse	5′-TCCCTTGATCATTTGCCATTGCTG-3′		
sdrC	Forward	5′-CGCATGGCAGTGAATACTGTTGCAGC-3′	731	ATCC 25904 Newman
	Reverse	5′-GAAGTATCAGGGGTGAAACTATCCACAAATTG-3′		
sdrD	Forward	5′-CCACTGGAAATAAAGTTGAAGTTTCAACTGCC-3′	467	ATCC 25904 Newman
	Reverse	5′-CCTGATTTAACTTTGTCATCAACTGTAATTTGTG-3′		
sdrE	Forward	5′-GCAGCAGCGCATGACGGTAAAG-3′	894	Sanger 476
	Reverse	5′-GTCGCCACCGCCAGTGTCATTA-3′		
spa[b]	Forward	5′-GATGACCCAAGCCAAAGCGCTAA-3′	200	NRS133 (RN0025, NCTC8325, ATCC 25923)
	Reverse	5′-TTTCTTTGCTCACCGAAGGATCGTC-3′		
Toxins				
eta	Forward	5′-CGCTGCGGACATTCCTACATGG-3′	676	NRS153 (RN8540, NRS266, HT 20020455)
	Reverse	5′-TACATGCCCGCCACTTGCTTGT-3′		
etb	Forward	5′-GAAGCAGCCAAAAACCCATCGAA-3′	419	NRS266 (HT 20020455)
	Reverse	5′-TGTTGTCCGCCTTTACCACTGTGAA-3′		
hlg	Forward	5′-TTGGCTGGGGAGTTGAAGCACA-3′	306	NRS133 (RN0025, NCTC8325)
	Reverse	5′-CGCCTGCCCAGTAGAAGCCATT-3′		
PVL	Forward	5′-TGCCAGACAATGAATTACCCCCATT-3′	894	NRS162 (HT 20000328, NRS248, HT 20020338)
	Reverse	5′-TCTGCCATATGGTCCCCAACCA-3′		
sea	Forward	5′-TTGCAGGGAACAGCTTTAGGCAATC-3′	252	NRS111 (FRI913, NRS162, HT 20000328, NRS266, HT 20020455)
	Reverse	5′-TGGTGTACCACCCGCACATTGA-3′		
seb	Forward	5′-GACATGATGCCTGCACCAGGAGA-3′	355	
	Reverse	5′-AACAAATCGTTAAAAACGGCGACACAG-3′		
sec	Forward	5′-CCCTACGCCAGATGAGTTGCACA-3′	602	NRS111 (FRI913, NRS248, HT 20020338)
	Reverse	5′-CGCCTGGTGCAGGCATCATATC-3′		
sed	Forward	5′-GAAAGTGAGCAAGTTGGATAGATTGCGGCTAG-3′	830	NRS110 (FRI472)
	Reverse	5′-CCGCGCTGTATTTTTCCTCCGAGAG-3′		
see	Forward	5′-TGCCCTAACGTTGACAACAAGTCCA-3′	532	NRS111 (FRI913)
	Reverse	5′-TCCGTGTAAATAATGCCTTGCCTGAA-3′		
seg	Forward	5′-TGCTCAACCCGATCCTAAATTAGACGA-3′	117	NRS110 (FRI472, NRS113, MNDON)
	Reverse	5′-CCTCTTCCTTCAACAGGTGGAGACG-3′		
seh	Forward	5′-CATTCACATCATATGCGAAAGCAGAAG-3′	358	NRS113 (MNDON, NRS248, HT 20020338)
	Reverse	5′-GCACCAATCACCCTTTCCTGTGC-3′		
sei	Forward	5′-TGGAGGGGCCACTTTATCAGGA-3′	220	NRS110 (FRI472, NRS113, MNDON)
	Reverse	5′-TCCATATTCTTTGCCTTTACCAGTG-3′		
sej	Forward	5′-CTCCCTGACGTTAACACTACTAATAACCC-3′	432	NRS110 (FRI472)
	Reverse	5′-TATGGTGGAGTAACACTGCATCAAAA-3′		
tst	Forward	5′-AGCCCTGCTTTTACAAAAGGGGAAAA-3′	306	NRS111 (FRI913, NRS162, HT 20000328)
	Reverse	5′-CCAATAACCACCCGTTTTATCGCTTG-3′		
Other genes				
agr genes[c]				
Group I	Forward	5′-ATCGCAGCTTATAGTACTTGT-3′	578	NRS133 (RN0025, NCTC8325)
	Reverse	5′-CTTGATTACGTTTATATTTCATC-3′		
Group II	Forward	5′-AACGCTTGCAGCAGTTTATTT-3′	814	NRS149 (502A, RN6607)
	Reverse	5′-CGACATTATAAGTATTACAACA-3′		
Group III	Forward	5′-TATATAAATTGTGATTTTTTATTG-3′	893	NRS162 (HT 20000328, NRS266, HT 20020455, NRS248, HT 20020338)
	Reverse	5′-TTCTTTAAGAGTAAATTGAGAA-3′		

Continued on following page

TABLE 1—*Continued*

PCR product[a]	Primer description	Primer sequence	Product length (bp)	Positive control (alias strain[s])
Group IV	Forward	5′-GTTGCTTCTTATAGTACATGTT-3′	757	NRS153 (RN8540)
	Reverse	5′-CTTAAAAATATAGTGATTCCAATA-3′		
chp	Forward	5′-AACGGCAGGAATCAGTACACACCATC-3′	479	NRS71 (Sanger 252)
	Reverse	5′-GGCAAGTTATGAAATGTCTGCCAAACC-3′		
efb	Forward	5′-CGGTCCAAGAGAAAAGAAACCAGTGAG-3′	303	NRS133 (RN0025, NCTC8325)
	Reverse	5′-TGTGCTTTTCTGTGTGCACTGACAGTATG-3′		
icaA	Forward	5′-TCAGACACTTGCTGGCGCAGTC-3′	936	NRS133 (RN0025, NCTC8325)
	Reverse	5′-TCACGATTCTCTCCCTCTCTGCCATT-3′		
V8	Forward	5′-CAACGAATGGTCATTATGCACCCGTA-3′	529	ATCC 49775
	Reverse	5′-TTTGGTACACCGCCCCAATGAA-3′		
arcA[b]	Forward	5′-CACGTAACTTGCTAGAACGAG-3′	724	NRS384
	Reverse	5′-GAGCCAGAAGTACGCGAG-3′		

[a] Except where noted otherwise, the conditions for the multiplex reaction were as follows: 95°C for 15 min; 32 cycles of 95°C for 1 min, 60°C for 1.3 min, and 72°C for 1 min; 72°C for 10 min; and a final hold at 4°C.

[b] Conditions for the uniplex reaction were as follows: 95°C for 5 min; 35 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min; 72°C for 10 min; and a final hold at 4°C.

[c] Conditions for uniplex reaction for *agr* genes were as follows: 95°C for 15 min; 35 cycles of 95°C for 1 min and 72°C for 1 min; 72°C for 10 min; and a final hold at 4°C. Annealing temperatures were the following: *agr* group I, 63.7°C; *agr* group II, 62.7°C; *agr* group III, 58°C; and *agr* group IV, 57°C.

toxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *tst*, *eta*, *etb*, *hlg*, and *PVL*), adhesins (*bbp*, *clfA*, *clfB*, *cna*, *ebpS*, *fnbA*, *fnbB*, *map/eap*, *sdrC*, *sdrD*, *sdrE*, and *spa*), *agr* groups I to IV, staphylococcal cassette chromosome *mec* (SCC*mec*) types I to IV, and other virulence genes (*chp*, *efb*, *icaA*, *V8*, and *arcA*). Primers and conditions used to amplify the genes of interest are described in Table 1. PCR amplifications were performed in a Dyad thermal cycler (Bio-Rad, Hercules, CA) with HotStart *Taq* polymerase (Qiagen, Valencia, CA). Genomic DNA (≈100 ng) was added to 1× multiplex PCR mix containing 3 mM $MgCl_2$, a 10 mM concentration of the deoxynucleoside triphosphates, a 0.2 μM concentration (each) of the forward and reverse primers, and *Taq* polymerase. PCR products were analyzed by 2% agarose gel electrophoresis. A positive control and a negative control (ATCC 6358) were included in each PCR run.

PCR was used to screen for a total of 33 genes. Of these, 30 genes were evaluated using multiplex PCR. To minimize the possibility of false-negative calls, genes that were not detected in the multiplex PCR assay were subsequently reanalyzed by uniplex PCR for confirmation of presence or absence of the gene. *spa*, *arcA*, and *agr* groups I to IV were detected using uniplex PCR alone. This was performed due to the presence of multiple repeats in the *spa* gene that interfered with the detection of other genes. *agr* groups I to IV were detected serially using primers described by Peacock et al. under conditions described in Table 1 (26). To verify the designation of the USA 300 genotype, the arginine catabolic mobile element-encoded *arcA* was evaluated using PCR (9). The positive control for the arginine catabolic mobile element was strain NRS384 from the Network on Antimicrobial Resistance in *Staphylococcus aureus*.

SCC*mec* typing. SCC*mec* typing was performed using multiplex PCR as described by Oliveira and de Lencastre (25). SCC*mec* types I and IV were further validated using uniplex PCR as previously described (24, 35).

Statistical methods. Categorical variables were analyzed using Pearson's chi-square test. All *P* values and confidence intervals (CIs) were two sided. Because there were no methicillin-resistant South African isolates, the evaluation of bacterial genotype associations with geographical location and methicillin resistance was limited to subgroups. Specifically, the association tests between genetic content and geography were restricted to methicillin-susceptible *S. aureus* (MSSA) isolates only, and the association tests between genetic content and methicillin susceptibility were restricted to isolates from the United States only. Significance levels were corrected for multiple tests using the false discovery rate (FDR) procedure (27). FDR thresholds of 5% and 20% were reported to balance the type I and type II error probabilities. Simpson's index of diversity and the corresponding CIs were calculated using methods previously described (13, 29). All analyses were conducted in R and verified in SAS, version 9.1 (SAS Institute Inc., Cary, NC).

RESULTS

S. aureus isolates were available from 90 study patients from 18 centers (11 from the United States and 7 from South Africa) who met criteria for the current analysis (Table 2). Most of these patients were white males. Forty-four of these 90 isolates (49%), all from U.S. sites, were methicillin-resistant *S. aureus* (MRSA). Deep abscesses were the most common form of infection in the study patients (73%), followed by cellulitis (17%). Overall, 93% of the patients were cured.

Presence of putative virulence determinants in clinical strains. The distribution of virulence genes is shown in Table

TABLE 2. Baseline characteristics of 90 clinically evaluable patients enrolled in the FAST II trial with an available pretreatment *S. aureus* isolate from a site of skin and soft tissue infection

Parameter	No. of patients (% of total)[a]
Patient demographics	
Age	42.1 ± 10.9[b]
Male	52 (58)
White race	64 (71)
Predisposing conditions	
Prior surgery	32 (36)
Diabetes	16 (18)
Trauma	15 (17)
Source of skin infection	
Major abscess	66 (73)
Infected burn	0 (0)
Deep/extensive cellulitis	15 (17)
Infected ulcer	2 (2)
Wound infection	7 (8)
Prior antimicrobial therapy	54 (60)
Infection with MRSA	44 (49)
Randomized antibiotic treatment assignment	
Telavancin	50 (56)
Standard therapy by investigator's choice	40 (44)
Vancomycin	38 (95)
Nafcillin, oxacillin, or cloxacillin	2 (5)
Geography	
United States (11 centers)	56 (62)
South Africa (7 centers)	34 (38)

[a] Percentages are based on a total patient population of 90.

[b] Mean (yr) ± standard deviation.

TABLE 3. Presence of putative virulence genes in *S. aureus* isolates from patients with cSSSI in the United States

Gene	No. of isolates positive for the gene (% of total [n = 56])	Methicillin resistance phenotype (no. of isolates [%])		P value[a]
		MRSA (n = 44)	MSSA (n= 12)	
Adhesins				
fnbA	50 (89)	40 (91)	10 (83)	0.60
clfA	56 (100)	44 (100)	12 (100)	NA
clfB	56 (100)	44 (100)	12 (100)	NA
cna	33 (59)	24 (55)	9 (75)	0.32
spa	56 (100)	44 (100)	12 (100)	NA
sdrC	49 (88)	41 (93)	8 (67)	0.03*
sdrD	53 (95)	44 (100)	9 (75)	<0.01**
sdrE	49 (88)	42 (95)	7 (58)	<0.01**
bbp	56 (100)	44 (100)	12 (100)	NA
ebps	56 (100)	44 (100)	12 (100)	NA
map/eap	45 (80)	38 (86)	7 (58)	0.05*
fnbB	53 (95)	43 (98)	10 (83)	0.11*
Toxins				
eta	35 (63)	32 (73)	3 (25)	<0.01**
etb	22 (39)	22 (50)	0 (0)	<0.01**
tst	36 (64)	32 (73)	4 (33)	0.02*
sea	34 (61)	30 (68)	4 (33)	0.04*
seb	8 (14)	7 (16)	1 (8)	0.67
sec	13 (23)	10 (23)	3 (25)	1
sed	19 (41)	18 (41)	1 (8)	0.04*
see	41 (73)	41 (93)	0 (0)	<0.01**
seg	25 (45)	17 (39)	8 (67)	0.11*
seh	26 (46)	22 (50)	4 (33)	0.35
sei	48 (86)	38 (86)	10 (83)	1
sej	50 (89)	41 (93)	9 (75)	0.11*
pvl	48 (86)	39 (89)	9 (75)	0.35
hlg	56 (100)	44 (100)	12 (100)	NA
agr group I vs all others	47 (84)	39 (89)	8 (67)	0.09*
Other genes				
efb	56 (100)	44 (100)	12 (100)	NA
icaA	56 (100)	44 (100)	12 (100)	NA
chp	55 (98)	43 (98)	12 (100)	1
V8	51 (91)	42 (95)	9 (75)	0.06*

[a] *, statistically significant result with FDR of 20%; **, statistically significant result with FDR of 5%. NA, not applicable.

TABLE 4. Distribution of virulence genes among MSSA isolates causing complicated skin and soft tissue infections in the United States and South Africa

Gene	No. of isolates positive for the gene (% of total)		P value[a]
	United States (n = 12)	South Africa (n = 34)	
Adhesins			
fnbA	10 (83)	31 (91)	0.59
clfA	12 (100)	34 (100)	NA
clfB	12 (100)	31 (91)	0.56
cna	9 (75)	31 (91)	0.32
spa	12 (100)	34 (100)	NA
sdrC	8 (67)	23 (68)	1
sdrD	9 (75)	34 (100)	0.01*
sdrE	7 (58)	23 (68)	0.73
bbp	12 (100)	31 (91)	0.56
ebps	12 (100)	34 (100)	NA
map/eap	7 (58)	13 (38)	0.31
fnbB	10 (83)	27 (79)	1
Toxins			
eta	3 (25)	16 (47)	0.31
etb	0 (0)	1 (3)	1
tst	4 (33)	17 (50)	0.5
sea	4 (33)	29 (85)	<0.01**
seb	1 (8)	11 (32)	0.14
sec	3 (25)	15 (44)	0.32
sed	1 (8)	9 (26)	0.25
see	0 (0)	11 (32)	0.04
seg	8 (67)	28 (82)	0.42
seh	4 (33)	21 (62)	0.11
sei	10 (83)	34 (100)	0.06
sej	9 (75)	32 (94)	0.1
pvl	9 (75)	34 (100)	0.01*
hlg	12 (100)	34 (100)	NA
agr group I vs all others	8 (67)	21 (62)	1
Other genes			
efb	12 (100)	34 (100)	NA
icaA	12 (100)	34 (100)	NA
chp	12 (100)	34 (100)	NA
V8	9 (75)	29 (85)	0.66

[a] *, statistically significant result with FDR of 20%; **, statistically significant result with FDR of 5%. NA, not applicable.

3. Several of these virulence genes were highly conserved among the overall group. For example, all isolates of *S. aureus* had genes for *spa* and *clfA*. As expected, significant differences were observed in the genotype of MRSA and MSSA isolates. Based on a 5% FDR threshold, MRSA isolates in the United States were significantly more likely than MSSA isolates in the United States to carry the genes *sdrD*, *sdrE*, *eta*, *etb*, and *see* (Table 3).

PFGE profiles. The PFGE profiles of MSSA isolates exhibited a larger index of diversity than MRSA isolates (Simpson's index of diversity, 0.90 [95% CI, 0.86 to 0.95] for MSSA and 0.29 [95% CI, 0.11 to 0.47] for MRSA). Among the 44 MRSA isolates, 38 (86%) were SCC*mec* IV and 6 (14%) were SCC*mec* II. Most MRSA isolates (37 isolates, or 84%) were USA 300, while the remaining isolates were USA 100 (2 isolates, or 5%), USA 400 (1 isolate, or 2%) or were untypeable (4 isolates, or 9%) (see Fig. S1 in the supplemental material). By contrast, among the 45 MSSA isolates, most (26, or 58%), were not previously identified PFGE types (see Fig. S2 in the supplemental material). PFGE on one MSSA isolate was unsuccessful. A significant proportion (10, or 29%) of MSSA isolates from South Africa belonged to a single clonal subtype (indicated as E in Fig. S2 in the supplemental material). By contrast, most MSSA isolates from the United States were either USA 300 (4, or 36%) or USA 200 (3, or 24%) (see Fig. S2 in the supplemental material). All USA 300 MRSA isolates contained the *arcA* element by PCR.

Associations of geographic region and bacterial gene distribution. Potential associations between geographic region and bacterial genetic content were considered. To do this, we compared the distribution of 33 virulence genes among MSSA isolates from the United States to that of MSSA isolates from South African patients. Since all MRSA iso-

TABLE 5. Association of putative virulence genes with clinical outcome among *S. aureus* isolates from 90 patients with skin and soft tissue infection

Gene	No. of patients (%) with the outcome:		*P* value[a]
	Cure (n = 84)	Failure or indeterminate (n = 6)	
Adhesins			
fnbA	77 (92)	4 (67)	0.11
clfA	84 (100)	6 (100)	NA
clfB	81 (96)	6 (100)	1
cna	59 (70)	5 (83)	0.67
spa	84 (100)	6 (100)	NA
sdrC	66 (79)	6 (100)	0.34
sdrD	81 (96)	6 (100)	1
sdrE	68 (81)	4 (67)	0.60
bbp	81 (96)	6 (100)	1
ebps	84 (100)	6 (100)	NA
map/eap	55 (65)	3 (50)	0.66
fnbB	75 (89)	5 (83)	1
Toxins			
eta	47 (56)	4 (67)	0.69
etb	22 (26)	1 (17)	0.69
tst	49 (58)	4 (67)	1
sea	59 (70)	4 (67)	1
seb	19 (23)	0 (0)	0.34
sec	27 (32)	1 (17)	0.66
sed	25 (30)	3 (50)	0.37
see	48 (57)	4 (67)	0.70
seg	47 (56)	6 (100)	0.08
seh	45 (54)	2 (33)	0.42
sei	77 (92)	5 (83)	1
sej	78 (93)	4 (67)	0.09
pvl	79 (94)	3 (50)	0.01*
hlg	84 (100)	6 (100)	NA
agr group I vs all others	63 (75)	5 (83)	1
Others			
efb	84 (100)	6 (100)	NA
icaA	84 (100)	6 (100)	NA
chp	83 (99)	6 (100)	1
V8	75 (89)	5 (83)	1

[a] *, statistically significant result with FDR of 20%.

lates were obtained from U.S. sites, they were excluded from this analysis. The results of these comparisons are provided in Table 4. Based on a 20% FDR threshold, South African MSSA isolates were significantly more likely to contain *sdrD* ($P = 0.01$), *sea* ($P < 0.01$), and *pvl* ($P = 0.01$) than U.S. MSSA isolates. South African MSSA isolates also demonstrated distinct genotypes by PFGE compared to U.S. MSSA isolates (see Fig. S2 in the supplemental material).

Association of bacterial genotype and patient clinical outcome. Next, we evaluated potential associations between the presence of putative virulence genes and clinical outcome (Table 5). The isolates from patients who were cured were significantly more likely to contain the *pvl* gene (79/84 isolates, or 94%, versus 3/6 isolates, or 50%; $P = 0.01$; 20% FDR threshold) than isolates from patients that failed or had an indeterminate outcome.

DISCUSSION

In the current investigation we used a collection of clinically well-characterized *S. aureus* isolates from patients with same type of infection (cSSSI) from different regions of the globe to evaluate potential associations between bacterial genotype and clinical outcome. Our investigation yielded several key observations.

First, our results demonstrated the impact of geography on the genetic composition of *S. aureus*, even among isolates associated with the same form of infection. In the current study, strains of MSSA causing skin and soft tissue infections in South Africa were significantly more likely to contain a variety of toxins or leukocidins, including *pvl* and *sea*, than MSSA isolates causing similar infections in the United States. Interestingly, these genes are known to be contained on mobile genetic elements, such as pathogenicity islands and bacteriophages (23). Thus, this observation could be due to regional dissemination of *S. aureus* clones containing these mobile genetic elements. In addition, the PFGE patterns of the South African and U.S. isolates were largely distinct. While our report is the first to compare detailed genotypic characteristics of *S. aureus* isolates causing soft tissue infections in different regions of the globe, our findings are consistent with prior observations (3, 28, 31).

Over 90% of the *S. aureus* isolates in this investigation contained the *pvl* gene. Although numerous studies have described the high rates of the *pvl* gene among strains of MRSA causing skin and soft tissue infections (5, 12, 17, 22, 34), its prevalence in the current study was higher than previously reported. There are two potential explanations for this observation. First, the investigation focused exclusively on *S. aureus* isolated from skin and soft tissue infections, a clinical condition in which *pvl* has been strongly associated. By contrast, many previous investigations included *S. aureus* from other sites of infection in which *pvl* is less frequently encountered. Second, the investigation was contemporary. As a result, the microbiology of the current investigation more accurately reflected the emergence of the USA 300 clone as the predominant cause of soft tissue infections (21). Interestingly, the role of the *pvl* product, a bi-component of leukocidin, in the pathogenesis of these infections is still a subject of debate. For example, one group of investigators concluded that *pvl* was not a major virulence factor in a murine model of *S. aureus* sepsis (32), while another group concluded that *pvl* plays a critical role in the pathogenesis of *S. aureus* necrotizing pneumonia (15). In the current study, a high prevalence of *pvl* was seen in both MSSA and MRSA isolates, providing further epidemiological evidence linking the presence of this gene and the occurrence of skin and soft tissue infections.

Interestingly, in the current study the presence of *pvl* was significantly associated with better cure rates than infections caused by *S. aureus* not containing the *pvl* gene. This observation may be due in part to the high frequency of abscesses among infections caused by the *pvl*-constituitive isolates (7), as these lesions may often be treated with surgical drainage alone (16). Patients infected with *pvl*-constitutive strains of community-associated MRSA (CA-MRSA) have been shown to experience similar hospitalization rates (6) and outcomes (18, 20) as patients infected with strains of MRSA not containing this

gene. Finally, skin and soft tissue infections produced by CA-MRSA and CA-MSSA are not clinically distinguishable (20). Taken together, these observations suggest that the presence of *pvl*, per se, does not confer a worse clinical course in skin and soft tissue infections caused by *S. aureus* in general and MRSA in particular.

Our results are consistent with a growing number of reports documenting the emerging importance of the USA 300 clone as a predominant cause of community-acquired skin and soft tissue infections in the United States (7, 14, 21). All of the MRSA isolates in this study were from the United States, and most (>80%) were strain USA 300.

This investigation was designed to evaluate potential associations between the presence of putative virulence genes in *S. aureus* and the outcome of skin and soft tissue infections caused by this bacteria. Our findings are consistent with prior studies. For example, Peacock and colleagues found that seven genes (*fnbA*, *cna*, *sdrE*, *sej*, *eta*, *hlg*, and *ica*) were significantly more common in *S. aureus* isolates associated with invasive infection than in nasal carriage isolates. (26). However, it is possible that our results may simply reflect the clonal nature of strains associated with these infections, or the genes identified in our analyses may be in linkage disequilibrium with other genes not tested in this analysis that influence the pathogenesis of these infections. Future studies should therefore focus on validating these findings using other collections of *S. aureus* isolates and on evaluating the biological relevance of these associations in vivo.

This study was limited by its relatively small sample size. These isolates were collected from a clinical trial and may not necessarily be representative of the epidemiology of infections in the study area. Additionally, at least in part due to the small sample size, there were no MRSA isolates within the South African subset of our collection, while more than three-quarters of the U.S. isolates were MRSA. For this reason, it was necessary to limit the evaluation of geographical differences in virulence factors to the MSSA subset in order to minimize the confounded effects of geography and MRSA/MSSA status in our sample. Other investigations currently under way using larger international collections of *S. aureus* isolates from soft tissue infections (4) and endocarditis (10) may be able to more fully characterize the geographical distribution of virulence factors and potentially validate the observations made in the current study. The current investigation evaluated only the presence or absence of particular genes based on PCR. Thus, it was not designed to evaluate expression of these genes or the presence of single nucleotide polymorphisms, which can influence the function of the gene products (33). Finally, this evaluation focused by design only upon infections in which the causative organism was available for culture. As a result, these observations cannot be generalized to soft tissue infections if the pathogen cannot be cultured.

Despite these limitations, however, the results of the current investigation offer several important observations. Although the *pvl* gene was found in the majority of MSSA and MRSA strains causing soft tissue infection in this study, its presence was associated with a better clinical outcome. The relative distribution of virulence genes differs significantly among *S. aureus* strains from different parts of the world—even when these bacteria are associated with same type of infection. The genetic variation in these clinical *S. aureus* isolates emphasizes the diversity of this emerging cause of human infections.

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