

# In vitro Activity of Vancaptin against Methicillin-Resistant *Staphylococcus aureus* from Periprosthetic Joint Infections

Hye-Kyung Cho, MD, PhD,<sup>1</sup> Melissa J. Karau, MS,<sup>1</sup> Kerryl E. Greenwood-Quaintance, MS,<sup>1</sup> Karl A. Hansford,<sup>2</sup> Matthew A. Cooper,<sup>2</sup> Mark A.T. Blaskovich,<sup>2</sup> Robin Patel, MD<sup>1,3</sup>

<sup>1</sup>Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic Rochester, MN

<sup>2</sup>Centre for Superbug Solutions, Institute for Molecular Bioscience, The University of Queensland, Australia

<sup>3</sup>Division of Infectious Diseases, Department of Medicine, Mayo Clinic, Rochester, MN

Contact Information:  
Robin Patel  
Mayo Clinic, Rochester, MN  
patel.robin@mayo.edu

## Abstract

**BACKGROUND:** The vancaptins are modified vancomycin derivatives developed by adding membrane targeting motifs to the C-terminus of vancomycin. We determined the *in vitro* activity of a lead vancaptin candidate against periprosthetic joint infection-associated methicillin-resistant *S. aureus* (MRSA) in the planktonic and biofilm states, and the effect of adding 0.002% polysorbate 80 (P-80; Sigma-Aldrich) on vancaptin susceptibility testing.

**METHODS:** Thirty-seven clinical isolates of MRSA collected at Mayo Clinic (Rochester, Minnesota) were studied. Vancaptin minimum inhibitory concentrations (MICs) were determined using Clinical and Laboratory Standards Institutes guidelines. Minimum biofilm bactericidal concentrations (MBBCs) were determined using a pegged lid microtiter plate assay. Vancaptin MIC and MBBC values were assessed with and without P-80. Vancaptin, vancomycin, and dalbavancin biofilm time-kill assays were performed using biofilms formed by 10 MRSA isolates on Teflon coupons.

**RESULTS:** Vancaptin MICs with and without P-80 ranged from 0.015 to 0.12 µg/mL and 0.25 to 1 µg/mL, respectively. Vancaptin MBBCs with and without P-80 ranged from 0.25 to 4 µg/mL and 1 to 8 µg/mL, respectively. Reductions of biofilm bacterial densities on Teflon coupons after 8 and 24 hours of incubation with vancaptin, vancaptin with P-80, vancomycin, or dalbavancin with P-80 were less than 3-log<sub>10</sub> cfu/cm<sup>2</sup> for all isolates tested.

**CONCLUSION:** Vancaptin has promising *in vitro* activity against planktonic MRSA and MRSA in a pegged lid biofilm assay, but was not bactericidal against biofilms on Teflon coupons. P-80 decreased vancaptin MICs and MBBCs.

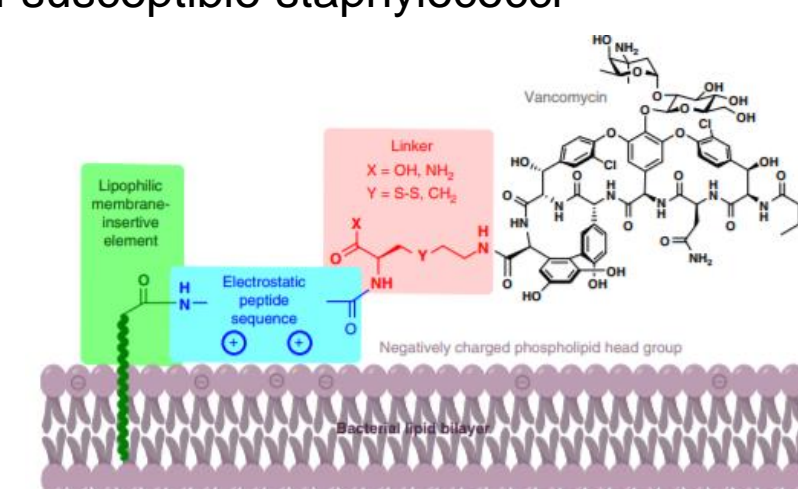
## Background

### ➤ Periprosthetic joint infections (PJI)

- Complication of arthroplasty associated with morbidity and mortality, most commonly caused by staphylococci
- Methicillin-resistant *Staphylococcus aureus* (MRSA) PJI related to increased rates of treatment failure, longer hospital stays, poorer clinical outcomes, and increased costs than PJI caused by methicillin-susceptible staphylococci

### ➤ Vancaptins

- Modified vancomycin derivatives prepared with membrane targeting motifs added to C-terminus of vancomycin
- Mechanism of action
  - ✓ Increased affinity for the target terminal *D*-Ala-*D*-Ala dipeptide of Lipid II
  - ✓ Enhanced dimerization leading to higher avidity for membrane-bound Lipid II
  - ✓ Increased membrane permeability
  - ✓ Enhanced drug concentration at the membrane surface by localizing drug



**Fig 1. Vancaptin structure**  
(Blaskovich MAT, et al. Nat Commun 2018;9(1):22.)

### ➤ Objectives:

- Evaluate *in vitro* activity of vancaptin (MCC5145) against planktonic and biofilm states of PJI associated-MRSA
- Determine effect of adding P-80 to vancaptin susceptibility testing

## Methods and Results

### (I. MIC, MBIC, and MBBC, 37 PJI-associated MRSA, 2000-2016)

#### ➤ Minimum inhibitory concentrations (MICs)

- Broth microdilution following CLSI guidelines

#### ➤ Minimum biofilm inhibitory concentrations (MBICs) and minimum biofilm bactericidal concentrations (MBBCs)

- Determined using pegged lid microtiter plate assay

- Tested with and without 0.002% polysorbate 80 (TWEEN®, Sigma-Aldrich, St. Louis, MO)

#### ▶ Table 1. Vancaptin MIC, MBIC, and MBBC of MRSA isolates (N = 37)

	MIC (µg/mL)										MIC <sub>50</sub>	MIC <sub>90</sub>
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8		
Vancaptin without P-80	0 (0)	0 (0)	0 (0)	0 (0)	18 (48.6)	17 (94.6)	2 (100)	0 (100)	0 (100)	0 (100)	0.5	0.5
Vancaptin with P-80	3 (8.1)	1 (10.8)	27 (83.8)	6 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0.06	0.125

	MBIC (µg/mL)										MBIC <sub>50</sub>	MBIC <sub>90</sub>
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8		
Vancaptin without P-80	0 (0)	0 (0)	0 (0)	0 (0)	5 (13.5)	21 (70.3)	9 (94.6)	2 (100)	0 (100)	0 (100)	0.5	1
Vancaptin with P-80	1 (2.7)	19 (54.1)	17 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0.06	0.125

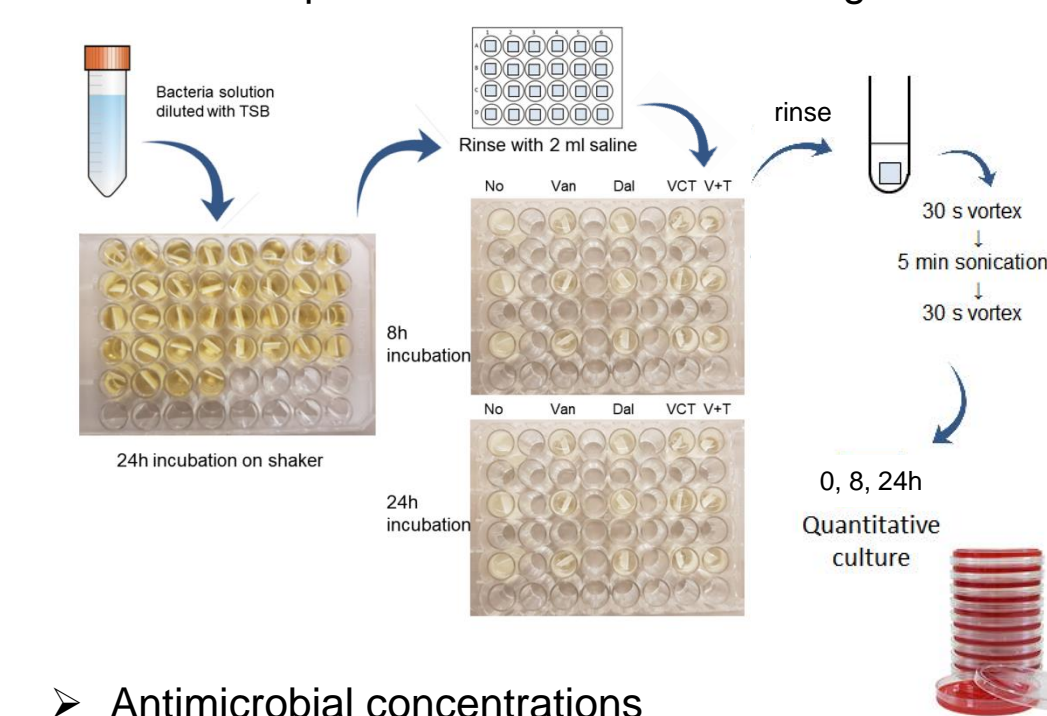
  

	MBBC (µg/mL)										MBBC <sub>50</sub>	MBBC <sub>90</sub>
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8		
Vancaptin without P-80	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	19 (54.1)	9 (76.3)	8 (100)	2	8
Vancaptin with P-80	0 (0)	0 (0)	0 (0)	0 (0)	2 (5.4)	11 (35.1)	15 (75.7)	8 (97.3)	1 (100)	0 (100)	1	2

## Methods and Results

### (II. Vancaptin, Vancomycin, and Dalbavancin Time-Kill Assays, 10 PJI-associated MRSA)

#### ➤ Teflon coupons used as biofilm-inducing surfaces



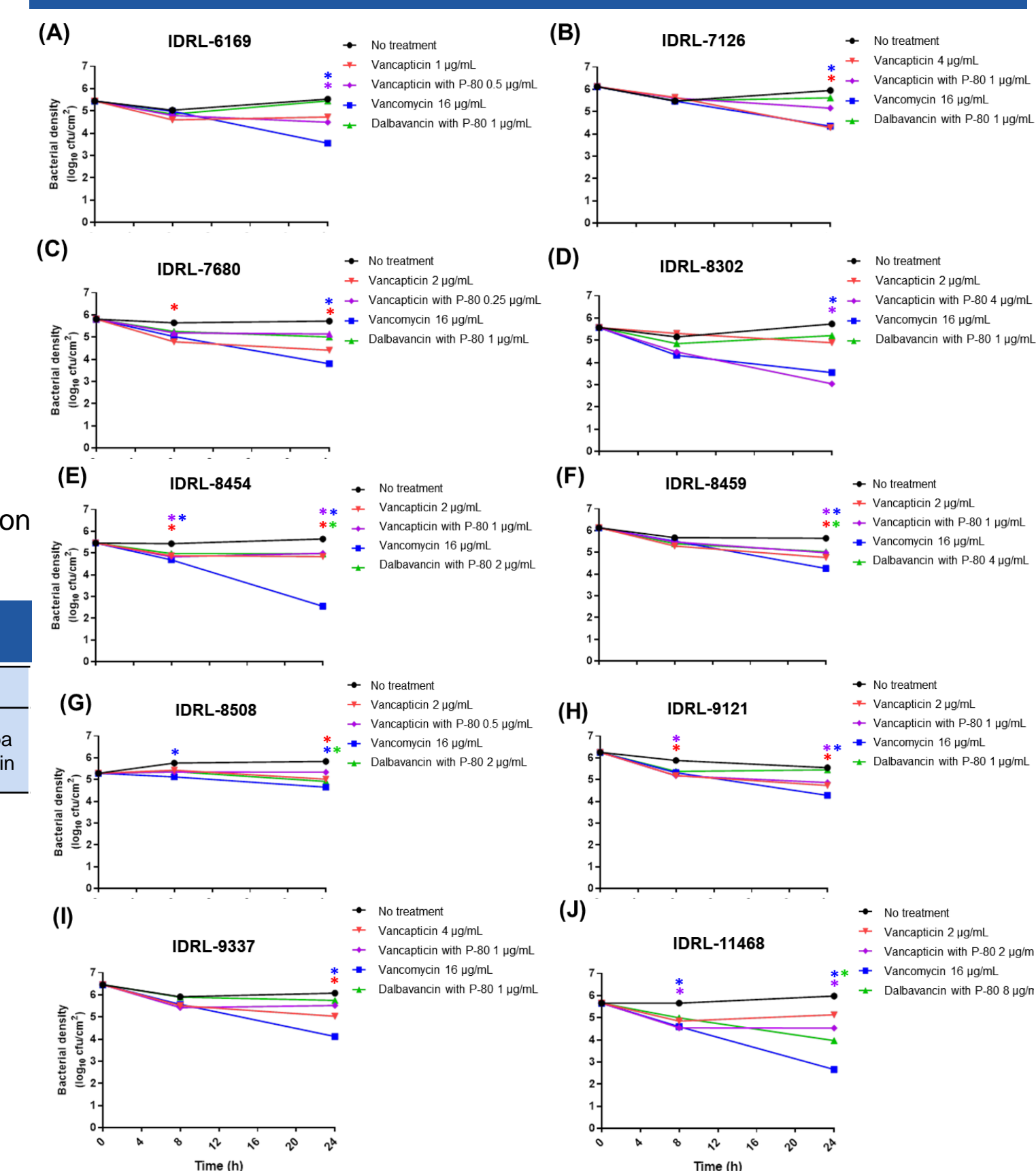
#### ➤ Antimicrobial concentrations = 1X MBBC or fCmax (when MBBC > fCmax)

- Bactericidal activity: Defined as 3-log<sub>10</sub> cfu/cm<sup>2</sup> reduction at 24 h compared to biofilm bacterial density at 0 h

**Table 2. MIC and MBBC values for 10 MRSA isolates**

Isolate (IDRL #)	MIC (µg/mL)				MBBC (µg/mL)			
	Vancaptin	Vancaptin with P-80	Vancomycin	Dalbavancin	Vancaptin	Vancaptin with P-80	Vancomycin	Dalbavancin
6169	0.25	0.015	1	0.03	1	0.5	>32	1
7126	0.25	0.06	1	0.03	4	1	>32	1
7680	0.25	0.06	2	0.03	2	0.25	>32	1
8302	0.5	0.06	2	0.03	2	4	>32	1
8454	0.5	0.06	1	0.03	2	1	>32	2
8459	0.25	0.06	1	0.06	2	1	>32	4
8508	0.25	0.06	1	0.03	2	0.5	>32	2
9121	0.25	0.06	1	0.03	2	1	>32	1
9337	0.5	0.06	1	0.25	4	1	>32	1
11468	0.25	0.06	2	<0.008	2	2	>32	8

**Fig 2. Biofilm time-kill curves against 10 MRSA isolates**



\*P < 0.05, in comparison with no treatment group at each time point

## Conclusions

- Vancaptin (MCC5145) has promising *in vitro* activity against PJI-associated MRSA in the planktonic state and biofilms state in pegged lid assays, but was not bactericidal against biofilms on Teflon coupons.
- The addition of P-80 decreased vancaptin MICs, MBICs and MBBCs.