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Supplementary information

Microtiter plate assays to assess antibiofilm activity against bacteria

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Supplementary Information for:

Microtitre plate assays to assess antibiofilm activity against bacteria

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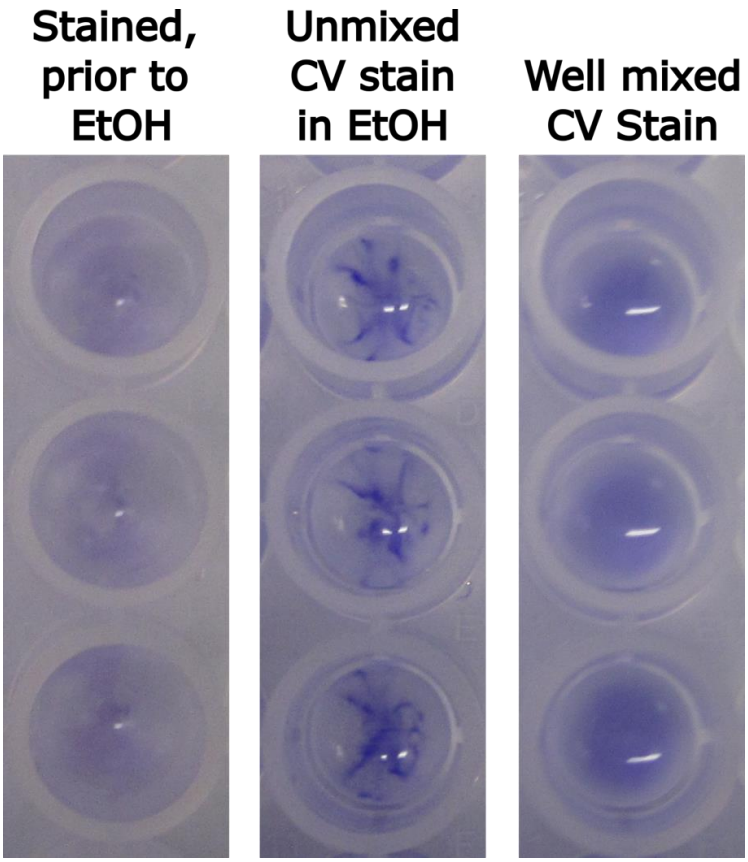
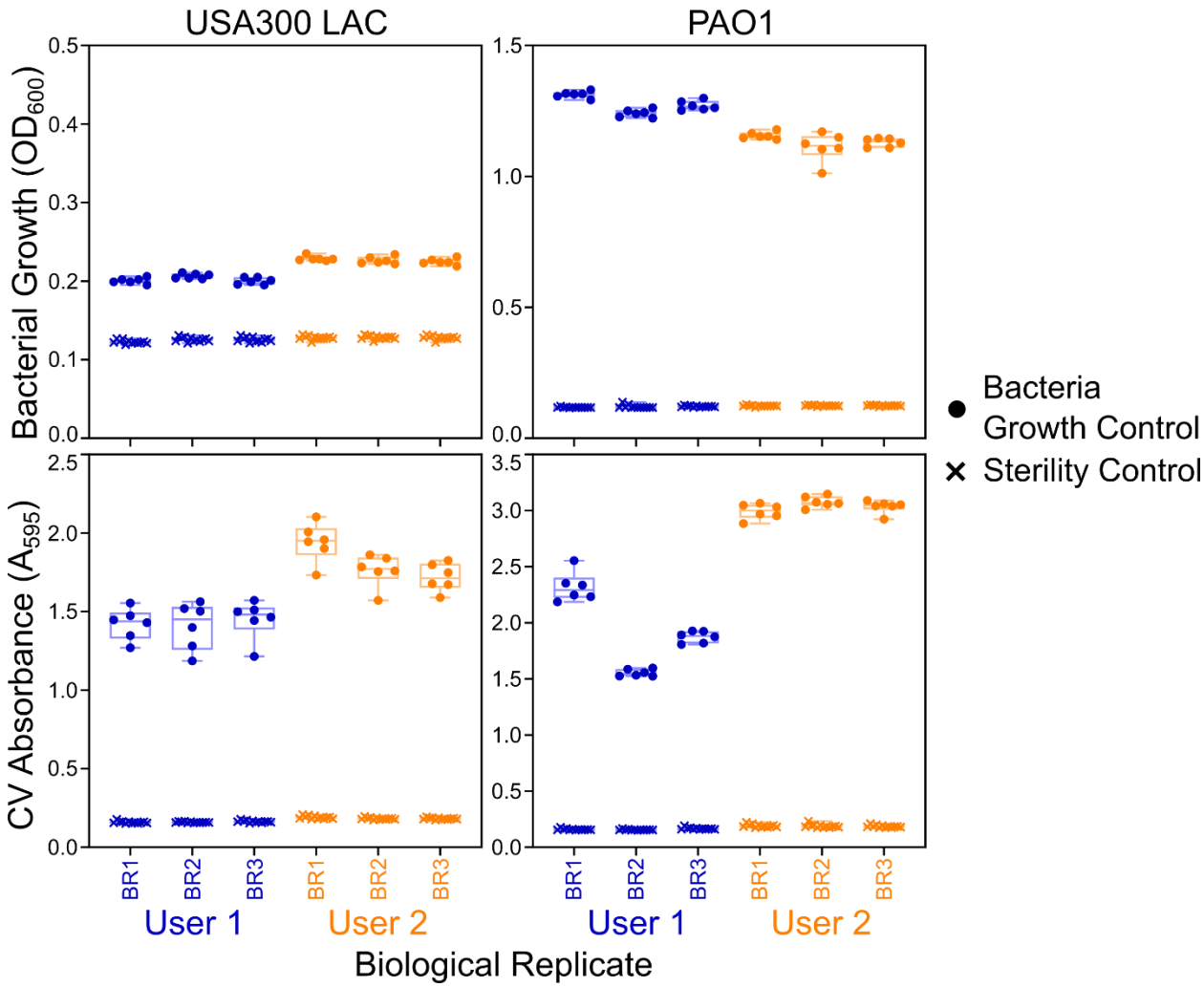
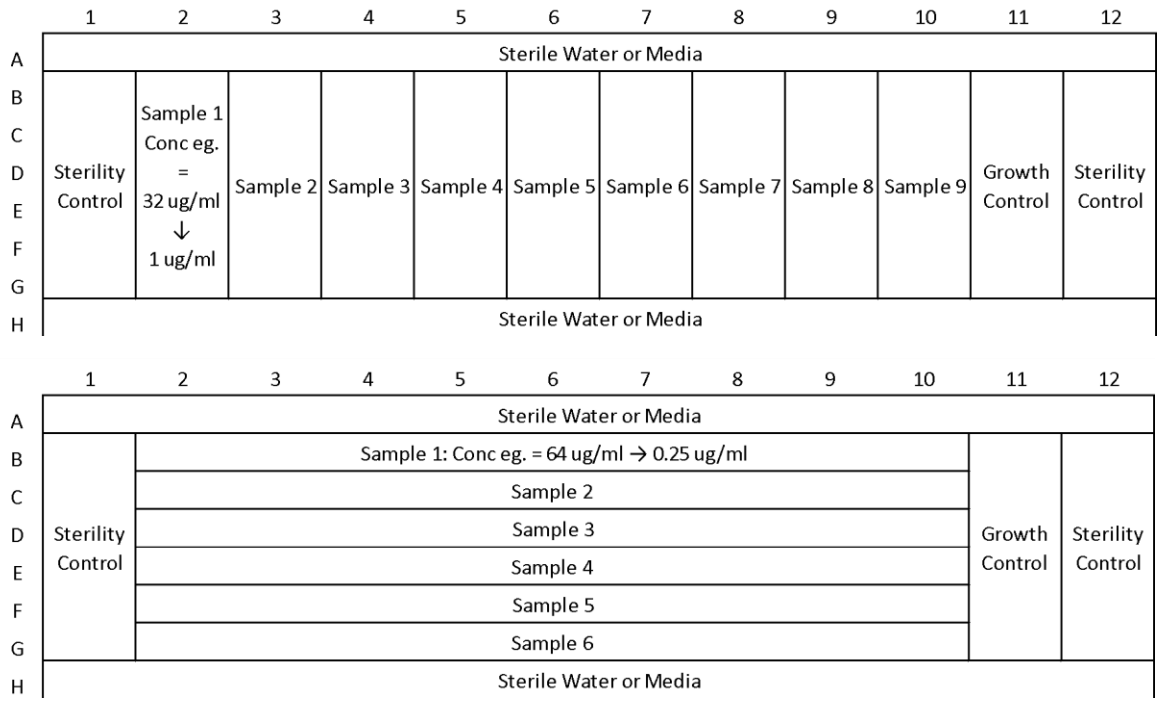


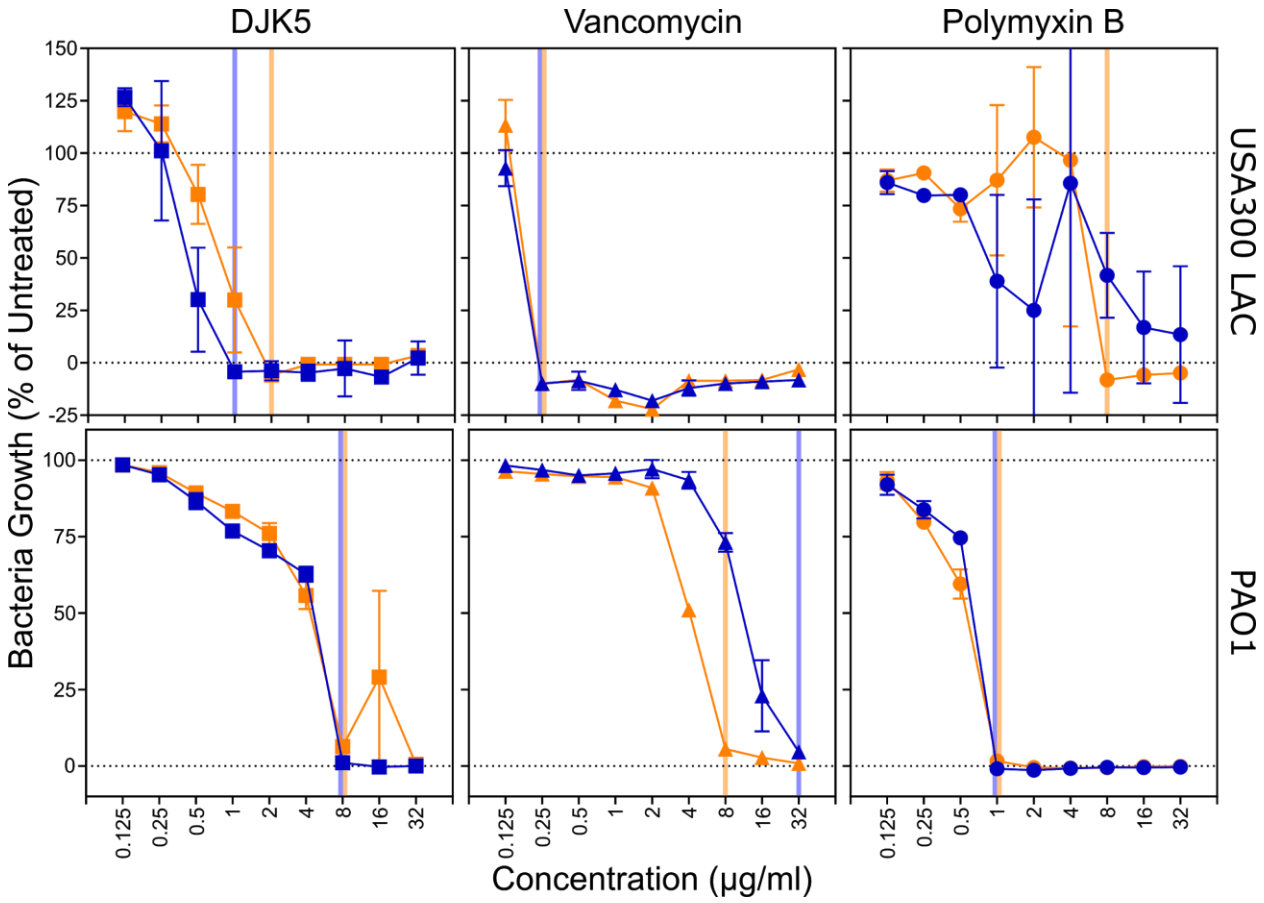
Supplementary Figure S1| Sample crystal violet staining profile of adhered *S. aureus* biofilms in a microtitre plate. The panel on the left shows the stained CV biomass on the surface of the well prior to the addition of 70% ethanol. Once ethanol is added, the CV dye is released from the biofilm but it will be unevenly distributed throughout the liquid in the well (middle panel). Following a 30 min incubation step with gentle shaking, the CV dye is evenly distributed throughout the ethanol in the well (right panel) and the absorbance at 595 nm can be recorded. If after incubation, samples still appear unevenly mixed, then a pipette can be used to evenly distribute the CV dye throughout the well.



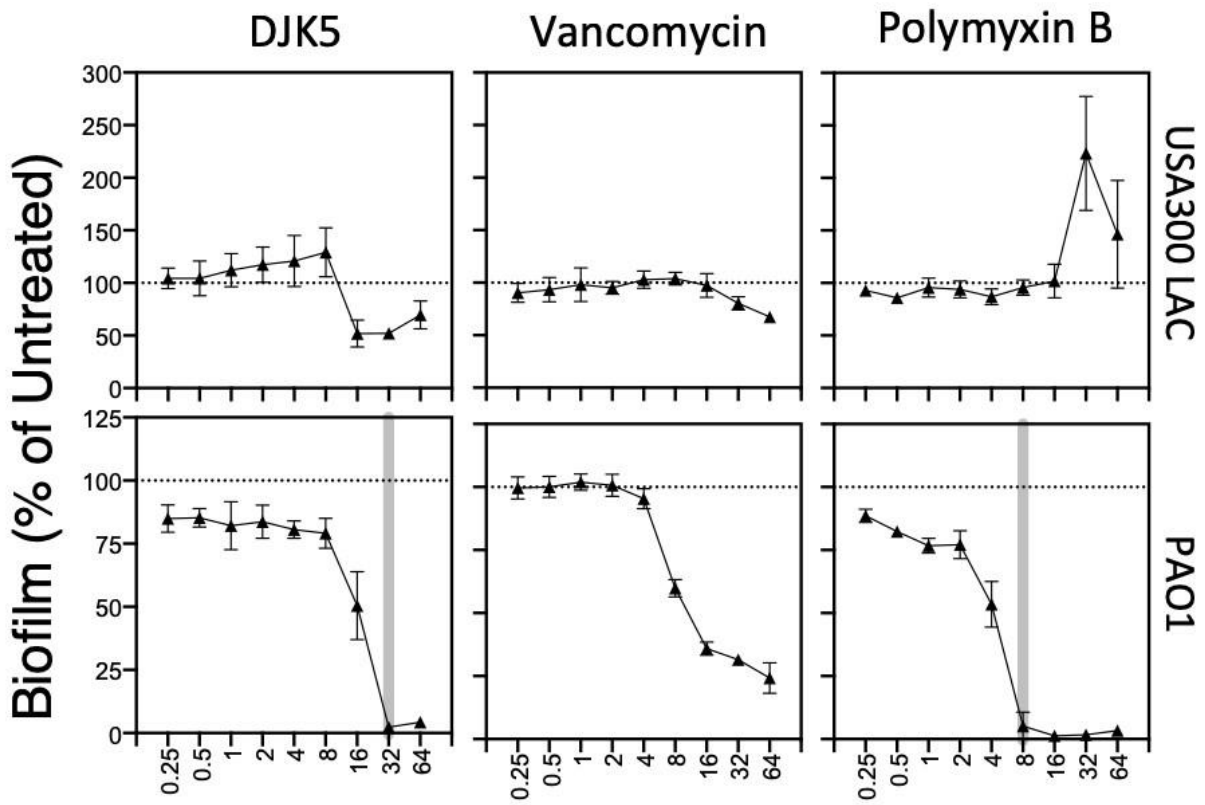
Supplementary Figure S2| Distribution of OD_{600} and A_{595} values for USA300 LAC and PAO1 biofilms grown in polypropylene microtitre plates. USA300 LAC samples were grown in 10% TSB supplemented with 0.1% glucose. PAO1 samples were grown in BM2 minimal media. Results shown are from two different users, indicated in blue and yellow respectively from three separate biological replicates (BRs). Each BR consists of 6 technical repeats for bacteria samples and 12 for sterility controls, consistent with suggested sample layouts shown in Supplementary Figure S3.



Supplementary Figure S3| Sample plate layouts for biofilm inhibition and/or eradication assays. The orientations of the concentration gradients for each sample to be evaluated (either vertically (top) or horizontally (bottom)) will dictate how many compounds can be evaluated in each plate for their antibiofilm activity in the microtitre plate. Wells along the edge of the microtitre plate should be avoided for measuring biofilm growth as some bacteria under certain conditions have been observed to yield variable crystal violet (CV) staining, likely due to increased gas exchange and evaporation of the media within these wells. Fill these wells with sterile media to serve as negative growth controls or sterile water to limit media evaporation from interior wells of the plate. Numerous positive growth control wells should be included to ensure that the biofilm growth under the conditions being evaluated is well defined.



Supplementary Figure S4| Bacterial growth in the presence of peptide/antibiotics for the biofilm inhibition assays (see Figure 2). The vertical blue and yellow bars correspond to the concentration resulting in greater than 90% reduction in bacterial growth (based on OD_{600} readings) determined for each user. They are identical to the vertical bars in the corresponding panels in Figure 2. Data shown are from two different testers (indicated in blue and gold respectively) and data points represent the average (± SD) of three independent biological replicates.

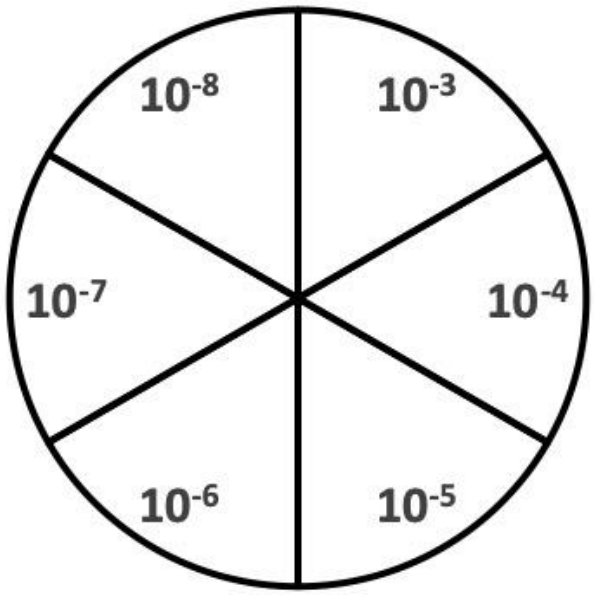


Supplementary Figure S5| Bacterial growth in the presence of peptide/antibiotics for the biofilm eradication assays (See Figure 3). The vertical gray bar corresponds to the concentration resulting in greater than 90% reduction in bacterial growth and is identical to the bars in the corresponding panels in Figure 3.

A

Drug [conc]/ Rows	64 ug/ml	32	16	8	4	2	1	0.5	0.25	Growth
B	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}
C	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}
D	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}
E	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}
F	10^{-7}	10^{-7}	10^{-7}	10^{-7}	10^{-7}	10^{-7}	10^{-7}	10^{-7}	10^{-7}	10^{-7}
G	10^{-8}	10^{-8}	10^{-8}	10^{-8}	10^{-8}	10^{-8}	10^{-8}	10^{-8}	10^{-8}	10^{-8}

B



Supplementary Figure S6| CFU serial dilution scheme and plate layout with corresponding dilutions. Dilutions in 96-well plate (A) are labeled in reference to the final plated dilution. When plating, transfer 10 µl from a well in the microtitre plate to the corresponding well on an LB-agar plate (B) and spread using a sterile loop.