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Characterization of a Strain of Community-Associated Methicillin-Resistant *Staphylococcus aureus* Widely Disseminated in the United States†

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A highly stable strain of *Staphylococcus aureus* with a pulsed-field gel electrophoresis type of USA300 and multilocus sequence type 8 has been isolated from patients residing in diverse geographic regions of the United States. This strain, designated USA300-0114, is a major cause of skin and soft tissue infections among persons in community settings, including day care centers and correctional facilities, and among sports teams, Native Americans, men who have sex with men, and military recruits. The organism is typically resistant to penicillin, oxacillin, and erythromycin (the latter mediated by *msrA*) and carries SCC*mec* type IVa. This strain is variably resistant to tetracycline [mediated by *tet*(K)]; several recent isolates have decreased susceptibility to fluoroquinolones. *S. aureus* USA300-0114 harbors the genes encoding the Panton-Valentine leucocidin toxin. DNA sequence analysis of the direct repeat units within the *mec* determinant of 30 USA300-0114 isolates revealed differences in only a single isolate. Plasmid analysis identified a common 30-kb plasmid that hybridized with *blaZ* and *msrA* probes and a 3.1-kb cryptic plasmid. A 4.3-kb plasmid encoding *tet*(K) and a 2.6-kb plasmid encoding *ermC* were observed in a few isolates. DNA microarray analysis was used to determine the genetic loci for a series of virulence factors and genes associated with antimicrobial resistance. Comparative genomics between USA300-0114 and three other *S. aureus* lineages (USA100, USA400, and USA500) defined a set of USA300-0114-specific genes, which may facilitate the strain's pathogenesis within diverse environments.

Staphylococcus aureus continues to be a major cause of health care-associated infections (1, 18, 19, 45, 53). Recently, strains of methicillin (oxacillin)-resistant *S. aureus* (MRSA) have been recovered from infections in community settings (8, 11, 27, 44, 49, 64) and among the urban poor in San Francisco, California (12). Most community MRSA strains harbored the *lukF-PV* and *lukS-PV* determinants, which encode the Panton-Valentine leucocidin (PVL) toxin (20, 26, 28, 35, 43). Using pulsed-field gel electrophoresis (PFGE), we recently described two major types of community-associated MRSA, designated USA300 and USA400 (46), both of which typically harbor PVL. USA400 isolates were associated with the deaths of four children in Minnesota and North Dakota in 1999 (6), all of whom were treated with cephalosporins. An isolate of the same lineage as USA400 (also known as *S. aureus* MW-2) was responsible for a series of infections in health care settings across Canada (61), infections in Native Americans (31) and among children in day care (33), and an outbreak of *S. aureus* infection on a maternity ward of a hospital in New York (62). USA300 isolates have been recovered from a variety of community populations, including children (5, 39), correctional facility inmates (7, 10), participants in sports teams (9, 40), men who have sex with men (8, 34, 42), and military recruits (72). Over the last 3 years, outbreak investigations conducted by the Centers for Disease Control and Prevention (CDC) in diverse geographic locations and with diverse patient populations often yielded the same USA300 PFGE pattern, designated USA300-0114, from wound cultures and other clinical specimens (40). These isolates yielded indistinguishable macrorestriction PFGE profiles with five restriction endonucleases, were consistently erythromycin resistant, clindamycin susceptible, and D-zone test (clindamycin induction) negative, harbored staphylococcal cassette chromosome *mec* (SCC*mec*) type IVa, and carried the genes for PVL (40). *S. aureus* USA300 strains have been isolated by Hidron et al. from patients admitted to an urban hospital in Atlanta, Georgia (34), and by Chavez-Bueno et al. from children in Dallas, Texas (13). This study further characterized the antimicrobial susceptibility patterns and genetic traits of this strain by using plasmid analysis, a series of PCR assays, and DNA microarrays.

MATERIALS AND METHODS

Bacterial strains. One hundred eighty-seven isolates of *S. aureus* from the CDC strain collection (from outbreak investigations, surveillance studies, and the *Staphylococcus* reference laboratory), identified on the basis of catalase, coagulase, and sugar fermentation patterns (4), demonstrated the SmaI PFGE profile identified as USA300 (46). These isolates underwent antimicrobial susceptibility testing, SCC*mec* typing, and testing for several genes encoding toxins and virulence factors. Thirty isolates from diverse geographic locations in the United States and showing variable antimicrobial susceptibility patterns were

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selected for further studies, including DNA sequence analysis of the *mec*-associated direct repeat unit (*dru*) region (48), *agr* grouping, and plasmid analysis. Fourteen of the 30 isolates, including 6 that were USA300-0114, underwent DNA microarray analysis; all 6 USA300-0114 isolates also underwent staphylococcal protein A (*spa*) typing, and 1 USA300-0114 isolate was analyzed by multilocus sequence typing (MLST).

Antimicrobial susceptibility testing. The antimicrobial susceptibility profiles of the isolates were determined by the broth microdilution method with cation-adjusted Mueller-Hinton broth (Becton Dickinson Microbiology Systems, Cockeysville, Md.), as described in the CLSI (formerly NCCLS) publication M7-A7 (52). The antimicrobial agents tested were clindamycin, chloramphenicol, erythromycin, gentamicin, levofloxacin, linezolid, oxacillin, penicillin, quinupristin-dalfopristin, rifampin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin. Quality control strains included *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 25922, and *S. aureus* ATCC 43300. Inducible clindamycin resistance was determined using a disk diffusion D-zone test as described by the Clinical and Laboratory Standards Institute (15).

Pulsed-field gel electrophoresis. PFGE was performed as described previously (46), using a contour-clamped homogeneous electric field apparatus DR-II, DR-III, or CHEF Mapper (Bio-Rad, Hercules, CA). Running parameters were as follows: volts, 200 (6 V/cm); temperature, 14°C; initial switch, 5 s; final switch, 40 s; and time, 21 h.

Gel pattern analysis. Gels were photographed and digitized using a FOTO/Analyst Archiver system (Fotodyne, Inc., Hartland, WI), and saved as TIFF images for use with BioNumerics software (Applied Maths, Kortrijk, Belgium). The reference standard *S. aureus* NCTC 8325, which was included in the 1st, 7th, 14th, 20th, and last lanes of each gel, was normalized to the global standard *S. aureus* NCTC 8325. Percent similarities were calculated using Dice coefficients, and the unweighted-pair group method using arithmetic averages was used for cluster analyses. Band position tolerance and optimization were set at 1.25% and 0.5%, respectively.

SCC*mec* typing. SCC*mec* typing was performed essentially as described by Okuma et al. (54).

PCR assays. PCR detection of *ermA*, *ermB*, *ermC*, and *msrA* was performed as described by Sutcliffe et al. (68). The PCR primers for *tet*(K) were tetKFwd, 5′ TAG GGG GAA TAA TAG CAC ATT 3′, and tetKRev, 5′ AAT CCG CCC ATA ACA AAT A 3′. Primers for *blaZ* were blaZfwd, 5′ GGC CCT TAG GAT AAA CAA AAG 3′, and blaZrev, 5′ CAG TTC ACA TGC CAA AGA GTT 3′. Isolates were screened via PCR for carriage of the genes encoding staphylococcal enterotoxin A (SEA), SEB, SEC, SED, SEE, SEH, and toxic shock syndrome toxin 1 as previously described (45a). The presence of the genes encoding PVL (*lukS-PV* and *lukF-PV*) was assessed using the PCR assay described by Lina et al. (43).

***agr* grouping.** Multiplex PCR-based *agr* grouping was performed using the primer sets for *agr* group I, *agr* group II, and *agr* group III described by Moore and Lindsay (47). The primers for *agr* group IV were agrIV forward, 5′ CAC TTA TCA TCA AAG AGC C 3′, and agrIV reverse, 5′ GTA TTT CAT CTC TTT AAG G 3′. Cycling conditions consisted of an initial denaturation step at 94°C for 2 min, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, followed by a primer extension period of 5 min at 72°C. The primers were validated against a set of 16 control strains prior to use in the study.

Plasmid analysis. Plasmid DNA was isolated using a modified protocol for the QIAGEN plasmid mini kit (Valencia, CA). A loopful of bacteria from a Trypticase blood agar plate incubated overnight at 35°C was suspended in 500 μl of cold QIAGEN cell suspension buffer. Fifty microliters of lysostaphin (0.5 mg/ml) was added, followed by a 30-min incubation at 37°C. Plasmid profiles were determined by agarose (0.75%) gel electrophoresis before and after restriction of DNA with HindIII. Digoxigenin-labeled DNA probes were generated by PCR using DNA from the following control organisms: *S. aureus* RN4220 (*msrA*), *S. aureus* RN2442 (*ermC*), *S. aureus* CDC82-7701 [*tet*(K)], and *S. aureus* 01057 (*blaZ*). Four identical agarose gels containing uncut plasmid DNA from 11 isolates of *S. aureus* USA300-0114 with different phenotypes and genotypes were prepared, and the DNA was transferred to Zeta-Probe membranes by vacuum blotting (Boekel Scientific, Feasterville, Pa.) and probed individually using the four probes listed above as previously described (45a).

Multilocus sequence typing and *spa* typing. MLST was performed as described by Enright et al. (23, 24). *spa* typing was performed as described by Shopsin et al. (67).

Microarray analysis. The genetic compositions of *S. aureus* pulsed-field types (PFTs) USA100-0022, USA300-0114, USA400-0051, and USA500-0004 isolates were determined using *S. aureus* Affymetrix GeneChips (Saur2a) (Affymetrix, Santa Clara, CA), as previously described (21); multiple isolates of the USA300 and USA400 PFTs were analyzed. Chromosomal DNA from each isolate was interrogated for the presence or absence of the 7,792 loci represented on the Saur2a chip (21). Briefly, chromosomal DNA was purified from each isolate, fragmented, and biotinylated at the 3′ end. Then, 1.5 μg of labeled DNA was hybridized to a GeneChip, and adjusted "present" and "absent" determinations were made for each locus represented on the array (21).

Direct repeat unit sequencing. Sequence analysis of the *mec*-associated *dru* region was performed as described by Goering et al. (29, 30), using the nucleotides 5′ GTT AGCATATTACCTCTCCTTGC 3′ and 5′ GCCGATTGTGCTTGATGAG 3′ as forward and reverse primers, respectively. PCR was performed with an initial denaturation step at 94°C for 2 min, followed by 30 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min. DNA sequencing was performed using an ABI PRISM 3100-Avant genetic analyzer (Applied Biosystems, Foster City, CA).

RESULTS

PFGE analysis. A dendrogram of SmaI macrorestriction fragment patterns showing the relationship of USA300-0114 to other isolates in the USA300 PFT and other USA PFTs is presented in Fig. 1. USA300-0114 is one of several PFGE patterns within the USA300 PFT. As previously reported, the closest PFT to USA300 is USA500, which is also sequence type (ST) 8 as determined by MLST (46). USA300 PFGE patterns are distinct from other MRSA PFGE patterns (e.g., USA100, USA200, and USA400).

USA300-0114 characteristics. The antimicrobial resistance patterns of 187 oxacillin-resistant USA300 isolates were determined by the broth microdilution method. The isolates were generally susceptible to chloramphenicol (100%), clindamycin (98%), gentamicin (100%), linezolid (100%), quinupristin-dalfopristin (100%), rifampin (99%), trimethoprim-sulfamethoxazole (100%), and vancomycin (100%) but were less susceptible to levofloxacin (84%) and tetracycline (83%). On the other hand, 97% of isolates were resistant to erythromycin. Of these, only three showed inducible clindamycin resistance by use of the D-zone test; one additional isolate was constitutively resistant to clindamycin. Of the 30 isolates selected for additional testing, all were positive by PCR for SCC*mec* type IVa. All six USA300-0114 isolates that underwent *spa* typing were *spa* type YHGFMBQBLO, and the one USA300-0114 isolate analyzed by MLST was identified as ST8.

Table 1 describes the 30 isolates selected for the additional studies. The geographic locations (states) of isolation (when known), antimicrobial resistance patterns, plasmid profiles, and resistance genotypes are listed. Multiplex PCR demonstrated that all 30 isolates were *agr* group I. The *dru* regions within the *mec* determinants of the 30 isolates were sequenced. All of the isolates were *dru* type 9g, except for one isolate from Colorado, which was type 9h (Table 2).

Plasmid characterization. Eighteen (60%) of the 30 isolates contained 30-kb plasmids that were similar by restriction endonuclease analysis. Each 30-kb plasmid hybridized with both the *blaZ* and *msrA* probes (Table 1). Twenty-eight of the 30 isolates contained a 3.1-kb cryptic plasmid. The two isolates that lacked the 30-kb plasmid were β-lactamase negative and erythromycin susceptible. Seven tetracycline-resistant isolates each had a 4.3-kb plasmid that hybridized with the *tet*(K) probe. Three isolates with inducible clindamycin resistance each had a 2.6-kb plasmid that hybridized with the *ermC* probe. Isolate 19 was positive by PCR for *ermC* and showed constitutive clindamycin resistance but lacked a 2.6-kb plasmid (Table 1). The location of the *ermC* determinant is presumed to be chromosomal. Isolate 29, which was negative for *msrA* by PCR, contained a 23-kb plasmid that hybridized only with the *blaZ* probe.

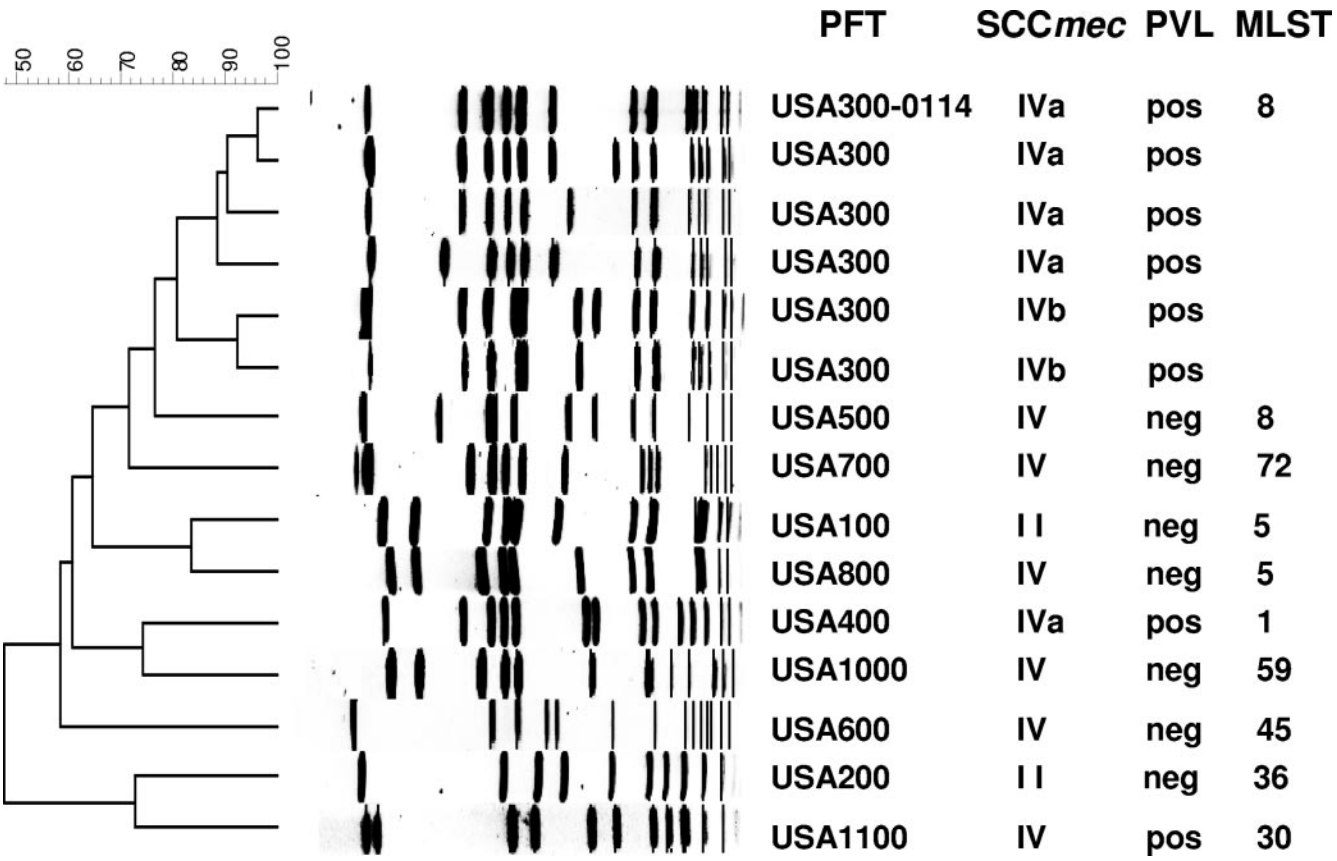

FIG. 1. PFGE profiles of SmaI macrorestriction patterns of *S. aureus* isolates of various USA types (Centers for Disease Control and Prevention, unpublished data); 46). PFTs, SCC*mec* types, results of the PCR assay for PVL toxin genes (pos, positive; neg, negative), and sequence types determined by MLST are shown.

Virulence factor characterization. All 30 isolates were positive by PCR for *lukS-PV* and *lukF-PV*, indicating the presence of the genes encoding PVL. All 30 were negative by PCR for the genes encoding SEA, SEB, SEC, SED, SEE, and SEH and toxic shock syndrome toxin 1.

By use of Affymetrix GeneChips (Saur2a), all of the six USA300-0114 isolates were highly related, with an average of only six genes differing between any two isolates [e.g., the presence or absence of *tet*(K)]. USA300-0114 isolates contained 20 of the 46 putative antimicrobial resistance genes and 117 of the 289 putative virulence factor or regulatory genes represented on the Saur2a chip (Table 3).

In addition to the plasmid-mediated resistance genes noted above, all USA300-0114 isolates carry the genes for arsenic (*arsB* and *arsC*) (37), bacitracin (*bacA*) (22), cadmium (*cadD*) (17), and fosfomycin (*fosB*) (71) resistance. The strain also contains a number of putative drug transporters, including members of the EmrB/QacA family of transporters (COL-SA2413), quinolone (*norA*) (70), an ABC transporter (*vga*), the Bcr/CflA subfamily of transporters (COL-SA2437), and the putative multidrug transport protein (COL-SA2348).

USA300-0114 isolates contain a number of virulence determinants, which can be broadly characterized as either cell surface components or extracellular elements (exoenzymes, exotoxins, and enterotoxins) (Table 3). Regarding cell surface components, USA300-0114 isolates contained all members of the capsule type 5 operon (*cap5A* to *cap5P*) and numerous adhesion genes, including clumping factor B (*clfB*) and clumping factor A (*clfA*) (Table 3). The *sdr* loci encode proteins with similarity to *clfA* and *clfB* that are involved in binding fibrinogen and bone (69). Both *sdrC* and *sdrE* were present, although *sdrD* was not. Likewise, the extracellular matrix-binding protein homologue, *ebh* (which is represented by several oligonucleotides on the microarray), and *fnbA* and *fnbB* (14, 32) (which encode fibronectin-binding protein) were present. All isolates tested also harbored the genes encoding eight LPXTG-containing proteins, protein A (*spa*), the elastin-binding protein (*ebpS*) (56), accumulation-associated protein (*sasG*) (60), and the intercellular adhesin (*icaA* to *icaD*) (16).

The USA300-0114 isolates also contained an array of extracellular virulence factors, including numerous exoenzymes, such as serine protease (*splA* to *splF*) (58), zinc metalloproteinase aureolysin (*aur*) (3), coagulase (*coa*) (57), and lipase (*geh*) (41) genes. The V8 protease (*sspA*) (59), cysteine protease (*sspB* and *sspC*) (59), and hyaluronidase (*hysA*) (25) genes were detected, in addition to four enterotoxin genes. These genes included *ent* (COL-SA0886), *entB* (COL-SA0172) (38), *sei* (COL-SA0887), and an enterotoxin family gene (COL-SA1657). USA300-0114 also harbored a number of exotoxin genes, including exotoxin 2 (COL-SA0472), exotoxin 3 (COL-SA0468), *set7*, *set8*, *set9*, *set10*, *set11*, *set12*, *set13*, and *set14*. In addition, isolates contained the genes required for α-,

TABLE 1. Description of USA300-0114 isolates characterized in this study

Isolate no.	State of isolation; population cluster (reference)	Antimicrobial resistance pattern[b]	Plasmid profile (kb)	Resistance genotype detected by PCR
1	Pennsylvania; college football team (9)	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
2	Pennsylvania; college football team (9)	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
3	Mississippi; prisoners (7)	Pen^r Oxa^r Ery^r Tet^r	30, 4.3, 3.1	*blaZ mecA msrA tet*(K)
4	Georgia; prisoners (10)	Pen^r Oxa^r Ery^r	30	*blaZ mecA msrA*
5	State unknown; nasal surveillance survey	Pen^r Oxa^r Ery^r Tet^r	30, 4.3, 3.1	*blaZ mecA msrA tet*(K)
6	State unknown; nasal surveillance survey	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
7	Tennessee; children (5)	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
8	Tennessee; children (5)	Pen^r Oxa^r Ery^r Tet^r Lvx^I	30, 4.3, 3.1	*blaZ mecA msrA tet*(K)
9	Texas; children	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
10	Texas; prisoners (10)	Pen^r Oxa^r Ery^r Tet^r	30, 4.3, 3.1	*blaZ mecA msrA tet*(K)
11	Texas; prisoners (10)	Pen^r Oxa^r Ery^r Cli^{Ind}	30, 3.1, 2.6	*blaZ mecA msrA ermC*
12	Texas; outpatient	Pen^r Oxa^r Ery^r Cli^{Ind}	30, 3.1, 2.6	*blaZ mecA msrA ermC*
13	Texas; outpatient	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
14	Colorado; fencer (9)	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
15	Colorado; fencer (9)	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
16	Washington; adult	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
17	Washington; adult	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
18	Nevada; child	Pen^r Oxa^r Ery^r Tet^r Lev^I	30, 4.3, 3.1	*blaZ mecA msrA tet*(K)
19	California; MSM[a] (8)	Pen^r Oxa^r Ery^r Cli^{Con}	30	*blaZ mecA msrA ermC*
20	California; college football team (8)	Pen^r Oxa^r Ery^r Lvx^I	30, 3.1	*blaZ mecA msrA*
21	California; prisoner (8)	Pen^r Oxa^r Ery^r Lvx^I	30, 3.1	*blaZ mecA msrA*
22	California; children (8)	Pen^r Oxa^r Ery^r Tet^r Lvx^I	30, 4.3, 3.1	*blaZ mecA msrA tet*(K)
23	California; MSM (8)	Oxa^r Tet^r Lvx^I; β-lactamase negative	4.3, 3.1	*mecA tet*(K)
24	Missouri; professional football team	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
25	California; professional football team (8)	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
26	California; professional football team (8)	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
27	California; professional football team (8)	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
28	Pennsylvania; young adult with necrotizing pneumonia	Pen^r Oxa^r Ery^r	30, 3.1	*blaZ mecA msrA*
29	Texas; patient with influenza	Pen^r Oxa^r Ery^r Cli^{Ind}	23, 3.1, 2.6	*blaZ mecA ermC*
30	Hawaii; seawater	Pen^r Oxa^r; β-lactamase negative	3.1	*mecA*

[a] MSM, men who have sex with men.
[b] Cli, clindamycin; Con, constitutive; Ery, erythromycin; I, intermediate; Ind, inducible; Lvx, levofloxacin; Oxa, oxacillin; Pen, penicillin; Tet, tetracycline.

γ-, and at least a portion of β-hemolysin production. PVL (*lukF-PV* and *lukS-PV*), *lukD* and *lukE* leucocidin, and *lukF* and *lukM* genes were also present within USA300-0114, confirming the PCR data.

Comparison of USA300-0114 and USA500-0004 lineages. Using GeneChips, we compared the composition of USA300-0114 to that of three other MRSA PFTs (USA100, USA400, and USA500). By microarray analysis, USA300-0114 is most closely related to USA500-0004. These two PFTs fall into the same ribogroup, and both are ST8 by MLST and *agr* group I, although the *spa* type of USA300-0114 differs by a single repeat (underlined) from that of USA500-0004 (YHGFMB QBLO versus YHGCMBQBLO, respectively) (46). Despite the relatedness of the two PFTs, USA300-0114 is most frequently associated with infections in the community and USA500-0004 is most frequently associated with infections in health care institutions (55, 65). Thus, a genome-wide comparison of the genetic compositions of USA300-0114 and USA500-0004 may identify genes that are unique to USA300-0114 or are associated with community- versus health care-associated infections.

As shown in Table 3, 131 of the 137 putative virulence or resistance determinants within USA300-0114 are also present within USA500-0004. However, six genes present within USA300-0114 were absent from USA500-0004. These genes include the *msrSA* locus (which is the COL annotation for the *msrA* erythromycin resistance gene), two hypothetical LPXTG motif-containing cell surface components, the PVL genes (*lukF-PV* and *lukS-PV*), and the COL *set9* exotoxin. Recognizing that genes other than defined resistance or virulence factors may contribute to the lineage's ability to cause disease and disseminate in community settings, we expanded our analysis to identify all putative open reading frames (ORFs) that are present with USA300-0114 but absent from USA500-0004. Fifty-nine USA300-0114 genes were absent from USA500-0004 (see the supplemental material). These genes included 22 putative ORFs with either very limited or no amino acid similarity to characterized proteins, a plasmid (pSR1) replication protein, and a transposase (N315-SA0062). Interestingly, the majority of the genes that differentiate these two lineages are bacteriophage encoded. More specifically, two hypothetical phi ETA proteins, three bacteriophage phi PVL genes, and 22 phi

TABLE 2. Sequences of *dru* regions found in 30 USA300-0114 isolates[a]

dru type	Sequence
9g	5′ ATAAGAGGAATAGTAAAAGCAATTCTAAGTAAAATTGCAG
	ATAAGAGGTTTGTTAAAAGCAGTTCTCAGTAAAATTACAG
	ATAAGAGGTACGTTAAAAGCAGTTCTAAGTAAAATTGCAG
	ATAAGAGGTTTGTTAAAAGCAGTTCTAAGTAAAATTGCAG
	ATAAGAGGTACGTTAAAAGCAATTCCATGCAAAATTGCTG
	ATAAGGGGTAAGTTAAAAGCAGTTCTCAGTAAAATTGCAG
	ATAAGAGGTACGTTAAAAGCAGTTCTAGGCAAAATTGCAG
	ATAAGAGGTGCGTTAAAAGCAGTTCTCAGTAAAATTGCTG
	ATAAGGGGTAAGTTAAAAGCAATCCTAAGTAAAATTGCAG 3′
9h	5′ ATAAGAGGAATAGTAAAAGCAATTCTAAGTAAAATTGCAG
	ATAAGAGGTTTGTTAAAAGCAGTTCTCAGTAAAATTACAG
	ATAAGAGGTACGTTAAAAGCAGTTCTAAGTAAAATTGCAG
	ATAAGAGGTTTGTTAAAAGCAGTTCTAAGTAAAATTGCAG
	ATAAGAGGTACGTTAAAAGCAGTTCTAAGTAAAATTGCAG
	ATAAGAGGTTTGTTAAAAGCAGTTCTAAGTAAAATTGCAG
	ATAAGAGGTACGTTAAAAGCAATTCCATGCAAAATTGCTG
	ATAAGGGGTAAGTTAAAAGCAGTTCTCAGTAAAATTGCAG
	ATAAGAGGTACGTTAAAAGCAATCCTAAGTAAAATTGCAG 3′

[a] As noted in the text, type 9g was found in 29 of the 30 isolates, while type 9h was found in only 1 isolate. Sequences (from top to bottom) are listed as they occurred in tandem in the 5′ to 3′ direction. Sequence analysis was based on using ATAAGAGGTA CGTTAAAAGC AGTTCTAAGT AAAATTGCAG as the consensus sequence (30).

N315 genes were present in USA300-0114 but missing from USA500-0004. It is possible that one (or more) of these phage-encoded proteins confers a selective advantage to USA300-0114 isolates in community settings.

Comparison of USA300-0114 and USA400 lineages. We next compared the genetic composition of USA300-0114 to that of representative isolates of USA400, a less frequently observed community-associated MRSA PFT (*spa* type UJJJJFE, *agr* group III, ST1 by MLST). There were 27 virulence or antimicrobial resistance genes present within USA300-0114 that were missing from USA400 (Table 3). These included several cell surface components that are involved in fibronectin binding (*fnbA*, *fnbB*, and *ebh*) and three exotoxins (MW-2 exotoxin 21 and two exotoxin 3 genes [COL-SA0478 and COL-SA0468]). USA300-0114 also harbored 155 other genes that were absent from USA400 (see the supplemental material). Of these 155, 106 ORFs encoded hypothetical proteins. The remaining genes included five SaPIn2 pathogenicity island proteins, a single SaPIbov pathogenicity island protein, a bacteriophage phi PVL antirepressor, and six bacteriophage phi N315 proteins. Interestingly, with the exception of the SaPIn2 components, each of these genes is also missing from USA500 isolates. Thus, it is possible that these genes encode proteins that facilitate USA300-0114's ability to circulate and cause disease in community settings.

Comparison of USA300-0114 and USA100 lineages. We also compared USA300-0114 to USA100 (*spa* type TJMBMD MGMK, *agr* group II, ST5 by MLST), the major health care-associated lineage in the United States (46). There were 228 genes that were present within USA300-0114 but absent from USA100, including 21 resistance or virulence determinants (Table 3) and 207 genes that have not been previously linked to pathogenesis (see the supplemental material). In comparison to USA300-0114, USA100 lacks several cell surface adhesion genes, including *fnbA*, *fnbB*, and *ebh*, all of which encode proteins that bind fibronectin. The USA100 isolate also appears to be missing several USA300-0114 extracellular virulence factor genes, such as PVL (*lukF-PV* and *lukS-PV*), *ent*, *sei*, and exotoxin 3 genes (COL-SA0468 and COL-SA0478).

USA300-0114-specific genes. By comparing the microarray-derived genetic composition of each PFT (USA300-0114, USA100, USA400, and USA500-0004), we identified a number of USA300-0114-specific genes. More specifically, a total of 20 genes were conserved across all USA300-0114 isolates but were absent from the other MRSA PFTs tested. None of these genes were previously recognized *S. aureus* virulence determinants. These included 10 hypothetical genes, the bacteriophage phi PVL antirepressor gene (AB009866-cds33), five bacteriophage phi N315 genes (N315-SA1802 to N315-SA1806), the plasmid pSR1 rep gene (AAF99572), a plasmid-associated gene (*mphBM*), a recombinase (AF053772-cds1), and a SaPIbov pathogenicity island gene (AF217235-cds17) (see the supplemental material).

DISCUSSION

During our initial investigations of clusters of MRSA infections in U.S. prisons, we were surprised that MRSA isolates with indistinguishable PFGE patterns were recovered from prisoners in Mississippi, Texas, and Georgia (7, 10, 46). We were further surprised to see additional MRSA isolates with the same PFGE pattern isolated from a variety of athletes and sports teams (9, 40), military recruits (72), children from Tennessee (5) and Texas (13, 39, 66), and a large county hospital in Atlanta (34). Clearly, this MRSA strain is widely disseminated within the United States and appears to be a major cause of community-associated infections.

USA300-0114 is ST8 by MLST, which differs by one or two loci from previously described representatives of the archaic (ST250) and Iberian (ST247) clones (24, 63, 65). The USA300-0114 isolates also share a common *spa* type motif (MBQBLO) and most likely evolved from a common ancestor with a genotype of the early archaic strains of the 1960s (55). All four SCC*mec* types have been identified among MRSA of ST8 (23, 24, 36), although USA300-0114 is exclusively type IVa. Most USA300-0114 isolates are resistant only to β-lactams and macrolides, although plasmid-mediated resistance markers, such as tetracycline and clindamycin resistance, mediated by *tet*(K) and *ermC*, respectively, are starting to appear.

Microarray analysis confirms that, in general, USA300 isolates are highly related to USA500 isolates. However, USA500 isolates are typically multiply resistant and are more likely to cause health care-associated infections than infections in community settings. When we compared the profiles of genes present within USA300 but missing from USA500, the community-associated lineage USA400, and the major health care-associated lineage USA100, several differences became apparent. First, USA300 harbors sequences from (i) a number of bacteriophages, including phi PVL and phi N315, (ii) the SaPIn2 pathogenicity island, (iii) the SaPIbov pathogenicity island, and (iv) the genes encoding a number of fibronectin-binding proteins that are missing either totally or in part from the isolates of the other PFTs surveyed. These factors may be key contributors to USA300's ability to cause infection in

TABLE 3. USA300-0114 putative antimicrobial resistance and virulence factor genes

Gene	COL ORF no.[a]	COL description	Result for[b]:				Comment
			USA300-0114	USA400	USA500-0004	USA100	
Resistance genes							
arsB	SA1823	Arsenical pump membrane protein	+	+	+	+	
arsC	SA1824	Arsenate reductase	+	−	+	−	
bacA	SA0743	Bacitracin resistance protein	+	+	+	+	
blaZ	AF086644-cds1	beta-Lactamase	+	+	+	+	
cadB	AF134905-cds1	Low-level cadmium resistance	+	+	+	−	
ddh	SA2535	2-Hydroxyacid dehydrogenase (associated with vancomycin resistance)	+	+	+	+	
fosB	SA2326	Fosfomycin resistance protein	+	−	+	+	
Hypothetical	SA2413	Drug resistance transporter	+	+	+	+	
Hypothetical	SA0772	Aluminum resistance protein	+	+	+	+	
Hypothetical	SA1327	Aluminum resistance protein	+	+	+	+	
Hypothetical	SA2348	Drug transporter	+	+	+	+	
Hypothetical	SA2437	Drug resistance transporter	+	+	+	+	
mecA	SA0033	Penicillin-binding protein 2	+	+	+	+	
msrA	SA1397	Peptide methionine sulfoxide reductase	+	+	+	+	
msrA	SA2683	Peptide methionine sulfoxide reductase	+	+	+	+	
msrA	SA1459	Peptide methionine sulfoxide reductase	+	+	+	+	
msrSA	AB016613-cds3	Erythromycin resistance protein	+	+	−	−	
norA	SA0754	Drug transporter	+	+	+	+	
semB	SA2347	Drug resistance transporter	+	+	+	+	
vga	SA2036	ABC transporter	+	+	+	+	
Virulence genes							
Regulatory genes							
agrA	SA2026	Accessory gene regulator protein A (type 1)	+	+	+	+	
agrB	SA2023	Accessory gene regulator protein B (type 1)	+	+	+	+	
agrB	SA2023	Accessory gene regulator protein B (type 1)	+	−	+	−	USA400 *agr* type III; USA100 *agr* type II
agrD		Accessory gene regulator protein D (type 1)	+	−	+	−	USA400 *agr* type III; USA100 *agr* type II
icaR	SA2688	Intercellular adhesion regulator	+	+	+	+	
sarA	SA0672	Staphylococcal accessory regulator A	+	+	+	+	
sarR	SA2287	Staphylococcal accessory regulator R	+	+	+	+	
sarS (sarH1)	SA0096	Staphylococcal accessory regulator S	+	+	+	+	
sarT	SA2506	Staphylococcal accessory regulator T	+	±	+	+	USA400 87.5% positive
sarU	SA2507	Staphylococcal accessory regulator U	+	+	+	+	
sarV	SA2258	Staphylococcal accessory regulator V	+	+	+	+	
saeS	SA0765	Sensor histidine kinase	+	+	+	+	
saeR	SA0766	Response regulator	+	+	+	+	
mgrA	SA0746	Transcriptional regulator	+	+	+	+	
arlS	SA1450	Sensor histidine kinase	+	+	+	+	
arlR	SA1451	Response regulator	+	+	+	+	
rot	SA1812	Transcriptional regulator	+	+	+	+	
svrA	SA0416	Transcriptional regulator	+	+	+	+	
srrA	SA1535	Response regulator	+	+	+	+	
TRAP	SA1891	RNAIII-activating protein	+	+	+	+	
yycG	SA0020	Sensory box histidine kinase	+	+	+	+	
Cell surface genes							
Amidase	SA2666	*N*-Acetylmuramoyl-L-alanine amidase domain protein	+	+	+	+	

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TABLE 3—*Continued*

Gene	COL ORF no.[a]	COL description	Result for[b]:				Comment
			USA300-0114	USA400	USA500-0004	USA100	
cap5A	SA0136	Capsular polysaccharide synthesis	+	+	+	+	
cap5B	SA0137	Capsular polysaccharide synthesis	+	+	+	+	
cap5C	SA0138	Capsular polysaccharide synthesis	+	+	+	+	
cap5D	SA0139	Capsular polysaccharide synthesis	+	+	+	+	
cap5E	SA0140	Capsular polysaccharide synthesis	+	+	+	+	
cap5F	SA0141	Capsular polysaccharide synthesis	+	+	+	+	
cap5G	SA0142	Capsular polysaccharide synthesis	+	+	+	+	
cap5H	SA0143	Capsular polysaccharide synthesis	+	−	+	+	USA400 capsule type 8
cap5I	SA0144	Capsular polysaccharide synthesis	+	−	+	+	USA400 capsule type 8
cap5J	SA0145	Capsular polysaccharide synthesis	+	−	+	+	USA400 capsule type 8
cap5K	SA0146	Capsular polysaccharide synthesis	+	−	+	+	USA400 capsule type 8
cap5L	SA0147	Capsular polysaccharide synthesis	+	+	+	+	
cap5M	SA0148	Capsular polysaccharide synthesis	+	+	+	+	
cap5N	SA0149	Capsular polysaccharide synthesis	+	+	+	+	
cap5O	SA0150	Capsular polysaccharide synthesis	+	+	+	+	
cap5P	SA0151	Capsular polysaccharide synthesis	+	+	+	+	
capA	SA2687	CapA protein	+	+	+	+	
capB	SA2686	CapB protein	+	+	+	+	
capC	SA2685	CapC protein	+	+	+	+	
clfA		Clumping factor A	+	+	+	+	
clfB	SA2652	Clumping factor B	+	+	+	+	
ebh	SA1472	Pathogenicity protein	+	−	+	−	
ebh	SA1472	Pathogenicity protein	+	−	+	−	
ebh	SA1472	Pathogenicity protein	+	−	+	−	
ebh	SA1472	Pathogenicity protein	+	+	+	−	
ebh	SA1472	Pathogenicity protein	+	±	+	+	USA400 87.5% positive
ebh	SA1472	Pathogenicity protein	+	+	+	+	
Hypothetical	(SA1267)	Similar to streptococcal adhesin *emb*	+	+	+	+	
icaA	SA2689	IcaA protein	+	+	+	+	
icaB	SA2691	IcaB protein	+	+	+	+	
icaC	SA2692	IcaC protein	+	+	+	+	
icaD	SA2690	IcaD protein	+	+	+	+	
isaA	SA2584	Immunodominant antigen A	+	+	+	+	
isaB	SA2660	Immunodominant antigen B	+	+	+	+	
sdrC	SA0608	SdrC protein	+	+	+	+	
sdrE	SA0610	SdrE protein	+	+	+	−	
sai-1	SA1139	29-kDa cell surface protein	+	+	+	+	
fnbA		Fibronectin-binding protein A	+	−	+	−	
fnbB		Fibronectin binding protein B	+	−	+	−	
Hypothetical	(SA2284)	Similar to accumulation-associated protein	+	±	+	+	USA400 87.5% positive
pls homolog	SA2505	LPXTG motif cell wall anchor domain protein	+	−	+	+	
Hypothetical	SA2676	LPXTG motif cell wall anchor domain protein	+	+	+	+	
Hypothetical	SA2676	LPXTG motif cell wall anchor domain protein	+	+	−	+	
Hypothetical	Predicted ORF	LPXTG motif cell wall anchor domain protein	+	+	−	+	
Hypothetical	Predicted ORF	LPXTG motif cell wall anchor domain protein	+	+	+	+	
Hypothetical	SA1781	LPXTG motif cell wall anchor domain protein	+	+	+	+	
Hypothetical	SA0119	LPXTG motif cell wall anchor domain protein	+	+	+	+	
Hypothetical	SA1141	LPXTG motif cell wall anchor domain protein	+	+	+	+	
spa	SA0095	Immunoglobulin G binding protein	+	+	+	+	
isdB	SA1138	LPXTG motif cell wall anchor domain protein	+	+	+	+	

Continued on following page

TABLE 3—*Continued*

Gene	COL ORF no.[a]	COL description	Result for[b]:				Comment
			USA300-0114	USA400	USA500-0004	USA100	
Hypothetical	SA1806	LPXTG motif cell wall anchor domain protein	+	+	+	−	
Hypothetical	SA1806	LPXTG motif cell wall anchor domain protein	+	+	+	+	
fmtB (*mrp*)	SA2150	Cell wall protein	+	+	+	−	
fmtB (*mrp*)	SA2150	Cell wall protein	+	−	+	−	
Exoenzymes							
aur	SA2659	Aureolysin	+	+	+	+	
coa	SA0209	Coagulase	+	+	+	+	
splA	SA1869	Serine protease	+	+	+	+	
splB	SA1868	Serine protease	+	+	+	+	
splC	SA1867	Serine protease	+	+	+	+	
splD	SA1866	Serine protease	+	+	+	+	
splE	SA1865	Serine protease	+	±	+	−	USA400 62.5% positive
splF		Serine protease	+	+	+	+	
hysA	SA2194	Hyaluronate lyase	+	−	+	−	USA400 and USA500 alleles present
geh	SA0317	Lipase precursor	+	+	+	+	
sspA	SA1057	V8 protease	+	+	+	+	
sspB	SA1970	Serine protease	+	+	+	+	
sspB	SA1056	Staphopain	+	+	+	+	
lip	SA2694	Lipase	+	+	+	+	
plc	SA0078	1-Phosphatidylinositol phosphodiesterase precursor	+	+	+	+	
Exotoxins and enterotoxins							
set10	(SA0386)	Exotoxin 10	+	+	+	+	
set11	(SA0387)	Exotoxin 11	+	+	+	+	
set12	(SA0388)	Exotoxin 12	+	+	+	+	
set13	SA0473	Exotoxin 5; N315 exotoxin 13	+	+	+	+	
set14	SA0474	Exotoxin 4; N315 exotoxin 14	+	+	+	+	
set7	SA0469	Exotoxin 2; N315 exotoxin 7	+	+	+	+	
set8	SA0470	Exotoxin 2; N315 exotoxin 8	+	+	+	+	
set9	(SA0385)	Exotoxin 9	+	+	−	+	
Hypothetical	BAB94252_1	Similar to MW-2 exotoxin 21	+	−	+	−	
Exotoxin 2	SA0472	Exotoxin 2	+	+	+	+	
Exotoxin 3	SA0468	Exotoxin 3	+	−	+	−	
Exotoxin 3	SA0478	Exotoxin 3	+	−	+	−	
ent	SA0886	Staphylococcal enterotoxin	+	±	+	−	USA400 87.5% positive
entB	SA0172	Isochorismatase	+	+	+	+	
Enterotoxin	SA1657	Similar to enterotoxin A	+	+	+	+	
sei	SA0887	Staphylococcal extracellular enterotoxin type I	+	±	+	−	USA400 87.5% positive
hld	SA2022	delta-Hemolysin	+	+	+	+	
hlb	(SA1811)	Truncated beta-hemolysin	+	+	+	+	
hlgA	SA2419	gamma-Hemolysin	+	+	+	+	
hlgB	SA2422	Leucocidin precursor	+	+	+	+	
hlgC	SA2421	Leucocidin precursor	+	+	+	+	
lukD	SA1880	Leucotoxin LukD	+	+	+	+	
lukF	SA2004	Leucocidin precursor	+	+	+	+	
lukF-PV	AB006796	PVL	+	±	−	−	USA400 62.5% positive
lukM	SA2006	Leucotoxin LukM	+	+	+	+	
lukS	SA1881	Synergohymenotropic toxin	+	+	+	+	
lukS-PV	AB006796	PVL	+	±	−	−	USA400 62.5% positive
Hypothetical	SA2295	Similar to secretory antigen precursor SsaA	+	+	+	+	
Hypothetical	SA2557	Similar to secretory antigen precursor SsaA	+	+	+	+	
Hypothetical	SA0507	Similar to autolysin	+	+	+	+	
ssaA	SA2291	Secretory antigen precursor SsaA	+	+	+	+	

[a] ORFs in parentheses refer to *S. aureus* strain N315.
[b] +, presence of gene; −, absence of gene; ±, gene was not present in any isolates of the PFT tested.

diverse patient populations. For instance, despite the high degree of relatedness between USA300 and USA500, the latter strain lacks members of the bacteriophage phi PVL and phi N315 gene sets. These factors are also absent from the major hospital PFT, USA100, suggesting that they may be essential for pathogenesis in the community setting. Indeed, phi PVL (*lukF-PV* and *lukS-PV*) genes are thought to distinguish community from health care isolates (2, 20, 43, 50). Moreover, a comparison of the two community-associated MRSA PFTs, USA300 and USA400, indicates that all of the USA400 isolates examined are missing the fibronectin-binding proteins, *fnbA*, *fnbB*, and *ebh*, as well as SaPIn2 and several but not all phi N315 genes, suggesting that these components may contribute to the virulence of the USA300-0114 isolates. These results also indicate that although both community-associated MRSA lineages harbor the PVL toxin genes (which health care-associated lineages typically do not), other factors distinguish these lineages from health care-associated isolates. Based on these comparisons, 20 genes or hypothetical genes unique to USA300-0114 isolates have been identified. Other PFTs contain some but not all of these 20 genes. It is likely that USA300 isolates contain additional virulence loci that are not represented on the Saur2a chip and that are yet to be elucidated.

The microarray-based procedure used here monitored the presence or absence of both well-studied virulence determinants (Table 3) and genes and ORFs that have not previously been shown to influence pathogenesis directly (see the supplemental material). It is likely that there are unrecognized virulence and other factors (51) in addition to latter collection of determinants. The genes that were identified in our analysis, including many bacteriophage-encoded proteins, may represent a series of new virulence factors which collectively facilitate the organism's ability to both circulate and cause infection within diverse environmental conditions and in diverse populations. Although 20 USA300-0114-specific and hypothetical genes have been identified in this study, it is difficult to know whether these genes are actively transcribed, translated, and functional or are in fact silent. These factors may be expressed only in specific environmental settings, such as those that are encountered during the course of infection. Further characterization of these genes and their protein products is expected to facilitate our understanding of the pathogenic potential of USA300-0114 and may lead to strategies that attenuate the strain.

In conclusion, USA300-0114 is a highly stable strain of *S. aureus* that is primarily responsible for community-associated infections in the United States. This strain continues to evolve its antimicrobial resistance profile through plasmid acquisition. Its natural reservoir remains an open question.

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