

Molecular Characterization of *Staphylococcus aureus* Isolates from a 2005 Clinical Trial of Uncomplicated Skin and Skin Structure Infections[∇]

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Received 20 December 2006/Returned for modification 10 March 2007/Accepted 13 June 2007

A clinical trial of uncomplicated skin and skin structure infections (39 locations in 19 states) observed that community-associated or community-onset methicillin-resistant *Staphylococcus aureus* (CO-MRSA) represented 23% of all pathogens at baseline culture and 53% of 190 *S. aureus* isolates. CO-MRSA strains typically were Panton-Valentine leukocidin (PVL) positive (95%), contained staphylococcal cassette chromosome *mec* type IVa (99%), were USA300 or USA400 clones (92%), and exhibited minimal coresistances (macrolides and/or fluoroquinolones). Clinical results remained identical (89% cures) regardless of the antimicrobial used or CO-MRSA molecular patterns, PVL production, or antimicrobial susceptibility profiles.

The treatment of uncomplicated skin and skin structure infections (uSSSI) consumes a significant proportion of national health care resources, as recently quantitated by McCaig et al. (10) using statistics from national ambulatory medical care and national hospital ambulatory care surveys. When data derived from physician offices and emergency departments from 2001 to 2003 and 1992 to 1994 were compared, the number of ambulatory care visits was 11.6 million in 2001 to 2003. From 1992 to 1994 to 2001 to 2003, rates increased 59% and 31% in the outpatient and emergency departments, respectively (10). The increase was attributed to the emergence of community-associated or community-onset methicillin-resistant *Staphylococcus aureus* (CO-MRSA) infections. Since the recognition of CO-MRSA in the 1990s (2, 20), the understanding of the dominant strains/clones has evolved through reports of dramatic epidemic clusters, some with fatal consequences (1), as well as by thoughtfully performed epidemiologic or molecular investigations (5, 8, 11, 12, 19). CO-MRSA has numerous characteristics that differentiate it from hospital-acquired MRSA, including (i) younger affected patient population (5, 12), (ii) methicillin resistance produced via staphylococcal cassette chromosome *mec* (SCC*mec*) type IVa (2, 11–13, 18, 20), (iii) high presence of Panton-Valentine leukocidin (PVL) (2, 9, 11, 20), and (iv) the presence of dominant epidemic clones, classified as USA300 or USA400 (18). The serious consequence of these uSSSI cases has been progression to complicated SSSI requiring hospitalization (4 to 23%) (5, 10), including potentially fatal necrotizing fasciitis or pneumonia (1). These features and concerns regarding limited treatment options among orally administered antimicrobial agents have led to adjusted treatment guidelines and suggested therapeutic paradigm shifts (7, 16).

To update our knowledge of CO-MRSA and facilitate an

understanding of clinical outcomes using contemporary treatment regimens, *S. aureus* isolates derived from a recent phase IV, prospective, investigator-blinded, randomized uSSSI clinical trial (6) were examined to determine the impact of antibiogram patterns and pathogen (*S. aureus*) molecular characteristics. These organisms were obtained from patients presenting in outpatient clinical practices or emergency departments with community-onset infections (39 locations in 19 states).

(The data summarized in this paper were presented at the 46th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 27 to 30 September 2006 [7a].)

A total of 190 *S. aureus* isolates were available from the multicenter uSSSI study (6). The isolates comprised 171 baseline isolates, 151 of which were from clinically and bacteriologically evaluable patients and 19 of which were posttreatment isolates; 2 baseline isolates were not available for testing. These pathogens were studied by molecular methods to characterize SCC*mec* type, the presence of PVL, *agr* type, and pulsed-field gel electrophoresis (PFGE) patterns. CO-MRSA isolates (101 strains [53%]) were detected by the reference broth microdilution method (3) using an oxacillin breakpoint of ≥ 4 $\mu\text{g/ml}$ as resistant (4). Other comparison agents (erythromycin, ciprofloxacin, penicillin, ampicillin, piperacillin-tazobactam, cephalothin [surrogate for cephalixin], cefdinir, clindamycin, quinupristin-dalfopristin, tetracyclines, trimethoprim-sulfamethoxazole, gentamicin, rifampin, imipenem, and vancomycin) were also tested to identify profiles of cross- or coresistance with results interpreted by Clinical and Laboratory Standards Institute (CLSI) criteria (4).

PCR amplification of PVL genes (*lukF-PV* and *lukS-PV*) was performed on 101 CO-MRSA strains and 89 methicillin-susceptible *S. aureus* (MSSA) strains. The following PCR primers and the procedures used were those described previously by Lina et al.: *lukF-PV-F* (ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A) and *lukF-PV-R* (GCA TCA AST GTA TTG GAT AGC AAA AGC) (9).

All isolates were characterized for the type of SCC*mecA*

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[∇] Published ahead of print on 18 June 2007.

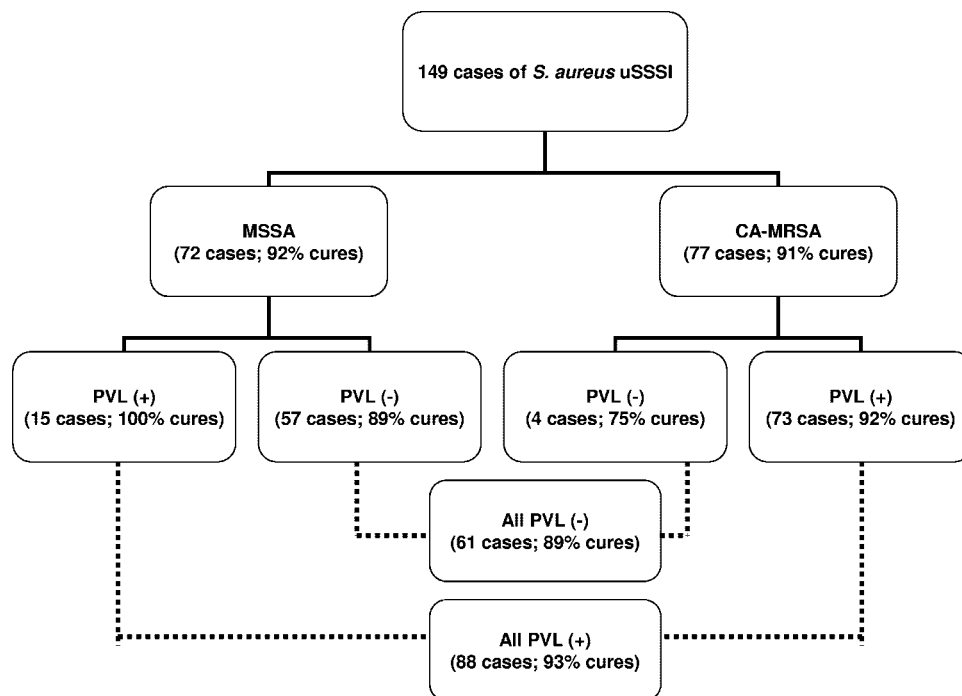


FIG. 1. Clinical response results for 149 evaluable cases of *S. aureus* uSSSI treated with either ceftin or cephalexin that were characterized by susceptibility to oxacillin (methicillin) and PVL production. CA-MRSA, community-acquired MRSA (6).

gene cassette using a multiplex PCR strategy (13). The primers amplified various DNA segments within *SCCmec* characteristic to each of types I, II, III, and IV. *mecA* was amplified as part of the multiplex PCR to serve as an internal control. PCR products were separated on 2% agarose gels in Tris-acetate-EDTA buffer on the Criterion Sub-cell GT system (Bio-Rad, Hercules, CA) and stained with ethidium bromide. *SCCmec* types were assigned based on the numbers and sizes of the amplicons obtained. The *agr* types were determined by use of a subset of PVL-producing and *SCCmec* type IVa-positive strains.

Epidemiologic typing of CO-MRSA and MSSA strains that had PVL-positive PCR tests was performed by PFGE using procedures described previously (18). Briefly, bacterial cells grown overnight were embedded in agarose, lysed, and deproteinized to isolate near-intact genomic DNA. The DNA was digested with *Sma*I restriction enzyme (New England Biolabs, Ipswich, MA). The restriction fragments were separated by electrophoresis on a CHEF DR II (Bio-Rad) apparatus under the following conditions: 1% agarose, 0.5× Tris-borate-EDTA, and 200 V with a switch interval of 5 to 40 s over a 21-h period. Ethidium bromide-stained gels were examined visually. PFGE patterns were compared to CO-MRSA clones that are prevalent in the United States (18). Strains were assigned the same PFGE pattern only when all bands matched. When there were one or two band differences, the strains were assigned as a subtype or variant of the major type (designated by a capital letter, e.g., A, B, C, etc.), which was assigned the same capital letter followed by an Arabic numeral (for example, A1, A2, and A3).

Among the 190 *S. aureus* isolates available for reference susceptibility testing, 101 (53%) were MRSA isolates, and

among the 149 evaluable cases (2 were not available for study), 77 (52%) were caused by CO-MRSA (Fig. 1). These CO-MRSA isolates were distributed across all participating geographic locations (6). CO-MRSA isolates (Table 1) were more likely to be PVL positive (95%), have *SCCmec* type IVa (99%) and *agr* type I, be resistant to macrolides (erythromycin) and/or

TABLE 1. Results of testing of 101 strains of CO-MRSA by molecular methods

PVL result (no. of strains, %)	<i>SCCmecA</i> type	<i>agr</i> type ^b	Antibiogram resistance(s) ^a	No. of strains (% USA300/ USA400 PFGE patterns)
Positive (96, 95.0)	IV	I	ER	49 (93.3)
	IV	NT	ER, CIP	23 (100.0)
	IV	NT	ER, CIP, TC	8 (87.5)
	IV	NT	CIP or none	4 (100.0)
	IV	I	ER, TC	3 (100.0)
	IV	I	ER, RIF	2 (100.0)
	IV	I	ER, CL, RIF	1 (0.0)
	IV	NT	ER, CIP, CL	1 (100.0)
	IV	NT	ER, CIP, CL, T/S	1 (100.0)
	IV	III	ER	1 (100.0)
	IV	I	Variable	3 (100.0)
Negative (5, 5.0)	IV	NT	ER, CIP	2 (100.0)
	IV	NT	None	1 (0.0)
	II	NT	ER, CIP	1 (0.0)
	— ^c	NT	QD, TC	1 (0.0)

^a ER, erythromycin; CIP, ciprofloxacin; TC, tetracycline; RIF, rifampin; CL, clindamycin; QD, quinupristin-dalfopristin; T/S, trimethoprim-sulfamethoxazole.

^b NT, not tested against all strains.

^c —, unable to type.

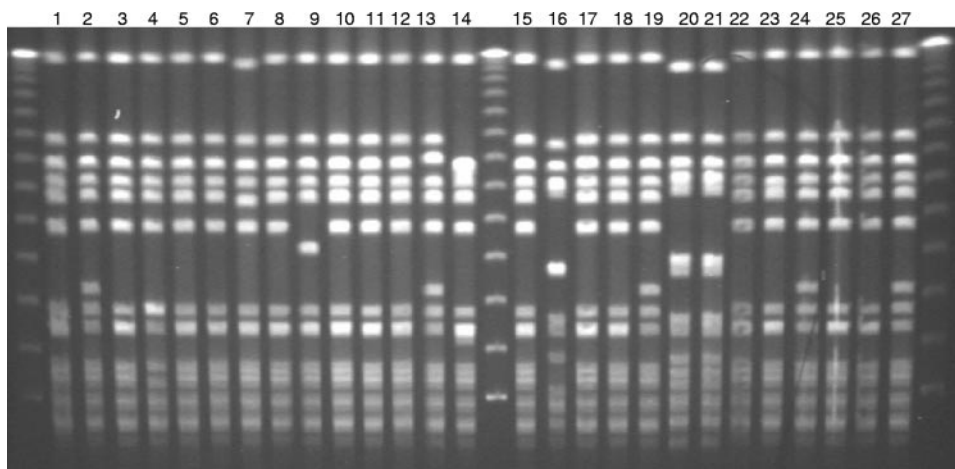


FIG. 2. Typical PFGE patterns of CO-MRSA uSSSI clinical trial strains showing dominant USA300 (lanes 1 to 13, 15, 17 to 19, and 22 to 27) and USA400 (lanes 16, 20, and 21) clonal patterns (18).

fluoroquinolones, and be clonally consistent with USA300 or USA400 (92%). All PVL-positive isolates contained SCC mec type IVa but varied slightly in their antibiograms. Over one-half of these CO-MRSA isolates were resistant only to erythromycin among the alternative non- β -lactam agents tested, and 37 were resistant to erythromycin and ciprofloxacin. Additional resistance rates for these tested agents were as follows (Table 1): erythromycin, 92.1%; ciprofloxacin or levofloxacin, 38.6%; tetracycline, 11.9%; clindamycin, 3.0%; rifampin, 3.0%, trimethoprim-sulfamethoxazole, 1.0%; and quinupristin-dalfopristin, 1.0%. The USA300 clonal type was highly represented (94% were usually USA300-0114, and 2% were USA400) in the PVL-positive CO-MRSA cases. In contrast, PVL-negative CO-MRSA isolates were more diverse in SCC mec types (II and IV), antibiograms, and occurrences of USA300 or USA400 clones (only 40%). An example of the PFGE patterns for 27 PVL-positive CO-MRSA isolates is shown in Fig. 2. The distribution of abscess or furuncle cases was found predominantly in the PVL-positive CO-MRSA isolates (69%) compared to only superficial wounds among the PVL-negative cases (small sample of four patients).

Results from the 89 MSSA strains indicated that only 15 (17%) were PVL positive and were quite diverse, with three different antibiograms and six unique PFGE patterns. The PVL-negative MSSA isolates displayed nine different antibiogram profiles, and the PVL-positive isolates were from abscesses or furuncles in 67% of cases compared to only 21% for the evaluable PVL-negative MSSA cases ($P < 0.05$).

The case outcomes of *S. aureus* uSSSI from clinically and bacteriologically evaluable patients treated with the two orally administered agents were analyzed by the oxacillin (methicillin) susceptibility patterns and the PVL molecular results (Fig. 1) of the baseline isolate. The clinical cure rates were not significantly different between these compounds (6), so all cases were combined for this analysis. The patient infection cure rates were essentially identical when MSSA- or MRSA-caused cases were compared (92% versus 91%, respectively). Similarly, PVL did not adversely influence the outcomes, with documented clinical cure rates of 89 to 93% (highest in PVL-positive cases).

These high levels of CO-MRSA isolates among all *S. aureus* isolates from year 2005 uSSSI cases confirms the elevated occurrence of MRSA (59 to 63%), high associated PVL production (98%), and clonality via USA300 strains (90 to 97%) reported previously by King et al. (8) and Moran et al. (11). The resistance patterns of these strains (erythromycin and/or ciprofloxacin) also conform to the antibiograms reported elsewhere previously (2, 5, 8, 11, 12, 14, 20) and illustrate the continued susceptibility to some older antimicrobials (clindamycin and trimethoprim-sulfamethoxazole) (2, 5, 8, 11, 12, 18). The use of clindamycin for infections caused by erythromycin-resistant *S. aureus* has been a concern because of inducible clindamycin resistance; however, the rates appear to be low ($\leq 33\%$; none detected here) compared to those for hospital-associated MRSA (14). These agents have been suggested for suspected CO-MRSA therapy (7), but national prescription audits for uSSSI therapy indicate persisting β -lactam use (usually oral cephalosporins) (3,558 prescriptions/10,000 visits/year, or one-half of all therapies) and treatment declines in the use of lincosamides-macrolides and sulfonamide or related compounds in the last decade (10). This pattern of prescription practice was also noted previously by Naimi and colleagues (12), where 61% of CO-MRSA uSSSI cases received a β -lactam agent, which is regarded as having limited therapeutic value (3, 4).

The results from a large uSSSI clinical trial presented in this report clearly demonstrate a level of successful outcomes against CO-MRSA cases that was not significantly different than those for MSSA cases (6) or divergent from a nearly identical trial of orally administered β -lactams reported in 1997 (17). These observations confirm those of others, where measured resistances to the antimicrobials used for treatment (active or inactive) did not correlate with compromised patient outcomes (5, 11). Similarly, the presence of PVL in CO-MRSA isolates was not associated with poor cure rates or persisting infections, questioning the role of PVL in virulence. Voyich et al. (19) concluded from studies of PVL-negative (*lukS/F-PV* knockout) strains of USA300 or USA400 in a sepsis model that PVL was not a major virulence determinant; however, in our CO-MRSA case series, the type of infection (abscess and fu-

runcle) was correlated with the presence of the PVL gene as reported previously (2, 9, 19).

Obviously, MRSA emergence in the community environment remains a high-priority clinical concern requiring well-constructed treatment guidelines and the promotion of continued searches for novel orally applied agents (16, 20). These CO-MRSA strains (USA300 clones) have been encountered among isolates from hospital-based bloodstream infections (34% of cases), expanding the range of public health concerns (15). These antimicrobial treatment regimens supplement the complete management of uSSSI that must consider local/topical wound care and surgical drainage (7, 16), with the consideration of expanded use of cultures to foster a better understanding of pathogen (CO-MRSA) frequency and local antibiogram patterns.

We express gratitude to the following individuals for their technical support and assistance in preparing the manuscript: N. D. O'Mara-Morrissey, T. R. Fritsche, H. S. Sader, D. J. Biedenbach, T. A. Busman, and M. G. Stilwell.

The molecular studies presented were sponsored by Abbott Laboratories.

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