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CF-301, a Phage Lysin, is a Potent Eradicator of *Staphylococcus aureus* Biofilms

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INTRODUCTION

Bacteriophage lysins are enzymes that degrade bacterial peptidoglycans. Lysin CF-301 is being developed to treat *S. aureus* (including methicillin-resistant *S. aureus* [MRSA]) because of its potent antibacterial effects, activity on drug-resistant strains, low resistance profile, and synergistic activity with antibiotics. These features of CF-301 make it an attractive candidate for antimicrobial development.

This poster presents in vitro data characterizing the anti-biofilm properties of CF-301 when tested on biofilms attached to polystyrene and catheter surfaces.

METHODS

Strain. MRSA strain ATCC BAA-42 was used in all experiments in the Figures. The MICs (μg/ml) are CF-301 (32), daptomycin (1), vancomycin (1), and linezolid (1).

Growth and treatment of bioflims. Biofilms were grown on polystyrene plates (1 day) or in the lumens of di(2-ethylhexyl)phthalate (DEHP) catheters (3 days). All washes were with PBS. Treatments were at 37ºC in MHB or CAMHB media (Figure 1) or Lactated Ringers buffer (all other experiments).

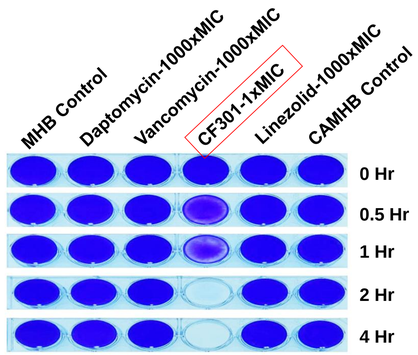
CFU determination. Catheters were washed and treated with 0.1 mg/ml lysostaphin to lyse cells, and the ATP content in the supernatant was determined by a luciferase assay (Promega). Conversion to CFU was made by comparing luminescence units to a standard curve of luminescence units vs CFU calibrated using known amounts of bacteria.

Table 1. MIC_{90} and BEC_{90} Determinations for Staphylococci and Streptococci

type	n	MIC_{90}	Range	BEC_{90}	Range
MSSA	45	32	2-32	1	0.125-4
MRSA	50	32	2-32	0.5	0.125-1
GrAS	27	4	0.5-32	0.06	0.03-1
GrBS	18	8	2-32	0.5	0.03-1

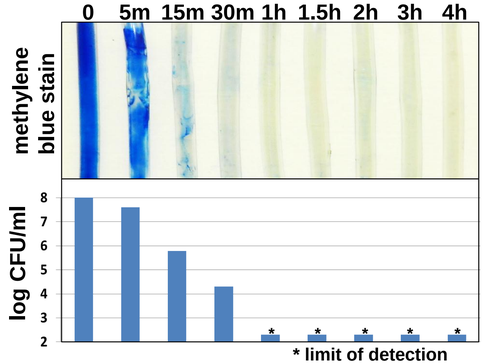
MIC values were determined using CLSI protocols (in the absence of reducing agent). BEC (biomass eradication concentration) values were determined by the microtiter plate assay [Merritt et al. Curr Prot in Microbiology 1B.1.1-1B.1.18, Aug 2011]. After 1 day growth in polystyrene 96-well plates, biofilms were washed, treated for 24 hr, washed, and developed using crystal violet followed by extraction and OD determination. The BEC cutoff criteria was ≥75% biomass removal.

Figure 1. CF-301 Removes *S. aureus* Biofilms to a Greater Extent and More Rapidly than Antibiotics



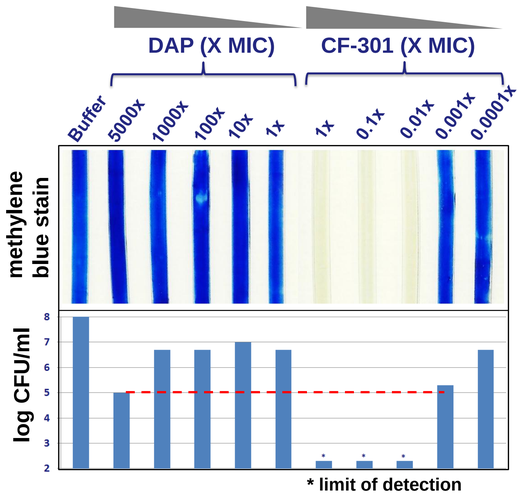
Biofilms in 24-well polystyrene plates were washed and treated as indicated. At time intervals, wells were washed and stained with crystal violet stain to visualize biofilms.

Figure 2. CF-301 Rapidly Removes Biofilm Biomass and Viable Bacteria from Catheter Surfaces



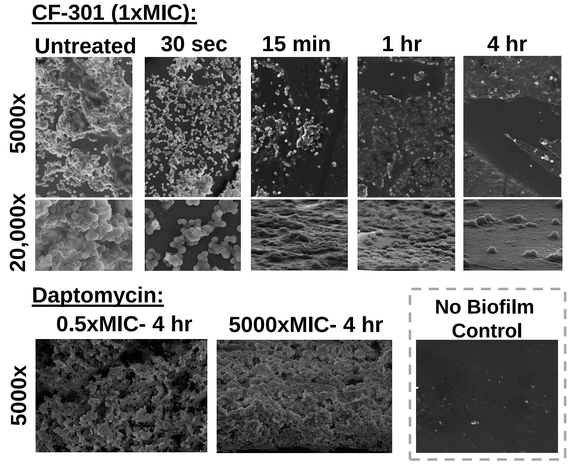
Biofilms were grown in catheter lumens, washed, and treated with 1xMIC CF-301. At indicated times, catheters were washed and residual biomass (methylene blue staining) and CFU/ml were determined.

Figure 3. CF-301 is Over a Million-Fold More Effective at Removing Viable Bacteria From Biofilm-Infected Catheters Compared to Daptomycin



Biofilms were grown in catheter lumens, washed, and treated with either daptomycin or CF-301. After 4 hr of treatment, catheters were washed an residual biomass and CFU/ml were determined. The dotted red line indicates the DAP and CF-301 levels needed for equivalent CFU removal.

Figure 4. Scanning Electron Microscopy (SEM) Analysis of Treated Biofilms



Biofilms were grown in catheter lumens in TSB+0.2% glucose, washed, and treated with either CF-301 or daptomycin. At the indicated times, samples were washed, fixed, and examined by SEM.

Lysin Killing Bacteria

Lysin Mechanism of Action

RESULTS

CF-301 rapidly and extensively removes biomass from polystyrene surfaces, compared to antibiotics

- Within 2 hours, CF-301 at 1xMIC removed all visibly staining biomass while antibiotics removed no biomass at 4 hours, even at 1000xMIC (**Figure 1**).
- CF-301 BEC_{90} values are 6 to 64 times lower than the corresponding MIC_{90} values (**Table 1**).

CF-301 rapidly and extensively removes biomass from catheter surfaces, compared to antibiotics

- CF-301 at 1xMIC removed all visible biomass and all detectable CFU from biofilm-infected catheters within 1 hour (**Figure 2**).
- CF-301 at 0.01xMIC removed all visible biomass and all detectable CFU within 4 hours (**Figure 3**).
- Compared to CF-301, 5 million-fold more daptomycin is required to achieve an equivalent amount of CFU removal (as measured in fold-MIC units) (**Figure 3**).

CF-301 action on biofilms is characterized by very rapid removal of an extracellular biomass followed by rapid cellular degradation

- The time-course of CF-301-mediated biomass removal seen with SEM (**Figure 4**) closely correlates with the kinetics of biomass removal as monitored by biomass staining and CFU counts (**Figure 2**).
- The most CF-301-sensitive biomass component appears to be the extracellular glycocalyx (see **Figure 4** – Untreated versus 30 sec). By the 15 min time-point, *S. aureus* cells are clearly degraded.

CONCLUSIONS

CF-301 is a potent and rapid-acting eradicator of *S. aureus* biofilms formed on abiotic surfaces including catheter tubing.

ACKNOWLEDGEMENTS

We acknowledge Berice Ruisi for help in poster preparations and Nadine Soplop and Kunihiro Uryo (Electron Microscopy Resource Center at The Rockefeller University) for support with electron microscopy.
Disclosures: VAF is a consultant to ContraFect Corporation.