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In Vitro Activity of Omiganan Pentahydrochloride Tested Against Vancomycin-Tolerant, -Intermediate (VISA and hVISA) and -Resistant (VRSA) *Staphylococcus aureus*

TR FRITSCHE, PR RHOMBERG, HS SADER, RN JONES
JMI Laboratories, North Liberty, IA, USA

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

ABSTRACT

Background: Omiganan, a novel topical cationic peptide active against a broad spectrum of bacteria and yeast, is currently in a pivotal phase 3 trial targeting prevention of local catheter site infections. Here we provide a spectrum evaluation of the agent against methicillin-(oxacillin [OXA])-resistant (R) *S. aureus* (MRSA), including subgroups displaying reduced susceptibility (S) to VAN.
Methods: SA strains (109) were selected from recent specialized surveillance collections. Strain phenotypes included: VAN-tolerant (MBC/MIC, ≥ 32-fold); intermediate-level MIC values (VISA; 4-8 µg/ml); heterogeneous VAN-intermediate (hVISA); and VRSA (≥ 16 µg/ml). S testing used CLSI methods and criteria against omiganan and comparator agents.
Results: All SA tested were inhibited by ≤ 64 µg/ml (range, 4 to 64 µg/ml) of omiganan, with MIC_{50}/MIC_{90} values of 16/32 µg/ml, respectively, consistent with prior studies (see Table).

	MIC (µg/ml)		Cumulative % inhibited at MIC (µg/ml)				
Organism (no. tested)	50%	90%	4	8	16	32	64
S. aureus							
OXA-S (21)	16	16	0	5	90	100	-
OXA-R (20)	16	16	0	0	100	-	-
CA-MRSA (22)	16	16	0	0	91	95	100
VAN-tolerant (20)	16	32	5	10	70	95	100
hVISA (11)	16	32	0	18	55	100	-
VISA (10)	16	32	0	10	50	100	-
VRSA (5)	16	-	0	0	100	-	-
All strains (109)	16	32	1	6	82	98	100

Compared to wild-type SA strains, MIC_{90} values were only two-fold greater for VAN-tolerant, hVISA and VISA strains. The 5 VRSA, representing the most R strains tested, were inhibited by 16 µg/ml (mode for all groups). Neomycin, the antistaphylococcal component of the widely utilized, nonprescription triple antibiotic ointment (TAO), and TAO, displayed decreased activity against all subsets except methicillin-S SA.
Conclusions: Omiganan demonstrated potent activity against all SA, regardless of R mechanisms. Given the worrisome emergence of SA with reduced S to VAN, the finding that omiganan remains equally active against all isolates of this important species below the clinical formulation concentration (1%; 10,000 µg/ml) is especially noteworthy.

INTRODUCTION

Omiganan pentahydrochloride is a novel cationic peptide analog of indolicidin that is being developed as a topical antimicrobial, with the first targeted indication being the prevention of local catheter-site infections. The compound has a broad spectrum of cidal activity including Gram-positive and -negative bacterial species and, importantly, yeast. The development of most catheter-related blood stream infections are thought to arise from colonization of the catheter and infection of tissues at the site of catheter placement; the most commonly occurring organisms include coagulase-negative staphylococci (CoNS), *Staphylococcus aureus, Candida* spp., *Pseudomonas* spp., and *Enterococcus* spp., among others. Given the importance of CoNS and *S. aureus* as the two most prevalent pathogens producing local catheter-site and catheter-related bloodstream infections, prevention of their occurrence can be expected to have significant impact on overall patient morbidity and mortality, and related health care costs (primarily extended hospital stays and additional treatment).

Omiganan has been shown to be highly active against all staphylococci, including against methicillin- (oxacillin)-susceptible (MSSA) and -resistant (MRSA) subsets with $MIC_{50/90}$ values for CoNS being 4 and 4-8 µg/ml, respectively, and for *S. aureus*, all 16 µg/ml.

The purpose of this study was to expand the analysis of omiganan activity to include *S. aureus* with worrisome emerging resistance profiles, specifically those strains displaying reduced susceptibility to vancomycin for which treatment failures are known to occur. Specifically, these phenotypes include strains displaying tolerance to vancomycin *in vitro* (defined as the MBC values being ≥ 32-fold higher than the corresponding MIC); strains that display intermediate-level MIC values (VISA; MIC, 4-8 µg/ml) or which are demonstrated to be heterogeneous vancomycin-intermediate *S. aureus* (hVISA); and strains that are resistant (MIC, ≥ 16 µg/ml) to vancomycin (VRSA) using current criteria.

The recent emergence of highly virulent community-associated methicillin-resistant *S. aureus* (CA-MRSA) infections are also being observed increasingly in the health-care setting, making their potential to produce catheter-related infections inevitable. The increased prevalence of all of these staphylococcal resistance phenotypes necessitates additional analyses of omiganan, to better characterize the compound's breadth of spectrum and potency against these unique subsets compared with wildtype strains.

MATERIALS AND METHODS

Organism collection: The collection of 109 *S. aureus* strains was selected from various international surveillance programs (JMI Laboratories, North Liberty, IA) and other specialized collections and included: wildtype MSSA (21 strains); wildtype MRSA (20); community-associated MRSA, including CDC type USA300 strain (22); vancomycin-tolerant MRSA (20); vancomycin-intermediate (VISA) MRSA (10); heterogeneous-vancomycin-intermediate (hVISA) MRSA (11); and vancomycin-resistant (VRSA) MRSA (five).

Susceptibility test methods: The susceptibility profiles of the strain collection were determined using validated broth microdilution test panels according to CLSI methods (M7-A7, 2006) and interpretive criteria (M100-S16, 2006). Quality control (QC) determinations for all antimicrobials were performed on each day of testing using *S. aureus* ATCC 29213; all QC MIC results were found to be within established ranges as specified by the current CLSI M100-S16 document.

RESULTS

- Omiganan MIC_{50} and MIC_{90} values of wildtype MSSA and MRSA were identical (16 µg/ml); the highest MIC observed was only 32 µg/ml and the range was inclusive of 8 to 32 µg/ml. These values are essentially identical to those reported in recent publications (Table 1).
- Analysis of CA-MRSA isolates demonstrated MIC_{50} and MIC_{90} values identical to those of wildtype strains; one isolate had a MIC of 64 µg/ml, the highest detected, but consistent with the highest MIC for *S. aureus* observed in a previously published study (Tables 1 and 2).
- Vancomycin-tolerant isolates are those that may be susceptible to vancomycin, but which usually have MIC values at the high end of the susceptible range (1 and 2 µg/ml) and which present possible therapeutic failure risks to seriously ill patients because of very high MBC values. Among these isolates, the MIC_{90} value was 32 µg/ml, only two-fold higher than for the other groups tested. No isolate in this group had a MIC exceeding 64 µg/ml (Table 2).
- hVISA isolates included those that appear to be vancomycin susceptible when tested by standard techniques, but which display elevated MICs (≥ 8 µg/ml for both vancomycin and teicoplanin) when tested using a high inoculum. Anecdotal reports suggest that hVISA isolates may be responsible for clinical failures despite appropriate vancomycin therapy. Omiganan activity against hVISA isolates was similar to that for vancomycin-tolerant isolates (MIC_{50} and MIC_{90} values, 16 and 32 µg/ml, respectively).

Table 1. MIC_{50}, MIC_{90}, and cumulative frequency distributions of seven *S. aureus* groups with varying susceptibility profiles when tested against omiganan pentahydrochloride (109 strains).

	MIC (µg/ml)		Cumulative % inhibited at MIC (µg/ml)						
Organism (no. tested)	50%	90%	2	4	8	16	32	64	≥128
S. aureus									
Oxacillin-susceptible (21)	16	16	0	0	5	90	100	-	-
Oxacillin-resistant (20)	16	16	0	0	0	100	-	-	-
Community-associated MRSA (22)	16	16	0	0	0	91	95	100	-
Vancomycin-tolerant (20)	16	32	0	5	10	70	95	100	-
hVISA (11)	16	32	0	0	18	55	100	-	-
VISA (10)	16	32	0	0	10	50	100	-	-
VRSA (5)	16	-	0	0	0	100	-	-	-
All strains (109)	16	32	0	1	6	82	98	100	-

- Those isolates having vancomycin-intermediate MIC values (4 to 8 µg/ml; VISA) had results identical to those of the hVISA isolates (Table 1).
- True VRSA isolates (five reported to date and included here, courtesy of NARSA) all had omiganan MIC values of 16 µg/ml, the mode for all isolates tested (Table 1).
- Among comparator antimicrobials, wildtype MRSA exhibited co-resistance to other classes of agents (ciprofloxacin, erythromycin, clindamycin), including having elevated MIC values to neomycin (the active component in triple antibiotic ointment [TAO]), unlike oxacillin-susceptible strains (Table 2).

Table 2. Activity (MIC values in µg/ml) of antimicrobial agents tested against *Staphylococcus aureus* with varying susceptibility profiles (109 strains).

Organism (no. tested)/ Antimicrobic	MIC_{50}	MIC_{90}	Range	% susceptible/ resistant[a]
Oxacillin-susceptible (21)				
Omiganan	16	16	8-32	- / -
Ciprofloxacin	0.25	1	≤0.03->4	95.2 / 4.8
Clindamycin	≤0.25	≤0.25	≤0.25->4	90.5 / 9.5
Erythromycin	0.25	>8	≤0.06->8	81.0 / 19.0
Gentamicin	≤2	≤2	≤2	100.0 / 0.0
Neomycin	0.5	0.5	0.25-0.5	- / -
Oxacillin	≤0.25	0.5	≤0.25-0.5	100.0 / 0.0
TAO	0.31	1.2	0.15-2.4	- / -
Teicoplanin	≤2	≤2	≤2	100.0 / 0.0
Tetracycline	≤2	≤2	≤2->16	90.5 / 9.5
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->2	95.2 / 4.8
Vancomycin	1	1	1-2	100.0 / 0.0
Oxacillin-resistant (20)				
Omiganan	16	16	16	- / -
Ciprofloxacin	>4	>4	0.25->4	15.0 / 85.0
Clindamycin	>4	>4	≤0.25->4	40.0 / 60.0
Erythromycin	>8	>8	0.25->8	5.0 / 95.0
Gentamicin	≤2	≤2	≤2	100.0 / 0.0
Neomycin	>16	>16	0.25->16	- / -
Oxacillin	>2	>2	>2	0.0 / 100.0
TAO	39	39	0.31-78	- / -
Teicoplanin	≤2	≤2	≤2	100.0 / 0.0
Tetracycline	≤2	>16	≤2->16	85.0 / 15.0
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5	100.0 / 0.0
Vancomycin	1	1	1-2	100.0 / 0.0
Community-associated oxacillin-resistant (22)				
Omiganan	16	16	16-64	- / -
Ciprofloxacin	0.25	0.5	0.25-2	95.5 / 0.0
Clindamycin	≤0.25	≤0.25	≤0.25	100.0 / 0.0
Erythromycin	>8	>8	0.5->8	18.2 / 81.8
Gentamicin	≤2	≤2	≤2	100.0 / 0.0
Neomycin	>16	>16	0.25->16	- / -
Oxacillin	>2	>2	>2	0.0 / 100.0
TAO	39	39	0.31-78	- / -
Teicoplanin	≤2	≤2	≤2-8	100.0 / 0.0
Tetracycline	≤2	≤2	≤2->16	90.9 / 9.1
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5	100.0 / 0.0
Vancomycin	1	1	1	100.0 / 0.0
Vancomycin-tolerant (20)				
Omiganan	16	32	4-64	- / -
Ciprofloxacin	>4	>4	4->4	0.0 / 100.0
Clindamycin	>4	>4	≤0.25->4	25.0 / 75.0
Erythromycin	>8	>8	0.25->8	15.0 / 85.0
Gentamicin	>8	>8	≤2->8	45.0 / 55.0
Neomycin	>16	>16	≤0.12->16	- / -
Oxacillin	>2	>2	>2	0.0 / 100.0
TAO	20	39	≤0.08-39	- / -
Teicoplanin	≤2	4	≤2-4	100.0 / 0.0
Tetracycline	≤2	>16	≤2->16	80.0 / 20.0
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->2	95.0 / 5.0
Vancomycin	1	2	0.5-2	100.0 / 0.0
hVISA (11)				
Omiganan	16	32	8-32	- / -
Ciprofloxacin	>4	>4	>4	0.0 / 100.0
Clindamycin	>4	>4	≤0.25->4	9.1 / 81.8
Erythromycin	>8	>8	8->8	0.0 / 100.0
Gentamicin	>8	>8	≤2->8	9.1 / 90.9
Neomycin	16	>16	0.25->16	- / -
Oxacillin	>2	>2	0.5->2	9.1 / 90.9
TAO	20	39	0.61-39	- / -
Teicoplanin	≤2	4	≤2-8	100.0 / 0.0
Tetracycline	≤2	>16	≤2->16	63.6 / 36.4
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5	100.0 / 0.0
Vancomycin	2	2	1-4	90.9 / 0.0
VISA (10 strains)				
Omiganan	16	32	8-32	- / -
Ciprofloxacin	>4	>4	>4	0.0 / 100.0
Clindamycin	>4	>4	≤0.25->4	10.0 / 80.0
Erythromycin	>8	>8	4->8	0.0 / 90.0
Gentamicin	>8	>8	≤2->8	40.0 / 60.0
Neomycin	>16	>16	≤0.12->16	- / -
Oxacillin	>2	>2	1->2	20.0 / 80.0
TAO	20	39	0.15-39	- / -
Teicoplanin	8	8	≤2-16	90.0 / 0.0
Tetracycline	≤2	>16	≤2->16	50.0 / 50.0
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->2	90.0 / 10.0
Vancomycin	8	8	4-8	0.0 / 0.0
VRSA (5 strains)				
Omiganan	16	-	16	- / -
Ciprofloxacin	>4	-	>4	0.0 / 100.0
Clindamycin	>4	-	>4	0.0 / 100.0
Erythromycin	>8	-	>8	0.0 / 100.0
Gentamicin	>8	-	≤2->8	20.0 / 80.0
Neomycin	>16	-	>16	- / -
Oxacillin	>2	-	>2	0.0 / 100.0
TAO	39	-	20-39	- / -
Teicoplanin	8	-	8-16	60.0 / 0.0
Tetracycline	>16	-	≤2->16	40.0 / 60.0
Trimethoprim/sulfamethoxazole	≤0.5	-	≤0.5->2	80.0 / 20.0
Vancomycin	>64	-	>64	0.0 / 100.0

a. Criteria as published by the CLSI [2006]. ß-lactam susceptibility should be directed by oxacillin test results. - = no criteria are found in CLSI documents.

- Unlike wildtype MRSA isolates, CA-MRSA isolates remained more susceptible to ciprofloxacin, clindamycin, gentamicin and tetracycline, while displaying greater resistance to erythromycin and neomycin (parenteral breakpoints only).
- Vancomycin-tolerant, hVISA, VISA and VRSA isolates all displayed some co-resistance to other class agents including ciprofloxacin, erythromycin, clindamycin, tetracycline and the aminoglycosides (including neomycin).

CONCLUSIONS

- All *S. aureus* isolates tested were inhibited by 64 µg/ml (range, 4 to 64 µg/ml) of omiganan, with MIC_{50} and MIC_{90} values of 16 and 32 µg/ml, respectively.
- Vancomycin-tolerant, hVISA and VISA strains had omiganan MIC_{90} values that were only two-fold greater than for wildtype strains.
- The five VRSA isolates, representing the most resistant strains tested, were all inhibited by 16 µg/ml of omiganan.
- Neomycin, the active antistaphylococcal component of the widely utilized, nonprescription triple antibiotic ointment (TAO), and TAO itself, displayed a marked decrease (64-fold) in activity against all subsets compared with MSSA.
- Omiganan demonstrates potent activity against all staphylococci, regardless of identified resistance mechanisms and may be a critical component in the prevention of catheter-related infections, including local catheter site infections.
- Given the recent, and worrisome, emergence of *S. aureus* with reduced susceptibility to vancomycin, the finding that omiganan remains equally active against all isolates of this important species below the clinical formulation concentration (1%) is especially noteworthy.

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