Relevance of levofloxacin-resistant MRSA and hVISA when utilizing directed antibiotic locks to treat stage four biofilm bone and joint infections

Martie L. Vicent[1 2], Kathryn E. Daffinee [1], and Kerry L. LaPlante[1 2 3]

1 Providence VAMC, Infectious Diseases Research Program, Providence, RI

2 University of Rhode Island, College of Pharmacy, Kingston, RI

3 The Warren Alpert School of Medicine, Brown University, Division of Infectious Diseases, Providence, RI

CONTACT INFORMATION

Address: 7 Greenhouse Road
Kingston, Rhode Island 02881

E-mail: martie.vicent@gmail.com; kerrylaplante@uri.edu

Phone: 954.235.1927

ABSTRACT

Background: Directed antibiotic locks have the potential to treat orthopedic infections due to the ability to bypass specific systemic absorption allowing for higher antimicrobial concentrations. With limiting antimicrobial options to treat biofilm commonly associated with bone and joint infections, our aim is to understand and further test the relevance of utilizing standard systemic MIC values to direct therapy.

Methods: Using our previously described biofilm assay with six unique spa-type (t004, t018, t062, t064, t1340, t008) MRSA isolates, biofilm was grown for 4, 6, and 24hrs starting at 5-6 log10 CFU/mL. MIC were run using CLSI guidance. Planktonic cells were removed by irrigation, simulating debridement. 24hr treatment assays, simulating antibiotic locks included levofloxacin (5 mg/mL) in d5W solution and vancomycin (5 mg/mL) in normal saline.

Results: Levofloxacin by definition of standard MIC values was found to be resistant in five out of the six MRSA isolates (MIC ranging from 1 – 312mcg/mL). However, at 16-25,000 times the MIC, levofloxacin demonstrated a decrease in biofilm growth on mature 24-hr established biofilm by 9-130%. Similarly, vancomycin heterogeneous vancomycin-intermediate Staphylococcus aureus (hVISA), at 3000-6000 the MIC demonstrated a decrease of 53-190% mature biofilm growth.

Conclusion: All isolates produced consistent biofilm as previously tested as a low or high biofilm-producer when there was no antibiotic present. Levofloxacin and vancomycin at least 1000X the MIC decreased all established MRSA biofilm. Our next steps will be to evaluate if the MIC values have any effect on additional antibiotics in a lock solution.

INTRODUCTION

Systemic application of antibiotics can limit therapeutic effectiveness when the anticipated needed dose is unachievable due to toxicities or if the site of infection is unreachable. Antibiotic lock therapy and intra-articular administration allows higher drug concentrations to reach directed sites of infection.[1,2] Our objective was to test if antibiotic selection intended for lock therapy needs to be based off of standard MIC values.

METHODS

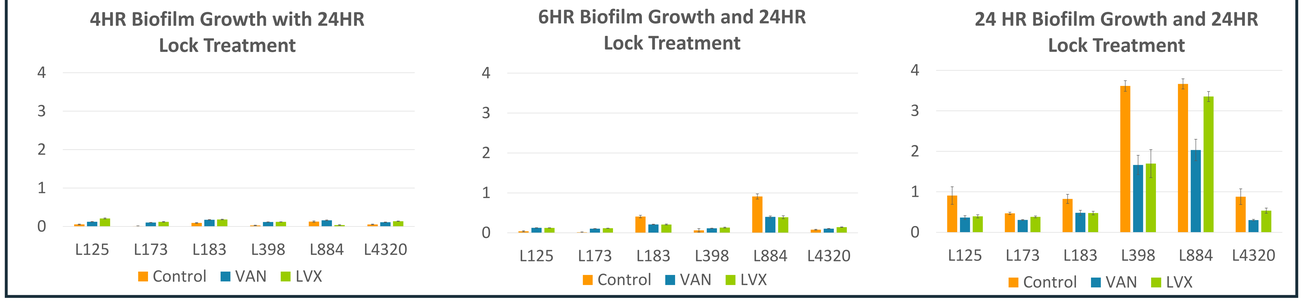
- Testing included six unique spa-type (t004, t018, t062, t064, t1340, t008) biofilm-producing methicillin-resistant (MRSA) and heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) isolates
- Biofilms were grown for 4, 6, and 24 hours with a starting inoculum of 5-6 log10 CFU/mL and diluted 1:100 in tryptic soy broth supplemented with calcium and magnesium per CLSI guidance and dextrose 1.25% to support biofilm production
- MICs were completed according to CLSI guidance[3]
- Planktonic cells were removed by irrigation three times to simulate debridement of an infected bone and/or joint
- Treatment assays included vancomycin (5 mg/mL) in normal saline solution and levofloxacin (5 mg/mL) in d5W solution added to the biofilm-containing wells for 24 hours
- Controls were grown and left untreated
- Following the control and treatment period, remaining biofilm was air-dried overnight in a biosafety cabinet, stained with 0.1% crystal violet for 15 minutes, and resolubilized with 33% glacial acetic acid before reading the optical density (OD) at an absorbance of 570

SELECTED MRSA ISOLATES

Isolate	Spa Type	Biofilm Producer	Source	VAN MIC[a] (µg/mL)	LVX MIC[b] (µg/mL)
L125 (hVISA)	t004	Low	Urine	0.75	9.7
L173	t018	Low	Tissue	0.75	9.7
L183	t062	Low	Tissue	1.5	39
L398	t064	High	Other	1	39
L884	t1340	High	Urine Catheter	1.5	312
L4320 (hVISA)	t008	Low	Tissue	1	0.19

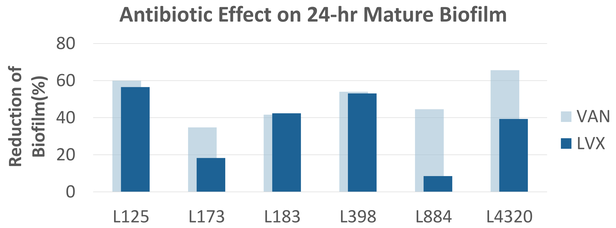
[a]Vancomycin MIC Breakpoints (µg/mL): ≤ 2 (S); 4-8 (I); ≥ 16 (R)
[b]Levofloxacin MIC Breakpoints (µg/mL): ≤ 1 (S); 2 (I); ≥ 4 (R)

TREATMENT RESULTS



RESULTS

- Following CLSI standard, levofloxacin was found to be resistant in five out of the six MRSA isolates with MIC values ranging from 0.2-312 mcg/mL and vancomycin was found to be susceptible in all of the tested isolates with MIC values ranging from 0.75-1.5 mcg/mL
- By using levofloxacin at 16-25,000 times the MIC, levofloxacin demonstrated a reduction of biofilm within every isolate, including the isolate with the highest MIC (L884)
- Despite using two hVISA isolates, vancomycin demonstrated the greatest decrease in biofilm growth compared to the control when using a concentration of 3,000-6,000 times the MIC
- Results from the control group allowed us to classify the isolates as either low (OD<2)or high (OD≥2) biofilm-producers (as shown in the table)
- Vancomycin had an average 50% reduction of biofilm across all isolates and levofloxacin demonstrated a 36% average reduction



CONCLUSIONS

- Following 24-hour antibiotic lock treatment, both vancomycin and levofloxacin failed to eradicate both young (4 and 6 hour) and mature (24 hour) biofilm but inhibited further growth compared to the stability control in stable, mature biofilm
- Due to the instability of the formation of biofilm in the early phases, we question if antibiotic treatment can potentially promote biofilm formation based on our treatment results
- Although we display a decrease of biofilm with levofloxacin, we do highlight that the least amount of reduction resulted from the isolate with the highest MIC (L884) while possibly having its greatest chance of eradication on the young biofilm (4-hr growth)
- Results from this study reinforce the known complications of treating existing biofilm infections and emphasize the importance of utilizing minimum biofilm inhibitory concentrations (MBIC) instead of solely using the MIC to make clinical decisions

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