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Staphylococcal Cassette Chromosome *mec* and Panton-Valentine Leukocidin Characterization of Methicillin-Resistant *Staphylococcus aureus* Clones[▽]

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Staphylococcal cassette chromosome *mec* (SCC*mec*) types and Panton-Valentine leukocidin (PVL) gene carriage were compared among suspected community-associated methicillin-resistant *Staphylococcus aureus* MRSA (CA-MRSA) and health care-associated MRSA (HA-MRSA) isolates. CA-MRSA isolates carried the SCC*mec* type IV complex, and most were PVL positive. The HA-MRSA isolates carried the SCC*mec* type II complex and did not harbor the PVL genes.

Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be a major cause of both health care-associated and community-associated infections. The resistance of the organism is due to acquisition of the methicillin resistance gene *mecA*, carried by the staphylococcal cassette chromosome *mec* (SCC*mec*). Recently, numerous studies have reported the emergence of community-associated MRSA (CA-MRSA) within the hospital setting, posing a significant public health threat (1, 2, 12). CA-MRSA isolates have several distinguishing characteristics that make them distinct from nosocomial strains. First, health care-associated MRSA (HA-MRSA) strains tend to carry SCC*mec* types I, II, and III, while the type IV element is generally carried by CA-MRSA (9, 10, 11, 16). Second, the Panton-Valentine leukocidin (PVL) genes have been demonstrated primarily among CA-MRSA strains (4, 8, 13, 14). PVL is an *S. aureus*-specific exotoxin often associated with severe skin infections and necrotizing pneumonia (4); however, its role as a virulence determinant has recently been disputed (15). Finally, CA-MRSA strains typically demonstrate resistance to fewer antimicrobials than strains acquired within hospitals (7). We sought to characterize the SCC*mec* types of isolates submitted for strain typing and, among these strains, to determine the prevalence and distribution of the PVL exotoxin. Antimicrobial susceptibility patterns were also compared among strains.

The original specimens used in this study were collected from central Florida hospitals during a 9-month period from March 2005 through November 2005 among two patient populations (institutional review board approval number 101457D). Surveillance specimens consisting of either nasal or rectal specimens were collected upon admission from patients considered to be at high risk for MRSA colonization. Specimens collected from inpatients for the purpose of infection control investigations included tracheal aspirate, skin/wound/abscess, blood, and cerebrospinal fluid (CSF) samples. Only one isolate from each patient was included in the study.

Strain typing was performed using the DiversiLab strain typing system (Bacterial Barcodes, Inc., Athens, GA). Genomic DNA was extracted (Ultra Clean microbial isolation kit; MoBio Laboratories, Carlsbad, CA) and amplified by repetitive sequence-based PCR (rep-PCR) methodology using the *Staphylococcus* kit for DNA fingerprinting (6). Analysis was performed using the Web-based DiversiLab software, which determines distance matrices to create dendrograms. Sample relationships were designated as follows: indistinguishable (no band differences), related (one band difference), or different (two or more band differences).

Sixty isolates were screened by real-time PCR to confirm the identification of *S. aureus* (*femA*) and the presence of the *mecA* gene (5). Traditional PCR, targeting the *mec* gene complex and the *ccr* gene complex, was then performed on all samples to identify SCC*mec* elements I to IV, including subtypes IVa and IVb (9). Controls were *S. aureus* isolates containing SCC*mec* types I to IV (including subtypes IVa and IVb). Finally, the PVL locus was detected by TaqMan PCR (3), using a 0.5 McFarland standard suspension of the organism added directly to the reaction mixture.

The rep-PCR patterns clustered the majority of isolates into four major clonal groups or clusters (clusters A, B, C, and D) as demonstrated in Fig. 1. Within these major clusters, samples were arranged based on similarities. Clusters A through D represent subsets of indistinguishable or related isolates. Of the 60 MRSA isolates that were tested, the majority possessed the type II cassette (57% [$n = 34$]). All but one of these samples had clustered by strain typing into cluster A or B, and most had met the criteria for HA-MRSA. A MRSA isolate was defined as health care associated if it was obtained 48 h after hospital admission or if the patient had been hospitalized within 2 years before the date of MRSA isolation. All 34 of the SCC*mec* type II isolates were PVL negative (100%). The single SCC*mec* type II sample that did not cluster within the two groups (sample 59) was not related to any other isolates yet was also PVL negative.

The subtype IVa cassette was identified in 37% of the isolates ($n = 22$). Three samples were identified as type IV (5%) but did not subtype using these primer sets. No isolates belonged to subtype IVb. Twenty-three of these type IV samples

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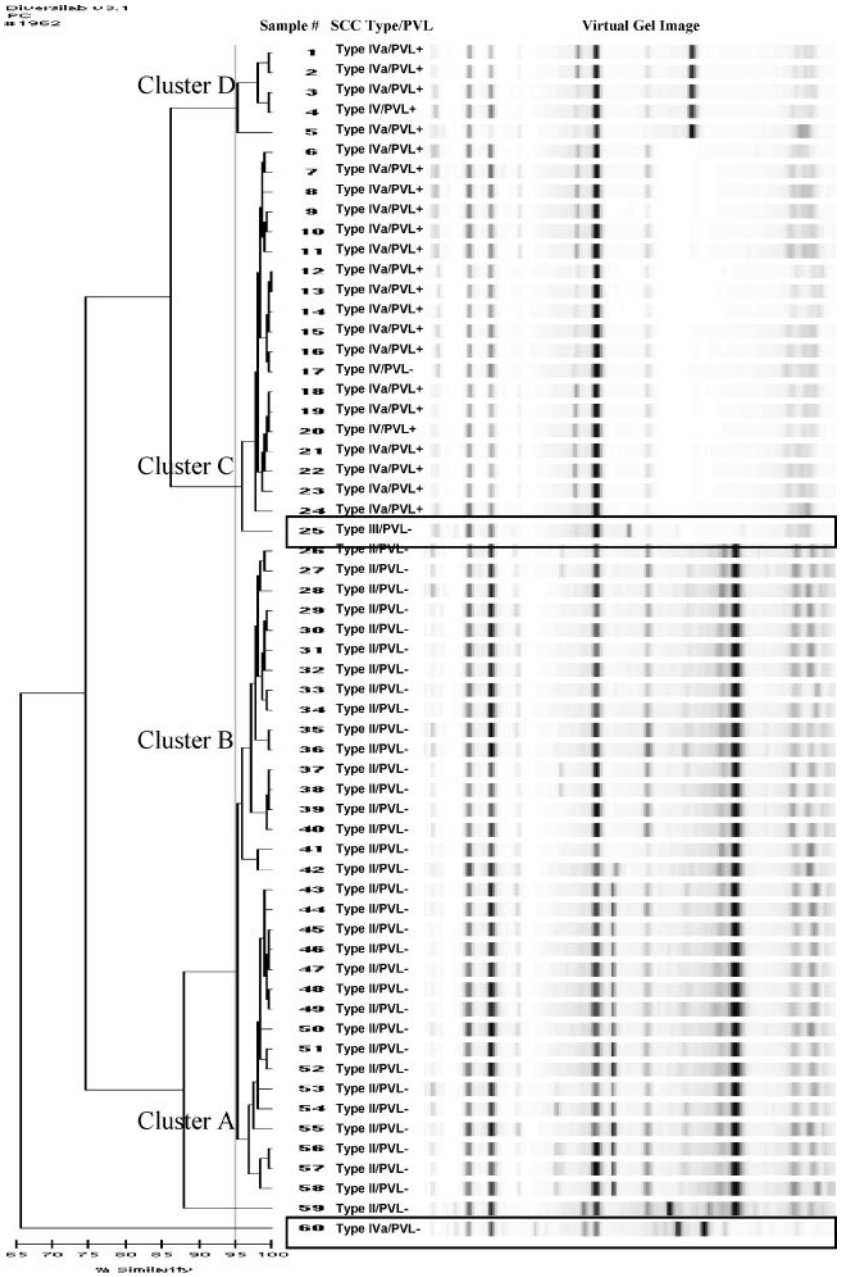

FIG. 1. Dendrogram representation and virtual gel image of rep-PCR fingerprints from MRSA isolates. Percentages of similarity are shown at the bottom left of the dendrogram. Clusters A, B, C, and D are shown along with the SCC*mec* type and PVL status.

grouped into cluster C or D, and most had been classified by infection control as CA-MRSA. An isolate was determined to be CA-MRSA when it had been obtained from a patient outside the hospital setting or within 48 h of hospital admission and if it was from a person who had not been hospitalized within 2 years before the date of MRSA isolation. Twenty-three of the 25 SCC*mec* type IV isolates were PVL positive (92%). Of the two type IV isolates that were PVL negative (8%), one had subtype IVa and a genetic fingerprint substantially different from those of the other type IV isolates. The other type IV, PVL-negative isolate (sample 17) did not subtype and grouped within cluster C.

Antibiogram analysis showed that overall, the type II, PVL-negative isolates demonstrated resistance to more antimicrobials than the type IV, PVL-positive isolates (Table 1). It appeared that the purported CA-MRSA isolates were more likely than the HA-MRSA strains to be susceptible to clindamycin, levofloxacin, and moxifloxacin.

One sample typed as III (1.7%) and was also PVL negative. This isolate did not fall within any of the major clusters, being distinguished from cluster C by a distinct, unique band. Interestingly, this isolate was resistant to more antimicrobials than any other sample studied; it was susceptible only to linezolid, rifampin, and vancomycin (data not shown). This strain may

TABLE 1. Antimicrobial susceptibility profile comparison of MRSA isolates with SCC*mec* types II and IV

Antimicrobial agent(s)	No. (%) of susceptible isolates[a]	
	Type II (n = 31)	Type IV (n = 19)
Sulbactam-ampicillin	0 (0)	0 (0)
Cefazolin	0 (0)	0 (0)
Clindamycin	18 (58)	18 (95)
Erythromycin	0 (0)	2 (11)
Gentamicin	30 (97)	18 (95)
Levofloxacin	0 (0)	11 (58)
Linezolid	30 (97)	19 (100)
Moxifloxacin	4 (13)	15 (79)
Oxacillin	0 (0)	0 (0)
Penicillin	0 (0)	0 (0)
Rifampin	31 (100)	19 (100)
Tetracycline	31 (100)	17 (89)
Trimethoprim-sulfamethoxazole	30 (97)	19 (100)
Vancomycin	31 (100)	19 (100)

[a] Data available for 50 of the 60 patients in the study.

represent a MRSA clone with prolonged exposure to the hospital setting, where the antibiotic selective pressure is high. None of the 60 isolates tested as SCC*mec* type I.

The majority (57%) of the 23 SCC*mec* type IV, PVL-positive isolates were taken from nasal or rectal collections for the purpose of MRSA screening upon admission. Other type IV, PVL-positive isolates were from skin/wound/abscess (35%), tracheal aspirate (4%), and CSF (4%) specimens. The type II, PVL-negative isolates were recovered predominately from skin/wound/abscess collections (65%), followed by nasal (20%), blood (9%), rectal (3%), and CSF (3%) specimens. These strains were generally recovered from sites with more invasive disease than the sites from which type IV isolates were recovered; however, our type IV patient population included many asymptomatic carriers. Still, it is noteworthy that 43% of the patients with what was determined to be CA-MRSA presented with significant infection within the hospital setting. Consequently, the data do not appear to indicate an association between severity of infection and the presence of PVL. In fact, many of the type IV strains characterized in this study represent colonizing strains as opposed to the type II infecting strains. For the purpose of this study, the presence of the toxin was used primarily as a marker for tracking community-associated strains rather than as an indicator of invasive disease.

It is apparent that CA-MRSA has established itself within the hospital environment. Community-associated strains that are carried into hospitals have the ability to eventually cause potentially serious infections. The presence of CA-MRSA within the hospital setting also complicates efforts on behalf of infection control departments to distinguish CA-MRSA from HA-MRSA infections. SCC*mec* typing and PVL analysis are valuable adjunctive tools for evaluating the molecular epidemiology of MRSA, further characterizing strains within the hospital as well as those circulating in the community.

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