Sporadic “Transitional” Community-Associated Methicillin-Resistant *Staphylococcus aureus* Strains from Health Care Facilities in the United States[▽]

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We describe phenotypic and genotypic traits of a group of methicillin-resistant *Staphylococcus aureus* (MRSA) clones that are either remnants of unsuccessful community-associated MRSA (CA-MRSA) clones or represent a transitional state with some yet-to-be-acquired characteristics of CA-MRSA. These rare strains ($n = 20$) were identified during a 10-year period (1990–1999) from 13 unrelated health care facilities in Wisconsin. The isolates were recovered from patients in nosocomial or long-term chronic care facilities (60%) and outpatient settings (40%). Sixty percent ($n = 12$) of the isolates were recovered from skin and soft tissue infections, whereas the remaining isolates ($n = 8$) were from invasive infections. Ninety percent of isolates were susceptible to all antibiotic classes tested or resistant to erythromycin and clindamycin. Pulsed-field gel electrophoresis, multilocus sequence typing, and *spa* typing clustered these isolates into 8, 8, and 14 clonal groups, respectively. Eight plasmid profiles were represented in these strains. All four *agr* types were represented, with type IV being predominant (40%). All strains harbored subtypes of type IV staphylococcal cassette chromosome *mec* but lacked genes for the virulence factor Panton-Valentine leukocidin (PVL). The strains harbored one or more of the following toxin genes: *sea*, *seb*, *sec*, *sed*, *see*, *seh*, *sej*, *sek*, *sel*, *seg*, *sei*, *sem*, *sen*, and *seo*. Individual clonal groups maintained the same set of enterotoxin genes even though they were isolated over extended time periods, suggesting significant genomic stability. The potential role of PVL-carrying phages and plasmids in the success of CA-MRSA clones has been discussed.

Since the first identification of methicillin-resistant *Staphylococcus aureus* (MRSA) in 1961, it has evolved over the last 45 years into primarily two separate genotypic entities, called community-associated MRSA (CA-MRSA) and hospital-associated MRSA (HA-MRSA), suggesting its origin from community and nosocomial environments, respectively (6). CA-MRSA was first described in the United States in the 1980s for intravenous drug users and eventually emerged in several communities throughout the 1990s (14, 28, 36, 37, 41). Since then, it has been reported for groups from Native Americans and aboriginal populations to professional athletic teams, prison inmates, and military recruits (7, 8, 14, 20, 28, 42). Lately, CA-MRSA has also been reported to cause several clinical syndromes, such as necrotizing fasciitis, purpura fulminans, and Waterhouse-Friderichsen syndrome, that were not typically associated with MRSA infections in the past. This expanding disease-causing ability is perhaps due to its evolving and increasing virulence arsenal (1, 22, 27, 38). CA-MRSA strains remain different from HA-MRSA strains in their phenotype, genotype, and risk factors. Unlike HA-MRSA strains, CA-MRSA strains are still susceptible to multiple classes of antimicrobials, except for beta-lactams and, occa-

sionally, erythromycin, ciprofloxacin, and clindamycin (6, 9). CA-MRSA strains are genetically distinguished by their distinct pulsed-field gel electrophoresis (PFGE) type, the presence of type IV or V staphylococcal cassette chromosome *mec* (SCC*mec*), and their genes for Panton-Valentine leukocidin (PVL) (10, 25, 45). HA-MRSA strains harbor type I, II, or III SCC*mec* elements which are relatively large in size and possess additional resistance genes (19). Based on genotypic properties of MRSA, five lineages have been recognized (12, 18, 32, 33). However, not all MRSA clones are created equal, and only a small number of MRSA clones have caused major epidemics in communities and hospitals across the United States (12). It has been reported that HA-MRSA and CA-MRSA are predominantly *agr* type II and type III, respectively (28, 43). There are also clinical isolates of MRSA that do not fit into any of the known epidemic clones of the CA-MRSA or HA-MRSA classification. Aires de Sousa and de Lencastre (2) described sporadic isolates of MRSA with diverse genotypic properties. As part of our long-term epidemiologic study of MRSA in Wisconsin, we have characterized several hundred MRSA isolates that include strains associated with CA-MRSA outbreaks in five American Indian communities during the early 1990s (40, 41). From the MRSA collection ($n = 600$) from that period (1989–1999), we serendipitously identified a number of MRSA strains that did not fit into either the HA-MRSA or CA-MRSA category based on current molecular criteria. We refer to these strains as “transitional” CA-MRSA because they possess some, but not all, phenotypic and genotypic characteristics of CA-

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TABLE 1. Transitional MRSA strains and their clinical, phenotypic, and genotypic characteristics^{a,c}

MRSA isolate	Date of isolation (mo/day/yr)	Source	Infection site	Age of patient (yr)	<i>mec</i> complex	SCC <i>mec</i> type ^b	Antimicrobial resistance
WI-250	10/03/95	CA	Sputum	77	B1	IVa	Ery, Cli
WI-398	05/18/98	CA	Groin	46	B1	IVc	
WI-356	11/24/97	CA	Foot	53	WT	IVa	Cip
WI-217	04/07/95	CA	Toe	45	B1	IVc	
WI-211	02/23/95	CA	Leg	12	B1	IVa	
WI-180	08/11/94	CA	Knee	16	B1	IV*	Ery, Cli, Tet, SXT
WI-591	12/13/99	CA	Hand	6	B1	IVa	
WI-339	08/06/97	NOS	Blood	87	B1	IV*	
WI-319	01/14/97	NOS	Sputum	89	B1	IV*	
WI-369	02/02/98	NOS	Sputum	4	B1	IVa	Ery, Cli
WI-503	04/26/99	NOS	Sputum	78	B1	IVc	Ery, Cli
WI-23	01/23/92	NOS	Trachea	1	WT	IV*	
WI-178	08/05/94	NOS	Trachea	2 mo	B1	IV*	Ery, Cli, Tet
WI-490	03/17/99	NOS	Trachea	85	B1	IV*	
WI-16	12/02/91	NOS	Rectum	44	B1	IV*	
WI-7	01/15/91	NOS	Hip	87	WT	IV*	Ery, Cli
WI-443	10/26/98	NOS	Catheter site	72	B1	IV*	
WI-548	09/22/99	NOS	NP	11	B1	IVa	
WI-585	11/22/99	LTC	NP	36	B1	IVc	
WI-380	03/10/98	LTC	Back	89	B1	IV*	

^a CA, community associated; Cip, ciprofloxacin; Cli, clindamycin; Ery, erythromycin; LTC, long-term care; NOS, nosocomial; SXT, trimethoprim-sulfamethoxazole; Tet, tetracycline; WT, wild-type *mec* complex.

^b *, Could not be subtyped.

^c All isolates were negative for the PVL genes.

MRSA. In this communication, we present their genetic characterization and speculate about why they failed to become successful clones.

MATERIALS AND METHODS

Strains. The strains used for this study were collected from 13 health care facilities in rural Wisconsin between 1991 and 1999. Their year-wise distribution is as follows: 1991 ($n = 2$), 1992 ($n = 1$), 1994 ($n = 2$), 1995 ($n = 3$), 1997 ($n = 3$), 1998 ($n = 4$), and 1999 ($n = 5$).

Antibiotic susceptibilities. The antimicrobial agents tested with a Vitek instrument included penicillin, oxacillin, ampicillin, cephalothin, erythromycin, ciprofloxacin, gentamicin, clindamycin, tetracycline, trimethoprim-sulfamethoxazole, and rifampin.

Molecular methods. The PFGE method used in this study was adapted from previously described methods (4, 26). The evaluation of genetic relatedness among the strains was based on the criteria of Tenover et al. (44) and a similarity index of $\geq 80\%$. Plasmid analyses were completed by previously described methods (34). SCC*mec* typing was done using the modified method of Oliveira and de Lencastre (31). Multilocus sequence typing (MLST) was done by following the methods described by Enright et al. (13). *spa* typing was done following the method of Koreen et al. (21), and the *spa* types were determined by the Ridom *spa* database (www.ridom.de/spaserver/) and analytical tools available at eGenomics (<http://tools.egenomics.com>). The genetic structure of the *mec* complex was determined by sequencing the entire *mec* complex as described previously (39). Specific *agr* allele types were determined using a slightly modified version of multiplex real-time PCR on an Applied Biosystems Prism 7000 instrument (Foster City, CA) as previously described (15). Modifications included multiplexing of only *agr-1*, *agr-3*, and *agr-4* and single-plex analysis of *agr* type 2. The PCR cycle number was increased from 30 to 40.

eBURST analysis. The clonal relationship of the isolates was also determined with the entire MLST database, using eBURST v3 (http://www.mlst.net/vz_new_features.asp).

Virulence factor genes. The following virulence genes were screened by newly developed multiplex or single-plex methods (data not shown): *sea*, *seb*, *sec*, *sed*, *see*, *she*, *sej*, *sek*, and *sel* (staphylococcal enterotoxin genes); *seg*, *sei*, *sem*, *sen*, and *seo* (enterotoxin gene cluster); *lukSF-PV* (leukocidin genes) (24); *cna* (collagen adhesion gene); *tst* (toxic shock syndrome toxin gene); and *clfA* and *clfB* (clumping factor genes). We also determined the range and median percentage of positivity of different virulence genes in all the clonal groups. In addition, we

determined the frequency of positivity of the virulence factors screened in the three common sequence types (STs) identified in this study.

Statistical analysis. All data analyses were carried out using Statistical Analysis System (SAS, Cary, NC) and StatXact (Cytel Software Corp., Cambridge, MA) statistical software. *P* values were obtained based on Fisher's exact test. A *P* value of <0.05 was considered statistically significant.

RESULTS

Fifty-five percent of the isolates came from noninvasive infections (skin and soft tissue infections), whereas 45% of the isolates came from invasive infections (blood, sputum, and urine). As expected, all strains were resistant to beta-lactams, but 90% of the isolates were sensitive to all antibiotics tested except, occasionally, erythromycin and inducible clindamycin. Two isolates (WI-178 and WI-180) were resistant to multiple antibiotics, including erythromycin, clindamycin, and tetracycline and erythromycin, clindamycin, tetracycline, and trimethoprim-sulfamethoxazole, respectively (Table 1). Sixty percent of the isolates were recovered from hospitalized patients or patients living in long-term elderly care facilities. The average age of patients from whom strains were recovered was 42.9 (± 33.7) years.

PFGE-based analysis clustered the 20 isolates into eight clonal groups, with 45% of isolates being represented by two main groups, namely, clonal groups 13 and 23 (Fig. 1 and Table 2). In general, multiple strains within a clonal group had at least 80% genetic similarity, except for clonal groups 2 and 9. Three clonal groups (CG6, CG16, and CG17) were represented by single isolates.

Eight different restricted plasmid profiles were observed for these MRSA strains. Frequently, more than one plasmid profile was associated with the same genomic background (Table 2). However, all seven strains representing clonal groups 20

FIG. 1. PFGE-based dendrogram of 20 “transitional” MRSA isolates, using the unweighted-pair group method using average linkages and the Dice similarity coefficient. Strain numbers and corresponding clonal groups (CG) are shown on the right.

and 23 had the same plasmid profile (plasmid type 4). Interestingly, plasmid type 4 was one of the types seen frequently among nosocomial MRSA strains from Wisconsin (data not shown).

Eight STs (STs 1, 5, 8, 12, 30, 51, 59, and 487) were represented within this collection of strains. Four STs were represented by single isolates. Fourteen *spa* types were identified, and their relationship with the PFGE and ST profiles is shown in Table 2. A search of the Ridom *spa* database (www.ridom.com) showed that 12 of the 14 *spa* types had frequencies of 0.5% or less. The *spa* types t012 and t002 had frequencies of 0.9 and 5%, respectively. eBURST analysis of representative STs with respect to the entire MLST database was done to determine the clonal complexes of the isolates. ST5 and ST487 were found to be single-locus variants of each other, with ST5 being the likely founder of the group. However, eBURST analysis of just our ST data set did not determine any founding relationship between the two STs. Again, as part of the entire MLST data set, several STs (e.g., STs 1, 5, 8, and 30) were represented by the respective major clonal complexes. STs 12, 51, and 59 appeared as part of the unrelated minor groups (Fig. 2).

Eighty-five percent of the isolates had truncated class B1-type *mec* complexes, and the remaining 15% had intact *mec* complexes. Notably, all MRSA isolates harbored variants of type IV SCC*mec* but lacked *lukSF-PV* (Table 1). Table 2 describes the virulence gene profiles of all the strains in the multiple STs. Each of the genes tested was randomly present among different STs. None of the individual genes tested was

present in all strains or within each ST. The genes for staphylococcal enterotoxin A (SEA), SEC, and SEK, which are frequently reported for CA-MRSA, were present in 55% of the strains, representing six STs. Only four of the STs (STs 5, 12, 30, and 51) harbored the enterotoxin gene cluster (*egc*), with ST30 being negative for *sem*. Strains in ST59 had either *sea*, *seb*, and *sek* or *egc* and accounted for 57% of the invasive isolates (Tables 1 and 2). *cna* was present only in STs 1, 8, 12, 30, and 59. *sec* and *sel* were present together in only two strains (WI-23 and WI-591). All four *agr* types (type I, 10%; type II, 25%; type III, 25%; and type IV, 40%) were represented (Table 2). There was only a moderate correlation between the *agr* and ST types, with 100% of ST59 isolates carrying *agr* type IV.

The range and median percent values for the rate of positivity of all the virulence genes screened across the eight PFGE-based clonal groups varied for different genes and are shown in Table 3. The ranges were summarized as 0% (absence), 0 to 50%, and 0 to 100% (Table 3). A range percent implies the observed range of percentages of a particular gene across the eight clonal groups identified. Range percentages for different genes varied widely from 0 (*seb*, *sec*, *sed*, *sej*, and *sem*) to 75% (*sei*). As expected, the range for *lukSF-PV* in the eight clonal groups was zero. Similarly, for *tst*, the range was 0 to 100%, with the median value being 10%. The median percent value (Table 4) (e.g., 10% for *sek*) reflects the observation that 50% of the clones had rates of positivity above and below that value. In strains belonging to ST5, genes for SED, SEG, SEI, SEJ, SEM, SEN, and SEO were always present, whereas

TABLE 2. Summary of STs, clonal groups, *spa* types, plasmid profiles, SCCmec types, and the presence (+) or absence (–) of different virulence genes in transitional MRSA strains

Isolate	PFGE type	ST	<i>agr</i> type	<i>spa</i> type	Plasmid profile ^a	<i>sea</i>	<i>seb</i>	<i>sec</i>	<i>sed</i>	<i>see</i>	<i>seg</i>	<i>seh</i>	<i>sei</i>	<i>sej</i>	<i>sek</i>	<i>sel</i>	<i>sem</i>	<i>sen</i>	<i>seo</i>	<i>cna</i>	<i>tst</i>	<i>clfA</i>	<i>clfB</i>
WI-591	2	1	III	t128	13	+	–	+	–	–	–	+	–	–	+	+	–	–	–	+	–	+	+
WI-23	2	12	II	t213	9	–	–	+	–	–	–	–	+	–	–	+	–	–	–	+	–	+	+
WI-7	6	51	IV	t272	0	–	–	–	–	–	+	–	+	–	–	–	+	+	+	+	–	+	+
WI-16	9	30	III	t012	42	+	–	–	–	–	+	–	+	–	–	–	–	+	+	+	+	+	+
WI-356	9	30	III	t338	0	+	–	–	–	–	+	–	+	–	–	–	–	+	+	+	+	+	+
WI-548	9	30	III	t012	0	–	–	–	–	–	+	–	+	–	–	–	–	+	+	+	+	+	+
WI-217	13	5	II	t443	1	–	+	–	+	–	+	–	+	+	–	–	+	+	+	–	–	+	+
WI-369	13	5	II	t242	16	–	–	–	+	–	+	–	+	+	+	–	+	+	+	–	+	+	+
WI-398	13	5	III	t088	1	–	+	–	+	–	+	–	+	+	–	–	+	+	+	–	–	+	+
WI-503	13	5	II	t002	16	–	+	–	+	–	+	–	+	+	–	–	+	+	+	–	–	+	+
WI-585	13	487	II	t442	0	–	–	–	+	–	+	–	+	+	–	–	+	+	+	–	–	+	+
WI-180	16	8	I	t064	17	+	+	–	–	–	–	–	–	–	+	–	–	–	–	+	+	+	+
WI-178	17	8	I	t068	14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+
WI-211	20	59	IV	t444	4	–	–	–	–	–	+	–	–	+	–	–	–	+	+	–	–	+	+
WI-250	20	59	IV	t216	4	–	–	–	+	–	+	+	+	–	–	–	+	+	+	–	–	+	+
WI-319	23	59	IV	t216	4	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–	+	+	+
WI-339	23	59	IV	t216	4	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+	+
WI-380	23	59	IV	t216	4	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+	+
WI-443	23	59	IV	t216	4	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+	+
WI-490	88	59	IV	t216	4	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+	+

^a Plasmid profile 0, devoid of detectable plasmids.

genes for SEA, SEC, SEE, SEH, SEL, CNA, and PVL were always absent. Similarly, in strains of ST30, genes for SEG, SEI, SEJ, SEN, SEO, CNA, and TSST were always present but the genes for SEB, SEC, SED, SEE, SEH, SEJ, SEK, SEL, SEM, and PVL were always absent. In strains of ST59, none of the genes were consistently present, but *sec*, *see*, *sel*, and *lukSF-PV* were always absent. Other genes were present at variable rates (Tables 3 and 4). All of the isolates were positive for *clfA* and *clfB* (Table 2).

Our analysis showed that there was a statistically significant difference ($P = 0.0246$) in the proportions of positivity for virulence factor genes among the eight PFGE clonal groups. In addition, the differences in frequencies of positivity for each virulence factor gene in ST5, ST30, and ST59 strains were also significantly different ($P < 0.0001$). Our data suggest that, with few exceptions, strains of transitional MRSA within individual clonal groups maintained the same set of virulence factor genes, even though they were isolated over extended periods.

FIG. 2. eBURST analysis of the eight sequence types identified in this study with respect to the entire MLST database.

TABLE 3. PFGE type-based range and median percentages for the virulence genes tested

Gene and range of positivities	Median %
0 to 100%	
<i>seb</i>	0
<i>sec</i>	0
<i>sed</i>	0
<i>sej</i>	0
<i>sel</i>	0
<i>sem</i>	0
<i>sek</i>	10
<i>tst</i>	10
<i>sea</i>	25
<i>seg</i>	50
<i>sen</i>	50
<i>seo</i>	50
<i>cna</i>	60
<i>sei</i>	75
0 to 50%	
<i>seh</i>	0
0%	
<i>see</i>	0
<i>lukSF-PV</i>	0

DISCUSSION

We have genetically characterized a group of epidemiologically unrelated, "transitional" CA-MRSA strains from Wisconsin that harbor type IV *SCCmec*, the characteristic CA-MRSA genotypic trait, but lack *lukSF-PV* genes, its second ubiquitous marker (40, 45). Rare reports of both CA-MRSA and non-CA-MRSA strains that harbor *SCCmec* IV but lack PVL in methicillin-sensitive and methicillin-resistant genetic backgrounds have been described (2, 29). The Western Australian CA-MRSA strains were of *SCCmec* type IV with a truncated *mecA* regulatory region in the *mec* complex but lacked PVL.

Significance of PVL gene-carrying phages in CA-MRSA clones. Since the report by Gillet et al. of necrotizing pneumonia in immunocompetent individuals due to PVL-containing CA-MRSA, this necrotizing factor has always been implied as a virulence factor in all CA-MRSA strains that carry the *lukSF-PV* genes (17). Indeed, the acquisition of PVL genes by CA-MRSA strains almost parallels the global emergence of these genotypes (45). The prevalence of PVL genes remains at 1 to 2% for clinical *S. aureus* isolates, compared to nearly 100% for CA-MRSA strains (40, 41, 45). The recent and more aggressive CA-MRSA clone (USA300) which appeared in the United States around the year 2000 also harbors PVL genes, pointing to the significance of this virulence factor to CA-MRSA strains (20). However, the exclusive role of this toxin in causing all CA-MRSA-associated diseases remains to be clarified. Two recent independently published mouse studies point to an important but nonexclusive role for this toxin in CA-MRSA virulence. Using PVL-positive and PVL-negative isogenic *S. aureus* strains and purified reconstituted PVL, Labandeira-Rey et al. showed a direct role for this toxin in severe necrotizing pneumonia in mice, whose features are mimicked in human necrotizing pneumonia cases (23). Such a virulence role has been described by Gillet et al. (17) for CA-MRSA-

TABLE 4. Frequencies of positivity of 17 virulence genes in the three common STs ST5, ST30, and ST59

Gene	ST frequency (%)		
	ST5 (n = 4)	ST30 (n = 3)	ST59 (n = 7)
<i>seg</i>	100	100	29
<i>sei</i>	100	100	29
<i>seo</i>	100	100	29
<i>sed</i>	100	0	14
<i>sej</i>	100	0	14
<i>sel</i>	100	33	86
<i>cna</i>	0	100	14
<i>tst</i>	25	100	14
<i>sek</i>	25	0	86
<i>seb</i>	75	0	71
<i>sem</i>	25	67	14
<i>sen</i>	25	67	14
<i>sea</i>	0	67	71
<i>seh</i>	0	0	14
<i>sec</i>	0	0	0
<i>see</i>	0	0	0
<i>lukSF-PV</i>	0	0	0

associated necrotizing pneumonia in immunocompetent patients from France. Voyich et al. showed, however, albeit in a mouse sepsis model, that isogenic PVL-negative strains of USA300 and USA400 were as virulent as the isogenic PVL-positive strains (46). In addition, there was little difference in neutrophil lysis activities and survival following phagocytosis for the two groups of strains. These two animal studies definitely point to a specific role for PVL in necrotizing pneumonia but perhaps a limited and/or host-dependent virulence in skin and soft tissue infections. However, the ubiquitous presence of PVL-carrying phages in the CA-MRSA strains, which are predominantly attributed to skin and soft tissue infections, does not contradict its limited role, because not all clinical MRSA strains transcribe the same level of *lukSF-PV* genes (35). Certainly, the ability of PVL to induce global transcriptional changes in different *S. aureus* genetic backgrounds needs to be investigated further. Keeping the above discussion in the context of the characteristics of transitional CA-MRSA strains, we surmise that besides virulence, these phages contribute to the stability and spread of PVL-carrying strains. These strains were represented by some of the common STs, such as STs 1, 5, 8, and 30, and possessed two new *spa* types.

There was a high level of concordance between the PFGE and MLST profiles, but *spa* types were quite divergent and more discriminatory, since six additional types (a total of 14) were represented from the same eight PFGE groups or STs. It was not surprising that we observed three new *spa* types (t442, t443, and t444). Surface protein A is encoded by the *spa* gene and interacts with the Fc domain of the host immunoglobulin, thereby influencing pathogenesis by being part of the immune evasion machinery (11, 16). *spa* types are a manifestation of variable nucleotide tandem repeats in surface protein A and can be expected to have variations due to genotypic influences of regional host diversity. Additional *spa* types compared to STs were not surprising because the discriminatory power of MLST relies on the nucleotide changes in seven housekeeping genes which are stable and evolve slowly. Therefore, *spa* is expected to go through more intense selection pressure than

that of the housekeeping genes. Indeed, strains belonging to the same MLST types have different *spa* types (Table 2). It is possible that *spa* typing may soon be the typing method of choice to study *S. aureus* because it is cheaper to analyze one gene and there is an ever-expanding database with which to make comparisons.

Seven of the eight MLST allelic profiles observed in our study have been described in the MLST database (www.mlst.net). Unlike previously reported CA-MRSA strains belonging to ST1 and ST8 in Wisconsin, strains WI-178, WI-180, and WI-591 lacked *lukSF-PV* genes (40, 42). Strains belonging to other STs, such as ST5 and ST59, have been described as CA-MRSA from other regions of the United States. Strains representing ST12 and ST51 are probably uncommon and have been reported only from the United Kingdom and The Netherlands. ST487, which was identified in this study, has not yet been reported elsewhere. Aires de Sousa and de Lencastre (2) have described what they called sporadic isolates of CA-MRSA, some of which appear to have evolved from nosocomial MRSA. Alternatively, Okuma et al. (30) have described SCCmec IV-harboring isolates from both the community and nosocomial settings, suggesting that isolate source is not a reliable criterion for grouping into the CA- or HA-MRSA category. The SCCmec IV isolates characterized in that study were represented by diverse genetic backgrounds, as also observed in our collection. eBURST analysis showed that these strains were members of multiple and mostly unrelated clonal complexes, suggesting their true sporadic nature. It is not clear if the genetic backgrounds of uncommon STs (STs 12, 51, and 59) play any role in restricting the efficient transduction of the phage carrying the *lukSF-PV* genes. These transitional strains were represented by all four *agr* types, suggesting their sporadic and diverse lineages. Unlike a previous report of *agr* type 1 association with invasive infections (5), our data suggest an association with *agr* type IV.

These strains were recovered during a 7-year period when USA400 was the predominant CA-MRSA clone within this geographic area (14, 28, 41). Since more than 99% of the USA400 strains from this period harbored both type IV SCCmec and PVL genes in our collection, it would appear that these sporadic strains could not compete with the MW2 (USA400) clonal strains for a successful ecological niche. This may be due to the lack of specific sustainability genes or the possibility of genomic incompatibility with associated plasmids.

Compatibility of plasmids in successful CA-MRSA clones.

Aires de Sousa and de Lencastre (2) suggested that sporadic isolates from their study put themselves in an advantageous position by "escaping" from the antibiotic-saturated environments of hospitals and moving into the community. While we agree that this is a plausible explanation, we propose an alternative explanation that suggests that these strains probably did not successfully compete with the predominant clones circulating during that time. Their failure to create a successful environmental niche may have been due to the lack of one or more yet-to-be-identified genes or compatible plasmids as part of their genetic makeup. This speculation is based on plasmid profile data for nearly 600 characterized CA-MRSA and HA-MRSA strains collected from Wisconsin during the early periods of emergence of MRSA in this region. We noticed a synergy between successful clones and their plasmid profiles.

For example, successful strains of Wisconsin clonal group 2 (MW2 clone) almost always harbored plasmid type 11 or the closely related type 13 (41). Similarly, two of the most successful nosocomial clones, clonal groups 4 ($n = 36$) and 7 ($n = 172$), harbored plasmid types 4 (86.1%) and 1 (69.2%), respectively. However, the PFGE profiles of the sporadic isolates that harbored plasmid type 4 were clonally unrelated to clonal group 4. This suggests that both the genomic backgrounds and the compatible plasmids contribute to the fitness of *S. aureus* clones. Interestingly, the PFGE type of strain 591 was the same as that of other successful ST1 strains, along with plasmid type 13, but lacked the PVL genes (3). In this case, one can speculate that several of the uncharacterized genes present on PVL-carrying phages probably also contribute to the success of a CA-MRSA strain (3). An alternative but not mutually exclusive explanation would be that there is a metabolic or phenotypic cost to the bacterial host for the presence of an extra and probably incompatible plasmid. If the extra plasmid did not confer any benefit on its host, then either it would be eliminated or it would have a deleterious effect on the success of that clone. We believe that this could also be the case for some of the sporadic isolates in this study.

In this group of isolates, the range of enterotoxin genes, *egc*, and the adhesion gene *cna* were not consistently present. The variable presence of virulence genes spanning multiple clones was due more to the different clonal lineages and diverse genomic backgrounds than to their association with the SCCmec genotype. In fact, the percentages of positivity for different virulence genes varied considerably. Genes such as *sen* and *seo* were more consistently present than other genes of the *egc* cluster, suggesting that not all genes of the *egc* cluster are equally distributed. This may contribute indirectly to the successful fitness and virulence of these isolates.

In conclusion, we have described unsuccessful PVL-negative CA-MRSA strains with some common and some uncommon genetic backgrounds. The fact that most of the isolates are not representative of epidemic clones suggests that (i) they may lack certain genetic markers needed to become successful clones of CA-MRSA; (ii) their genetic background is not receptive to yet-to-be-identified CA-MRSA-specific traits, possibly some of the genes residing on ϕ Sa2; or (iii) they lack the appropriate plasmids. Not surprisingly, enterotoxin genes such as *sea*, *sec*, and *sek* were not consistently present in these isolates. Since little is known about the genotypic features of many early CA-MRSA-like strains, further characterization of these strains will perhaps shed light on how successful CA-MRSA may have evolved over the last 20 years.

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