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Antimicrobial Activity of Tigecycline against Community-Acquired Methicillin-Resistant *S. aureus* (CA-MRSA) Isolated in North American Medical Centers

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ABSTRACT

Background:
CA-MRSA, characterized by the presence of Panton-Valentine leukocidin (PVL) gene and PFGE pattern US-300, has disseminated very rapidly in the United States (US) and became endemic in many parts of the country. CA-MRSA infections require prompt initiation of effective antimicrobial therapy. We evaluated the in vitro activity of tigecycline (TIG) against a well-characterized collection of PVL-positive CA-MRSA strains.

Methods:
Among 2,898 MRSA isolates collected by the SENTRY program from 33 medical centers (MC) in the 2000-2004 period, 951 (33%) were categorized as CA-MRSA based on the epidemiologic data provided by the participant MCs. All strains were susceptibility (S) tested against TIG and >30 antimicrobials by broth microdilution methods according to CLSI guidelines. Isolates resistant (R) only to erythromycin (ERY) were evaluated for the presence of PVL genes by PCR. All PVL positive strains were typed by PFGE and had the SCC*mec* gene cassette characterized.

Results:
107 MRSA strains (3.7%) were R only to ERY and screened for PVL gene along with 33 controls. A total of 90 strains (including 9 controls) from 20 MCs were PVL positive. All of them harbored the SCC*mec*IV gene cassette and 84 (93.3%) showed a PFGE pattern related to US-300 clone. PVL-positive strains showed S rates generally higher compared to the entire MRSA collection. TIG activity is summarized in the table:

Organism	Cumulative % inhibited at TIG MIC (µg/ml) of:				% S to	% S to
(no. tested)	≤0.12	0.25	0.5	1	clindamycin	ciprofloxacin
All MRSA (2,898)	56.1	85.1	98.5	100.0	36.7	15.0
CA-MRSA (951)	52.6	81.2	98.2	100.0	38.0	19.3
PVL-positive (90)	82.2	96.7	100.0	-	100.0	93.3

Conclusions:
PVL-producing CA-MRSA is widely disseminated in the US and TIG is very potent (MIC_{90}, 0.25 µg/ml; 100.0% S) against this pathogen. TIG was also very active against MRSA strains in general (MIC_{90}, 0.5 µg/ml; >98% S). TIG may represent an excellent option for the treatment of MRSA infections in the US, including PVL-producing CA-MRSA.

INTRODUCTION

The emergence and rapid dissemination of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in the United States (US) necessitates reconsideration of current empirical treatment with ß-lactam antimicrobials for community-acquired *S. aureus* infections and current control strategies for MRSA in hospitals. MRSA strains harbour the *mecA* gene, which is carried on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*). Five types of SCC*mec* have been identified, which differ in size and genetic composition, and SCC*mec* type IVa is most commonly found in clones from patients with CA-MRSA infections.

CA-MRSA strains differ from the major pandemic clones of hospital-associated MRSA (HA-MRSA) strains in SCC*mec* types, the presence of additional antimicrobial resistance genes, bacterial growth rate and the distribution of toxin genes. Recent studies have indicated that well-defined CA-MRSA strains carry SCC*mec* type IVa, whereas the majority of HA-MRSA strains carry SCC*mec* type I, II or III. SCC*mec* type IVa is relatively small in size, this appears to have resulted in its increased mobility and therefore greater potential for horizontal spread to diverse *S. aureus* genetic backgrounds, compared with other SCC*mec* types. In addition, the presence of Panton–Valentine leukocidin (PVL) genes is common among CA-MRSA strains from different genetic backgrounds, whereas these genes are more uncommon among HA-MRSA strains.

Although CA-MRSA infections are commonly mild, they may also be severe and require prompt initiation of effective parenteral antimicrobial therapy. For example, necrotising fasciitis caused by CA-MRSA has recently been reported as an emerging clinical entity and severe necrotising pneumonia due to CA-MRSA has occasionally been described in young patients without known healthcare-associated risk factors for the acquisition of MRSA. Furthermore, the observed clinical spectrum of infections caused by CA-MRSA has been associated with the presence of PVL genes, but other exotoxin genes or combinations of genes could also be important pathogenic factors.

The glycylcyclines represent a new class of antimicrobial agents with a documented expanded spectrum of activity against resistant Gram-positives, Enterobacteriaceae, anaerobic wound pathogens, *Haemophilus influenzae*, *Neisseria gonorrhoeae,* chlamydiae and mycoplasmas. In this study, we evaluated the in vitro activity of tigecycline against a well-characterized collection of PVL-positive CA-MRSA strains and other populations of MRSA.

MATERIALS AND METHODS

Bacterial Strains: MRSA isolates collected by the SENTRY Antimicrobial Resistance Surveillance Program from 33 medical centers located in North America (USA and Canada) were evaluated. The strains were isolated in the 2000-2004 period. Among 2,898 strains collected in this period, 951 (32.8%) stated to be CA-MRSA based on the epidemiologic data provided by the participant medical center. Isolates resistant only to erythromycin were evaluated for the presence of PVL gene by PCR, had the SCC*mec* characterized and were molecular typed by pulsed field-gel electrophoresis (PFGE).

Antimicrobial Susceptibility Testing: All 2,898 strains were susceptibility tested against tigecycline and comparator antimicrobials using validated, dry-form broth microdilution panels with cation-adjusted Mueller-Hinton medium (TREK Diagnostics Inc., Cleveland, OH) according to Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) guidelines. The results were interpreted as specified by the M100-S16 CLSI document (2006). For tigecycline, breakpoints approved by the USA Federal Drug Administration (US-FDA) were applied, i.e. ≤0.5 µg/ml for susceptibility with no resistant breakpoint.

Molecular Characterization of CA-MRSA Strains: All organisms resistant to erythromycin and susceptible to clindamycin, levofloxacin, tetracycline trimethoprim/sulfamethoxazole, and chloramphenicol, and 32 MRSA strains with distinct susceptibility patterns (controls) were tested for PVL and SCC*mec*I, II, III and IV genes by PCR using primers described by Lina et al. (2006) and Oliveira and de Lencastre (2002), respectively.

Pulsed Field-Gel Electrophoresis (PFGE): All PVL-positive strains were typed by PFGE. Chromosomal DNA was digested with SmaI and electrophoresis was performed using the conditions described by Tenover et al. (2006). PFGE patterns were then compared to those of the USA clones. The PFGE pattern was designated by a capital letter (C, F, G and K). Strains were assigned with the same PFGE pattern 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).

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RESULTS

- Among 2,898 MRSA strains tested (Table 1), 951 were said to be CA-MRSA with only 107 (3.7%) having the single macrolide (erythromycin)-resistant antibiogram. These strains along with 32 "control" antibiogram strains were tested for PVL; 90 (64.7%) were positive from 20 medical centers.
  - 84 (93.3%) had PFGE patterns consistent with USA300-0114 clone
  - 4 (4.4%) had the USA400 clonal pattern
  - 2 strains had unique PFGE patterns (Table 2)
- Tigecycline was very active against all MRSA (98.5% susceptible; Table 1), CA-MRSA (98.2% susceptible); and PVL-positive CA-MRSA (100.0% susceptible) strains.

Table 1. Antimicrobial activity of tigecycline and comparator agents tested against oxacillin-resistant *S. aureus* (MRSA) strains including community-associated (CA-MRSA) strains.

Antimicrobial (no. tested)	MIC (µg/ml) MIC_{50}	MIC_{90}	Range	% Susceptible	% Resistant
All MRSA strains (2,898)					
Tigecycline	≤0.12	0.5	≤0.12-1	98.5	-[a]
Erythromycin	>8	>8	≤0.06->8	4.9	94.8
Clindamycin	>8	>8	≤0.06->8	36.5	63.2
Ciprofloxacin	>4	>4	≤0.25->4	15.0	84.4
Levofloxacin	>4	>4	≤0.03->4	15.8	82.7
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->2	95.4	4.6
Tetracycline	≤4	≤4	≤4->8	92.9	6.8
Chloramphenicol	8	16	<2->16	86.1	0.6
Linezolid	2	2	≤0.25-16	>99.9	-
Non-nosocomial CA-MRSA (951)					
Tigecycline	≤0.12	0.5	≤0.12-1	98.2	-
Erythromycin	>8	>8	≤0.06->8	6.1	93.8
Clindamycin	>8	>8	≤0.06->8	38.0	61.8
Ciprofloxacin	>4	>4	≤0.25->4	19.3	80.0
Levofloxacin	>4	>4	0.06->4	20.2	77.9
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->2	95.4	4.6
Tetracycline	≤4	≤4	≤4->8	90.7	8.9
Chloramphenicol	8	16	<2->16	85.9	0.4
Linezolid	2	2	0.5-16	99.9	-
PVL-positive CA-MRSA (90)					
Tigecycline	≤0.12	0.25	≤0.12-0.5	100.0	-
Erythromycin	>8	>8	>8	0.0	100.0
Clindamycin	0.12	0.12	≤0.06-0.12	100.0	0.0
Ciprofloxacin	≤0.25	0.5	≤0.25->4	93.3	4.4
Levofloxacin	0.12	0.25	0.06-4	95.6	4.4
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5	100.0	0.0
Tetracycline	≤4	≤4	≤4->8	94.4	5.6
Chloramphenicol	8	8	4-8	100.0	0.0
Linezolid	2	2	1-2	100.0	-

a. - = No breakpoint has been established by the CLSI or US-FDA.

- The tigecycline MIC_{90} was very consistent at 0.25-0.5 µg/ml (Table 1).
- Generally the well characterized PVL-positive CA-MRSA (USA300 or 400 clones) strains were more susceptible to tested agents except erythromycin.
- Other agents having the best activity versus all CA-MRSA were: linezolid (>99.9% susceptible) > tigecycline (98.5%) > trimethoprim/sulfamethoxazole (95.4%) > tetracyclines (92.9%); fluoroquinolone and clindamycin susceptibility was quite variable.

Table 2. PFGE patterns among PVL-positive strains.

PFGE pattern	No. of strains	No. of medical centers
All C subtypes (USA300)	84	18
C (USA300-0114)	58	17
C1	1	1
C2	1	1
C3	14	10
C4	3	1
C7	1	1
C8	2	1
C9	2	2
C10	1	1
C12	1	1
All F subtypes (USA400)	4	3
F	1	1
F1	3	2
Other types		
G	1	1
K	1	1
Total	90	20

CONCLUSIONS

- PVL-producing CA-MRSA of the USA300 and USA400 clonal types are widely disseminated in the USA and tigecycline was very potent (MIC_{90}, 0.25 µg/ml; 100.0% susceptible) against these pathogens.
- Tigecycline was also very active against MRSA strains in general (MIC_{90}, 0.5 µg/ml; 98.5% susceptible), regardless of the origin of the infection (CA or HA).
- Tigecycline should represent an excellent option for the treatment of MRSA infections in the hospital setting, including patients with PVL-producing CA-MRSA admitted from the outpatient setting.