

# Minimum Biofilm Eradication Concentration of Tobramycin and Vancomycin in Bone and Muscle *In Vitro*

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## Introduction

Bacterial biofilms cause chronic orthopaedic infections. Surgical debridement to remove biofilm can be ineffective without adjuvant local antimicrobials because undetected biofilm fragments may remain in the wound and reestablish the infection if untreated. However, the concentrations and duration of antimicrobial exposure necessary to eradicate bacteria from clinical biofilms remain largely undefined. In this study, we determined the minimum biofilm eradication concentration (MBEC) of tobramycin and vancomycin for bacterial biofilms grown on bone and muscle *in vitro*.

## Research Question

Does the Minimum Biofilm Eradication Concentration (MBEC) of orthopaedic pathogens, both gram(+) and gram(-) organisms, grown on skeletal muscle and bone decrease with increasing exposure time and is their susceptibility different than on previously studied polystyrene (PS) dishes?

## Materials and Methods

- *S. aureus*, *S. epidermidis*, *E. faecalis*, *P. aeruginosa*, and *E. coli* biofilms were established for 72 hr on rabbit muscle and bone specimens *in vitro*
- Biofilms were characterized by SEM imaging and CFU counts
- Biofilm-covered tissue specimens were exposed to serial log<sub>2</sub> dilutions (4000 to 31.25 µg/mL) of tobramycin, vancomycin, or a 1:1 combination of both drugs (tob/vanc)
- Exposure times were 6, 24, or 72 hrs
- Tissues were subcultured following antimicrobial exposure to determine bacterial survival
- The breakpoint concentration with no surviving bacteria was defined as the MBEC for each pathogen-antimicrobial-exposure time combination

- MBECs decreased with exposure time
- Tobramycin/vancomycin (1:1) was the most effective antimicrobial regimen
- Tob/vanc MBEC's of 10/10 pathogens on muscle and 7/10 pathogens on bone were between 100-750 µg/mL at 24 or 72 hr exposure
- MBECs on bone were significantly higher than corresponding MBECs on muscle tissue (p < 0.05)

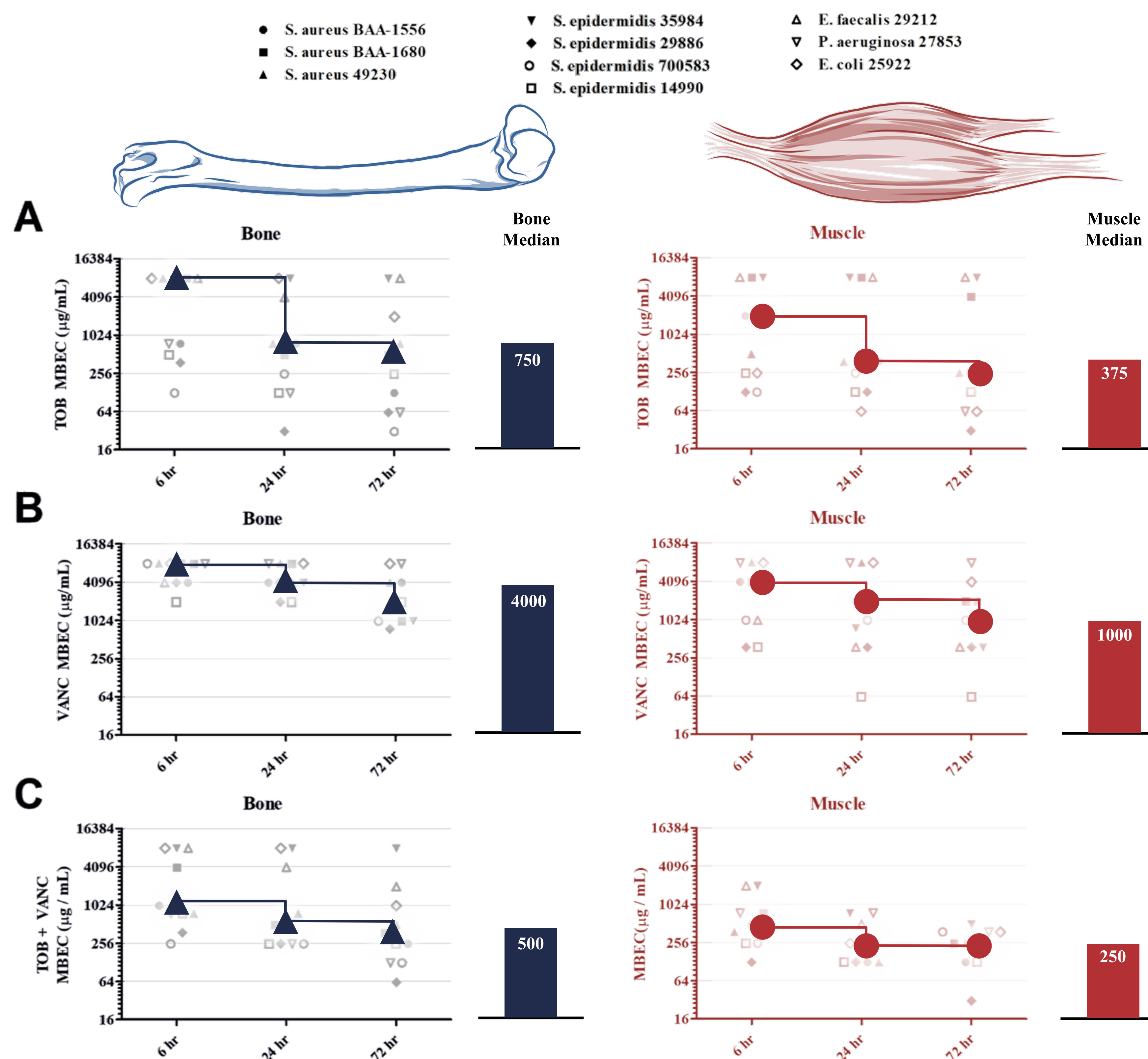


Figure 1: MBEC for 10 musculoskeletal biofilms on bone (left column) and muscle (right column) tissue treated with (A) tobramycin, (B) vancomycin, or (C) tobramycin/vancomycin combination for 6 to 72 hr. Median values emphasized

## Conclusion

The majority of MBECs for orthopaedic infections on bone and muscle are on the order of 100-750 µg/mL of vancomycin+tobramycin when sustained for at least 24 hr, which may be clinically achievable using high-dose antimicrobial-loaded bone cement (ALBC).

## Summary

- The MBECs of orthopaedic pathogens is lower on muscle than bone
- MBECs of both muscle and bone decrease with time
- MBECs of both muscle and bone are lower than on PS

## Results

- All tested pathogens formed biofilm on tissue
- In most cases, tissue MBECs were lower compared to previously published MBECs for the same pathogens on polystyrene tissue-culture plates

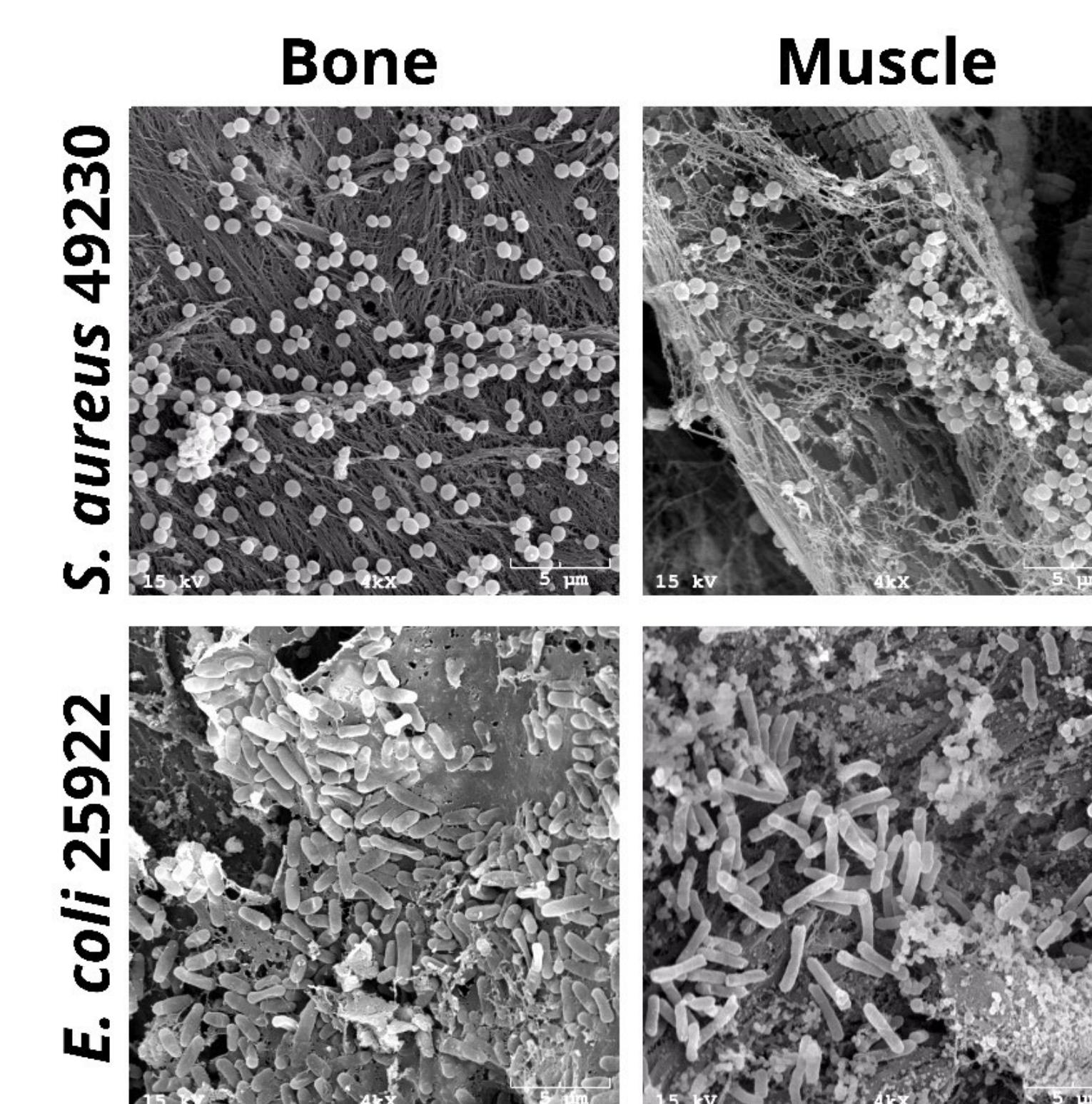


Figure 2: Biofilm characterization with SEM analysis following 72 hr biofilm growth on bone and muscle tissue. SEM images were taken at 4000X magnification and show two representative biofilm strains

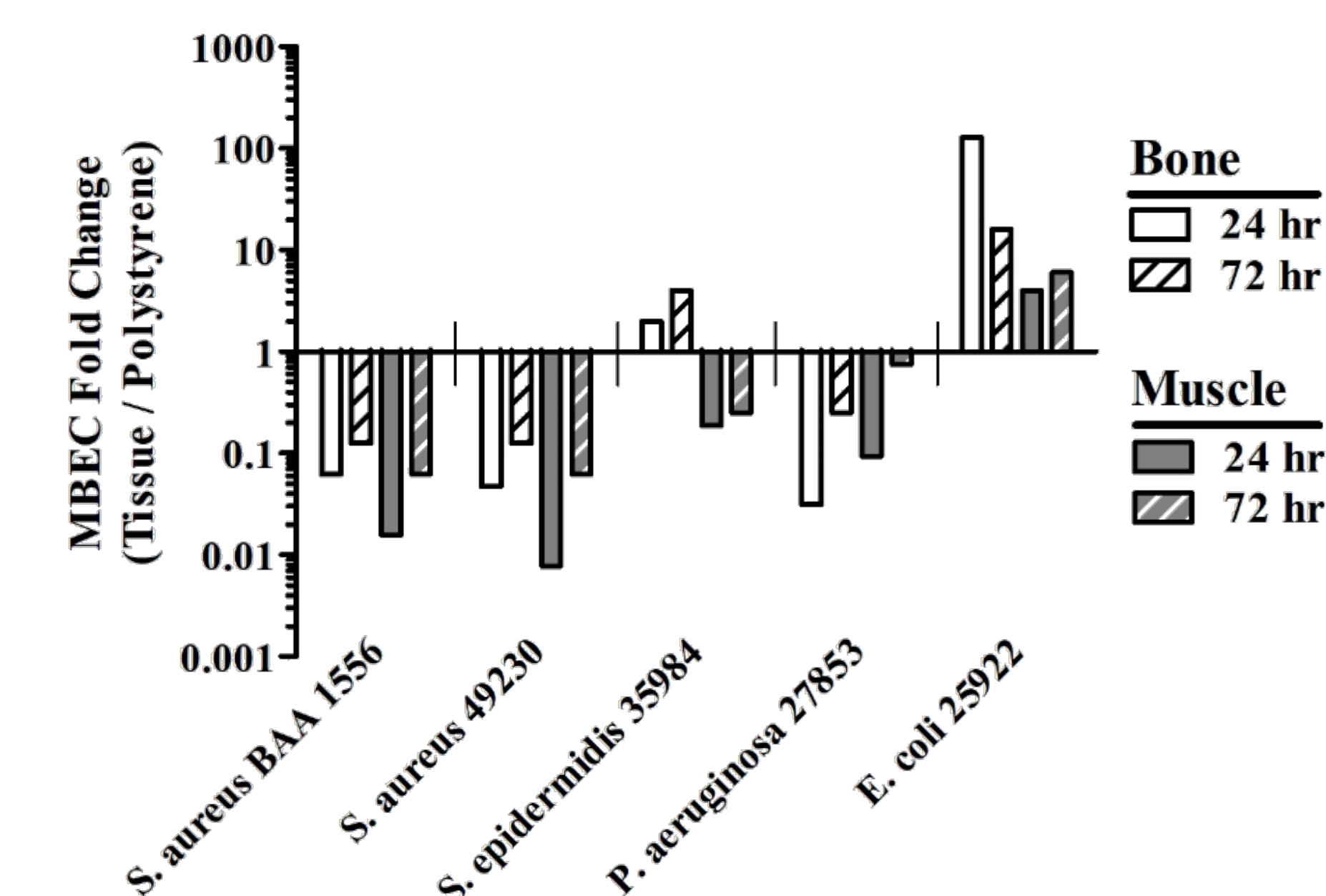


Figure 3: Fold change in MBEC for musculoskeletal biofilms on muscle or bone treated with tobramycin/vancomycin compared to MBEC on polystyrene.

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