Supplementary Materials: Alpha-Toxin Contributes to Biofilm Formation among Staphylococcus aureus Wound Isolates

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Figure S1. Total CFU recovered from explants infected with different S. aureus isolates were similar. Explants were infected and cultured for 72 h then transferred to 1.7 mL snap cap tubes containing 250 µl of PBS for CFU recovery. Tubes were vortex mixed for 4 min on medium-high setting, serially diluted in PBS and plated on TSA-B to enumerate CFU. All isolates grew up to a similar density, near $1 \times 10^8 \log_{10}$ CFU/explant.

Figure S2. Less than 1% of exogenous alpha-toxin is recovered. Porcine vaginal mucosa (PVM) explants were treated with 0.1 – 5 µg/explant with wild-type (WT) alpha-toxin (AT) and incubated for 24 h. Explants were vortex mixed and the vortexates analyzed for AT recovery via ELISA. Only ~0.3% of AT was recovered from the higher applied doses. Data (n=3) are represented as mean ± SD; * denotes significance from non-AT treated control explants; p<0.05 as determined by one-way analysis of variance followed by Dunnett’s multiple comparisons post-test.
Figure. S3. Recovery of biofilm CFU is similar when explants are vortex mixed or sonicated followed by vortex mixing. Explants were infected with *S. aureus* and cultured for 72 h then transferred to 1.7 mL snap cap tubes containing 250 µl of PBS for CFU recovery. Tubes were either vortex mixed for 4 min on medium-high setting or sonicated in a water bath for 5 min followed by vortex mixing for 4 min on medium-high setting. No statistical difference was observed, n=4, p=0.19.