JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2004, p. 3752–3757 Vol. 42, No. 8
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.8.3752–3757.2004
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Molecular Characteristics of Nosocomial and Native American Community-Associated Methicillin-Resistant *Staphylococcus aureus* Clones from Rural Wisconsin

Sanjay K. Shukla,[1]* Mary E. Stemper,[2] Srinivas V. Ramaswamy,[3] Jennifer M. Conradt,[1] Robert Reich,[3] Edward A. Graviss,[3] and Kurt D. Reed[1,2]

Molecular Microbiology Laboratory, Marshfield Clinic Research Foundation,[1] and Microbiology Section, Marshfield Laboratories,[2] Marshfield, Wisconsin, and Department of Pathology, Baylor College of Medicine, Houston, Texas[3]

Received 17 February 2004/Returned for modification 16 April 2004/Accepted 9 May 2004

In central and northern Wisconsin methicillin-resistant *Staphylococcus aureus* (MRSA) was first detected in 1989. Over the next 10-year period, 581 MRSA isolates were collected, 17.2% of which came from patients who were treated at five Native American clinics. These isolates were typed by SmaI-macrorestricted pulsed-field gel electrophoresis (PFGE). The PFGE patterns clustered the isolates into six major clonal groups (MCGs), i.e., MCGs 1 to 6, and 19 minor clonal groups (*m*CGs). The 25 clonal groups were represented by 109 unique PFGE types. Sixty-five percent of the MCG-2 isolates were recovered from patients who were treated at Native American clinics. Ninety-four percent of the MCG-2 isolates harbored the staphylococcal cassette chromosome *mec* (SCC*mec*) IVa. These isolates also had PFGE profiles that were clonally related to the midwestern community-associated MRSA (CA-MRSA) strain, MW2. The representative isolates from MCG-2 had the multilocus sequence type allelic profile 1-1-1-1-1-1-1 and contained *pvl* genes. They were also susceptible to various antibiotics, a finding consistent with the CA-MRSA phenotype. SCC*mec* IV was also present in other *m*CGs. Unlike MCG-2, isolates from the remaining five MCGs harbored SCC*mec* II and were resistant to multiple antibiotics, suggesting their nosocomial origin. The 19 *m*CGs were represented by diverse SCC*mec* types and three putative new variants referred to as SCC*mec* Ib, IIa, and IIb.

Since the first reported case of methicillin-resistant *Staphylococcus aureus* (MRSA) from the United Kingdom in 1961, MRSA has continued to spread in different parts of the world (9, 22, 26, 37, 44). In U.S. hospitals, MRSA infections have been steadily increasing. Surveillance data have suggested that the percentage of staphylococcal hospital infections due to MRSA has increased from 2.4% in 1975 to 54.5% in 1999 (32, 35). Genotyping of MRSA is important in order to determine outbreaks, the dissemination of virulent strains, and the understanding of its changing epidemiology. In the past, MRSA was confined to nosocomial settings, but in recent years, community-associated MRSA (CA-MRSA) has become a significant concern in the United States and other parts of the world (8, 11, 26, 44). The problem is further compounded by the fact that the ecologic distinction between hospital-acquired MRSA (HA-MRSA) and CA-MRSA seems to be decreasing (8, 39).

A number of molecular tools, such as pulsed-field gel electrophoresis (PFGE), ribotyping, and multilocus sequence typing (MLST) have been utilized to type MRSA strains (4, 12, 25, 36, 42). A great deal of epidemiologic studies on MRSA has been reported from different parts of the world. These studies have focused largely on characterizing different circulating MRSA clones and discussing their evolutionary relationships (5, 9, 13, 19–21, 23, 28). Molecular and epidemiologic distinctions have been made between MRSA strains that are typically acquired in a nosocomial or community-associated setting (30). Nosocomial MRSA isolates are represented by numerous genomic backgrounds; harbor primarily staphylococcal cassette chromosome *mec* (SCC*mec*) types I, II, III, and occasionally IV; have resistance to multiple antibiotics; and produce multiple staphylococcal toxins (38). CA-MRSA isolates, on the other hand, are represented by diverse PFGE-based genetic profiles, harbor SCC*mec* IV, and are susceptible to most antibiotics, except for β-lactams (10, 38). The majority of CA-MRSA strains that cause necrotizing pneumonia produce Panton-Valentine-leukocidin (PVL) toxins (24). The *pvl* genes also have been reported to be present in 77% of the CA-MRSA isolates (30).

Cases of CA-MRSA in the United States have been reported from Alaska (3), Chicago (15, 18, 41), Dallas (1, 17), Los Angeles (7), Minnesota and North Dakota (6, 29), and Nebraska (14). In central and northern Wisconsin, a relatively rural area in the United States, MRSA was not seen until 1989. Many clinical specimens containing MRSA that were sent to Marshfield Laboratories for identification reportedly came from five Native American clinics in central and northern Wisconsin. We undertook a comprehensive molecular epidemiologic study of these MRSA isolates collected between 1989 and 1999 from Wisconsin because specimens dating back to the early 1990s reflect the early phases of the CA-MRSA problem in the United States. We characterized and determined the clonal relationships of CA- and HA-MRSA isolates by PFGE, SCC*mec* typing, MLST, and the presence or absence of the *pvl* genes.

(A portion of the data discussed here was presented at the

* Corresponding author. Mailing address: Clinical Research Center, Marshfield Clinic Research Foundation, 1000 North Oak Ave., Marshfield, WI 54449. Phone: (715) 389-5363. Fax: (715) 389-3808. E-mail: shukla.sanjay@mcrf.mfldclin.edu.

2002 International Conference on Emerging Infectious Diseases meeting in Atlanta, Ga.)

MATERIALS AND METHODS

***S. aureus* collection and their identification.** MRSA clinical strains (n = 581) were collected from 77 healthcare facilities that included clinics (64%) and hospitals and nursing homes (36%) located in northern and central Wisconsin. The isolates were collected from 1989 to 1999. The frequency distribution of isolates in each year of the study was as follows: 1989 (n = 1), 1990 (n = 5), 1991 (n = 10), 1992 (n = 44), 1993 (n = 89), 1994 (n = 44), 1995 (n = 62), 1996 (n = 53), 1997 (n = 49), 1998 (n = 95), and 1999 (n = 129). The geographic origin of these isolates and other demographic data are described elsewhere (M. E. Stemper, S. K. Shukla, and K. D. Reed, submitted for publication). *S. aureus* strains were identified by colony morphology, Gram stain, and positive tests for catalase and coagulase. The SCC*mec* control strains—COL, PER34, BK2464, ANS46, and HDE288—were kindly provided by Herminia de Lencastre of Rockefeller University. The MW2 strain was kindly provided by Timothy Naimi of the Minnesota Department of Health. This study was reviewed and approved by the Institutional Review Board of Marshfield Clinic Research Foundation.

Antimicrobial susceptibilities. An antibiogram for seven drugs—erythromycin (ERY), gentamicin (GEN), rifampin (RIF), ciprofloxacin (CIP), clindamycin (CLI), tetracycline (TET), and trimethoprim-sulfamethoxazole (SXT)—was determined by using the Vitek System (bioMérieux, Inc., Durham, N.C.). Isolates were considered methicillin resistant if the MIC for oxacillin was ≥4 μg/ml as determined by the E-test method according to the National Committee on Clinical Laboratory Standards (31).

PFGE. PFGE was done on 581 MRSA isolates. The PFGE method used in the present study was essentially adapted from previously described methods (4, 27). Briefly, plugs were prepared with 250 μl of cell suspension, 4 μl of lysostaphin enzyme (1 U/μl), and an equal volume of molten 1.8% SeaPlaque agarose. Plugs were placed in lysis buffer for 4 h at 37°C. Incubation proceeded overnight at 55°C in proteinase K buffer, followed by four washes with Tris-EDTA buffer. A portion of the plug was digested with 35 U of SmaI enzyme. Separation was performed in 1% SeaKem agarose in a CHEF-DRIII system (Bio-Rad Laboratories, Hercules, Calif.) for 20 h at 200 V with switching times ramped from 5 to 40 s. Gels were poststained with ethidium bromide solution, imaged by using GelDoc 2000 (Bio-Rad Laboratories), and saved as TIFF files for analysis. The genetic relatedness of strains, shown as a dendrogram, was based on analysis of PFGE patterns of isolates by Multianalyst Fingerprinting Plus software version 1.1 (Bio-Rad Laboratories). The digested DNA from a global standard control strain, *S. aureus* NCTC 8325, was run in every fifth lane to normalize the PFGE band pattern of clinical strains. The dendrogram was created by using the Dice coefficient and the unweighted pair group method with a 1.5% position tolerance. The tolerance, expressed as a percentage, is the numerical value for the positional difference between two bands that are considered matching for defining relatedness.

SCC*mec* typing. A total of 169 isolates were characterized for their SCC*mec* genotype. This included all unique PFGE types (n = 109), 32.9% (n = 155) of the strains in major clonal group 2 (MCG-2), and nine random isolates from other clonal groups. The determination of SCC*mec* types was done by modifying the multiplex PCR assay described by Oliveira and de Lencastre (34). The modified SCC*mec* multiplex protocol was as follows. The assay was carried out in a 50-μl reaction that contained 1.4× Tris buffer, 2 mM Mg^{2+}, 200 mM concentrations of deoxynucleoside triphosphates, 0.5 U of *Taq* polymerase, a 50 nM concentration (each) of primer set KDPF1 and KDPR1, 100 nM concentrations of the primer sets mecAP4-mecAP7, RIF4F3-RIF4F9, DCSF2-DCSR1, and IS431P4-pUB110R1, 150 nM concentrations of the primers mecIP2-mecIP3, IS431P4-pT181R1, 200 nM concentrations of RIF5F10-RIF5R13 and CIF2F2-CIF2R2, and 3 μl of the template DNA. The primer sequences are described by Oliveira and de Lencastre (34). The reaction mix was set up in a PE 9700 thermocycler for initial denaturation at 94°C for 4 min; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 30 s, and elongation at 68°C for 90 s; with a final extension at 72°C for 4 min. PCR products were resolved in 2.5% SeaKem agarose gels and run at 80 V for 4 h. The following MRSA strains were used as a positive control for SCC*mec* types: COL (type I), PER34 (type Ia), BK2464 (type II), ANS46 (type III), and HDE288 (type IV). The definitions of the SCC*mec*-specific DNA loci A to G that were used to define the SCC*mec* variants are described by Oliveira and de Lencastre (34). The SCC*mec* subtyping was done by the method of Okuma et al. (33).

***pvl* PCR and sequencing.** A total of 156 isolates that included all isolates from the six MCGs and representative isolates from the minor clonal groups (*m*CGs) were screened for the presence of the *pvl* genes. The *pvl* PCR assay was performed as described by Lina et al. (24). Two independent *pvl* amplicons were randomly selected for sequencing to confirm their identity.

MLST. Two representative strains from each of the six MCGs were selected for MLST to see how well they correlated with the strain clonality determined by PFGE. MLST was done by the procedure described by Enright et al. (12). The sequencing was done on an ABI 3100 genetic analyzer (Applied Biosystems, Foster City, Calif.).

Case definitions of CA-MRSA and HA-MRSA. Since we had no access to information about the patients' prior medical records, we were not able to determine the timeline of the exposure to MRSA and/or whether the source patients were significantly exposed to nosocomial settings prior to acquiring MRSA. Therefore, we were limited to define the terms, CA-MRSA and HA-MRSA, based on the currently described molecular characteristics associated with these phenotypes. Thus, an isolate was referred to as a CA-MRSA if it met the following criteria: (i) its SmaI-digested PFGE pattern was identical to or within one to six bands difference (one or two mutation events) with that of the well-described midwestern CA-MRSA strain, MW2, (ii) the isolate harbored both the type IV SCC*mec* and the *pvl* genes, and (iii) the isolate was sensitive to multiple antibiotics. An isolate was considered to be a HA-MRSA if (i) it was resistant to multiple antibiotics; (ii) the isolate harbored SCC*mec* type I, II, III, or sometimes IV; and (iii) the isolate lacked the *pvl* genes. As expected, we did find a small number of isolates (3.4%, n = 581) that did not clearly meet either one of the criteria. It is possible that some true CA-MRSA cases may have been missed by our strict molecular definition.

RESULTS

Clonality by PFGE. Eighty-two percent of the Wisconsin MRSA strains were clustered into six MCGs, referred to as MCG-1, MCG-2, MCG-4, MCG-7, MCG-10, and MCG-18, based on six DNA band difference criteria (42). Isolates within each MCG had a genetic similarity index of ≥80%. An MCG was defined as a clonal group that was represented by at least 5% (n = 581) of the total isolates. In addition to MCGs, 19 *m*CGs were identified. An *m*CG was defined as a group with sporadic isolates that were represented by <5% of the total isolates. Of 581 MRSA isolates, 109 unique PFGE types were identified based on a single band difference (42).

Clonality by MLST. The MLST allelic profile was determined on two representative isolates from all six MCGs. Five sequence types (STs)—ST1, ST5, ST45, ST225, and ST373—were identified, of which the first four have been previously reported (Table 1). Individual isolates from the same or closely related MCGs had similar allelic profiles. For example, isolates from MCGs 1, 7, and 18, which had a similarity index value of ~75% by PFGE analysis (Fig. 1), had an MLST allelic profile of 1-4-1-4-12-1-10 (ST 5). Interestingly, the MCG-10 was represented by two STs, ST225 and the newly identified ST373 (Table 1). The allelic profiles of ST225 and ST373 were identical except for the different alleles in the *gmk* (4 versus 49) and the *tpi* (25 versus 1) locus (Table 1). The two MCG-4 isolates had the ST45. The MCG-2 isolates had an allelic profile of 1-1-1-1-1-1-1, the same as the profile for a midwestern hypervirulent strain, MW2.

Analysis of SCC*mec* types. We determined the SCC*mec* types of 169 isolates that included all 109 unique PFGE types, 32.9% of MCG-2 isolates (n = 155), and 9 random isolates from other clonal groups. The aim was to determine (i) whether there was a preponderance of a specific SCC*mec* type in a majority of the unique PFGE types, including the major clonal types, and (ii) whether the majority of the MCG-2 isolates were of SCC*mec* type IV. SCC*mec* types II, III, and IV, subtypes Ia, IIIb, and IVa, and putative new variants of type I (named Ib) and type II (named IIa and IIb) were identified from this collection of isolates based on

TABLE 1. Representative multilocus STs from each major clonal group and their relationships to SCC*mec* types

Isolate	MCG	Allelic profile	ST	SCC*mec* type(s)	
				n[a]	*mec* type(s) (%)
WI-MRSA 33	2	1-1-1-1-1-1-1	1	51	IVa (94.1), IV (2), Var[b] (3.9)
WI-MRSA 34	2	1-1-1-1-1-1-1	1		
WI-MRSA 24	1	1-4-1-4-12-1-10	5	9	II (33.3), IIa (66.7)
WI-MRSA 363	1	1-4-1-4-12-1-10	5		
WI-MRSA 214	7	1-4-1-4-12-1-10	5	19	II (78.9); IIa (21)
WI-MRSA 507	7	1-4-1-4-12-1-10	5		
WI-MRSA 207	18	1-4-1-4-12-1-10	5	12	II (91.7); IIa (8.3)
WI-MRSA 440	18	1-4-1-4-12-1-10	5		
WI-MRSA 75	4	10-14-8-6-10-3-2	45	10	II (80); IV (20)
WI-MRSA 290	4	10-14-8-6-10-3-2	45		
WI-MRSA 302	10	1-4-1-4-12-25-10	225	11	II (100)
WI-MRSA 280	10	1-4-1-49-12-1-10	373		

[a] *n* = number of isolates from six major clonal groups that were typed for the presence of SCC*mec*.
[b] Var, SCC*mec* variants.

the presence or absence of expected SCC*mec* locus-specific PCR products in a multiplex assay to determine SCC*mec* types (34). Two isolates (WI-MRSA 291 and WI-MRSA 434) harbored SCC*mec* variants that could not be assigned to any known SCC*mec* due to the lack of enough specificity with any one type. Among isolates with unique PFGE types ($n = 109$), the type II SCC*mec* was the most common form (51.38%), followed by putative new variants (14.68%), IV (13.8%), IVa (12.8%), Ia (2.75%), type III (0.9%), and IIIb (0.92%). Of these, 2.75% did not give any bands when tested for SCC*mec* type. As shown above, SCC*mec* II and IIa together were found in 62.2% of the unique PFGE types, more than twice the number of the PFGE types that harbored SCC*mec* IV. Two isolates showed SCC*mec* profiles that did not match with any known I to IV SCC*mec* types (data not shown).

Identification of new SCC*mec* variants. Twenty-one MRSA isolates clustered into four new SCC*mec* variants (Table 2). Fifteen isolates were identified as SCC*mec* IIa since they lacked the DNA locus G present in the left junction between IS*431* and pUB110 (35). One isolate was positive for loci B (internal to the *kdp* operon) and D, a region in the *dcs* gene only. We referred to this isolate as SCC*mec* IIb clone. Four genetic loci—B, C (a *mecI* region), D, and G—are distinctive features of the SCC*mec* II. Three isolates were positive for loci D and G only, the specific markers for SCC*mec* I and Ia. Since these isolates lacked the locus A, a region downstream of the *pls* gene, an additional specific marker for SCC*mec* I, we have tentatively named the clone as SCC*mec* Ib. Two isolates belonging to the MCG-2 were positive for loci D, a region in the *dcs* gene, and E, a region between the integrated plasmid pI258 and transposon Tn*554*. The locus D is one marker for SCC*mec* types I, II, and IV, whereas the locus E is the marker for SCC*mec* II. We consider this clone a new *mec* type. All of these SCC*mec* variants were positive for the *mecA* internal control.

Determination of CA- and HA-MRSA based on the genotypic properties. A representative group of Wisconsin MRSA isolates from the 6 MCGs ($n = 30$) and 19 *m*CGs ($n = 23$) was compared to the available SCC*mec* types, *pvl* genes, and antibiogram data (Fig. 1). Available data from six reference strains—ANS46, BK2464, COL, HDE288, MW2, and PER34—were also included for comparison. The dendrogram was created on the basis of the PFGE profile only. This comparison created a clustering of isolates in three distinct groups referred to as CA-MRSA, HA-MRSA, and mixed types. The figure shows that the isolates in four PFGE-based related MCGs—MCG-18, MCG-7, MCG-1, and MCG-10—harbored either SCC*mec* II or IIa and were resistant to CIP, CLI, and ERY. They were also negative for the *pvl* genes by PCR. The MCG-4, which was somewhat distantly related to the four previous MCGs, had the same genetic markers and antibiogram with the exception of the WI-35 strain that harbored SCC*mec* IV and *pvl* genes. The genotypic trait, such as the presence of SCC*mec* II, multidrug resistance, and the absence of *pvl* genes suggest that the MRSA isolates in these MCGs were HA-MRSA. The strain WI-35 probably represents an example of a HA-MRSA strain that had acquired the phenotypic and some genotypic features typically associated with CA-MRSA.

TABLE 2. Characteristics of SCC*mec* variants

New SCC*mec* variant	Loci present[a]	No. of isolates	Antibiogram	*mec* type
Ib	D, G	1	CIP, CLI, ERY, TET	Class B1 *mec*
Ib	D, G	1	CIP, CLI, ERY, TET, SXT	WT[c]
Ib	D, G	1	CIP, CLI, ERY, TET, SXT, GEN, RIF	WT
IIa	B,C,D	15	CIP, CLI, ERY	WT
IIb	B, D	1	CLI, ERY	Class B1 *mec*
New	D, E	2	–[b]	Class B1 *mec*

[a] Definitions of loci A to G are based on the criteria of Oliveira and de Lencastre (34).
[b] Sensitive to all seven antibiotics tested (see Materials and Methods).
[c] WT, wild type.

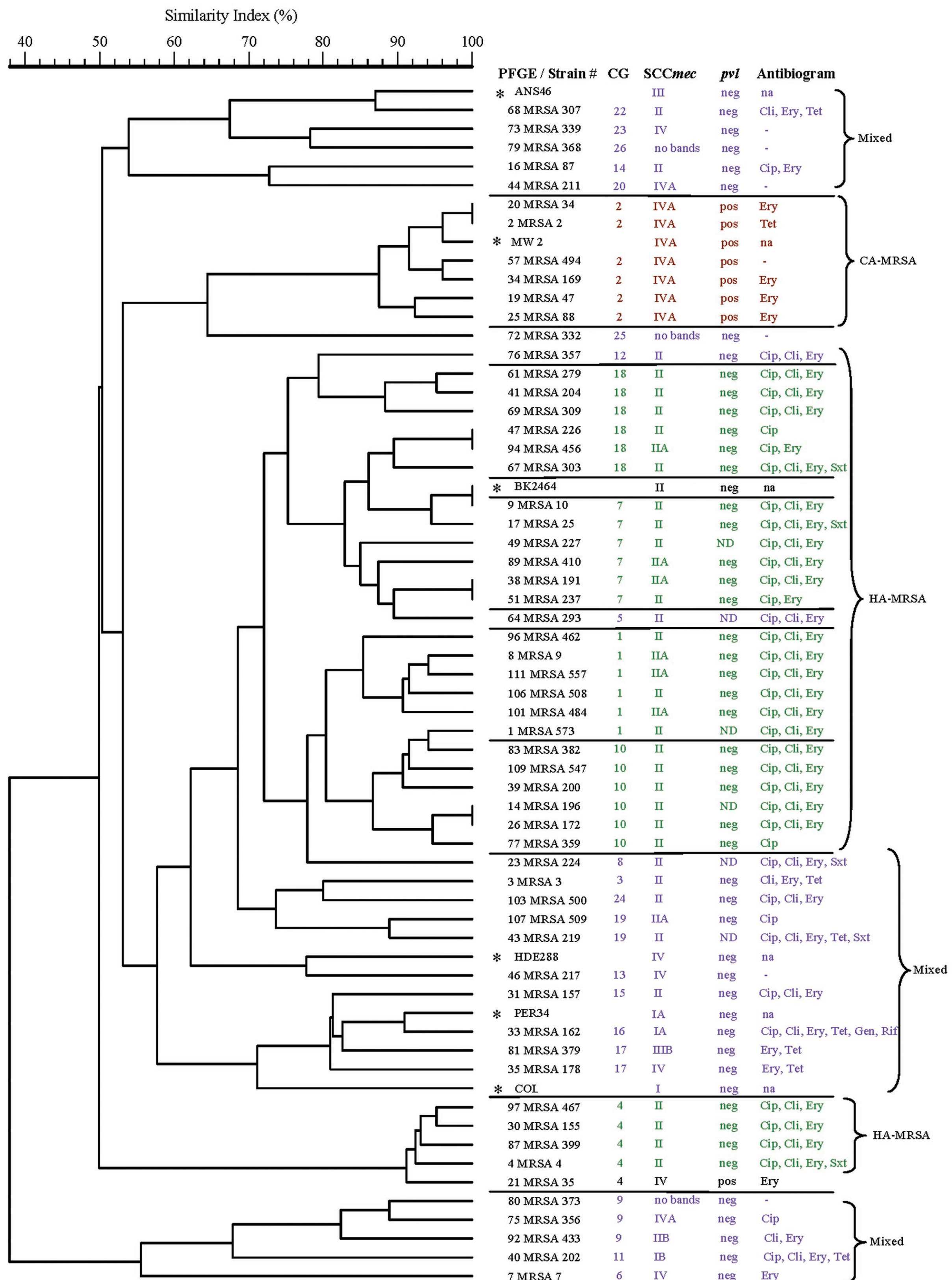

FIG. 1. Dendrogram showing the genetic relatedness of representative isolates from 6 MCGs and 19 *m*CGs based on the SmaI macrorestriction pattern of genomic DNA. The corresponding SCC*mec* genotypes, antibiograms, and the presence or absence of *pvl* features are presented next to the strain numbers. PFGE types and strain identification for each isolate are shown in the first column. The scale above the dendrogram shows the percentage of the similarity index among different strains. Strain numbers with asterisks represent SCC*mec* MRSA positive control or reference strains. The MRSA isolates belonging to MCGs and *m*CGs are demarcated by horizontal lines. Properties associated with CA-MRSA, HA-MRSA, and *m*CGs are in red, green, and blue, respectively. No bands indicates that no amplicons in the SCC*mec* multiplex assay were detected with the primers used in the present study. Abbreviations: CG, clonal group; ND, not determined; neg, negative; pos, positive; na, not available.

The molecular characteristics of MCG-2 isolates shown in Fig. 1 were different from other MCGs. Of the 51 MCG-2 isolates that were SCC*mec* genotyped, 94% were type IVa, 4% were SCC*mec* variants, and 2% were not subtyped beyond type IVa. These isolates also had *pvl* genes and were sensitive to multiple antibiotics, including CLI and fluoroquinolones. Most strains from the MCG-2 had the SmaI-restricted PFGE pattern that were indistinguishable from, or closely related to, the MW2 strain, the prototype of a CA-MRSA strain. The remaining isolates of MCG-2 had a PFGE banding pattern within the six bands difference of MW2. Like MW2, representative isolates belonging to the MCG-2 had the allelic profile 1-1-1-1-1-1-1 (Fig. 1 and Table 1). The majority of these isolates came from five outpatient clinics in Wisconsin, which primarily served Native American patients (Stemper et al., submitted). The presence of the SCC*mec* IV and the *pvl* genes and the lack of resistance to multiple antibiotics are features that are strongly suggestive of CA-MRSA (30). The fact that Wisconsin MCG-2 isolates had these features suggested that they were indeed CA-MRSA.

The representative isolates from the 19 *m*CGs (Fig. 1, shown in the blue font) were clustered into three unrelated PFGE based groups. All four SCC*mec* types or their variants such as Ia, Ib, IIIb, and IVa were represented in these three groups. The antibiogram profiles of MRSA isolates in these groups were variable and ranged from being susceptible to all antibiotics tested to resistant to several antibiotics such as CIP, CLI, ERY, TET, GEN, and RIF. Interestingly, isolates in these three clusters lacked the *pvl* genes. Thus, these three clusters show a heterogeneous mixture of MRSA that have molecular characteristics representative of both the CA-MRSA and the HA-MRSA.

DISCUSSION

Our PFGE data show the presence of several MRSA major and minor clones circulating in the rural communities of northern and central Wisconsin. The representative isolates from MCGs 1, 7, and 18 had the same ST, ST5, suggesting those strains may have originated from a single clone. Additional evidence that supports this conclusion was based on the presence of a similar antibiogram, the same SCC*mec*, and the lack of *pvl* genes. The MLST allelic profiles of the representative isolates of MCG-10 were represented by STs 225 and 373 due to differences in alleles of *gmk* and *tpi* genes. A single base change (in WI-MRSA 280) at nucleotide position 329 (T→A) in the *gmk* gene created the new ST373 (Table 1).

MLST data also suggest the presence of at least two international clones, ST5 and ST45, described as the New York/Japan clone and the Berlin clone, respectively, in Wisconsin (13, 43). ST45 has been reported from several European countries and in a CA-MRSA from western Australia (http://saureus.mlst.net/). The ST225, which is related to ST5, has been reported in MRSA isolates from the United States (13). Our ST373 is a new ST represented in the midwestern United States. When we combine the MLST and SCC*mec* data to define clonality, we could identify five clonal groups: ST1-IV, ST5-II, ST45-II, ST225-II, and ST373-II. Of these five, ST1-IV (CA-MRSA), ST5-II (New York/Japan clone), and ST45-11 have been described before (2, 13). To the best of our knowledge, the remaining two combinations have not been reported yet. Therefore, they may well represent some of the HA-MRSA clones still limited to the midwestern United States.

One of the significant features that emerged from the molecular characterization of MRSAs from rural Wisconsin was the presence of a single clonal group, MCG-2, that had very distinct genotypic features of CA-MRSA. The majority of these isolates came from clinics primarily visited by Native American communities. This fact strongly suggests the presence of predominantly a single CA-MRSA clone circulating in the Native American communities of Wisconsin. Our molecular data show that almost all CA-MRSA present in Wisconsin are identical or clonally related to the hypervirulent strain, MW2, the strain that caused fatal septicemia and septic arthritis in a 16-month-old girl in North Dakota, in 1998 (6). The presence of CA-MRSA in the late 1990s in a rural Native American community in the midwestern United States has been described previously based on the patient record (16). However, our data suggest the presence of the MW2 related clone in a Native American population in the midwestern United States since the early 1990s. Another marker for the Wisconsin CA-MRSA is the presence of the class B1 *mec* complex in *mec* DNA. About 25% of these isolates had this deleted version of the *mec* complex (40).

The fact that SCC*mec* IV was present in several PFGE types suggests the possibility of multiple and independent acquisition of this *mec* genotype in different MRSA genetic backgrounds. However, a high percentage of the SCC*mec* IV had the same *mecA* promoter mutation and the same type of deletion in the *mec* complex suggests that source(s) of SCC*mec* IV in Wisconsin MRSA isolates may be limited (40). It is likely to be from a single or a very small number of source organism(s).

In conclusion, several important observations were made from the present study of a collection of temporal isolates from a predominantly rural environment where the dynamics of nosocomial and community-based spread of infectious diseases is expected to be different from the coastal U.S. cities and hospitals. First, the molecular evidence that CA-MRSA coming out of the Native American clinics in northern and central Wisconsin were clones of the virulent strain MW2. Second, it seems that there are three closely related clones of HA-MRSA circulating in hospitals and long-term care institutions in Wisconsin. Third, almost all CA-MRSA isolates contain SCC*mec* IV and *pvl* genes, but not all SCC*mec* IV harboring MRSA would be CA-MRSA. It appears that the *pvl* genes and SCC*mec* IV together offers some selective advantage for CA-MRSA (43). In addition, SCC*mec* type IV was integrated into some isolates of other *m*CGs representing multidrug-sensitive nosocomially related isolates. Whether the acquisition of SCC*mec* IV by nosocomial isolates provides them any advantage in colonization remains to be seen. Genetically, it should be more favorable for an *S. aureus* strain to acquire an approximately 21- to 24-kb genetic element with two functional recombinase genes than for an approximately 67-kb element to gain *mecA* based methicillin resistance. Smaller genetic elements, such as SCC*mec* IV, can be packaged in phage more efficiently than larger genetic elements.

ACKNOWLEDGMENTS

The Marshfield Clinic Research Foundation provided financial support for this study.

We thank Alice Stargardt and Linda Weis for help with preparation of the manuscript.

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