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Title: Comparison of Telavancin and Vancomycin Antibiotic Lock Solutions in the Eradication of Biofilm-Producing Staphylococci and Enterococci from Central Venous Catheters

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ABSTRACT

PURPOSE: Antibiotic lock solutions (ALS) are used for management of catheter-related bloodstream infections. We compared activity of vancomycin and telavancin against biofilm-forming *Staphylococcus epidermidis*, *Enterococcus faecalis,* and *Staphylococcus aureus*.

METHODS: An established *in vitro* central venous catheter antibiotic lock model was used to evaluate: vancomycin (5 mg/mL) and telavancin (5 mg/mL), with and without preservative-containing heparin sodium (benzyl alcohol 0.45%) 2500 units/mL; and heparin and normal saline. ALS were introduced after 24h bacterial growth in catheters incubated at 35°C. After 72h exposure to lock solution, catheters were drained, flushed, and cut into segments for CFU/mL quantification.

RESULTS: Against *S. epidermidis*, vancomycin and telavancin (with and without heparin) demonstrated similar activity. Against *E. faecalis*, vancomycin alone (no heparin) was more active than telavancin alone ($p<0.01$). Against *S. aureus*, vancomycin plus heparin demonstrated activity similar to vancomycin alone. Both demonstrated greater activity than telavancin ($p<0.02$). When heparin was added to the vancomycin lock, activity against *S. epidermidis* and *E. faecalis* was reduced ($p<0.01$). Telavancin activity was not significantly changed with addition of heparin.

CONCLUSION: Both telavancin and vancomycin significantly reduced biofilm burden against biofilm-forming *S. epidermidis*, *E. faecalis*, and *S. aureus*, but were unable to completely eradicate these bacteria in the *in vitro* catheter model.

INTRODUCTION

Staphylococcal and enterococcal infections are a major problem in hospital settings, especially among patients with indwelling devices.[1] These infections are often caused by biofilm-producing strains which are difficult to eradicate and which may progress to bacteremia. Vancomycin therapy is one of the recommendations in the Infectious Diseases Society of America catheter-related infection management guidelines when systemic antibiotics are used in combination with antibiotic lock solutions (ALS) to treat catheter-related bacteremia while attempting to retain the catheter.[2]

Telavancin is a lipoglycopeptide antibiotic with a core chemical structure similar to the glycopeptide vancomycin. Unlike vancomycin, telavancin possesses a second mechanism of action that causes a rapid depolarization and loss of the functional integrity of the bacterial membrane.[3, 4] Clinical data support the use of telavancin in the treatment of complicated skin and soft-tissue infections and nosocomial pneumonia [5-8], while animal model data suggest efficacy in the treatment of bacteremia, endocarditis, osteomyelitis, and meningitis caused by gram-positive pathogens.[9-13] Of great interest is the activity of telavancin against biofilm-producing staphylococci and enterococci.[6, 14] We previously described the activity of daptomycin and vancomycin on formed biofilms in an in vitro central venous catheter model.[15]

We have used a validated catheter modeling system to assess the activity of telavancin or vancomycin alone or in combination with the anticoagulant heparin (containing benzyl alcohol preservative) in the eradication of biofilm-forming staphylococci and enterococci. This model uses 72 hour lock times that are useful in clinical settings, particularly in the management of hemodialysis catheter infections.[16, 17]

MATERIALS AND METHODS:

Bacterial strains. Known biofilm-producing strains of *Staphylococcus epidermidis* (ATCC 35984), methicillin-susceptible *Staphylococcus aureus* (MSSA, ATCC 35556), and *Enterococcus faecalis* (ATCC 29212; vancomycin- susceptible) were evaluated. In addition, biofilm-forming clinical isolates of *S. epidermidis* (L369D, from urine), vancomycin-resistant *E. faecalis* (VRE, L2022, from tissue), methicillin-resistant *S. aureus* (MRSA; L32 and L83, from blood), and MSSA (L2, from blood), were evaluated. The biofilm forming ability of each strain, as determined by optical density measurements, has been previously described.[14] Minimum inhibitory concentrations of vancomycin (1 to 2mg/L except the VRE strain at 256mg/L) and telavancin (0.03-0.25 mg/L) were also previously tested. [14]

Lock solutions. Vancomycin hydrochloride[a] (5mg/mL final concentration) and telavancin[b] (5mg/mL final concentration) were obtained from commercial pharmacy stock. Telavancin drug product (telavancin for injection, 250mg vial) also contains mannitol (312.5mg) and hydroxypropylbetadex (2500mg) to increase solubility. Stock solutions of each antibiotic were freshly prepared each day. Heparin sodium solution (5000units/mL with 0.9% benzyl alcohol; Hospira, Lake Forest, IL) was obtained from commercial pharmacy stock and diluted with normal saline (NS; without preservatives) or antibiotic solution to a final concentration of 2500units/mL which contained 0.45% benzyl alcohol. These lock solutions have demonstrated compatibility and stability up to 72h at 37°C.[18]

[a] Hospira, Lake Forest IL, lot # 943903A, 12070DD
[b] Astellas Pharma, Deerfield IL, lot# 2029222

Medium. Supplemented Tryptic soy broth (STSB, Difco, Becton Dickinson Co., Sparks, MD) with 1% dextrose, 2% sodium chloride, 25 mg/L calcium chloride, and 12.5 mg/L magnesium was used for all catheter models.[15, 19] Colony counts were determined using tryptic soy agar (TSA, Difco, Becton Dickinson Co., Sparks, MD).

Device inoculation and treatment. Two sets of catheters were processed. For all catheters, a 0.5 McFarland standard of each test organism (noted above) was diluted in STSB and added to the lumen of triple-lumen polyurethane central venous catheters (Arrow-Howes CVC® 15703, Reading, PA).[15, 19] Starting inocula were ~10^6 CFU/mL, verified by colony count on TSA. After 24h biofilm development at 35°C, one set of catheters was processed for CFU/mL to determine a baseline of biofilm formation. The other catheters were drained and lock solution instilled. Under sterile conditions, each drug was injected into each access port sufficient to fill the catheter lumen. Catheters were then clamped at the distal end. This procedure was also repeated with separate CVC containing anticoagulant (preservative-containing heparin sodium) or NS. Each catheter was then incubated at 35°C for an additional 72h. Each organism was tested against each agent at least in triplicate.

Recovery of treated organisms. Catheter fluid was removed and discarded following incubation. A sterile needle was introduced into the open lumen and 1 mL of sterile NS was flushed through each lumen and collected. To optimize yield of viable bacteria, the flushed saline was sonicated at 60Hz for 1 minute, then vortexed for 15 seconds as previously described.[15] Additionally, 3 cm cut pieces of each catheter were sonicated and vortexed in 3 mL of sterile NS. Sonication served to separate clusters of cells for quantification, as well as removing biofilm from the catheter surface. Serial dilutions of

the flushed saline and saline used to sonicate and vortex the cut segments were plated on TSA for colony count enumeration. The limit of detection for the flushed, sonicated, and vortexed cultures from the lumen and the sonicated and vortexed saline of the catheter segment is 2.0 $\log_{10}$ CFU/mL.[15]

Drug Stability. Concentrations of vancomycin and telavancin with and without heparin were evaluated before and after 72h incubation in CVC at 35°C. Vancomycin concentrations were determined by a homogeneous particle-enhanced turbidmetric immunoassay (PETIA; Architect, Multigent®; Abbott Diagnostics Abbott Park, IL, USA) at the Providence Veteran Affairs Medical Center.[20] The vancomycin assay has a detection range of 0.5 to 80.0 µg/mL, and an intra- and inter-day CV% of <2.0% and <5.0%, respectively. Telavancin concentrations were determined by Theravance, Inc. using liquid chromatography mass spectrometry.[21, 22]

Data analysis. Each catheter was used to test one lock bacterial strain combination in triplicate. The $\log_{10}$ CFU/mL from the flushed saline and saline used to sonicate and vortex the cut catheter segment were added together to give a total $\log_{10}$ CFU/mL result. This allowed for quantification of the total biofilm remaining in the catheter and allowed for combining catheter lumens of different gauges. These triplicate total results were subtracted from the baseline catheter (0h lock solution, ~10^8 CFU/mL, also in triplicate) for each strain (n=9 per strain), to determine antimicrobial activity (reduction in $\log_{10}$CFU/mL, or kill). Average activity and standard error of the mean were calculated for each species and lock solution. Activity was compared between groups using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. All statistical

112 analyses were performed using SPSS statistical software (release 20; SPSS, Inc.
113 Chicago, IL.). A p value of < 0.05 indicates statistical significance.

RESULTS

Antimicrobial activity in a catheter model. Activity of tested lock solutions are shown in **Figure 1a-c**. The activity of each lock solution was averaged for *S. epidermidis*, *E. faecalis*, and *S. aureus*, as the results were similar for each species. The catheters processed after 24h incubation with media and bacteria, with no lock solution, yielded 10^7-10^8 CFU/mL for each strain. This served as the baseline biofilm formation for calculating activity of the lock solutions. Against *S. epidermidis*, all antibiotic lock solutions were more active than normal saline ($p<0.01$). Telavancin plus heparin demonstrated the most activity, but was not significantly more active than telavancin alone or vancomycin alone. The addition of heparin to vancomycin, however, reduced activity compared to vancomycin alone (mean difference 0.79, 95%CI 0.15-1.42, $p<0.01$).

Against *E. faecalis*, telavancin demonstrated minimal activity. Vancomycin alone was more active than the other lock solutions ($p<0.01$). Vancomycin activity was reduced by the addition of heparin (mean difference 2.89, 95%CI 2.42-3.36, $p<0.01$). Normal saline was more active than any heparin-containing lock solution or telavancin alone ($p<0.01$), suggesting that heparin reduces antimicrobial activity of the lock solution.

Against *S. aureus*, antibiotic lock solutions demonstrated more activity than heparin alone ($p<0.02$), however telavancin and telavancin plus heparin were not significantly more active than normal saline. Vancomycin plus heparin demonstrated the most activity, but not significantly different from vancomycin alone. The addition of

136 heparin to the antibiotic lock solution did not have a significant effect on the activity of
137 either antibiotic against these strains.
138
139 **Drug stability.** Telavancin and vancomycin solutions were evaluated for concentration
140 at least in duplicate before and after incubation. Lock solutions increased in
141 concentration after 72h incubation (Table 1). We attempted measuring concentrations
142 of heparin-containing lock solutions after 72h incubation, however, the added heparin
143 interfered with interpretation of these results. (data not shown).

DISCUSSION

As large numbers of patients continue to be dialyzed through long-term intravascular catheters and long-term intravascular catheter use continues to be important in caring for many patients; a niche exists for the ideal antimicrobial agent to be used as an ALS for catheter salvage. If successful, ALS use should reduce the cost and complications of catheter removal and reinsertion.

In a previous study, telavancin prevented biofilm formation by biofilm-forming staphylococci and enterococci at concentrations below the MIC.[14] In the current study testing eradication of formed biofilms, telavancin at concentrations 20,000-166,666x the MIC and vancomycin at concentrations 2,500-5,000x the MIC (except for the VRE strain which was ~20x the MIC) reduced the bacterial burden, but did not completely eradicate these strains. While the concentrations of both vancomycin and telavancin increased over the 72h period, we believe this was due to losses in volume from the lumen during incubation.

The activity of vancomycin against vancomycin-resistant enterococci (VRE) may reflect the high concentration used (~20x the MIC). Of note, the VRE strain produced less biofilm than the other strains, making it less difficult to eradicate. We hypothesize that the decreased activity of telavancin against *S. aureus* and *E. faecalis* may be partially due to the presence of mannitol (125% w/w telavancin) in the drug formulation.[23] Mannitol is a sugar alcohol that can be fermented by *S. aureus* and some *Enterococcus* strains, but not by *S. epidermidis*. Mannitol increases *S. aureus* biofilm formation,[24]

which may lead to reduced ALS activity. The activity of the ALS tested against these strains may be isolate-specific, likely reflecting the amount of biofilm produced.

Activity of the telavancin lock solutions may have been reduced by drug binding to the catheter surface. Recent recommendations have suggested adding polysorbate 80 (P-80) and dimethyl sulfoxide (DMSO) to telavancin for MIC testing to decrease binding to the polystyrene surface resulting in a decreased MIC.[25] MICs were previously tested without P-80 and DMSO and may appear falsely elevated compared to MICs with the more recent method. Addition of P-80 and DMSO may increase activity of lock solutions by decreasing binding to the polyurethane catheter; however, clinical utility is limited unless a commercial product containing these additives is available.

Addition of benzyl alcohol-containing heparin to the antibiotic solution significantly reduced antimicrobial activity of vancomycin against *S. epidermidis* and *E. faecalis*, which we hypothesize may be due to stimulated biofilm growth. The influence of heparin and benzyl alcohol on staphylococcal biofilm growth has been reported.[26-29] Data on the influence of heparin and benzyl alcohol on enterococcal biofilms are lacking. The minimal activity for all locks containing heparin may demonstrate heparin-induced biofilm growth in enterococci. We did not observe a reduction in antibiotic activity with heparin against *S. aureus*, which may be concentration-dependent. A previous study by our laboratory demonstrated significant reductions in activity of vancomycin 2mg/mL, linezolid 1mg/mL and 2mg/mL in combination with heparin 5000 units/mL (benzyl alcohol 0.45%). (unpublished data)

Normal saline demonstrated greater activity in our *in vitro* assay than expected. This is likely due to disturbance of biofilm and removal of planktonic bacteria during lock solution instillation and removal, which was not quantified. Normal saline has previously demonstrated a reduction in formed biofilms of ~1$\log_{10}$ CFU/mL over 24h.[30] Detachment of some planktonic bacteria from the biofilm would be expected due to the lack of nutrients in the lock solutions and a 72h incubation period. High antibiotic concentrations or osmotic stress present in the ALS can stimulate biofilm formation,[31] but as these are absent in saline solutions, detachment may be greater. Planktonic bacteria that detached from the biofilm would either be removed during lock solution withdrawal or potentially killed in an ALS; however these bacteria were not quantified in our study. This exemplifies the clinical importance of lock withdrawal instead of flushing lock solutions (and any planktonic bacteria within them) into the patient.

We hypothesize that the activity of normal saline is related to the amount of biofilm produced by each strain tested, as demonstrated by saline having particularly more activity than expected against the VRE strain that produced less biofilm. It is also important to note, that activity of the solutions average ~2-3 $\log_{10}$ CFU/mL, even for the antibiotic-containing lock solutions. This reduction from a baseline biofilm of 10^8 CFU/mL, left ~10^5-10^6 CFU/mL remaining in the catheter lumen. Activity may be increased by repeated lock instillation and removal, as in clinical practice.

There are some limitations to this work. A small number of isolates were tested. There are multiple possible explanations for the inability of the ALS tested to eradicate the microbial load which was not explored further, such as additives inhibiting bactericidal activity of antibiotics tested or stimulating biofilm formation. Increased biofilm formation

209 could also be interpreted as larger biomass growth, and/or stronger attachment, both of
210 which would result in more recovered cells at 72h.

211

212

CONCLUSIONS

Telavancin and vancomycin are active in reducing biofilm-forming staphylococci and enterococci in a central venous catheter model, but were unable to completely eradicate the biofilm-forming strains evaluated. Addition of preservative-containing heparin sodium 2500 units/mL to vancomycin reduces activity against *S. epidermidis* and *E. faecalis*. Finding the ideal ALS with antibiofilm activity and a minimal side-effect profile remains of great interest to investigators and to the clinical community.

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Conflict of Interest and Disclosures

The views expressed are those of the authors and do not necessarily represent the position or policy of the United States Department of Veterans Affairs. All data collection, extraction, and analyses were carried out by the Department of Veterans Affairs study team.

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REFERENCES

1 Mermel LA, Allon M, Bouza E et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2009; 49(1): 1-45. Erratum: *Clin Infect Dis.* 2010;50(3):457; *Clin Infect Dis.*10;50(7):1079.

2 Manierski C and Besarab A. Antimicrobial locks: putting the lock on catheter infections. *Adv Chronic Kidney Dis.* 2006; 13(3): 245-58.

3 Higgins DL, Chang R, Debabov DV et al. Telavancin, a multifunctional lipoglycopeptide, disrupts both cell wall synthesis and cell membrane integrity in methicillin-resistant Staphylococcus aureus. *Antimicrob Agents Chemother.* 2005; 49(3): 1127-34.

4 Lunde CS, Hartouni SR, Janc JW et al. Telavancin disrupts the functional integrity of the bacterial membrane through targeted interaction with the cell wall precursor lipid II. *Antimicrob Agents Chemother.* 2009; 53(8): 3375-83.

5 Stryjewski ME, Chu VH, O'Riordan WD et al. Telavancin versus standard therapy for treatment of complicated skin and skin structure infections caused by gram-positive bacteria: FAST 2 study. *Antimicrob Agents Chemother.* 2006; 50(3): 862-7.

6 Stryjewski ME, O'Riordan WD, Lau WK et al. Telavancin versus standard therapy for treatment of complicated skin and soft-tissue infections due to gram-positive bacteria. *Clin Infect Dis.* 2005; 40(11): 1601-7.

7 Krause KM, Barriere SL, Kitt MM et al. In vitro activity of telavancin against gram-positive isolates from complicated skin and skin structure infections: results from 2 phase 3 (ATLAS) clinical studies. *Diagn Microbiol Infect Dis.* 2010; 68(2): 181-5.

8 Dunbar LM, Tang DM and Manausa RM. A review of telavancin in the treatment of complicated skin and skin structure infections (cSSSI). *Ther Clin Risk Manag.* 2008; 4(1): 235-44.

9 Madrigal AG, Basuino L and Chambers HF. Efficacy of telavancin in a rabbit model of aortic valve endocarditis due to methicillin-resistant Staphylococcus aureus or vancomycin-intermediate Staphylococcus aureus. *Antimicrob Agents Chemother.* 2005; 49(8): 3163-5.

10 Reyes N, Skinner R, Kaniga K et al. Efficacy of telavancin (TD-6424), a rapidly bactericidal lipoglycopeptide with multiple mechanisms of action, in a murine model of pneumonia induced by methicillin-resistant Staphylococcus aureus. *Antimicrob Agents Chemother.* 2005; 49(10): 4344-6.

11 Stucki A, Gerber P, Acosta F et al. Efficacy of telavancin against penicillin-resistant pneumococci and Staphylococcus aureus in a rabbit meningitis model and determination of kinetic parameters. *Antimicrob Agents Chemother.* 2006; 50(2): 770-3.

12 Reyes N, Skinner R, Benton BM et al. Efficacy of telavancin in a murine model of bacteraemia induced by methicillin-resistant Staphylococcus aureus. *J Antimicrob Chemother.* 2006; 58(2): 462-5.

13 Yin LY, Calhoun JH, Thomas TS et al. Efficacy of telavancin in the treatment of methicillin-resistant Staphylococcus aureus osteomyelitis: studies with a rabbit model. *J Antimicrob Chemother.* 2009; 63(2): 357-60.

14 LaPlante KL and Mermel LA. In vitro activities of telavancin and vancomycin against biofilm-producing Staphylococcus aureus, S. epidermidis, and Enterococcus faecalis strains. *Antimicrob Agents Chemother.* 2009; 53(7): 3166-9.

15 LaPlante KL and Mermel LA. In vitro activity of daptomycin and vancomycin lock solutions on staphylococcal biofilms in a central venous catheter model. *Nephrol Dial Transplant.* 2007; 22(8): 2239-46.

16 Shah CB, Mittelman MW, Costerton JW et al. Antimicrobial activity of a novel catheter lock solution. *Antimicrob Agents Chemother.* 2002; 46(6): 1674-9.

17 Diskin CJ, Stokes TJ, Dansby LM et al. Is systemic heparin a risk factor for catheter-related sepsis in dialysis patients? An evaluation of various biofilm and traditional risk factors. *Nephron Clin Pract.* 2007; 107(4): c128-32.

18 LaPlante KL, Woodmansee S and Mermel LA. Compatibility and stability of telavancin and vancomycin in heparin or sodium citrate lock solutions. *Am J Health Syst Pharm.* 2012; 69(16): 1405-9.

19 Luther MK, Mermel LA and LaPlante KL. Comparison of ML8-X10 (a prototype oil-in-water micro-emulsion based on a novel free fatty acid), taurolidine/citrate/heparin and vancomycin/heparin antimicrobial lock solutions in the eradication of biofilm-producing staphylococci from central venous catheters. *J Antimicrob Chemother.* 2014; 69(12): 3263-7.

20 LaPlante KL and Woodmansee S. Activities of daptomycin and vancomycin alone and in combination with rifampin and gentamicin against biofilm-forming methicillin-resistant Staphylococcus aureus isolates in an experimental model of endocarditis. *Antimicrob Agents Chemother.* 2009; 53(9): 3880-6.

21 Shaw JP, Seroogy J, Kaniga K et al. Pharmacokinetics, serum inhibitory and bactericidal activity, and safety of telavancin in healthy subjects. *Antimicrob Agents Chemother.* 2005; 49(1): 195-201.

22 McConeghy KW, Liao S, Clark D et al. Variability in telavancin cross-reactivity among vancomycin immunoassays. *Antimicrob Agents Chemother.* 2014; 58(12): 7093-7.

23 Jaffer Y, Selby NM, Taal MW et al. A meta-analysis of hemodialysis catheter locking solutions in the prevention of catheter-related infection. *Am J Kidney Dis.* 2008; 51(2): 233-41.

24 Tang J CJ, Liu J, Zhang R, Yang R, Chen L. Effects of different cultivation conditions on Staphylococcus aureus biofilm formation and diversity of adhesin genes. *J Food Saf.* 2012; 32(2): 210-18.

25 Farrell DJ, Mendes RE, Rhomberg PR et al. Revised reference broth microdilution method for testing telavancin: effect on MIC results and correlation with other testing methodologies. *Antimicrob Agents Chemother.* 2014; 58(9): 5547-51.

26 Shanks RM, Donegan NP, Graber ML et al. Heparin stimulates Staphylococcus aureus biofilm formation. *Infect Immun.* 2005; 73(8): 4596-606.

27 Shanks RM, Sargent JL, Martinez RM et al. Catheter lock solutions influence staphylococcal biofilm formation on abiotic surfaces. *Nephrol Dial Transplant.* 2006; 21(8): 2247-55.

28 Milisavljevic V, Tran LP, Batmalle C et al. Benzyl alcohol and ethanol can enhance the pathogenic potential of clinical Staphylococcus epidermidis strains. *Am J Infect Control.* 2008; 36(8): 552-8.

29 Redelman CV, Maduakolam C and Anderson GG. Alcohol treatment enhances Staphylococcus aureus biofilm development. *FEMS Immunol Med Microbiol.* 2012; 66(3): 411-8.

30 Shenep LE, Shenep MA, Cheatham W et al. Efficacy of intravascular catheter lock solutions containing preservatives in the prevention of microbial colonization. *J Hosp Infect.* 2011; 79(4): 317-22.

31 Wang X, Niu C, Sun G et al. ygs is a novel gene that influences biofilm formation and the general stress response of Staphylococcus epidermidis. *Infect Immun.* 2011; 79(3): 1007-15.

Figure 1. Log reduction (CFU/mL; mean ± standard error of the mean) of an inoculated catheter containing biofilm-forming A) *Staphylococcus epidermidis,* B) *Enterococcus faecalis,* and C) *Staphylococcus aureus* locked for 72h with antibiotic, heparin, or normal saline.[c]

A *S. epidermidis* (2 strains: ATCC35984 and clinical strain L369D; n=18)

B *E. faecalis* (2 strains: ATCC29212 and clinical strain L2022; n=18)

C *S. aureus* (4 strains: 2 MSSA: ATCC35556 and clinical strain L2, and 2 MRSA: clinical strains L32 and L83; n=36)

[c] NS= normal saline

Hep = heparin sodium 2500units/mL with 0.45% benzyl alcohol

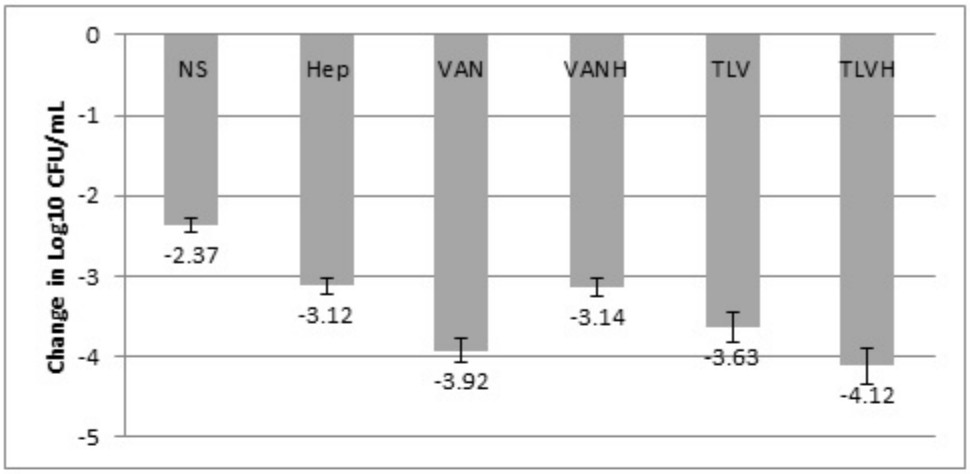
TLV = telavancin 5mg/mL

TLVH= telavancin 5mg/mL with heparin

VAN = vancomycin 5mg/mL

VANH= vancomycin 5mg/mL with heparin

A


Change in Log10 CFU/mL
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-2
-3
-4
-5
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Hep
VAN
VANH
TLV
TLVH
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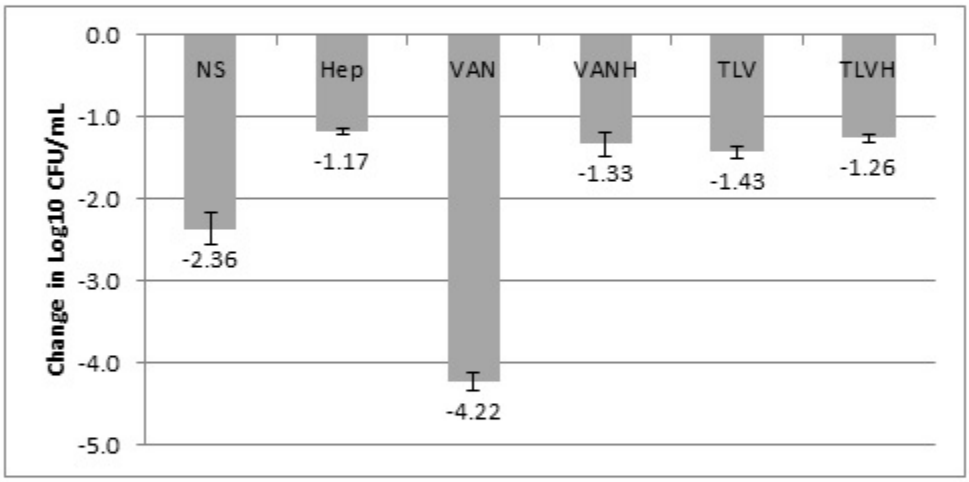
Change in Log10 CFU/mL
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NS
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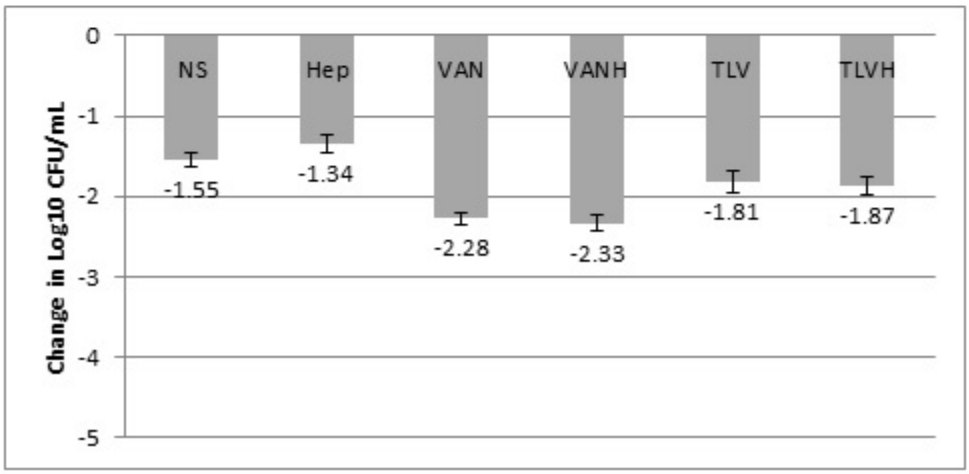
Change in Log10 CFU/mL
0
-1
-2
-3
-4
-5
NS
Hep
VAN
VANH
TLV
TLVH
-1.55
-1.34
-2.28
-2.33
-1.81
-1.87

Table 1. Concentrations of antibiotic lock solution stock and after 72 hour incubation in a central venous catheter. Targeted concentrations were 5 mg/mL.

Antibiotic	Stock Concentration (mg/mL)	72 hour Incubation (mg/mL)
Telavancin	4.5 ± 0.8	4.9 ± 0.1
Vancomycin	5.0 ± 0.2	5.8 ± 0.02