Molecular and Epidemiologic Characterization of Community-Associated Methicillin-Resistant *S. aureus* (CA-MRSA) Isolated from North American Medical Centers: Report from The SENTRY Program (2000-2004)

SENTRY
ANTIMICROBIAL SURVEILLANCE

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AMENDED ABSTRACT

Background: The epidemiology of MRSA is changing due to rapid emergence and dissemination of CA-MRSA. Although generally susceptible to many antimicrobials other than oxacillin (OXA) and erythromycin (ERY), CA-MRSA exhibit greater virulence by means of Panton-Valentine leukocidin (PVL) and are able to become multidrug-resistant (R) by acquisition of R markers in the SCC*mec* cassette. Prevalence of PVL, SCC*mec* type as well as clonality of CA-MRSA isolates was evaluated in the SENTRY Program.

Methods: CA-MRSA isolates (n=1994, 33 medical centers) submitted by SENTRY Program participants were susceptibility (S) tested against >30 antimicrobials by broth microdilution methods according to CLSI guidelines. PCR for PVL genes was performed in isolates R only to OXA and ERY (ORER, n=161) with 61 controls with other antibiograms (OABG). All PVL (+) isolates were characterized for the SCC*mec* gene cassette, and typed by PFGE. PFGE patterns were compared to USA-300 and -400 clones (CA-MRSA clones prevalent in the USA).

Results: Characterization of CA-MRSA isolates is summarized in the table:

Susceptibility pattern (no. tested)	PVL (no. of strains / %)	SCC*mec* type (no. of strains)[1]	PFGE Type(s) (no. of strains)[1]
ORER (161)	Positive (118 / 73.3)	IV (118)	C (111), F (5), G (1), K (1)
	Negative (43 / 26.7)[2]	IV (15)	A (3), L (4), unique (8)
		II or NT (7)	R (4), A (2), unique (1)
OABG (61)	Positive (14 / 23.0)	IV (14)	C (12), unique (2)
	Negative (47 / 77.0)	ND	ND

1. PFGE patterns: C=USA-300, F=USA-400 (MW2) clone, ND = not determined and NT = not typeable.
2. 21 isolates were further characterized.

Conclusions: PVL gene was more common among ORER (73.3%) than among OABG (23.0%) strains of CA-MRSA. PFGE USA-300 or -400 clonal patterns were highly prevalent among PVL (+), SCC*mec* type IV (+) strains both in the ORER (98.3%) and OABG (85.7%) groups. Among PVL (-) strains with or without SCC*mec* type IV, USA-300 or -400 clones were not observed in this series regardless of antibiogram. Clonal CA-MRSA with a defined geno-phenotype has become ubiquitous among clinical *S. aureus* isolates in the USA since the year 2000.

INTRODUCTION

Infections caused methicillin(oxacillin)-resistant *Staphylococcus aureus* (MRSA) pose a significant therapeutic challenge worldwide. They are particularly a problem with nosocomial infections caused by a few pandemic circulating clones. However, in the recent years, MRSA have been isolated from infections or body sites of individuals in the community, and termed "community-acquired (CA) MRSA". In contrast to the hospital-acquired (HA) strains, CA-MRSA are generally more susceptible to antimicrobials other than ß-lactams and macrolides (erythromycin).

The most commonly cited characteristics of CA-MRSA strains include 1) the production of Panton-Valentine leukocidin (PVL), 2) the SCC*mec* gene cassette (20-24Kb; termed SCC*mec*IVa), and 3) a unique macrorestriction (PFGE) pattern designated as USA300-0114 or a variant subtype. Production of PVL has been associated with virulence and a potential for epidemic spread among healthy individuals in the community, but its role is not totally understood. PVL-positive CA-MRSA also has been implicated in complicated skin and soft tissue infections, sepsis, necrotizing fasciitis and pneumonia with higher mortality. Presence of the SCC*mec* gene cassette (type IV) provides higher mobility (small size) allowing for widespread dissemination, as well as the potential to acquire more resistance markers for the evolution from a CA-MRSA to a multidrug-resistant epidemic isolate.

MRSA strains with these characteristics are frequently isolated from individuals in institutional settings such as day care children, correctional facility inmates, sports team members and military recruits. They are also detected in varied geographic regions in the United States (USA), as well as from Canada; and one report from Europe was due to trans-Atlantic dissemination. Moreover, isolates with this phenotype are increasingly being reported from infected patients admitted in hospitals in the USA. Currently, infections caused by CA-MRSA carrying PVL and SCC*mec*IVa genes located in a geography-specific clonal background, are being reported from all continents around the world.

This study was conducted to determine the rate of occurrence of the typical CA-MRSA "phenotype" among North American medical centers and the longitudinal pattern from 2000 to 2004. The SENTRY Antimicrobial Surveillance Program has a large collection of MRSA strains, and these strains were screened for CA-MRSA and associated molecular markers.

MATERIALS AND METHODS

Study Design: The *S. aureus* isolates from North America medical centers were screened for MRSA revealing 1,994 isolates; and these organisms were further selected for the protocol as follows:

- Strains with the commonly cited single-resistance pattern to erythromycin in addition to oxacillin (ORER) were selected.
- All strains from each geographic site (33 laboratories, 30 from the USA) with an ORER pattern were tested for SCC*mec* type, PVL genes and PFGE pattern (161 strains).
- Control MRSA having different or other antibiogram patterns (OABG) were randomly selected from the same medical centers, where available (61 strains; ratio of 1 control per 5 ORER). A total of 10 non-ß-lactam agents were used to define the antibiograms using CLSI methods (M7-A7 and M100-S16, 2006).
- Microbiology/molecular results were compared over time and between nations (USA versus Canada). Very low MRSA rates in Canada with ORER patterns precluded comparisons and only USA results are presented.

Detection of PVL genes: PCR amplification of PVL genes (*lukF-PV* and *lukS-PV*) was performed on 161 ORER strains and 61 OABG controls. PCR primers listed below and procedures used were those described by Lina et al (1999). luk-PV-F: ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A, luk-PV-R: GCA TCA AST GTA TTG GAT AGC AAA AGC.

Characterization of SCC*mec* gene cassette: All PVL-positive isolates were characterized for the type of SCC*mec* gene cassette using a multiplex PCR strategy (Oliveira & de Lencastre, 2002). The primers amplified various DNA segments within SCC*mec* characteristic to each of the types I, II, III, and IV. *mecA* gene was amplified as part of the multiplex PCR to serve as an internal control. PCR products were separated on 2% agarose gel in TAE buffer on Criterion Sub-cell GT system (Bio-rad, Hercules, CA) and stained with ethidium bromide. SCC*mec* types were assigned based on the number and sizes of the amplicons obtained.

Epidemiologic typing of CA-MRSA: PVL-positive CA-MRSA isolates were also subjected to pulsed-field gel electrophoresis (PFGE). Bacterial cells grown overnight were embedded in agarose, lysed and deproteinated to isolate near intact genomic DNA. The DNA was digested with SmaI (New England Biolabs, Ipswich, MA). The restriction fragments were separated by electrophoresis on CHEF DR II (Bio-rad, Hercules, CA) with the following conditions: 1% agarose, 0.5 X TBE, 200V with switch interval of 5-40 seconds over 21 hour period. Ethidium bromide stained gels were examined visually. PFGE patterns were compared to CA-MRSA clones prevalent in the USA (Tenover et al, 2006). The PFGE patterns were designated by a capital letter (eg. A, C, F). Strains were assigned with the same PFGE pattern only when all bands matched. When there was one or two bands difference, the strains were assigned as a sub-type or variant of the major type, which was designated with the same capital letter followed by an Arabic number (Example: C1, C2, C3).

RESULTS

- Antimicrobial susceptibility profile of 1994 CA-MRSA isolates is presented in Table 1. Rank order of highly active antimicrobials is as follows: Synercid® (99.9% susceptible) > trimethoprim/sulfamethoxazole (96.9%) > rifampin (93.7%) > tetracycline (90.0%). Highest resistance rates were observed for erythromycin (94.2%) followed by ciprofloxacin (84.4% resistant) and clindamycin (65.6%).

- In general, the OABG strains used as the control group showed lower resistance rates to ciprofloxacin (70.0%) and clindamycin (60.0%) when compared to the 1,994 CA-MRSA (Table 1).

- Results of molecular tests performed on CA-MRSA isolates are summarized in Table 2. Among ORER strains PVL was present in 73.3% isolates; and all these isolates had a SCC*mec* IV gene cassette; all from USA medical centers. Also, no PVL-positive strains were isolated from Canada, only two isolates were screened, of which one showed poor *mecA* expression.

Table 1. Antimicrobial susceptibility profile of CA-MRSA isolates tested in the SENTRY Program (2000-2004).

Antimicrobial tested (no. isolates)	MIC (µg/ml) 50%	MIC (µg/ml) 90%	% by category:[a] Susceptible	% by category:[a] Resistant
All CA-MRSA (1,994)				
Erythromycin	>8	>8	5.7	94.2
Chloramphenicol	8	16	82.6	0.7
Ciprofloxacin	>2	>2	15.1	84.4
Cindamycin	>8	>8	34.2	65.6
Gentamicin	≤2	>8	86.6	12.8
Rifampin	≤0.5	≤0.5	93.7	4.4
Tetracycline	≤4	≤4	90.0	9.1
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	96.9	3.1
Synercid®	0.5	1	99.9	0.0
Control/OABG (61)[b]				
Erythromycin	>8	>8	0.0	100.0
Chloramphenicol	8	16	85.0	1.7
Ciprofloxacin	>2	>2	25.0	70.0
Cindamycin	>8	>8	36.7	60.0
Gentamicin	≤2	>8	66.7	31.7
Rifampin	≤0.5	>2	81.7	13.3
Tetracycline	≤4	>8	61.7	33.3
Trimethoprim/sulfamethoxazole	≤0.5	2	93.3	6.7
Synercid®	0.5	1	100.0	0.0

a. Susceptibility criteria per CLSI M7-A7 and M100-S16 (2006).
b. At least one isolate per medical center that also contributed an ORER isolate was randomly chosen to serve as an OABG control.

- The vast majority of ORER, PVL-positive, SCC*mec* IV isolates (94%) belonged to PFGE pattern designated C (USA300-0114) or its variant clonal types. PFGE type designated F (USA400; MW2) was observed in only 4.2% isolates, while two isolates exhibited unique PFGE patterns unrelated to both clonal types (Table 2 and Figure 1).

- In contrast, PFGE patterns C or F were not observed among PVL-negative ORER strains with either SCC*mec* types IV (15/22 isolates, 71.4%) or II (6/22 isolates, 27.3%).

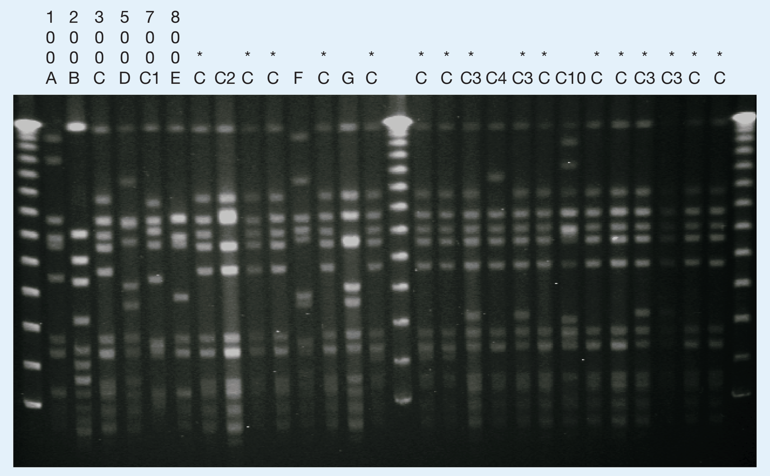
- Twenty-three USA sites contributed isolates conforming to USA300 clonal patterns. Three participant medical centers in Texas contributed a large fraction (31%) of isolates in this category followed by one site each in Arkansas (12.4%) and Ohio (8.1%). PFGE pattern F and its variant subtypes (USA400) were observed in isolates from New York, Texas, Wisconsin and Indiana.

Table 2. Molecular characterization of ORER and OABG subsets of CA-MRSA isolates (SENTRY Program; 2000-2004).

Susceptibility pattern (no. tested)	PVL (no. of strains / %)	SCC*mec* type[a] (no. of strains)	PFGE pattern(s)[b] (no. of strains)
Erythromycin resistant only (161)	Positive (118/ 73.3)	IV (118)	C (111), F (5), G (1), K (1)
	Negative (43/ 26.7)[c]	IV (15)	A (3), L (4), unique (8)
		II or NT (7)	R (4), A (2), unique (1)
Other antibiogram pattern (61)	Positive (14/ 23.0)	IV (14)	C (12), unique (2)
	Negative (47/ 77.0)	ND	ND

a. ND = not determined and NT = not typeable.
b. PFGE patterns: C=USA300 and F=USA400 (MW2) clones.
c. Only 22 isolates were further characterized.

Figure 1. PFGE patterns of CA-MRSA isolates in comparison to selected USA clonal patterns.



A= USA100, B= USA200, C= USA300, C1= USA700, D= USA500, E= USA800. Lanes 7-13 and 15-29, SmaI digests of CA-MRSA isolates from SENTRY Program showing subtle variations of PFGE pattern C (USA300 clonal type). C and C3 were the most commonly observed patterns.

- Among CA-MRSA isolates in the control group (OABG) with PVL-positive and SCC*mec* type IV, five PFGE patterns were observed. Three of the five patterns were USA300 or related clonal variants and accounted for 12 of the 14 isolates. Two isolates showed distinct PFGE patterns unrelated to either USA300 or 400 clonal types (Table 2).

CONCLUSIONS

- PVL-positive strains were more common among ORER (73.3%) than OABG (23.0%) isolates.
- PVL-positive, SCC*mec* type IV strains with PFGE type related to USA300 or 400 clonal types were highly prevalent among ORER strains (98.3%), as well as OABG strains (85.7%).
- USA300 or 400 clonal patterns were not observed among PVL-negative strains with and without SCC*mec* IV, indicating strong association of PVL with these clonal types, whereas SCC*mec* IV appears to be transferring horizontally among genetically unrelated strains irrespective of PVL determinants.
- Commonly defined CA-MRSA gene/phenotype has become widespread and ubiquitous among clinical *S. aureus* isolates the USA.

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