

Comparative Molecular Analysis of Community- or Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus*

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Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is a growing public health concern that has been associated with pediatric fatalities. It is hypothesized that the evolution of CA-MRSA is a recent event due to the acquisition of *mec* DNA by previously methicillin-susceptible strains that circulated in the community. This study investigated the genetic relatedness between CA-MRSA, hospital-associated MRSA (HA-MRSA), and nonmenstrual toxic shock syndrome (nmTSS) isolates. Thirty-one of 32 CA-MRSA isolates were highly related as determined by pulsed-field gel electrophoresis and *spa* typing yet were distinguishable from 32 HA-MRSA strains. The 31 related CA-MRSA isolates produced either staphylococcal enterotoxin B ($n = 5$) or C ($n = 26$), and none made TSS toxin 1. All CA-MRSA isolates tested contained a type IV staphylococcal cassette chromosome *mec* (SCC*mec*) element. In comparison, none of the HA-MRSA isolates ($n = 32$) expressed the three superantigens. Antibiotic susceptibility patterns were different between the CA-MRSA and HA-MRSA isolates; CA-MRSA was typically resistant only to β -lactam antibiotics. Six of twenty-one nmTSS isolates were indistinguishable or highly related to the CA-MRSA isolates. MnCop, an nmTSS isolate obtained in Alabama in 1986, was highly related to the CA-MRSA isolates except that it did not contain an SCC*mec* element. These data suggest that CA-MRSA strains may represent a new acquisition of SCC*mec* DNA in a previously susceptible genetic background that was capable of causing nmTSS. CA-MRSA poses a serious health risk not only because it is resistant to the antibiotics of choice for community-acquired staphylococcal infections but also because of its ability to cause nmTSS via superantigen production.

Staphylococcus aureus is a versatile human pathogen causing infections ranging from relatively mild involvement of skin and soft tissue to life-threatening sepsis, pneumonia, and toxic shock syndrome (TSS). The organism causes illness through production of numerous cell surface and secreted virulence factors, and disease is facilitated by its propensity to develop resistance to multiple antibiotics (8, 39). Infections in the community and hospital settings are common.

Among the secreted virulence factors of *S. aureus* are the superantigen toxins (SAGs), which include TSS toxin 1 (TSST-1) and staphylococcal enterotoxin (SE) serotypes A to Q (SEA to SEQ), excluding F (11, 22). These toxins cause TSS and related illnesses through their capacity to induce massive cytokine release both from macrophages and T cells (18, 22). Despite high variability in primary amino acid sequences, the SAGs are related in three-dimensional structures (18, 21, 22).

Another attribute of *S. aureus*, which complicates treatment, is resistance to multiple antibiotics (39). Nearly all isolates today in the United States are resistant to penicillin through production of β -lactamases. In recent years, more than 50% of

hospital-associated (HA) *S. aureus* isolates were resistant to all β -lactam antibiotics (including methicillin and oxacillin) through the production of an altered penicillin binding protein, PBP2a (31). HA methicillin-resistant *S. aureus* (HA-MRSA) isolates are also typically resistant to multiple, non- β -lactam antibiotics (8, 39). In addition, the first report of *vanA*-mediated vancomycin-resistant *S. aureus* was recently published (6).

Recently, the appearance of community-acquired MRSA (CA-MRSA) strains has been described (5, 14, 15, 24). In contrast to HA-MRSA, these strains are commonly susceptible to the majority of other non- β -lactam antistaphylococcal antibiotics and have a common pulsed-field gel electrophoresis (PFGE) pattern. Baba and colleagues have recently sequenced the entire genome of one CA-MRSA isolate (strain MW2; called C99-459 in the present study) obtained from North Dakota in 1998 (1, 5). The sequence analysis identified a type IVa staphylococcal cassette chromosome *mec* (SCC*mec*) element and 19 virulence genes, including SEC and SEH and panton-valentine leukocidin. The type IV SCC*mec* element is much smaller than both SCC*mec* types II and III and similar in size to the archaic SCC*mec* type I. In contrast to the archaic SCC*mec* type, which harbors an altered *ccrB1* gene, the recombinases are wild type in the type IV SCC*mec*, possibly explaining its apparent movement. SCC*mec* type IV has been found in at least four different genomic backgrounds, suggesting the

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TABLE 1. CA-MRSA isolates obtained from the Native American reservation and analyzed for this study^a

Isolate	Resistance phenotype ^b	Presence of SAg ^c			PFGE type	Source
		SEB	SEC	TSST-1		
REF541	POx	-	+	-	A3	Boil
REF542	POxE	-	+	-	A3	Right nipple
REF548	POx	-	-	-	Unique ^d	Wound
REF549	POx	-	+	-	A8	Boil, right arm
REF553	POx	+	-	-	A7	Unknown
REF559	POx	+	-	-	A4	Abscess
REF571	POx	+	-	-	A2	Wound
REF576	POx	-	+	-	A1	Unknown
REF592	POx	-	+	-	A1	Left thumb
REF595	POx	-	+	-	A1	Left thigh
REF596	POx	-	+	-	A1	Wound
REF597	POxC	+	-	-	A6	Wound
REF599	POx	-	+	-	A1	Left ear
REF606	POx	-	+	-	A1	Left shin
REF607	POx	-	+	-	A1	Urine
REF609	POxC	+	-	-	A6	Unknown
REF1169	POx	-	+	-	A3	Wound
REF1170	POx	-	+	-	A3	Left elbow
REF1171	POx	-	+	-	A1	Right ear
REF1182	POx	-	+	-	A1	Right ankle
REF1187	POx	-	+	-	A1	Buttocks
REF1188	POx	-	+	-	A1	Right foot
REF1193	POx	-	+	-	A1	Right wrist
REF1194	POx	-	+	-	A3	Right foot
REF1223	POxECl	-	+	-	A1	Face
REF1225	POx	-	+	-	A3	Right ear
REF1228	POx	-	+	-	A1	Buttocks
REF1236	POx	-	+	-	A1	Abdomen
REF1239	POx	-	+	-	A8	Stool
REF1246	POxE	-	+	-	A8	Wound
REF1247	POx	-	+	-	A8	Left knee
REF1277	POxECl	-	+	-	A8	Boil

^a REF = Native American reservation isolates.

^b P, penicillin; Ox, oxacillin; E, erythromycin; C, ciprofloxacin; Cl, clindamycin; Ch, chloramphenicol; G, gentamicin; Sxt, trimethoprim-sulfamethoxazole; and R, rifampin.

^c +, positive; -, negative.

^d "Unique" represents a PFGE pattern that was distinguishable from all other isolates in the present study, as represented by Fig. 1.

ease at which it may transfer compared to types I, II, and III (1, 20, 29).

In December 1998, the Nebraska Health and Human Services System was notified regarding an apparent increase in laboratory-confirmed CA-MRSA infections in a rural Nebraska county containing a community of Native Americans. This study was undertaken to compare a group of CA-MRSA with respect to their clonal relatedness and SAg production to HA-MRSA and to *S. aureus* strains from nonmenstrual TSS (nmTSS) patients from 1986 to the present.

MATERIALS AND METHODS

Strain collection. The strains used in the study are listed in Tables 1 to 3. One hospital serves as the major health care provider to the Native American residents, and one laboratory provided routine microbiologic testing for the hospital. Thirty-two CA-MRSA isolates from individual patients were obtained for further study (Table 1). All CA-MRSA isolates collected were isolated in 1998. CA-MRSA was defined as those MRSA isolates identified by the laboratory as collected from outpatients. In addition, 32 HA-MRSA isolates from individual patients were collected at random (two or three per month) from the University of Nebraska Medical Center (UNMC), Omaha, during 1998 (Table 2). This represents 40% of the total MRSA isolates collected at UNMC in 1998. These

isolates were collected from patients whose infections occurred at least 3 days after they were admitted into the hospital. UNMC is distinct from the hospital that serves the Native American residents, which is in a rural area approximately 100 miles from Omaha. An additional 21 *S. aureus* isolates collected throughout the United States from nmTSS cases, most of which produced either SEB or SEC, were compared with the aforementioned isolates (Table 3). Eighteen of these 21 isolates contained SCCmec. Both *S. aureus* COL and NCTC8325 were used as reference strains (Table 3).

Microbiologic and molecular methods. Antimicrobial susceptibility testing was performed using disk diffusion methodology according to NCCLS methodology (25). Resistance to oxacillin was confirmed by amplifying a 1,026-bp *mecA* fragment using the following primers: forward, 5' GGGTACAAGATGATACC 3'; and reverse, 5' GGTGCGTTAATATTGCC 3'. Primers and conditions used to amplify *seb*, *sec*, and *seh* have been previously described (23). The primers (designed from the *S. aureus* MW2 genomic sequence [1]) used to amplify a 1,554-bp region from *lukS-PV* and *lukF-PV* were as follows: forward, 5' GGCC TTTCCAATACAATATTGG 3'; and reverse, 5' CCCAATCAACTTCATAAA TTG 3'. A 183-bp portion of a truncated *seo* was amplified by using the following primers: forward, 5' GTATGATTTCGAAACTGGAG 3'; and reverse, 5' CTT GTTAAACAAATAGATATC 3' (1). PFGE was performed according to previously described methods (2). PFGE patterns were analyzed using Bionumerics software (Applied Maths, Kortrijk, Belgium) with a 1% molecular weight position tolerance. *spa* typing and SCCmec typing were performed using the protocols described previously by Shopsin et al. (37) and Oliveira et al. (27), respectively. Southern blotting was performed using standard methods (34). Primers used to amplify *gyrA* are previously described (13). *mecA* and *gyrA* DNA probes were prepared using digoxigenin-labeled dideoxy-UTP (Roche, Indianapolis, Ind.) and the primers listed above. TSST-1, SEB, and SEC production was assessed by a double-immunodiffusion assay (35). All isolates were verified as being positive or negative for *seb* or *sec* by PCR using primers described by Monday et al. (23).

RESULTS

Antimicrobial resistance and SAg typing of MRSA. Table 1 shows the antimicrobial susceptibility pattern for the 32 CA-MRSA isolates (identified with the REF prefix). Twenty-six of 32 isolates (81%) were resistant only to penicillin and oxacillin (methicillin). Methicillin resistance was confirmed by PCR in all isolates. Four of 32 isolates (13%) were resistant to erythromycin, while two isolates (6%) were resistant to clindamycin. An additional two isolates were resistant to ciprofloxacin (6%). No isolates were defined as multiresistant (resistant to more than three non-β-lactam antibiotics). In addition, nearly all isolates were obtained from skin and soft tissue infections. Twenty-four percent of the nmTSS *S. aureus* isolates were multiresistant; 86% contained SCCmec. In contrast, 28 of 32 (87.5%) HA-MRSA isolates were multiresistant and 56% were isolated from either blood or sputum.

SAg typing demonstrated that 31 of 32 CA-MRSA isolates produced either SEB or SEC (Table 1). Five isolates (15%) produced SEB, while 26 (81%) produced SEC. No isolates produced TSST-1. In contrast, no MRSA isolates from UNMC produced SEB, SEC, or TSST-1. Sixteen of 21 nmTSS isolates made either SEB or SEC; none made TSST-1.

PFGE. PFGE analysis demonstrated that 31 of 32 MRSA isolates from the Native American community were highly related to one another yet were divergent from the MRSA isolates collected at UNMC (Fig. 1). The one CA-MRSA isolate (REF548) that was divergent by PFGE was the same isolate that did not produce SEB, SEC, or TSST-1. Figure 2 shows a representative CA-MRSA isolate from each PFGE group (A1 to A8) shown in Fig. 1. Twenty-one nmTSS *S. aureus* isolates were also compared by PFGE with the CA-MRSA isolates; 18 of these isolates were MRSA (Table 1). *S.*

TABLE 2. HA-MRSA isolates analyzed in this study

Isolate ^a	Resistance phenotype ^b	Presence of SAg ^c			PFGE type	Source
		SEB	SEC	TSST-1		
U634	POxECIC	—	—	—	B1	Right knee
U655	POxECIC	—	—	—	B1	Body fluid
U652	POxECIC	—	—	—	Unique	Right eye
U639	POxECIC	—	—	—	Unique	Nasal wash
U661	POxECIC	—	—	—	Unique	Wound
U660	POxEC	—	—	—	Unique	Wound
U640	POxECIC	—	—	—	Unique	Blood
U609	POx	—	—	—	Unique	Sputum
U657	POx	—	—	—	Unique	Finger
U667	POxECICGSxt	—	—	—	B2	Bronchoalveolar lavage
U473	POxECICGSxt	—	—	—	Unique	Sputum
U480	POxECIC	—	—	—	B6	Blood
U479	POxECIC	—	—	—	B2	Sputum
U494	POxECICRCh	—	—	—	B5	Blood
U492	POxECICR	—	—	—	B5	Sputum
U487	POxECICRChGSxt	—	—	—	B5	Sputum
U601	POxECICGSxt	—	—	—	B2	Blood
U605	POxECIC	—	—	—	B4	Sputum
U674	POxECIC	—	—	—	B3	Wound
U472	POxECIC	—	—	—	B4	Catheter tip
U622	POxECIC	—	—	—	Unique	Sputum
U621	POxECIC	—	—	—	Unique	Blood
U630	POx	—	—	—	Unique	Wound
U628	POxECIC	—	—	—	Unique	Sputum
U604	POxECIC	—	—	—	B6	Sputum
U619	POxECIC	—	—	—	B2	Blood
U625	POxECIC	—	—	—	B2	Blood
U627	POxECIC	—	—	—	B2	Abdomen
U599	POxECICG	—	—	—	B3	Blood
U657	POx	—	—	—	Unique	Finger
U613	POxECIC	—	—	—	Unique	Sputum
U632	POxECIC	—	—	—	B2	Abdomen

^a U = hospital-acquired isolates.

^b P, penicillin; Ox, oxacillin; E, erythromycin; C, ciprofloxacin; Cl, clindamycin; Ch, chloramphenicol; G, gentamicin; Sxt, trimethoprim-sulfamethoxazole; and R, rifampin.

^c +, positive; —, negative.

^d "Unique" represents a PFGE pattern that was distinguishable from all other isolates in the present study, as represented by Fig. 1.

aureus COL and NCTC8325 were used as PFGE controls. Six of these nmTSS isolates were highly related to CA-MRSA, one of which (MnCop) is a methicillin-susceptible *S. aureus* isolate, as it does not contain *mecA*. Four of six isolates (C99-193, C99-529, C99-459, and C98-370) were involved with pediatric fatalities in Minnesota and North Dakota (5); of the additional two isolates, which were obtained from nmTSS patients in Minnesota (MnKn) and Alabama (MnCop) (32), one resulted in death.

***spa* typing and SCCmec typing.** *spa* typing was performed on 22 isolates, including 17 isolates that represent seven of eight PFGE groups identified among the Native American population. There was strong concordance between the PFGE groupings and the groupings based on the variable number tandem repeat arrays in the protein A gene. DNA sequence results revealed that all isolates tested had one of five highly related repeat arrays (*spa* types 131, 227, 194, 35, and 175 [Fig. 1]). With one exception, isolates within the same PFGE group had identical *spa* types. The three *spa* types identified in the A3 PFGE group had related repeat arrays (227, 131, and 194) in support of their genetic relatedness. The *spa* typing also confirmed the genetic relatedness between a geographically distinct isolate, MnEy, with a PFGE pattern similar to those of

the CA-MRSA isolates (Table 1). It was found that MnEy had *spa* type 131, which is identical to 9 of 17 CA-MRSA isolates tested. And finally, the *spa* typing also revealed that strains U674, MnMo, NCTC8325, and COL (types 2, 3, 59, and 1, respectively) are genetically distinct from CA-MRSA.

SCCmec typing was performed on 11 isolates to confirm the existence of a type IV *mec* element in each CA-MRSA isolate as previously reported for C99-459 (MW2) (1) (Fig. 1). Eleven isolates, representing five of the eight PFGE A groups, were typed. All isolates found within PFGE group A had a type IV SCCmec element as predicted. MnEy also had a type IV SCCmec element confirming its relationship to the CA-MRSA from Nebraska, whereas MnMo, which also had a PFGE pattern, but not *spa* type, highly related to that of CA-MRSA contained a type II SCCmec element.

Genetic relationship between MnCop and C99-459. The relationship between MnCop and C99-459 was further studied. MnCop was highly related to CA-MRSA as assessed by PFGE, *spa* typing, and production of SEC; however, this isolate did not contain *mecA* by PCR and was phenotypically susceptible to oxacillin. As the genome of C99-459 has recently been sequenced (1), it was determined whether other virulence genes found in C99-459 were also found in MnCop. C99-459 is

TABLE 3. Reference and nmTSS isolates analyzed in this study

Isolate	Resistance phenotype ^a	Presence of SAg ^b				PFGE type ^c	Origin (yr)	Location	Reference or source
		mec	SEB	SEC	TSST-1				
COL	POx	+	+	-	-	Unique	United Kingdom	Unknown	*
NCTC8325	None	-	-	-	-	Unique	United Kingdom	Unknown	17
MnSe	POx	+	-	-	-	Unique	Ohio (1998)	Unknown	TS
MnNj	PECSxt	-	+	-	-	Unique	California (1997)	Unknown	TS
MnLo	POxC	+	+	-	-	Unique	Michigan (1985)	Wound	TS
MnKn	POx	+	-	+	-	A3	Minnesota (2001)	Lungs	TS
MnOg	PECIGCh	-	-	-	-	Unique	Texas (1998)	Unknown	TS
MnCor	POx	+	-	-	-	Unique	California (1997)	Unknown	TS
MnEd	POxECICh	+	+	-	-	Unique	Pennsylvania (2000)	Wound	TS
MnKo	POx	+	+	-	-	Unique	Minnesota (1998)	Blood	TS
MnOe	POxE	+	+	-	-	Unique	Massachusetts (1993)	Wound	TS
MnHo	POxE	+	+	-	-	Unique	New Jersey (1985)	Leg bursa	TS
MnEy	POx	+	+	-	-	Unique	Minnesota (1993)	Blood	TS
MnMi	POx	+	+	-	-	Unique	Minnesota (1986)	Trachea	TS
MnDon	POx	+	-	+	-	Unique	North Carolina (1984)	Heel blister	4
MnMu	POx	+	-	-	-	Unique	Minnesota (1993)	Throat	TS
MnCop	P	-	-	+	-	A5	Alabama (1986)	Lungs	32
MnJa	POXEGCh	+	+	-	-	Unique	Connecticut (1998)	Stool	TS
MnMo	POxECICGR	+	-	-	-	Unique	California (1997)	Throat	TS
C99-529	POx	+	+	-	-	A3	North Dakota	Pleural tissue	5
C99-193	POx	+	+	-	-	A6	Minnesota	Pleural tissue	5
C99-459	POx	+	-	+	-	A3	North Dakota	Blood	5
C98-370	POx	+	-	+	-	A3	Minnesota	Unknown	5

^a P, penicillin; Ox, oxacillin; E, erythromycin; C, ciprofloxacin; Cl, clindamycin; Ch, chloramphenicol; G, gentamicin; Sxt, trimethoprim-sulfamethoxazole; and R, rifampin.

^b +, positive; -, negative.

^c "Unique" represents a PFGE pattern that was distinguishable from all other isolates in the present study, as represented by Fig. 1.

^d TS, this study; *, *S. aureus* COL genomic sequence can be accessed through The Institute for Genomic Research databases (<http://www.tigr.org>).

known to contain genes that encode the bicomponent toxin panton-valentine leukocidin as well as SEH and a truncated *seo* gene. Interestingly, both *seh* and the truncated version of *seo* were found just downstream of the SCC*mec* insertion point (1). PCR was performed using primers specific for each toxin, which demonstrated that all three genes, including the truncated *seo*, were found in MnCop (data not shown).

As the only detectable PFGE difference between MnCop and C99-459 was a two-band change (Fig. 3A), it was hypothesized that the band shift between the two strains was the insertion of SCC*mec* DNA in C99-459. Southern hybridization demonstrated that a *mecA* probe hybridized to an approximately 190-kb band in C99-459 (Fig. 3B). Since *mecA* is linked to *gyrA* on the same *Sma*I fragment (13, 17), a similar blot was probed with *gyrA*. This demonstrated that *gyrA* hybridized to the *Sma*I G fragment of NCTC8325 (175 kb) and a band of similar size in MnCop (Fig. 3B). *gyrA* also hybridized to the same band that *mecA* hybridized to in C99-459, as predicted (Fig. 3C). Therefore, *gyrA*, a gene linked to *mecA* on the same *Sma*I fragment, hybridized to both the DNA bands, which made C99-459 and MnCop distinguishable by PFGE. Furthermore, the difference in size between the *gyrA*-positive bands in both MnCop and C99-459 is consistent with the addition of an SCC*mec* element in C99-459.

DISCUSSION

Since the initial reports of CA-MRSA appeared in the literature, it has not been clear whether the strains in question were nosocomial strains that had "escaped" the hospital environment or whether these strains represented a new acquisition of SCC*mec* DNA. Studies have shown that patients who

have acquired CA-MRSA infections do not have typical MRSA risk factors, such as recent hospitalization, kidney dialysis, residence in a long-term health care facility, or intravenous drug use. Although it is clear that in many cases recent hospitalization is a major risk factor for MRSA found in the community (7), studies have clearly shown that patients in the northern Great Plains of the United States are acquiring MRSA in the community without attributable risk factors (5, 14, 15, 24).

PFGE analysis of the MRSA strains from the Native American community demonstrated that 32 of 33 were highly related to one another, suggesting clonal expansion in this population. These isolates were also highly related or indistinguishable from strains that caused deaths in Minnesota, North Dakota, and Alabama. The CA-MRSA strains from the Native American community were also indistinguishable from or highly related to CA-MRSA strains discussed in two recent papers by Naimi et al. and Groom et al. (data not shown) (14, 24). The *spa* typing results on representative isolates confirmed the genetic relatedness indicated by the PFGE patterns and more importantly provided an objective "subtype" to directly compare these strains to other MRSA and methicillin-susceptible *S. aureus* populations. As an example, MnCop, a methicillin-susceptible isolate that was isolated from a patient who clearly had nmTSS in Alabama in 1986 (32), had a *spa* type related to both the Minnesota-North Dakota (5) cluster of cases and to the CA-MRSA isolates presented in this study. PFGE and toxin typing using *sec*, *seh*, *seo*, and *lukS-PV/lukF-PV* suggests that MnCop is highly related to C99-459 or MW2. Southern hybridization using *mecA* and *gyrA* strongly suggests that the only detectable difference between C99-459 and MnCop as

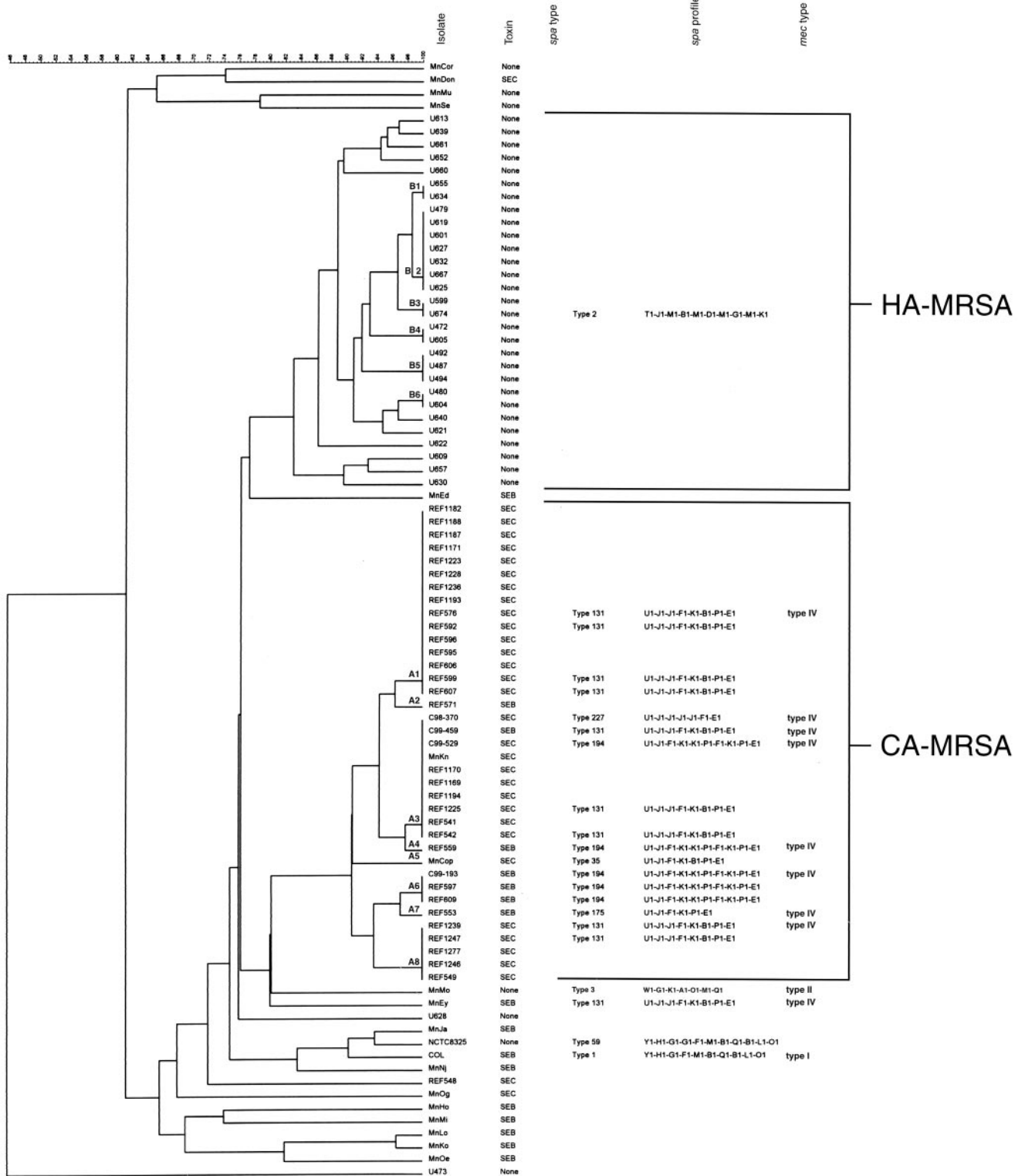


FIG. 1. PFGE subtyping and dendrogram of CA-MRSA, HA-MRSA, and nmTSS isolates. A PFGE pattern seen in more than one isolate was given a letter-and-number designation (e.g., A1). PFGE groups are noted on the far right end of the tree. The *spa* type, *spa* profile, and *SCCmec* type, if testing was performed, are shown on the right end of the figure.

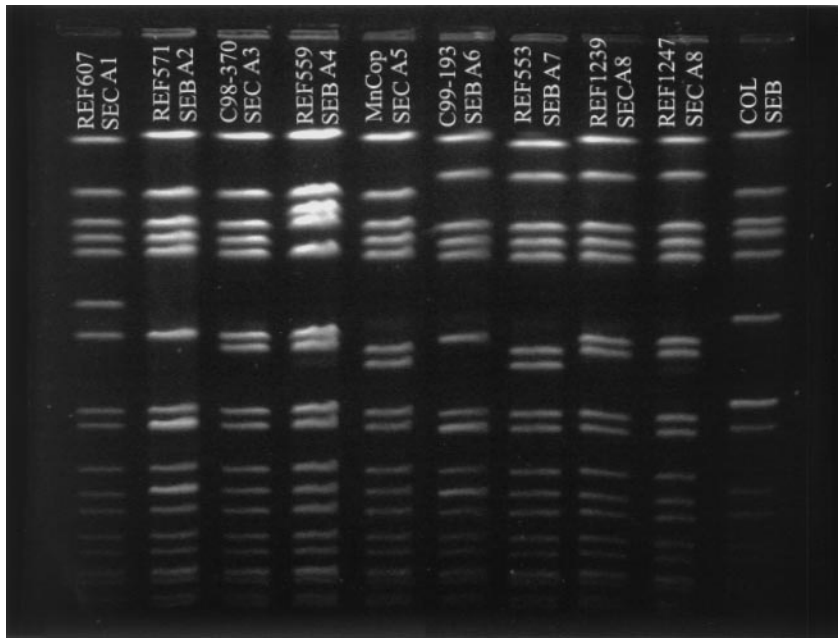


FIG. 2. Results of PFGE of a representative isolate from each CA-MRSA PFGE group (A1 to A8). Lanes: 1, REF607 (group A1); 2, REF571 (group A2); 3, C98-370 (group A3); 4, REF559 (group A4); 5, MnCop (group A5); 6, C99-193 (group A6); 7, REF553 (group A7); 8, REF1239 (group A8); 9, REF1247 (group A8); and 10, COL.

assessed by PFGE is the insertion of *SCCmec* DNA within C99-459. This suggests that MnCop may represent a progenitor CA-MRSA strain before it had acquired *SCCmec*. It is interesting that the *spa* type of MnCop, type 35, was also found in pansusceptible *S. aureus* isolated in Denmark in 1966 to 1968, suggesting that this strain may have been circulating worldwide for some time (29).

The CA-MRSA isolated in this study was divergent from the HA-MRSA isolated at UNMC using PFGE, *spa*, and SAg typing. HA-MRSA isolates from the hospital that serves the

Native American community were not available for analysis, and the prevalence of MRSA in this hospital was apparently quite low. Therefore, the CA-MRSA was compared to HA-MRSA isolated from the closest tertiary-care center (UNMC). The HA-MRSA isolated at UNMC had a *spa* type 2, which is a very common *spa* type in the United States (B. Kreiswirth, unpublished observations).

Five of the 32 strains studied from the Native American community expressed SEB, while 27 expressed SEC. Both SEC and SEB are typically found encoded on pathogenicity islands

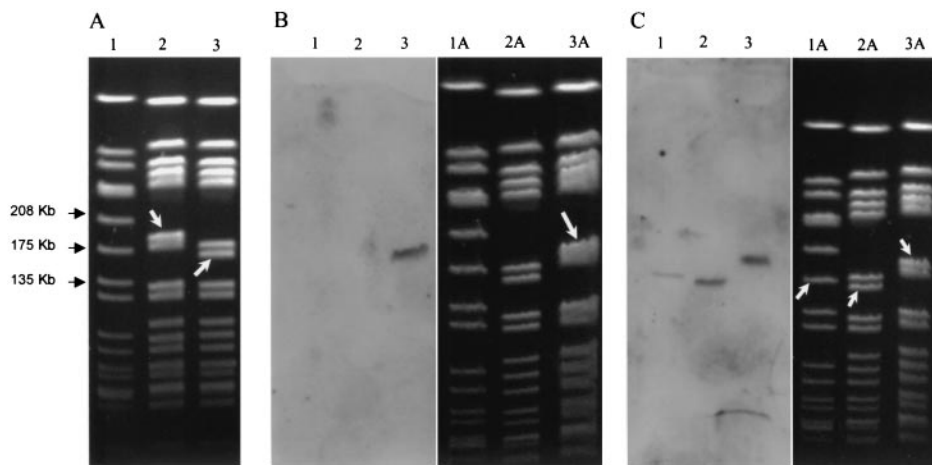


FIG. 3. Results of PFGE and subsequent hybridization with *mecA* and *gyrA* DNA probes. (A) Lanes: 1, NCTC8325; 2, C99-459; and 3, MnCop. White arrows show bands in both C99-459 and MnCop that differentiate these strains by PFGE. (B) Hybridization with a *mecA* probe. Lanes: 1 and 1A, NCTC8325; 2 and 2A, MnCop; and 3 and 3A, C99-459. White arrows show bands that hybridize with a *mecA* probe in C99-459. (C) Hybridization with a *gyrA* probe. Lanes: 1 and 1A, NCTC8325; 2 and 2A, MnCop; and 3 and 3A, C99-459. White arrows show bands that hybridize with a *gyrA* probe in all three strains. Black arrows in panel A show the size of the *SmaI* bands in NCTC8325, which approximates the size of the bands that hybridize to *gyrA* in MnCop and both *mecA* and *gyrA* in C99-459.

(19, 26, 33). In some cases, such as C99-529 (SEB producer) and C99-459 (SEC producer), the PFGE patterns are indistinguishable (PFGE group A3). In other cases, strains that produced SEB grouped together or alone (PFGE groups A4, A6, and A7). Baba et al. reported that SEC is encoded on a pathogenicity island called *vSa3* along with *ear* and *sel2* in strain C99-459 (1). DNA sequence analysis demonstrated that the *sec* gene found in C99-459 is a variant, which was named *sec4* (1). Further work is being performed to determine whether CA-MRSA isolates that produce SEB instead of SEC contain *vSa3* or a variant thereof.

Genetic analysis combining ribotyping, multilocus sequence typing, *spa* typing, and SCCmec typing has suggested that SCCmec has inserted into only five methicillin-susceptible genomic backgrounds worldwide (10, 16, 28, 29). It is now becoming quite clear that CA-MRSA found in the northern Great Plains of the United States represents a new genetic lineage of MRSA with a different SCCmec element (type IV). The genetic background represented by CA-MRSA represents the sixth genetic background that is known to contain SCCmec DNA. Due to the small size of the type IV SCCmec DNA, more genetic backgrounds may be soon discovered that harbor this SCCmec element.

The observations in this study have great clinical significance. Because of its high level (50 to 100 $\mu\text{g/ml}$ in culture fluids) of SEB and SEC production, CA-MRSA may cause nmTSS (9, 36). SEB and SEC, together with TSST-1, cause nearly all of TSS cases (3, 11, 22). Since only 3 to 5 μg of streptococcal pyrogenic exotoxin A is sufficient to induce TSS in humans (3, 11, 22) and since SEB and SEC are in the same subfamily of SAGs as streptococcal pyrogenic exotoxin A (3, 11, 12, 22, 30, 38), SEB and SEC are likely to have the same potency in humans. Clinicians associate CA staphylococcal disease with an antibiotic-susceptible phenotype and routinely prescribe penicillinase-stable penicillins or first-generation cephalosporins as antibiotics of choice. Therefore, some CA-MRSA patients may receive inappropriate treatment. Finally, our study and others indicate that CA-MRSA isolates, as opposed to HA-MRSA, remain susceptible to other antistaphylococcal agents, such as macrolides, trimethoprim-sulfamethoxazole, and fluoroquinolones (5, 14, 15, 24).

In conclusion, the prevalence of CA-MRSA in the United States is unknown, but this report and others indicate that it is likely increasing. Surveillance programs should be implemented by public health laboratories to determine the extent of CA-MRSA dissemination. Until the scope of this problem is better defined, clinicians should consider the use of non- β -lactam antistaphylococcal antibiotics in the empirical treatment of CA-MRSA.

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