

Bone & Joint Research

Supplementary Material

10.1302/2046-3758.133.BJR-2023-0038.R2

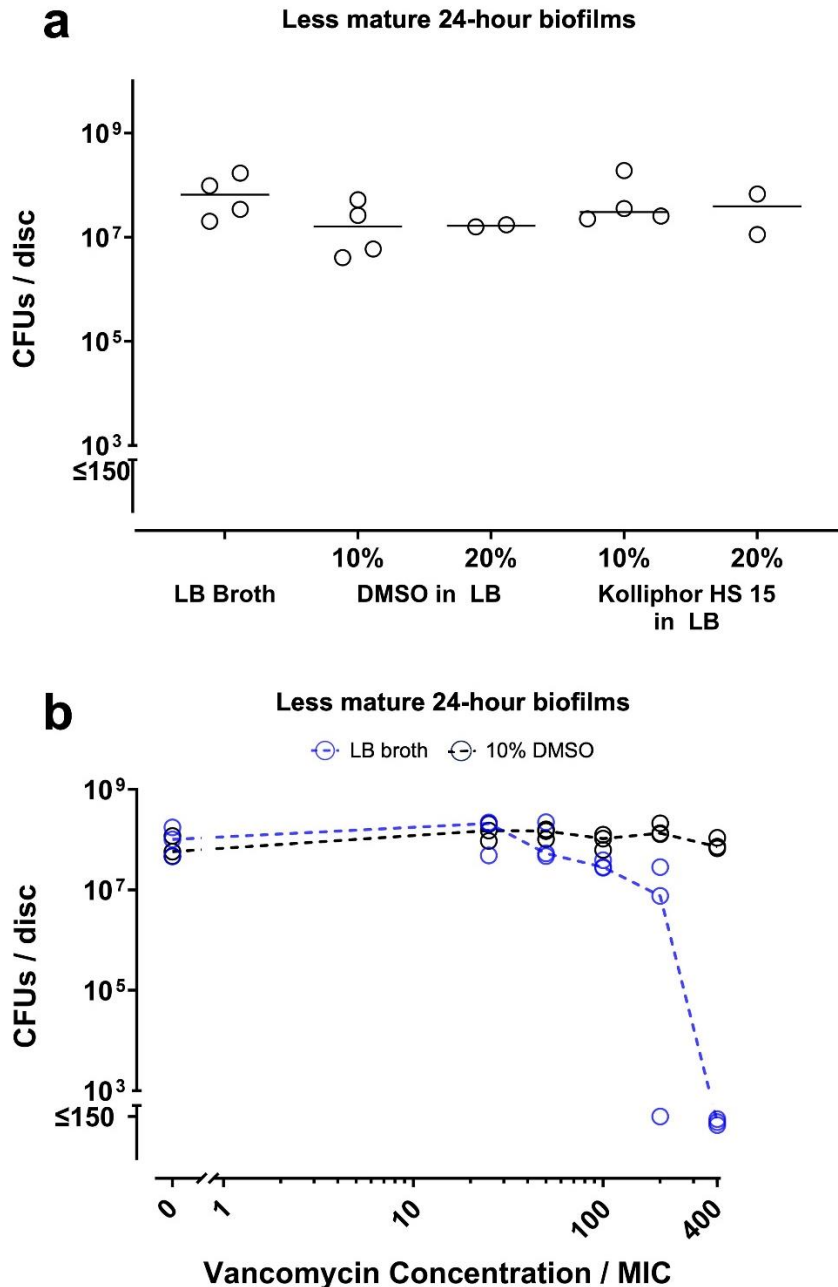


Fig. a. a) Vehicles used in this study alone do not alter bacterial viability in the biofilms. Less mature 24-hour biofilms on Ti6Al4V discs were exposed to the indicated concentrations of dimethyl sulfoxide (DMSO) (red symbols) or Kolliphor HS 15 (green symbols) in LB broth without kanamycin for 20 hours. Effects on biofilm viability were determined by colony-forming unit (CFU) assays. Horizontal lines indicate medians of two to four independent experiments for each group. Each symbol denotes the median for each drug concentration from an independent experiment, with four

Ti6Al4V discs per symbol. b) 10% DMSO inhibits the anti-biofilm activity of vancomycin. Less mature 24-hour biofilms on Ti6Al4V discs were exposed to the indicated concentrations of vancomycin in LB broth either without (blue symbols) or with 10% DMSO (black symbols) for 20 hours. Effects on biofilm viability were determined by CFU assays. Dashed lines connect medians of three independent experiments for each drug concentration. Each symbol denotes the median for each drug concentration from an independent experiment, with four Ti6Al4V discs per symbol. MIC, minimal inhibitory concentration.

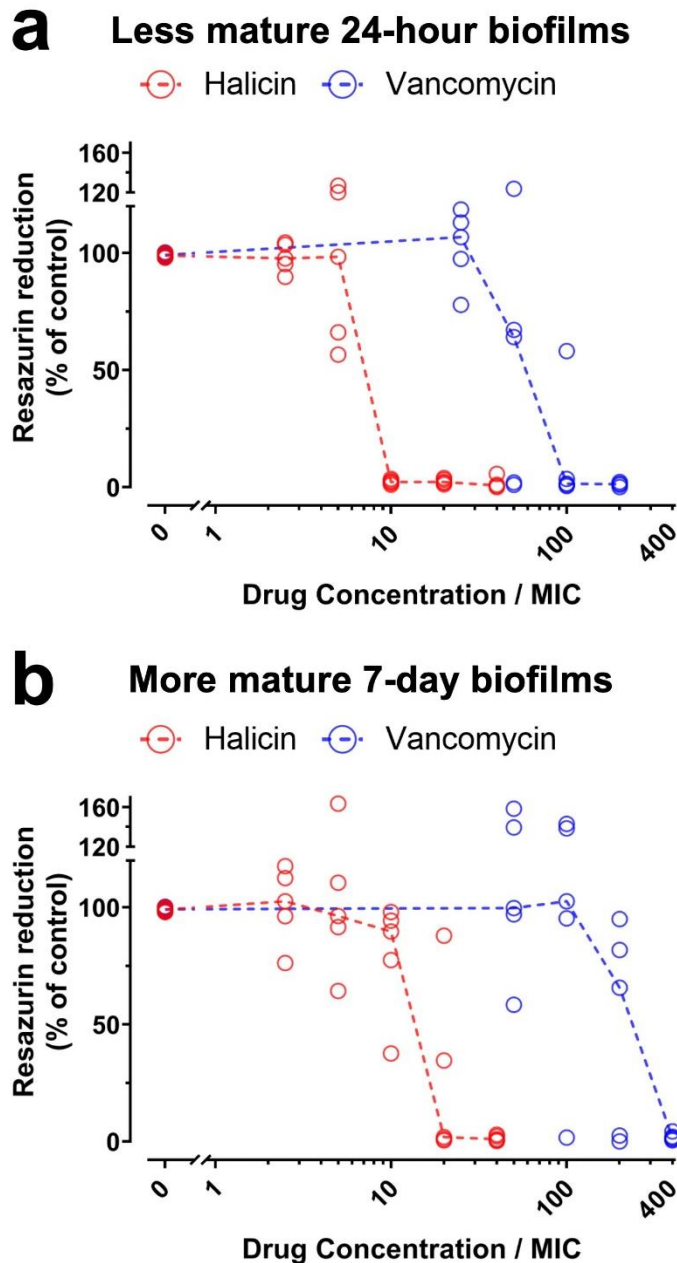


Fig. b. Halicin retains activity against *Staphylococcus aureus*-Xen36 biofilms grown on Ti6Al4V discs. a) Less mature 24-hour biofilms and b) more mature seven-day biofilms on Ti6Al4V discs were exposed to the indicated concentrations of halicin (red symbols) or vancomycin (blue symbols) for 20 hours. Effects on biofilm viability were determined by resazurin reduction assays. Dashed lines connect medians of five independent experiments for each drug concentration. Each symbol denotes the median for each drug concentration from an independent experiment, with four Ti6Al4V discs per symbol. MIC, minimal inhibitory concentration.

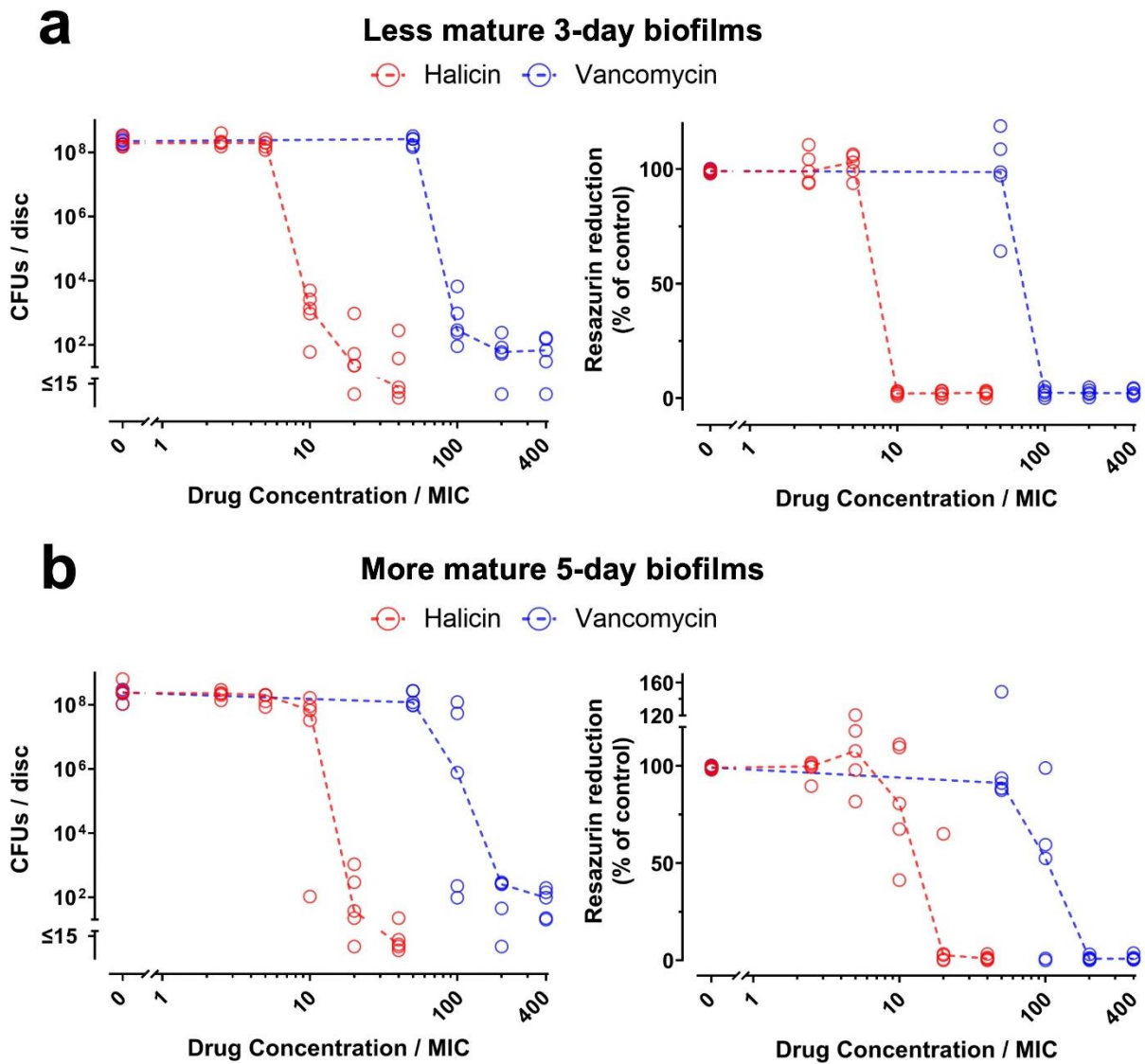
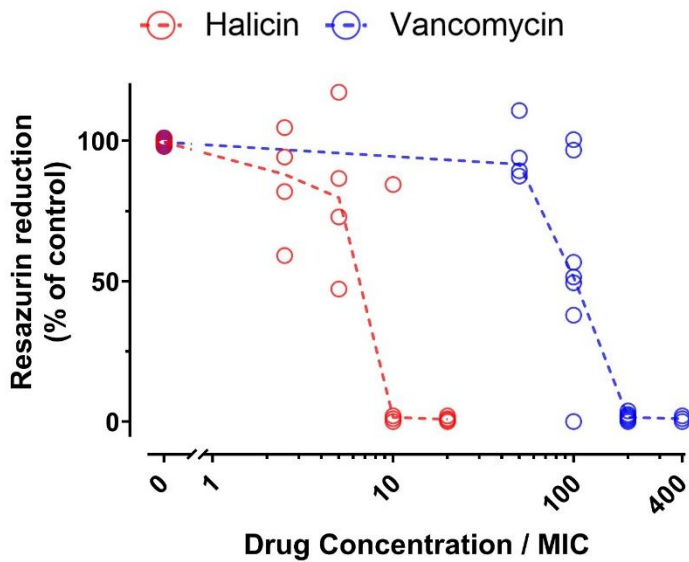


Fig. c. Halicin retains activity against *Staphylococcus aureus*-Xen36 biofilms grown for three or five days on Ti6Al4V discs. a) Less mature 24-hour biofilms and b) more mature seven-day biofilms on Ti6Al4V discs were exposed to the indicated concentrations of halicin (red symbols) or vancomycin (blue symbols) for 20 hours. Effects on biofilm viability were determined by colony-forming unit (CFU) assays (left panels) and resazurin reduction assays (right panels). Dashed lines connect medians of five independent experiments for each drug concentration. Each symbol denotes the median for each drug concentration from an independent experiment, with four Ti6Al4V discs per symbol. MIC, minimal inhibitory concentration.

a Less mature 24-hour biofilms



b More mature 7-day biofilms

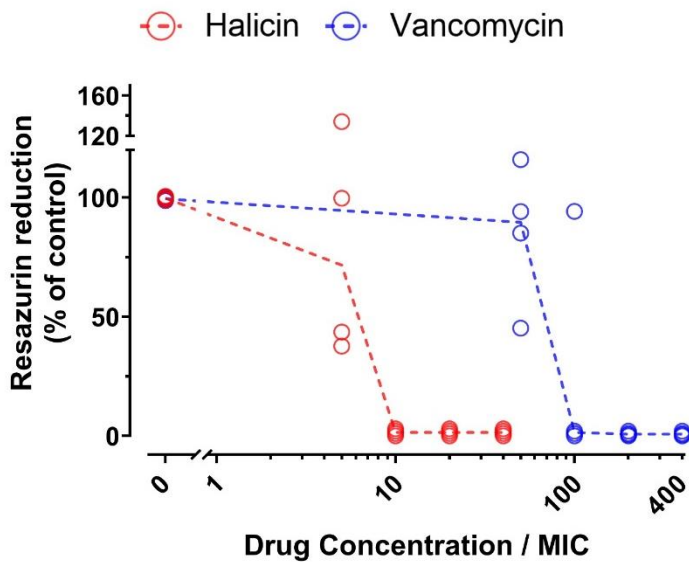


Fig. d. Halicin retains activity against *Staphylococcus aureus*-Xen36 biofilms grown on cobalt-chromium (Co-Cr) discs. a) Less mature 24-hour biofilms and b) more mature seven-day biofilms on Co-Cr discs were exposed to the indicated concentrations of halicin (red symbols) or vancomycin (blue symbols) for 20 hours. Effects on biofilm viability were determined by resazurin reduction assays. Dashed lines connect medians of four to seven independent experiments for each drug concentration. Each symbol denotes the median for each drug concentration from an independent experiment, with two to three Co-Cr discs per symbol. MIC, minimal inhibitory concentration.

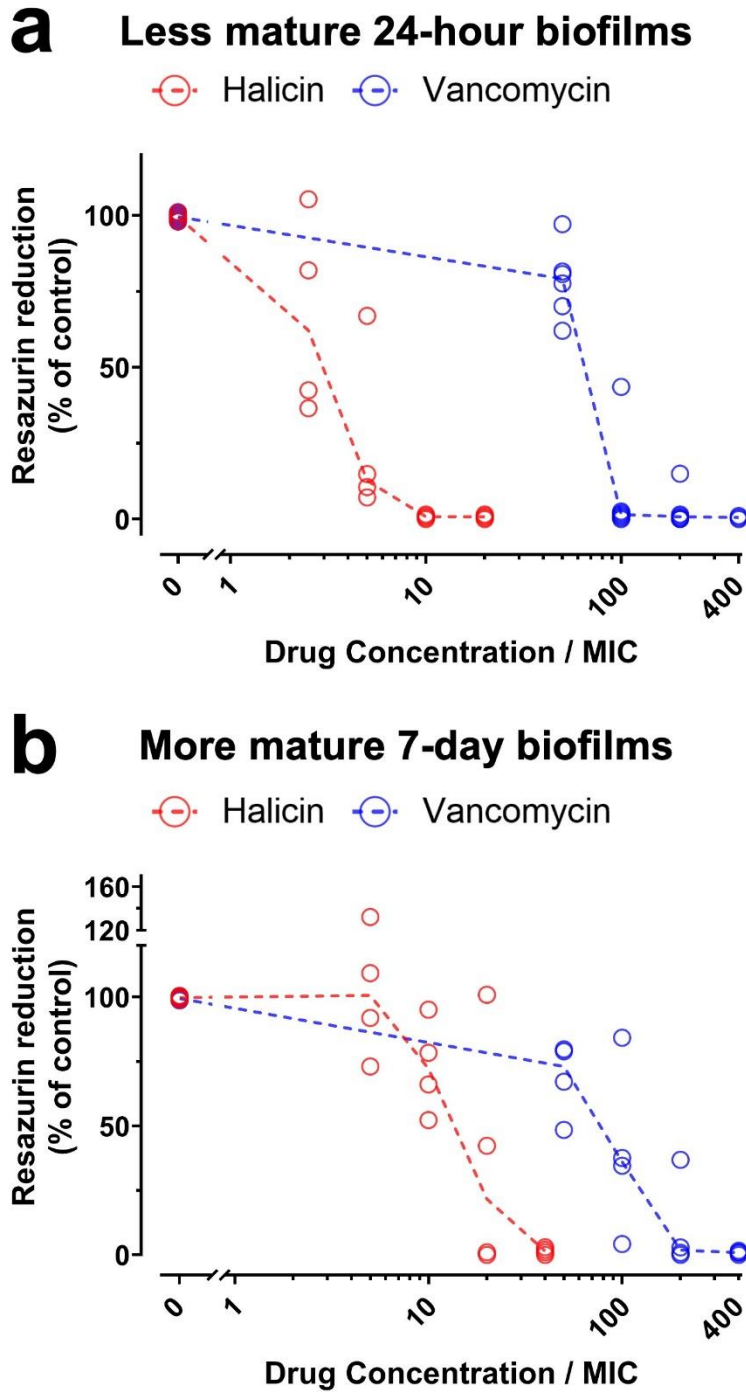


Fig. e. Halicin retains activity against *Staphylococcus aureus*-Xen36 biofilms grown on ultra-high molecular weight polyethylene (UHMWPE) discs. a) Less mature 24-hour biofilms and b) more mature seven-day biofilms on UHMWPE discs were exposed to the indicated concentrations of halicin (red symbols) or vancomycin (blue symbols) for 20 hours. Effects on biofilm viability were determined by resazurin reduction assays. Dashed lines connect medians of four to seven independent experiments for each drug concentration. Each symbol denotes the median for each drug concentration from an independent experiment, with two to three UHMWPE discs per symbol. MIC, minimal inhibitory concentration.

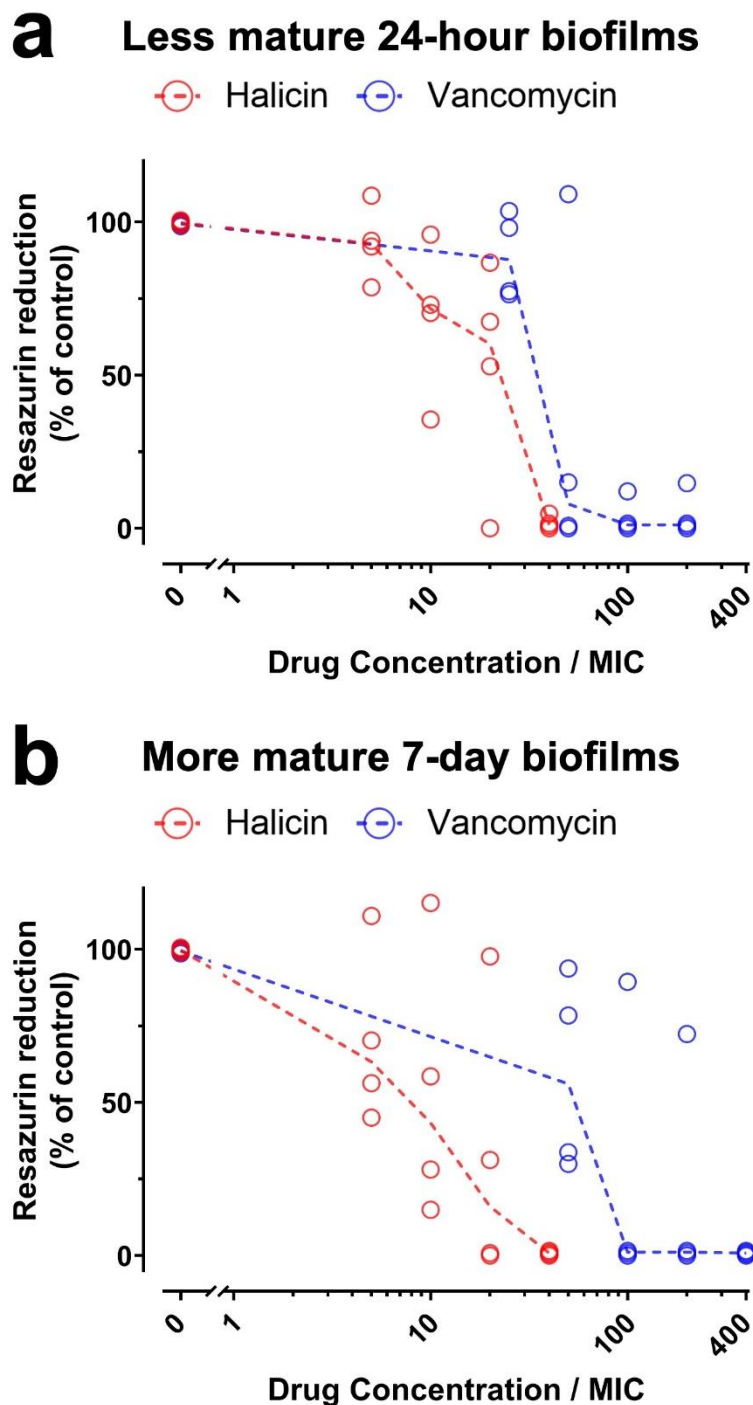
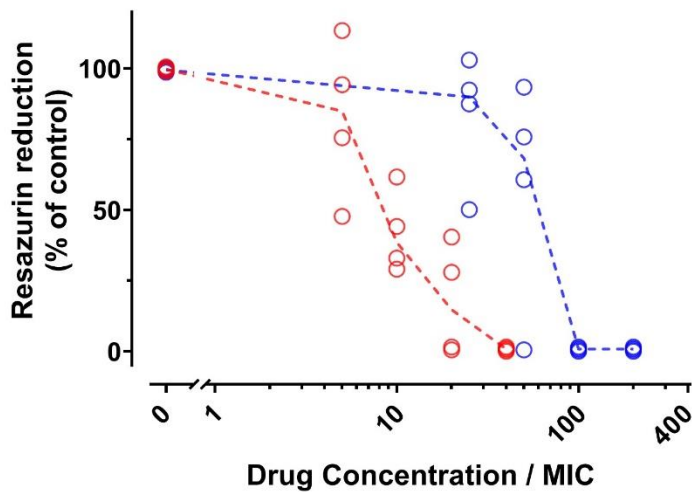


Fig. f. Halicin retains activity against *Staphylococcus aureus*-Xen36 biofilms grown on devitalized muscle. a) Less mature 24-hour biofilms and b) more mature seven-day biofilms on muscle samples were exposed to the indicated concentrations of halicin (red symbols) or vancomycin (blue symbols) for 20 hours. Effects on biofilm viability were determined by resazurin reduction assays. Dashed lines connect medians of four independent experiments for each drug concentration. Each symbol denotes the median for each drug concentration from an independent experiment, with three muscle samples per symbol. MIC, minimal inhibitory concentration.

a Less mature 24-hour biofilms

○ Halicin ○ Vancomycin



b More mature 7-day biofilms

○ Halicin ○ Vancomycin

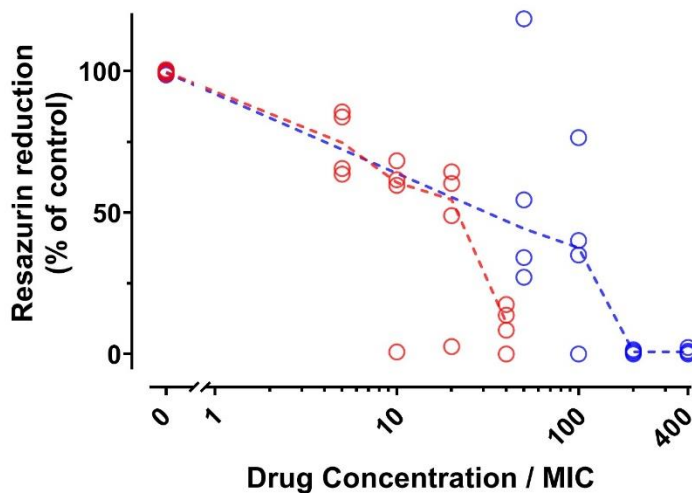


Fig. g. Halicin retains activity against *Staphylococcus aureus*-Xen36 biofilms grown on devitalized cortical bone. a) Less mature 24-hour biofilms and b) more mature seven-day biofilms on bone samples were exposed to the indicated concentrations of halicin (red symbols) or vancomycin (blue symbols) for 20 hours. Effects on biofilm viability were determined by resazurin reduction assays. Dashed lines connect medians of four independent experiments for each drug concentration. Each symbol denotes the median for each drug concentration from an independent experiment, with three bone samples per symbol. MIC, minimal inhibitory concentration.