F-647

CF-301, a Phage Lysin, Demonstrates Rapid Bactericidal and Synergistic Activity Against *Staphylococcus aureus*

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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. CF-301 also demonstrates activity on drug-resistant strains, has a low resistance profile, and eradicates biofilms.

Lysin Killing Bacteria	Lysin Mechanism of Action

This poster uses time kill curves and microscopy to demonstrate that CF-301 is bactericidal against *S. aureus*, and is distinguished from standard-of-care (SOC) antibiotics by the rapidity of its bactericidal activity. CF-301 is also shown to synergize with SOC antibiotics – at sub-minimum inhibitory concentrations (MICs) – against contemporary MRSA and methicillin-sensitive *S. aureus* (MSSA) clinical isolates. Finally, evidence is presented that suggests CF-301 can accelerate antibiotic binding to the cell envelope.

METHODS

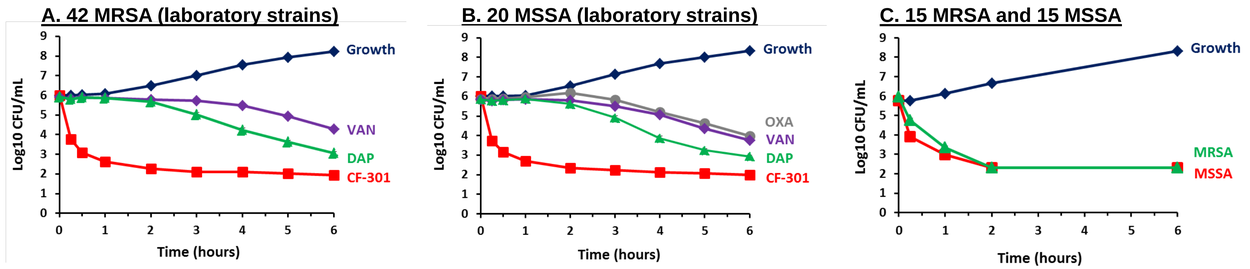
Time-kill Assays. CLSI methods were used. Briefly, CAMHB cultures (5x10^5 cells/ml) were treated with CF-301 and/or antibiotics and survivors were enumerated at indicated time points. Bactericidal activity was defined as a decrease of ≥3 log10 CFU/mL relative to the initial inoculum. Contemporary clinical isolates were obtained from JMI Laboratories. All other strains were from ATCC.

Electron Microscopy. MRSA strain MW2 was suspended in PBS, treated for 15 sec with and without CF-301 (8 µg/ml), fixed, stained and visualized by transmission electron microscopy (TEM).

Daptomycin binding. Daptomycin-resistant *S. aureus* strain CFS 955 was suspended in CAMHB containing BODIPY-FL-daptomycin (DAP; 4 µg/ml) with and without CF-301 (1 µg/ml) for 15 min. Cells were fixed, washed, stained with Marina Blue-wheat germ agglutinin (WGA; 2 µg/ml), and visualized by fluorescence microscopy.

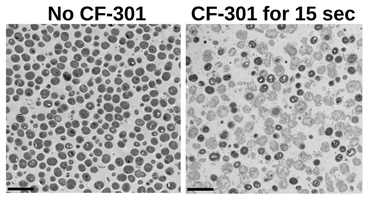
Vancomycin binding. Strain MW2 was pre-incubated with vancomycin (VAN; 30 µg/ml) for 30 min, washed, and suspended in CAMHB containing BODIPY-FL-VAN (0.25 µg/ml) with and without CF-301 (1 µg/ml). At time points, cells were fixed, stained with Alexa Fluor 350-WGA (2 µg/mL), and visualized by confocal microscopy.

Figure 1. CF-301 rapidly kills *S. aureus* in vitro.



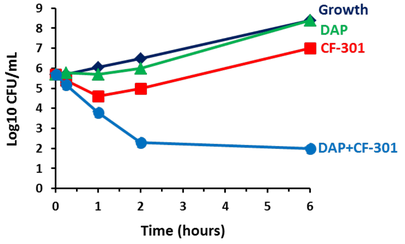
[A,B] Time-kill analysis of CF-301 compared to growth control, oxacillin (OXA), VAN, and DAP. Curves are composite results from 42 MRSA and 20 MSSA strains. Drug concentrations are strain-specific MICs and mean values (±SEM) are shown; **[C]** Composite curves for CF-301 against contemporary clinical *S. aureus* isolates (15 MRSA and 15 MSSA).

Figure 2. CF-301 causes rapid lysis.



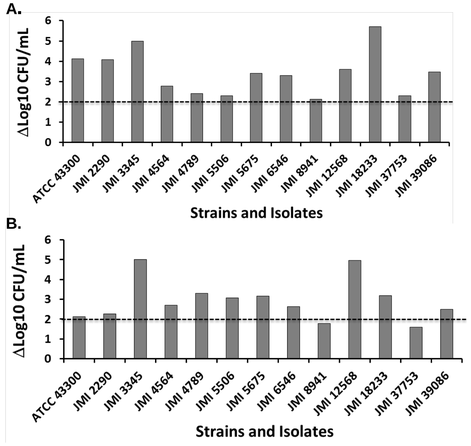
Lysis causes loss of darkly stained cytoplasm. Scale bars are 2 µm.

Figure 3. CF-301 synergizes with DAP.



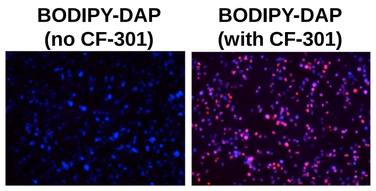
MRSA JMI 3345 treated with buffer (growth), sub-MIC CF-301 (1 µg/ml, ¼ MIC) or DAP (0.25 µg/ml, ½ MIC), or CF-301 and DAP combination. A >2-log10 difference between single-drug and combination indicates synergy.

Figure 4. Multi-strain analysis of synergy.



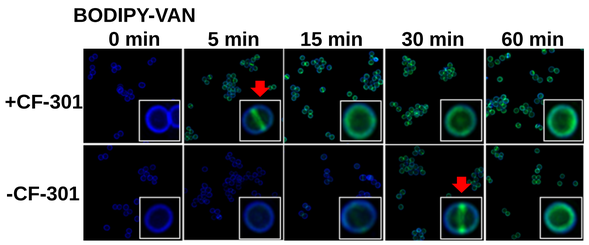
Log changes in CFU/ml between combination and single-drug treatments for 13 MRSA **(A)** and 13 MSSA **(B)**. Dotted lines indicate >2-log10 difference (cutoff value for synergy) between most active single drug and combination.

Figure 5. CF-301 promotes DAP binding.



CFS 955 labeled with BODIPY-DAP (1/4 MIC) for 15 min with and without CF-301 (1/32 MIC). Red: BODIPY-DAP; Blue: WGA.

Figure 6. CF-301 promotes rapid VAN binding.



MW2 labeled with BODIPY-VAN (1/4 MIC) over 60 min with and without CF-301 (1/32 MIC). Green: BODIPY-DAP; Blue: WGA. Arrows show equivalence.

RESULTS

CF-301 exerts a rapid bactericidal effect in vitro, compared to SOC antibiotics:

- CF-301 was bactericidal against 92 *S. aureus* strains (**Figure 1**), reaching 99.9% killing in 30 min against all strains tested. SOC antibiotics required at least 6 hr.
- In TEMs, CF-301 was bacteriolytic upon contact with *S. aureus* (**Figure 2**).

CF-301 synergizes with SOC antibiotics in vitro:

- Time-kill curves show synergy between CF-301 and DAP against 88.2% of MSSA and 100% of MRSA strains tested (**Figures 3 and 4**).
- Time-kill curves show synergy between CF-301 and VAN against 93.7% of MSSA and 84.6% of MRSA and between CF-301 and OXA against 93.7% of MSSA (data not shown).
- For all synergistic combinations, CF-301 and antibiotic were in sub-MIC ranges.

CF-301 accelerates antibiotic binding to cells:

- In the presence of sub-MIC CF-301, BODIPY-DAP stained *S. aureus* within 15 minutes, whereas without CF-301, no staining was observed (**Figure 5**).
- In the presence of sub-MIC CF-301, BODIPY-VAN stained *S. aureus* within 5 minutes, whereas without CF-301, staining required 30 minutes (**Figure 6**).

CONCLUSIONS

CF-301 has a potent bacteriolytic activity and is distinguished from antibiotics by its very rapid action. CF-301 also synergizes with SOC antibiotics – at sub-MIC levels – against clinical MSSA and MRSA isolates. A mechanism for synergy is hypothesized, whereby CF-301 cleaves peptidoglycan, resulting in a permeable structure that enables more rapid antibiotic penetration. Overall, our findings suggest that combinations of CF-301 and antibiotics will provide potent alternatives for treating *S. aureus* infections.

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