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Widespread Skin and Soft-Tissue Infections Due to Two Methicillin-Resistant *Staphylococcus aureus* Strains Harboring the Genes for Panton-Valentine Leucocidin

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Infections caused by community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) are emerging as a major public health problem. CA-MRSA has been associated previously with skin and soft-tissue infection (SSTI) and with carriage of staphylococcal cassette chromosome *mec* (SCC*mec*) type IV and the Panton-Valentine leucocidin (PVL) virulence factor. To assess the clonal distribution of PVL-carrying strains and the association with SSTI in the San Francisco Bay area, we surveyed six collections of *S. aureus* isolates—671 isolates in all—collected between 1997 and 2002 originating from inpatient and outpatient clinical specimens and from a community-based sampling. Isolates were genotyped by pulsed-field gel electrophoresis, multilocus restriction fragment typing, and multilocus sequence typing and assayed for the PVL virulence factor. The *S. aureus* populations showed a high proportion of PVL-carrying strains, with frequencies ranging up to 70% in MRSA isolated from jail inmate patients and 69% in MRSA from patients receiving surgical treatment at an outpatient clinic specializing in treating SSTIs. PVL-carrying isolates were identified in nine clonal groups, but 88.5% of the PVL-carrying MRSA isolates belonged to only two clonal groups. These two clonal groups carried the SCC*mec* type IV resistance determinant and were more likely than other clonal groups to be recovered from SSTI sites than from other sites ($P < 0.0001$). There is evidence of clonal replacement over the period from 1999 to 2002, with one of these two clonal groups being supplanted by the other.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infection worldwide. Hospital-associated MRSA strains are typically resistant to multiple antibiotics, compounding the challenge of finding effective treatment options. It is commonly accepted that MRSA strains can flourish in the hospital environment due to the selection pressure of antibiotic use but have low prevalence outside the health care setting due to the "fitness cost" of resistance (21). Recently, however, there have been reports indicating an increased incidence of MRSA infections, mostly skin and soft-tissue infections (SSTIs), afflicting individuals with no apparent risk factors for hospital acquisition (6, 13, 17, 33). These reports have raised questions regarding the origin of these presumed community-acquired MRSA (CA-MRSA) strains and the possibility of their increasing prevalence in hospital and community populations.

The newly described CA-MRSA strains possess several features that distinguish them from nosocomial MRSA. First, most are non-multidrug resistant, in contrast to the multidrug-resistant MRSA strains typically found in the hospital setting (1, 17). Second, methicillin resistance is conferred by carriage of the *mecA* gene on the recently identified type IV staphylococcal chromosomal cassette *mec* (SCC*mec*) element (7, 22). Third, they carry the genes for Panton-Valentine leucocidin (PVL), a bicomponent cytotoxin virulence factor associated with SSTIs as well as with more serious infections, e.g., severe necrotizing pneumonia (10, 15, 37). PVL is encoded by two cotranscribed genes, *lukS-PV* and *lukF-PV*, present in a prophage segment integrated in the *S. aureus* chromosome (32). Heretofore, the PVL genes have been detected infrequently (<5%) in hospital and community *S. aureus* isolates (20, 31). Last, this combination of features appears on geographically dispersed *S. aureus* strains with unrelated genetic backgrounds, indicative of multiple independent clonal origins (37). Taken together, these observations suggest the emergence of new strains of *S. aureus* bearing the type IV SCC*mec* element and PVL that are particularly well adapted for survival in the community setting (10, 37).

To gain a better understanding of the prevalence, clonal distribution, and population dynamics of PVL^+ MRSA, we surveyed isolates collected over a period of 6 years from hospital and community populations in the San Francisco area. Our findings indicate that even at a local level, PVL is circulating in multiple clonal lineages, mostly associated with MRSA bearing the SCC*mec* type IV element but in some cases in methicillin-sensitive *S. aureus* (MSSA) strains. Two clonal groups, both significantly associated with SSTIs, dominate the survey.

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TABLE 1. PVL$^+$ *S. aureus* in six sample populations

Sample source[a]	Sampling period	No. (%) of isolates		No. of clonal groups[b]	% PVL$^+$ for SSTI vs other sites (P)[c]
		Total	PVL$^+$		
SFGH/MRSA-1	Aug. 1999 to Mar. 2000	101	37 (37)	9	57 vs 13 (<0.0001)
SFGH/MSSA	Nov. 1999 to Dec. 1999	89	6 (6.7)	24	ND
SFGH/MRSA-2	Jan. 2002 to Dec. 2002	82	29 (35.4)	7	58 vs 17 (<0.0001)
ISIS Clinic	Jul. 2000 to Dec. 2000	64	44 (68.8)	5	ND
SF urban poor	Aug. 1999 to Apr. 2000	184	3 (1.6)	23	ND
SF county jails/MRSA	1997 to 2002	151	106 (70.2)	6	86 vs 27 (<0.0001)
Total		671	225 (33.5)		

[a] SF, San Francisco. The sample collections are described in more detail in Materials and Methods.
[b] Clonal groups are defined by MLRFT and PFGE.
[c] ND, significance tests were not performed: the MSSA population has inadequate numbers to test, the ISIS clinic samples are strongly biased toward SSTI, and the SF urban poor population samples contained no isolates from infection sites.

MATERIALS AND METHODS

Bacterial isolates. Six distinct collections of *S. aureus* isolates were surveyed in this study. These collections originated from the Molecular Epidemiology Research Laboratory (MERL), which provides molecular epidemiologic services to the San Francisco Community Health Network. The network includes San Francisco General Hospital (SFGH, a university-affiliated acute-care public hospital with a regional trauma center), 13 citywide outpatient clinics, a publicly funded long-term care facility, a chronic mental health care facility, a home health care network, and a patient clinic for the San Francisco County jails. MERL has archived over 90% of MRSA isolates cultured from inpatient and outpatient clinical *S. aureus* specimens submitted to the Clinical Microbiology Laboratory at SFGH since 1996; MSSA are typically not archived. Of the MRSA, about 20% are from blood or other sterile sites, 20% are of respiratory origins, 10% are from urinary tract infections, and 50% are from wounds and cutaneous abscesses.

Five collections originated from inpatient and outpatient populations with *S. aureus* infections (Table 1). The first, SFGH/MRSA-1, originated from inpatient and outpatient sources at SFGH and consisted of 101 MRSA isolates recovered from unique patient clinical specimens (abscess or wound, $n = 47$; blood, bone, and tissue, $n = 22$; respiratory, $n = 17$; urine, $n = 12$; and catheter, $n = 3$) between August 1999 and March 2000; the 101 isolates were randomly selected from 182 MRSA isolates archived in the MERL strain bank during this period. A comparison collection consisted of 89 consecutive MSSA clinical isolates recovered from hospitalized patients identified during November and December 1999, the middle of the sampling period of the first MRSA collection. To assess temporal changes in the distribution of MRSA clonal groups among hospitalized patients at SFGH, a sample set (SFGH/MRSA-2) consisting of 82 MRSA isolates was selected at random from 563 unique patient isolates collected during 2002. The fourth collection consisted of MRSA isolates collected from outpatients receiving surgical treatment at the Integrated Soft-tissue Infection Services (ISIS) Clinic between July and December 2000 (16; E. D. Charlebois, D. R. Bangsberg, N. Moss, and F. Perdreau-Remington, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother. p. 120, abstr. C2-306, 2001). Isolates originated from the first 64 consecutive unique patients who presented with culture-positive abscesses and wounds. The fifth collection consisted of clinical MRSA isolates from 151 unique incarcerated patients from five San Francisco County jails collected between January 1997 and December 2002; of these, 85% were recovered from abscesses or wounds (30). Overall, these five collections contained isolates from 487 unique patients.

To contrast community carriage against the clinical isolates, we surveyed 184 *S. aureus* isolates from nasal swabs collected as part of a population-based community prevalence study among the urban poor in San Francisco (5). These samples were collected between August 1999 and April 2000; of these, 23 were MRSA and the remainder were MSSA.

Bacterial characterization. *S. aureus* isolates were tested for phenotypic resistance to oxacillin by the salt agar method (35). Susceptibility to ciprofloxacin, tetracycline, gentamicin, erythromycin, cotrimoxazole, rifampin, clindamycin, and vancomycin was tested using broth microdilution with the MicroScan WalkAway 96 instrument (Dade Behring), and results were interpreted in accordance with the National Committee for Clinical Laboratory Standards guidelines (3). The structural features unique to each of the four major allotypes of the SCC*mec* element, types I through IV (18, 22), were determined by a previously described PCR-based multiplex assay (29).

All isolates were genotyped by PFGE with SmaI as previously described (5); the guidelines of Tenover et al. were used to interpret the PFGE patterns for genetic relatedness (36). Multilocus restriction fragment typing (MLRFT) was performed to provide an assessment of restriction site variation in seven housekeeping gene loci dispersed around the genome (11). Unique allelic combinations of the seven housekeeping genes defines a San Francisco (SF) type number (e.g., SF13). The combination of MLRFT and PFGE pattern type defines a clonal group (e.g., SF13:Z). Multilocus sequence typing (MLST) was performed on representative isolates from each clonal group (11); sequence types (ST) were assigned with reference to the MLST database (http://www.mlst.net). Some samples were additionally typed at the *spa* locus (34). PVL genes were detected by coamplification of *lukS-PV* and *lukF-PV* genes as described by Lina et al. (20). A subset of the PVL PCR amplicons were sequenced to confirm the specificity of the *lukS-PV* and *lukF-PV* primers.

Statistical analysis. Associations between categorical variables were tested by Fisher's exact test using the statistical program STATA (Stata Corp LP, College Station, Tex.) (2).

RESULTS

High prevalence of PVL in SSTI-associated MRSA isolates. Six *S. aureus* collections consisting of 671 isolates were tested for the prevalence of PVL. Overall, 33.5% (225 of 671) of the isolates were positive for PVL, although frequencies differed widely between collections (Table 1). Among two clinical SFGH MRSA collections from inpatient and outpatient sources, 37% (37 of 101) of isolates recovered in 1999-2000 and 35.4% (29 of 82) obtained in 2002 were positive for PVL. PVL prevalence among two clinical outpatient collections, originating from San Francisco jail inmate patients in the period from 1997 to 2002 and ISIS Clinic patients in 2000, was 70.2% (106 of 151) and 68.8% (44 of 64), respectively. In contrast to the four MRSA collections, the two collections containing predominantly MSSA isolates had significantly lower PVL prevalence: 6.7% (6 of 89) among consecutive clinical MSSA isolates from SFGH in 1999 and 1.6% (3 of 184) among a mixed set of MSSA ($n = 161$) and MRSA ($n = 23$) isolates from the nares of San Francisco urban poor in 1999-2000. PVL was found to be associated with SSTIs in the three MRSA collections with samples isolated from diverse sites of infection, i.e., the two clinical SFGH/MRSA collections and the clinical jail inmate collection, as well as in the collection from the ISIS Clinic population, which consisted almost entirely of patients with SSTIs ($P < 0.0001$) (Table 1).

The PVL$^+$ isolates were distributed among nine clonal groups as defined by MLRFT, MLST, and PFGE and included 9 MSSA and 216 MRSA isolates (Table 2). All the MRSA

TABLE 2. Differentiation of PVL$^+$ *S. aureus* isolates into clonal groups according to isolate population

Clonal group	Genetic typing markers				No. of PVL$^+$ *S. aureus* strains in sample collection[a]					Proportion of PVL$^+$ strains within clonal group
	MLRFT	MLST	PFGE group	SCC*mec*	SFGH/MRSA-1, 1999–2000 (n = 101)	SFGH/MRSA, 1999 (n = 89)	SFGH/MRSA-2, 2002 (n = 82)	ISIS Clinic MRSA, 2000 (n = 64)	SF urban poor, 1999–2000 (n = 184)	SF county jails MRSA, 1997–2002 (n = 151)	
SF1:K	AAAAAAA	ST1	K	IV	2			2		2	6/18
SF6:D	AAACCAA	ST5	D	Negative					(1)		1/72
SF7:B	AAACCAC	ST72	B	IV	1						1/15
SF13:Z	BBBBBAB	ST30	Z	IV	34	(1)	2	35		57	129/155
SF16:C	CAAACAC	ST8	C	IV		(3)		3	(2)	1	9/111
SF16:S	CAAACAC	ST8	S	IV			27	1		40	68/68
SF19:N	CAABCCB	ST121	N	Negative		(1)					1/5
SF23:O	CAACCAA	ST88	Unique	Negative		(1)					1/6
SF25:P	CAFBCDB	ST59	P	IV				3		6	9/70

[a] Numbers of *mecA*-negative strains are shown in parentheses.

FIG. 1. PFGE of SmaI-digested DNA from representative PVL$^+$ strains from different clonal groups. Lane 1, SF13:Z (ST30); lanes 2 and 4, SF16:S (ST8); lane 3, SF16:C (ST8); and lane 5, SF25:P (ST59).

isolates carried the type IV SCC*mec* element. As shown in Table 2, most of the 225 PVL$^+$ isolates fell into two clonal groups: 57% in SF13:Z and 30% in SF16:S. The former belonged to ST-30 complex, and the latter belonged to the ST-8 complex, both successful pandemic MRSA complexes (12, 26, 28, 37). Most isolates identified as belonging to these two clonal groups were PVL$^+$: 83% of the SF13:Z isolates and 100% of the SF16:S isolates. Within SF13:Z, 129 of the 155 isolates were MRSA, and of these, 128 (99%) were PVL$^+$. SF16:S has been classified as a distinct clonal group based on its PFGE pattern; although the SF16:S and the SF16:C clonal groups have the same sequence type (ST8) and *spa* type (YHGFMBQBLO), they share only 9 of 15 PFGE bands (Fig. 1). In contrast to the 100% PVL$^+$ presence in SF16:S, PVL genes were detected in only 9 of 111 SF16:C isolates, and of these, 5 were MSSA. Of the remaining six PVL$^+$ clonal types, only SF1:K and SF25:P, corresponding to ST1 and ST59 (Table 2), have been associated previously with PVL (37).

Most of the PVL$^+$ isolates were susceptible to all non-beta-lactam antibiotics. The exceptions were all in the SF16:S clonal group: 70% of isolates were resistant to ciprofloxacin, 94% were resistant to erythromycin, 27% were resistant to tetracycline, and 24% were resistant to all three.

Evidence of clonal replacement between 2000 and 2002. The San Francisco jail inmate MRSA isolates were collected over a 6-year period, 1997 to 2002, thereby allowing temporal trends in clonal distributions to be assessed (32). As noted in Table 1, 106 (70.2%) of the 151 MRSA isolates in this population were PVL positive; 97 of the 106 belonged to the SF13:Z and SF16:S clonal groups. PVL$^+$ SF13:Z isolates first appeared in 1997, increased to 50% of isolates through the years 1998 to 2000, and then declined to 32% in 2001 and 14% in 2002. The SF16:S

clonal group did not appear in the jail population until April 2001, but then it increased dramatically, ultimately accounting for 11 (31%) of the 36 MRSA isolates recovered that year and 29 (67%) of 43 MRSA isolates collected in 2002.

The same shift in clonal distribution was observed in comparing the clinical MRSA isolates from SFGH collected during 1999-2000 with those collected in 2002. The PVL^+ SF16:S clonal group was not detected in the 1999-2000 isolate population but accounted for 26 of 82 (32%) of the isolates sampled in 2002 (Tables 1 and 2). Concurrently, the occurrence of PVL^+ SF13:Z isolates declined from 34 of 101 (34%) in 1999-2000 to 2 of 82 (2.4%) in 2002. The SF16:S clonal group first appeared in an inpatient isolate in June 2001 (F. Perdreau-Remington, unpublished data), and the data presented here clearly show that the SF16:S clonal group is emergent in the hospital setting.

DISCUSSION

There has been recent speculation that PVL in combination with the type IV SCC*mec* element contributes to the spread of MRSA in the community, particularly in the context of SSTI (10). The findings reported here are consistent with this idea. Over one-third of the hospital MRSA isolates and over two-thirds of the outpatient MRSA isolates were found to carry the *lukS-PV–lukF-PV* gene complex; in contrast, less than 7% of the MSSA isolates were PVL^+. Most of the PVL^+ isolates (96%) were MRSA, and all the PVL^+ MRSA isolates carried the type IV SCC*mec* element. The majority of the PVL^+ MRSA strains were associated with SSTIs.

Two clonal groups, SF13:Z and SF16:S, dominated the survey, accounting for 49% of the isolates in the four MRSA collections and for 33% of all the isolates surveyed. That 91% of all the PVL^+ MRSA isolates were SF13:Z or SF16:S is consistent with the idea that PVL (or some other shared factor) contributes to the success of these two clonal groups. Of additional note, the SF16:S clonal group, a group that is 100% PVL^+ type IV SCC*mec* MRSA, has in the course of 2 years effectively supplanted the SF13:Z clonal group as the predominant strain among clinical samples from both inpatients and outpatients at SFGH.

The SF13:Z clonal group belongs to the ST30 clonal lineage. ST30 strains harboring the SCC*mec* type IV element have been recovered in Sweden, Germany, Spain, Argentina, and Greece (8, 12). The SF13:Z clonal group is indistinguishable in sequence type, SCC*mec* type, and PVL carriage from an outbreak PVL^+ MRSA strain recovered from Polynesian patients seen at an Australian hospital in 1998 (26, 28). It is possible that the Oceania strain and the SF13:Z clonal group share a common origin.

The SF16:S and SF16:C clonal groups possess distinctive PFGE banding patterns (Fig. 1) despite belonging to the same MLST clonal lineage, ST8. SF16:S and SF16:C appear to correspond to the USA300 and USA500 groups described recently by McDougal et al. (23). ST8 is a well-established lineage with worldwide distribution that includes MSSA strains and MRSA strains carrying each of the four SCC*mec* types (12); ST8 MRSA strains carrying the SCC*mec* type IV element have been recovered previously in both Europe and the United States. PVL genes were detected in both SF16:S and SF16:C lineages, although at different frequencies; relatively few SF16:C isolates were PVL^+, and these were split between MRSA and MSSA, whereas all the SF16:S were PVL^+ MRSA. There is a recent report indicating PVL carriage by an ST8 strain (37); from the data given, it is not possible to determine whether this corresponds to an SF16:C or SF16:S strain. However, given the recent appearance of the SF16:S clonal group in this area and its rapid increase in prevalence, we speculate that SF16:S is recently derived from an SF16:C-like ancestor that underwent substantial genomic reorganization and a significant gain in fitness. Wherever the origin of the SF16:S clonal group, it is evident that this PVL^+ type IV SCC*mec* MRSA has spread to multiple sites throughout the United States (23, 30), including the large MRSA outbreak in Los Angeles county in 2002 (4; F. Perdreau-Remington, E. Pan, H. Carleton, B. Diep, S. Harvey, and G. Sensabaugh, Abstr. 43rd Intersci. Conf. Antimicrob. Chemother., p. 382, abstr. K-1393, 2003).

The *lukS-PV–lukF-PV* gene complex was detected in six clonal groups in addition to SF13:Z, SF16:C, and SF16:S. Two clonal groups, SF1:K and SF25:P, were found to carry PVL at intermediate frequency (Table 2). SF1:K corresponds in sequence type to ST1, and the PVL^+ strains found in this area are thus linked to the PVL^+ ST1 strains responsible for CA-MRSA infections in the U.S. Midwest (14, 23), of which the fully sequenced MW2 strain is representative (3). PVL^+ ST1 strains have been recently reported in the Northeastern United States, and their detection here indicates that they have achieved nationwide distribution. SF25:P belongs to the ST59 clonal lineage and is ubiquitous in the San Francisco area (9); it has been associated with both community- and hospital-acquired infections. The remaining four PVL^+ isolates detected in this survey are singletons within their clonal groups; the presence of PVL in these clonal groups has not been reported previously.

The detection of PVL genes in nine different clonal groups raises the question of their origin. The *lukS-PV–lukF-PV* gene complex is carried on a prophage integrated in the *S. aureus* genome. Three PVL-bearing prophage sequences—ϕPVL, ϕSLT, and ϕMW2—have been determined, and apart from the region carrying the PVL gene complex, each differs considerably in gene organization and sequence (3, 19, 25). Narita et al. (25) provide evidence of multiple additional PVL bearing prophages. It is thus likely that the PVL-bearing prophages in the clonal groups identified here have independent origins. The presence of high-prevalence PVL^+ strains such as SF13:Z and SF16:S, however, raises the possibility that these strains can serve as reservoirs for the propagation of PVL to other clonal groups.

Finally, the emergence of PVL^+ type IV SCC*mec* MRSA strains in the community raises the concern that these strains will migrate into the hospital setting (27), and indeed, this study provides evidence that this is already happening. Heretofore, CA-MRSA strains have been susceptible to antibiotics other than the β-lactams, allowing alternative treatment options (24). However, in the hospital setting, it likely that multidrug-resistant strains will emerge, and the appearance of SF16:S isolates resistant to ciprofloxacin, erythromycin, and tetracycline may represent the first wave of multidrug-resistant CA-MRSA.

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