P1459

Characterization of Baseline Methicillin-resistant *Staphylococcus aureus* Isolates from a Phase IV Clinical Trial of Complicated Skin and Soft Tissue Infections

RE MENDES, HS SADER, LM DESHPANDE, BA DIEP, HF CHAMBERS, RN JONES

JMI Laboratories, North Liberty, IA, USA; UCSF School of Medicine, CA, USA

ECCMID 2008

JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
rodrigo-mendes@jmilabs.com

ABSTRACT

Objective: To characterize methicillin-resistant *S. aureus* (MRSA) associated with complicated skin and soft tissue infections (cSSTI) collected during a Phase IV clinical trial comparing linezolid with vancomycin for the treatment of cSSTI due to MRSA.

Methods: 532 MRSA baseline isolates were collected from subjects with cSSTI in Latin America (LA; 5 countries, 68 isolates), Europe (EU; 6 countries, 112), Asia (Singapore [1] and Malaysia [2]), South Africa (13) and the United States (USA; 336). Susceptibility testing was performed by CLSI broth microdilution method. Isolates were screened for heterogeneous resistance to vancomycin (hVISA) using the macro Etest method. The presence of inducible clindamycin (CC) resistance (R) phenotype was assessed by D-test; PVL genes and SCC*mec* types by PCR and clonality was evaluated by PFGE. Dominant PFGE type strains from each country were further evaluated by *spa* typing and multilocus sequencing typing (MLST).

Results: Most active antimicrobial agents were: linezolid = glycopeptides (teicoplanin and vancomycin, 0% R) > quinupristin/dalfopristin (0.2% R) > trimethoprim/sulfamethoxazole (4.7% R) > tetracycline (12.6% R) > CC (45.9% R; 18.6% inducible R plus 27.3% constitutive R) > gatifloxacin (67.1%; Table 1). CC-R rates were highest in Asia and South Africa (100.0%), followed by LA (86.8%), EU (78.6%) and the USA (24.1%). Most isolates were R to erythromycin (ERY; 92.9%) and 29.2% of ERY-R, CC susceptible (S) isolates had a positive D-test (inducible CC-R). Five (0.9%) isolates were characterized as hVISA, 4 from EU and 1 from LA. 278 (52.3%) isolates were PVL-positive and 96.8% of those were from the USA. The remaining PVL-positive isolates were from Colombia (4) and Venezuela (5). 373 (70.1%) isolates were SCC*mec* type IV, 89 (16.7%) type II, 44 (8.3%) type I and 26 (4.9%) type III. Isolates from the USA showed SCC*mec* type IV (81.2%) or II (18.8%). The majority of USA isolates clustered within the USA300 PFGE type (77.1%) or USA100 (15.8%). Most countries had a dominant and unique clone.

Conclusions: All MRSA isolates were S to linezolid and glycopeptides, and resistance rates were high for ERY, CC and gatifloxacin. The prevalence of hVISA was low overall and this phenotype was not observed in the USA. MRSA in the USA were SCC*mec* IV and PVL positive, previously associated with community acquired-MRSA; while in the rest of the world the distribution of SCC*mec* types and PVL genes varied by geographical region.

INTRODUCTION

Skin and soft tissue infections (SSTIs) are common cause of morbidity in both the community and hospital settings. Superficial or uncomplicated SSTIs are often benign and usually treated with orally administered antimicrobials and/or local wound care, including incision and drainage. However, some cases may worsen and require hospitalization due to the extension into deeper tissue levels and/or development of sepsis or other systemic symptoms which may rapidly progress to fatal outcomes. In these latter cases, prompt and effective life-saving antimicrobial therapy must be initiated.

SSTIs are typically caused by *Staphylococcus aureus*, including methicillin-(oxacillin) resistant strains (MRSA) that have emerged from both the hospital and community environments. This scenario has created the need for additional Gram-positive directed therapeutic agents. In this study, we evaluated the in vitro activity of linezolid and commonly used anti-Gram-positive agents tested against 532 baseline MRSA isolates collected during a Phase IV clinical trial comparing linezolid with vancomycin for the treatment of cSSTI. MRSA isolates were also screened for Panton-Valentine leukocidin (PVL) genes and heterogeneous resistance to vancomycin (hVISA). Furthermore, SCC*mec* type was characterized and clonality assessed by PFGE, multilocus sequence typing (MLST) and single-locus sequence typing (*spa* gene).

MATERIALS AND METHODS

Bacterial strains: A total of 532 MRSA baseline isolates were collected from subjects with cSSTI in Latin America (5 countries, 68 isolates), Europe (6 countries, 112), Asia (Singapore [1] and Malaysia [2]), South Africa (13) and the United States (USA; 336). *S. aureus* identification was confirmed by routine methods (catalase, coagulase, colony morphology, etc.) and automated procedures when needed (Vitek System, bioMerieux, Hazelwood, MO, USA).

Susceptibility testing: MIC values were determined using broth microdilution panels with cation-adjusted Mueller-Hinton medium (CLSI M7-A7, 2006). Testing, incubation and MIC interpretations were performed using the recommendations of the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS). Quality control strains utilized included *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212; all results were within ranges specified by the CLSI (M100-S18, 2008).

Definition of clindamycin resistance: Inducible clindamycin resistance was detected using the D-test disk diffusion method according to the CLSI recommendations. Briefly, a 2-µg clindamycin disk was placed 15mm from the edge of a 15-µg erythromycin disk. Following incubation, isolates that showed flattening of the clindamycin zone were considered D-test positive (indicative of the presence of *erm*).

Screening for hVISA: The hVISA phenotype was screened using the macro Etest method. This test consists of determining the Etest MIC values for vancomycin and teicoplanin using a 2.0 McFarland inoculum. Briefly, BHI agar was inoculated with 200 µL of a 2.0 McFarland standard cell suspension in BHI broth and streaked evenly on the agar surface with a swab. Etest strips (AB BIODISK) were applied and incubated for 48 hours at 35°C. MRSA isolates showing vancomycin and teicoplanin MIC values ≥8 mg/L or only a teicoplanin MIC value ≥12 mg/L were considered hVISA.

SCC*mec* type and detection of PVL genes: SCC*mec* types (I through VI) were characterized using a multiplex PCR strategy. Screening for PVL determinants (*lukF-PV* and *lukS-PV*) were performed by PCR amplification using the primers described below: luk-PV-F – ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A and luk-PV-R – GCA TCA AST GTA TTG GAT AGC AAA AGC.

Epidemiologic typing of MRSA: Chromosomal DNA was subjected to pulsed-field gel electrophoresis (PFGE) following digestion with SmaI. Additionally, one representative MRSA isolate belonging to the dominant clone from each country was further characterized by MLST and *spa* typing. Two additional isolates, one belonging to the dominant clone from Portugal and one from Colombia were also evaluated.

RESULTS

- The most active antimicrobial agents were linezolid = glycopeptides (teicoplanin and vancomycin, 0% resistant) > quinupristin/dalfopristin (0.2% resistant) > trimethoprim/sulfamethoxazole (4.7% resistant) > tetracycline (12.6% resistant) > clindamycin (45.9% resistant; 18.6% inducible resistance plus 27.3% constitutive resistance) > gatifloxacin (67.1% resistant; Table 1).
- Clindamycin resistance was highest in Asia and South Africa (100.0%), followed by Latin America (86.8%), Europe (78.6%) and the USA (24.1%). Most isolates were resistant to erythromycin (92.9%) and 29.2% of erythromycin-resistant, clindamycin-susceptible isolates were positive in the D-test assay.
- Overall, only five (0.9%) isolates were characterized as hVISA and were recovered in the UK (1 of 35 isolates; 2.8%), Venezuela (1 of 36 isolates; 2.8%), and Italy (3 of 9 isolates; 33.3%). hVISA isolates were not detected in the USA.
- 278 (52.3%) MRSA isolates were PVL-positive and 96.8% of those were from the USA. The remaining PVL-positive isolates were from Colombia (4) and Venezuela (5). PVL-positive strains were not observed in the participating European countries, Malaysia, Singapore or South Africa (Table 2).
- Overall, 373 (70.1%) isolates were SCC*mec* type IV, 89 (16.7%) type II, 44 (8.3%) type I and 26 (4.9%) type III.
- The majority of countries demonstrated predominant MRSA clones. Most USA isolates clustered within the USA300 (77.1%) or USA100 (15.8%) PFGE type (Table 2).
- The representative isolate of the dominant PFGE type found in the UK (EUR-P) was found to be ST22 and *spa* type TJJEJNI2MNI2MOMO, which correspond to the UK EMRSA-15 clone (Table 2). This clone was also predominant in patients from Spain.

Table 1. Susceptibility profile and $MIC_{50/90}$ of the MRSA clinical isolates recovered during a Phase IV clinical trial of complicated skin and soft tissue infections (cSSTI).

	MIC (mg/L)		Percentage by category:	
Antimicrobial agents	MIC_{50}	MIC_{90}	Susceptible	Resistant
Linezolid	2	4	100.0	-[a]
Erythromycin	>16	>16	7.1	92.1
Clindamycin	0.25	>4	54.1	45.9[b]
Gatifloxacin	2	>8	32.2	67.1
Tetracycline	0.25	>16	86.8	12.6
Trimethoprim/ sulfamethoxazole	≤0.5	≤0.5	95.2	4.7
Quinupristin/dalfopristin	0.25	0.5	99.8	0.2
Teicoplanin	1	1	100.0	0.0
Vancomycin	1	1	100.0	0.0

a. No resistance breakpoint has been established by the CLSI.
b. Includes constitutive and inducible resistance phenotypes.

- Isolates belonging to the EUR-K PFGE type from Portugal matched ST5 and ST105. ST105 is a single-locus variant of ST5. In addition, these two clones showed a *spa* motif similar to that of the New York/Japan clone (USA100/ST5-MRSA-II; Table 2).
- The dominant clone detected among the MRSA isolates from Russia (EUR-N, ST8-MRSA-IV) showed an allelic profile and *spa* type identical to USA300; however, the PFGE patterns were distinctly different.
- The dominant MRSA clone from Mexico (LAT-I, MRSA-II) was found to be associated with ST5.
- Isolates recovered from the remaining Latin American countries were associated with ST5 (Venezuela and Chile) or ST8 (Colombia).
- Isolates belonging to the LAT-C PFGE type, which correspond to the Cordobes/Chilean clone, were highly prevalent in patients from Latin American countries, mainly Chile and Venezuela (82% and 75%, respectively).

Table 2. Epidemiologic typing results obtained from the MRSA isolates evaluated in the clinical trial.

Region/country (no. tested)	PFGE typing results		SCC*mec* type	PVL	MLST[a]							
					Allelic profile							
	Pattern	No. of subtypes (%)			ST	*arcC*	*aroE*	*glpF*	*gmk*	*pta*	*tpi*	*yqiL*	*spa*
Latin America													
Argentina (4)	LAT-A	1 (25.0)	III	-									
	LAT-B	1 (25.0)	II	-									
	LAT-C	2 (50.0)	I	-									
Chile (11)	LAT-C	9 (81.8)	I	-	ST5	1	4	1	4	12	1	10	TIMEMDMGMGMK
	LAT-A	1 (9.1)	III	-									
	LAT-E	1 (9.1)	II	-									
Columbia (7)	LAT-G	2 (28.6)	IVE/F	+	ST8	3	3	1	1	4	4	3	YHGFMBQBLO
		2 (28.6)	IV	+	ST8	3	3	1	1	4	4	3	GFMBQBLO
	LAT-H	1 (14.3)	I	-									
	LAT-C	2 (28.6)	I	-									
Mexico (10)	LAT-I	10 (100)	II	-	ST5	1	4	1	4	12	1	10	TJMAGMK
Venezuela (36)	LAT-C	27 (75.0)	I	-	ST5	1	4	1	4	12	1	10	TIMEMDMGMGMK
	LAT-F	3 (8.3)	IV	-									
	LAT-G	4 (11.1)	IV	+									
	LAT-K	1 (2.8)	IV	+									
	LAT-L	1 (2.8)	I	-									
Europe													
Belgium (2)	EUR-A	1 (50.0)	IV	-									
	EUR-B	1 (50.0)	IV	-									
Italy (9)	EUR-A	1 (11.1)	IV	-									
	EUR-B	1 (11.1)	IV	-									
	EUR-C	2 (22.2)	I	-	ST228	1	4	1	4	12	24	29	TIMBMDMBMDMGMK
	EUR-D	1 (11.1)	III	-									
	EUR-E	2 (22.2)	I	-									
	EUR-G	1 (11.1)	IV	-									
	EUR-P	1 (11.1)	IV	-									
Portugal (40)	EUR-D	7 (17.5)	III	-									
	EUR-J	6 (15.0)	IV	-									
	EUR-K	2 (5.0)	II	-	ST105	1	4	1	4	12	1	28	TJMBMDMGMK
		12 (30.0)	IVE/F	-	ST5	1	4	1	4	12	1	10	TMK
	EUR-L	4 (10.0)	II	-									
	EUR-P	3 (7.5)	IV	-									
	EUR-Others	6 (15.0)											
Russia (20)	EUR-M	4 (20)	III	-									
	EUR-N	16 (80)	IV	-	ST8	3	3	1	1	4	4	3	YHGFMBQBLO
Spain (6)	EUR-O	6 (100.0)	IV	-	ST22	7	6	1	5	8	8	6	JEJNI2MOMOKR
UK (35)	EUR-P	20 (57.1)	IV	-	ST22	7	6	1	5	8	8	6	TJJEJNI2MNI2MOMO
	EUR-Q	1 (2.9)	II	-									
	EUR-R	11 (31.4)	IV	-									
	EUR-T	3 (8.6)	IV	-									
North America													
USA (336)	USA-A[b]	259 (77.1)	IV	+	ST8	3	3	1	1	4	4	3	YHGFMBQBLO
	USA-B[c]	53 (15.8)	II	-									
	USA-C[d]	2 (0.6)	IV	+									
	USA-Others	22 (6.5)	II/IV	-/+									
Others													
Malaysia (2)	ASI-A	1 (50.0)	III	-									
	ASI-B	1 (50.0)	III	-									
Singapore (1)	ASI-C	1 (100.0)	III	-									
South Africa (13)	AFR-A	1 (7.7)	IV	-									
	AFR-B	5 (38.5)	III	-									
	AFR-C	7 (53.8)	II	-	ST1071	2	2	2	19	3	3	2	WGKAKAO

a. Performed only on selected clones.
b. PFGE profile equivalent to the USA300.
c. PFGE profile equivalent to the USA100.
d. PFGE profile equivalent to the USA400.

CONCLUSIONS

- All MRSA isolates were susceptible to linezolid and the glycopeptides, whereas resistance rates were high for erythromycin, clindamycin and gatifloxacin.
- The prevalence of hVISA in cSSTIs was low overall and not observed in the USA.
- MRSA in the USA were SCC*mec* IV and PVL positive, previously associated with community acquired-infections; in other countries, the distribution of SCC*mec* types and PVL genes varied markedly by region.
- Although previous studies have shown a low prevalence of the EMRSA-15 clone in Spanish hospitals (2 to 3% of isolates), no other clone was detected from that country (Table 2).
- The presence of a dominant ST5-MRSA-II in Mexico confirms previous reports of its replacement of ST30-MRSA-IV between 2001 and 2002.
- Previous studies have shown replacement of the Brazilian clone by EMRSA-15 (ST22-MRSA-IV) in Portuguese hospitals. However, the EMRSA-15 clone was detected at low prevalence in this clinical trial (EUR-P; 7.5%) and the Brazilian clone (EUR-D; 17.5%) seems to have been slowly displaced by another clone, PFGE type EUR-K (New York/Japan clone; 35.0%).
- Heterogeneity of SCC*mec* was noted among isolates associated with ST5 (SCC*mec* type IVE/F or II) from Portugal and ST8 from Colombia (SCC*mec* type IVE/F or IVa), suggesting different acquisition events of SCC*mec*.
- The Cordobes/Chilean clone (LAT-C) has progressively displaced the Brazilian clone (LAT-A) since 1999 and become the dominant clone since 2001 in Argentina. This clone has been detected in several countries from Latin America.

ACKNOWLEDGEMENT

The study was supported by a grant from Pfizer, Inc.

SELECTED REFERENCES

1. Alcoceba E, Mena A, Cruz Perez M, Ruiz de Gopegui E, Padilla E, Gil J, Ramirez A, Gallegos C, Serra A, Perez JL, Oliver A (2007). Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Majorcan hospitals: High prevalence of the epidemic clone EMRSA-15. *Clin Microbiol Infect* 13: 599-605.
2. Amorim ML, Faria NA, Oliveira DC, Vasconcelos C, Cabeda JC, Mendes AC, Calado E, Castro AP, Ramos MH, Amorim JM, de Lencastre H (2007). Changes in the clonal nature and antibiotic resistance profiles of methicillin-resistant *Staphylococcus aureus* isolates associated with spread of the EMRSA-15 clone in a tertiary care Portuguese hospital. *J Clin Microbiol* 45: 2881-2888.
3. Clinical and Laboratory Standards Institute. (2006). *M7-A7, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - seventh edition.* Wayne, PA: CLSI.
4. Clinical and Laboratory Standards Institute. (2008). *M100-S18, Performance standards for antimicrobial susceptibility testing, 18th informational supplement.* Wayne, PA: CLSI.
5. Deurenberg RH, Vink C, Kalenic S, Friedrich AW, Bruggeman CA, Stobberingh EE (2007). The molecular evolution of methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 13: 222-235.
6. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG (2002). The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proc Natl Acad Sci U S A* 99: 7687-7692.
7. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J (1999). Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* 29: 1128-1132.
8. McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC (2003). Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: Establishing a national database. *J Clin Microbiol* 41: 5113-5120.
9. Perez-Roth E, Lorenzo-Diaz F, Batista N, Moreno A, Mendez-Alvarez S (2004). Tracking methicillin-resistant *Staphylococcus aureus* clones during a 5-year period (1998 to 2002) in a Spanish hospital. *J Clin Microbiol* 42: 4649-4656.
10. Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN (1999). Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol* 37: 3556-3563.