Molecular Characterization of *S. aureus* (SA) Isolates from a Contemporary (2005) Clinical Trial of Uncomplicated Skin and Skin Structure Infections (uSSSI)

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AMENDED ABSTRACT

Background: uSSSI, a common community-associated (CA) infection, is generally of mild-moderate severity and primarily caused by SA and ß-haemolytic streptococci (BHS). These infections are most often treated with local wound care and/or orally-administered cephalosporins (OAC). Interest in CA-MRSA involvement in uSSSI is increasing. SA from 39 USA locations (19 states) from a 2005 Phase 4 uSSSI clinical trial of 2 OACs were characterized.

Methods: 391 patients (≥13 years) were randomized and approximately 80% of cases were bacteriologically and clinically evaluable. 189 of 190 SA isolates (100 CA-MRSA) were available for molecular characterization of Panton-Valentine leukocidin (PVL), agr and SCC*mec* types, and PFGE. Antibiograms were determined to 16 agents (12 classes) including cefdinir and cephalexin. Resistance (R) was defined by CLSI criteria.

Results: Among 89 methicillin-susceptible (MS) SA, 15 (17%) were PVL (+) having 3 different antibiograms and 6 PFGE patterns. The PVL (-) MSSA showed 9 different antibiograms. The major CA-MRSA analysis groups are shown in the Table.

SA group (no. strains / %)	Resistance pattern (%)[a]	%USA-300/400 PFGE patterns
PVL (+)/SCC*mec*IVa/agrI (96 / 96%)	ER (51)	94
	ER, CIP (24)	100
	ER, CIP, TC (6)	88
	ER, TC (3)	100
	Other, 7 patterns (13)	92
PVL (-)/SCC*mec*IV or II (4 / 4%)	ER, CIP (50)	67
	Other (50)	0

a. ER = erythromycin, CIP = ciprofloxacin, TC-tetracycline

Overall, 83 of 88 evaluable patient isolates (94%) with PVL (+) CA-MRSA were USA300 or 400 clonal types (92% of all CA-MRSA strains), and SCC*mec*IVa was nearly universal among CA-MRSA with ER and/or CIP-R antibiograms.

Conclusions: A uSSSI clinical trial of OAC treatment in 2005 observed that CA-MRSA isolates were found frequently (53%). The 100 CA-MRSA typically were PVL (+; 96%), contained SCC*mec*IVa, were USA 300 or 400 clones (95%), and exhibited minimal co-R (ER, CIP). Clinical outcomes remained favorable (approximately 90%) for the OACs in the investigation, regardless of CA-MRSA or MSSA molecular patterns or PVL production.

INTRODUCTION

Uncomplicated skin and skin structure infections (uSSSI) such as impetigo, erysipelas, cellulitis, folliculitis, furunculosis, wound infection and simple abscesses are frequently encountered in the ambulatory care setting. These infections are commonly caused by *Staphylococcus aureus*, *Streptococcus pyogenes* and other ß-haemolytic streptococci. In clinical practice, empiric treatment for uSSSI is typically initiated at the time of the first visit, regardless of whether a culture is performed. Abscesses are treated by incision and drainage, with orally administered antimicrobial agents playing a secondary role. Because uSSSIs are confined to the superficial layers of skin and seldom result in hospitalization, they can generally be treated with antimicrobials with coverage against the most common Gram-positive pathogens.

Among the antimicrobial agents recommended for treating uSSSIs, penicillinase-stable ß-lactam agents possess acceptable microbiological activity against methicillin(oxacillin)-susceptible *S. aureus* (MSSA) and streptococci. Oral cephalosporins (cefadroxil, cefdinir, cefprozil, cefuroxime axetil and cephalexin) with proven efficacy and indications for uSSSIs remain the most common class of antimicrobials used for the treatment of uSSSIs and other agents used less frequently have been: ß-lactamase inhibitor combinations (amoxicillin/clavulanate), fluoroquinolones, tetracycline, trimethoprim/sulfamethoxazole, macrolides and clindamycin.

Since most treatments of uSSSI continue to be empiric, culture and susceptibility testing with subsequent modification of therapy, if indicated, has been generally reserved for patients with recurrent or recalcitrant disease or for those patients at high risk for infection with methicillin-resistant *S. aureus* (MRSA). However, strains of MRSA are emerging as an increasingly common cause of community-acquired (CA) skin infections and they can differ from the hospital-acquired (HA) strains. CA-MRSA are more likely to present as purulent infections such as furunculosis or abscess, and to occur in patients without typically described risk factors for HA-MRSA. These CA-MRSA are genetically distinct from HA strains and contain the virulence gene coding for Panton-Valentine leukocidin, which has been associated with sometimes fatal necrotizing pneumonia and necrotic abscesses.

This report describes CA-MRSA appearing in a uSSSI clinical trial performed in 2005 (39 USA locations; 19 states) and correlates outcomes in clinically evaluable subjects to methicillin (oxacillin) susceptibility and PVL status of the *S. aureus* strains isolated from baseline cultures of clinically evaluable patients (189 strains; 149 evaluable cases, see Figure 2).

MATERIALS AND METHODS

Study Design: The *S. aureus* isolates from an investigator-blinded, multicenter study in patients ≥13 years of age with uSSSI (Trial number M04-699) were studied by molecular methods to characterize SCC*mec*A type, presence of PVL, *agr* and the PFGE patterns.

- 189 of 190 isolates of *S. aureus* were available for the follow-up studies.
- 100 strains were CA-MRSA by reference laboratory MIC test results with oxacillin using the Clinical and Laboratory Standards Institute (CLSI; formerly the NCCLS) method M7-A7, 2006.
- All PVL (+) strains were tested for PFGE pattern, and all CA-MRSA had SCC*mec*A type determined. PFGE patterns were compared to those published by Tenover et al. (2006).
- *agr* type was determined on selected PVL (+) and SCC*mec*IV (+) strains.
- Results of the molecular tests were then compared to outcomes provided by the sponsor (Abbott Laboratories, North Chicago, IL) for clinically evaluable cases as published by Giordano et al. (2006). Overall clinical cure rates in clinically evaluable patients were identical for cefdinir and cephalexin at 89%. Eradication rates for all *S. aureus* were 91.0% for cefdinir (71 of 78 cases) and 87.7% for cephalexin (64 of 73 cases).

Detection of PVL genes: PCR amplification of PVL genes (*lukF-PV* and *lukS-PV*) was performed on 100 MRSA strains and 89 MSSA. 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 and selected PVL-positive MSSA isolates were also subjected to pulsed field gel electrophoresis (PFGE) using procedures described earlier. 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. The PFGE patterns were designated by a capital letter (eg. A, B, C). 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: A1, A2, A3).

RESULTS

- Among 189 of 190 *S. aureus* isolates available for testing:
  - 149 baseline isolates from clinically evaluable patients (72 MSSA, 77 MRSA)
  - 21 baseline isolates from non-evaluable patients (9 MSSA, 12 MRSA)
  - 2 baseline isolates were not available
  - 19 post-treatment isolates (9 MSSA, 10 MRSA)
  - 1 isolate was unassignable (MRSA) to an analysis group
- MSSA were initially divided by PVL test results with only 17% of testable isolates being (+). Antibiograms and PFGE patterns were varied among these MSSA, regardless of PVL result (Table 1).
- Table 2 lists the CA-MRSA molecular test results and antibiograms. For the PVL(+) strains (96) the following highlights were observed:
  - PVL(+) rate for CA-MRSA was 96%
  - All PVL(+) isolates were SCC*mec*IV type
  - When tested, *agr*I predominated
  - Many antibiograms were observed with erythromycin and fluoroquinolone resistances most prevalent
  - More modest resistances to clindamycin, tetracycline, rifampin and trimethoprim/ sulfamethoxazole were noted
  - PFGE clonal types USA300-0114 and variants accounted for 94% of all strains tested (2% were USA400; see Figure 1)

Table 1. Results of molecular characterization of *S. aureus* isolates having susceptibility to oxacillin (89 strains).

PVL result (no. strains; %)	Antibiograms	PFGE pattern
Negative (74; 83)	9 patterns	NT[a]
Positive (15; 17)	3 patterns	6 patterns

a. NT = not tested.

Table 2. Results of testing 100 strains of oxacillin-resistant *S. aureus* by molecular methods.

PVL result (no. strains; %)	SCC*mec*A type	*agr* type	Antibiogram[a]	No. strains (% USA300/400 PFGE pattern)
Positive (96; 96.0)	IV	I	ER	49 (93.3)
	IV	NTb	ER, CIP	23 (100.0)
	IV	NT	ER, CIP, TC	8 (85.7)
	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 (4; 4.0)	IV	NT	ER, CIP	1 (100.0)
	IV	NT	None	1 (0.0)
	II	NT	ER, CIP	1 (0.0)
	-[c]	NT	QD, TC	1 (0.0)

a. Resistances listed: ER = erythromycin, CIP = ciprofloxacin, TC = tetracycline, RIF = rifampin, CL = clindamycin, QD = quinupristin/dalfopristin and T/S = trimethoprim/sulfamethoxazole.
b. NT = not tested.
c. - = unable to type.

- Results for PVL(-) strains (only four cases) are also found in Table 2:
  - SCC*mec* types were usually IV (50%), but type II and untypeable strains were found
  - Antibiograms were more diverse, and the USA300 clonal pattern was only detected in two strains
- Figure 2 shows the correlations of molecular test results of baseline evaluable *S. aureus* isolates with the cure rates found in Abbott Trial M04-699 performed in early 2005 (isolates from 149 evaluable cases available).
  - MSSA and CA-MRSA cure rates were 92 and 91%, respectively
  - All PVL(+) and (-) subsets of MSSA and MRSA had comparable cure rates (75-100%), with the lowest rate associated with only one failure in four total cases (PVL[-] CA-MRSA)
  - Combining all PVL(+) and PVL(-) cases in this collection, the cure rates were 93 and 89%, respectively

Figure 1. Two typical PFGE patterns of CA-MRSA uSSSI clinical trial showing dominant USA300 (A1 and A4) and USA400 (A7) clonal patterns. Pattern A1 represents USA300-0114 as described by Tenover et al., 2006.

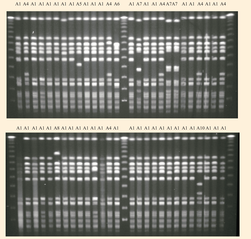
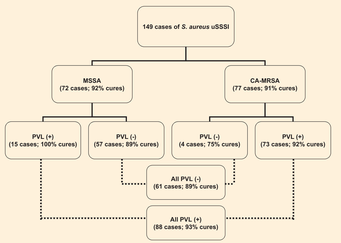


Figure 2. Clinical response results for 149 evaluable cases of *S. aureus* uSSSI treated with either cefdinir or cephalexin that were characterized by susceptibility to oxacillin (methicillin) and Panton-Valentine leukocidin production. MSSA = methicillin-susceptible *S. aureus*, MRSA = methicillin-resistant *S. aureus* and CA = community-acquired.



CONCLUSIONS

- In a year 2005 clinical uSSSI trial using 39 study sites across the USA (19 states), CA-MRSA accounted for 23% of all infections and 53% of *S. aureus* isolates at baseline (91/171 isolates in intent-to-treat patients; Giordano).
  - Most CA-MRSA were isolated from purulent infections (Akinlade et al, poster L-1552)
- Molecular analysis was performed on 100 CA-MRSA isolates, including 77 baseline isolates from clinically evaluable patients.
  - PVL(+) rates were very high among testable CA-MRSA strains at 96%; lower for MSSA (17%)
  - SCC*mec*IV and USA300 or 400 clonal strains were extremely common (95%)
- No adverse impact of oxacillin resistance or PVL(+) characteristics was observed on the clinical cure rates for *S. aureus* in this investigator blinded trial of two orally administered cephalosporins (cefdinir and cephalexin; see Figure 2).
  - Local care, such as incision and drainage, was allowed at the investigators clinical discretion and is an important therapeutic intervention for purulent uSSSI
  - Spontaneous drainage and microbiological sampling may also have contributed to successful clinical outcomes
- Oral ß-lactams, in conjunction with routine local wound care, continues to demonstrate acceptable eradication and clinical cure rates for mild-moderate uSSSI as noted in this blinded clinical trial having a high prevalence of well defined CA-MRSA strains.

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