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CF-301, a Phage Lysin, is a Potent Antimicrobial Agent Against *Staphylococcus aureus*

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INTRODUCTION

Bacteriophage lysins are enzymes that degrade bacterial peptidoglycans. Lysin CF-30 is being developed as an antimicrobial to treat blood stream infections due to *S. aureus* (methicillin-sensitive and -resistant *S. aureus* [MSSA and MRSA]) because of its potent, specific, and rapid bacteriolytic effects. CF-301 also demonstrates activity on drug-resistant strains, has a low resistance profile, and eradicates biofilms. The various features of CF-301 make it an attractive candidate for antimicrobial development.

Lysin Killing Bacteria	Lysin Mechanism of Action

In this poster we show that CF-301 is a potent antimicrobial agent against a wide range of contemporary clinical *S. aureus* isolates including antibiotic-resistant *S. aureus* strains. We also demonstrate that *S. aureus* resistance to CF-301 in vitro is less than that observed for other antibiotics.

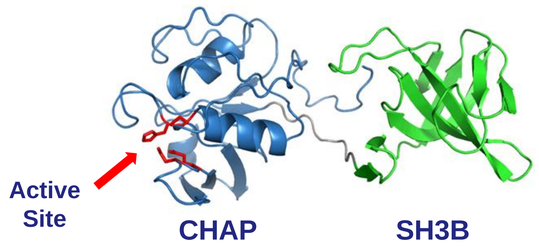
METHODS

Strains. Contemporary clinical *S. aureus* isolates recovered from hospitals throughout the United States in 2011 were obtained from JMI Laboratories, Network on Antimicrobial Resistance in *Staphylococcus aureus*, ATCC, and the CDC.

Testing. MIC for CF-301 and antibiotics (daptomycin [DAP], vancomycin [VAN], and linezolid) were determined in triplicate by broth microdilution using a method described by the CLSI (1). CF-301 was supplemented with 1 mM DL-dithiothreitol, a reducing agent, to remove an Eagle effect to facilitate inter-observer reliability.

Selection for decreased CF-301 or antibiotic susceptibility. Six cultures of *S. aureus* MW2 were serially passaged over 26 days in either CF-301, DAP or VAN. Bacteria were inoculated into CAMHB containing no drug or 1.1-fold serial dilutions of drug. Ranges bracketed the MIC value at each daily time-point. The daily inoculum was taken from the previous day passage; specifically, the well containing the highest concentration supporting growth was the next day's inoculum. Mutants generated at each step were passaged twice in the absence of drug prior to a final MIC analysis.

Figure 1. Lysin CF-301 Homology Model



Lysin CF-301 (a 26 kDa protein) consists of a two domain structure:

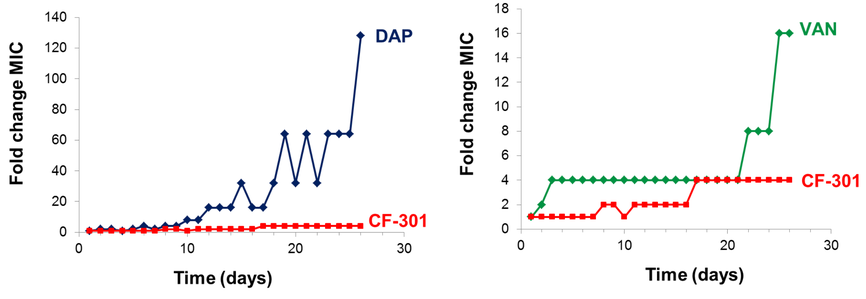
- The N-terminal cysteine-histidine-dependent amidohydrolase/peptidase (CHAP) domain contains the catalytic activity of the lysin
- The C-terminal cell binding domain (SH3B) binds with high affinity to bacterial cell wall-associated substrates essential for bacterial growth & viability

Table 1. MIC_{90} values for CF-301 and antibiotics against *S. aureus*

Strains (n=250)	CF-301 (mw=26,000 Da)		Daptomycin (mw=1,620 Da)		Vancomycin (mw=1,486 Da)		Linezolid (mw=337 Da)	
	µg/mL	µM	µg/mL	µM	µg/mL	µM	µg/mL	µM
MSSA (103)	8	0.31	1	0.62	1	0.67	1	3.0
MRSA (120)	8	0.31	1	0.62	1	0.67	2	5.9
DRSA (8)	4	0.15	16	9.88	1	0.67	2	5.9
VRSA (14)	4	0.15	1	0.62	>16	>10.8	2	5.9
LRSA (5)	2	0.08	1	0.62	1	0.67	>64	>190

The antibiotic resistance profile of each *S. aureus* group is shown (number of strains examined in parentheses). Abbreviations include: DRSA, daptomycin-resistant *S. aureus*; VRSA, vancomycin-resistant *S. aureus*; and LRSA, linezolid-resistant *S. aureus*. The molecular weight (mw) of each drug is shown. MIC_{90} values are tabulated in µg/mL and µM for each strain-drug combination.

Figure 2. Resistance of *S. aureus* to CF-301 is low relative to resistance to antibiotics



Selection for decreased susceptibility to CF-301, DAP, or VAN over the 26 day serial passage experiment. Initial MICs were: CF-301, 8 µg/mL; DAP, 0.5 µg/mL; and VAN, 1 µg/mL. Although six cultures were passaged for each drug, for clarity only one representative replicate is plotted for each drug treatment.

RESULTS

CF-301 was active against 250 different contemporary clinical *S. aureus* isolates and strains including antibiotic-resistant *S. aureus* strains (**Table 1**). CF-301 was very potent against all sampled species with MIC_{90} results ranging from 0.08 µM (LRSA) to 0.31 (MSSA and MRSA).

When cultures of *S. aureus* were subjected to serial passage in the presence of increasing amounts of either CF-301, DAP, or VAN for 26 days (**Figure 2**):

- CF-301 MIC increased from 8 to 32 µg/mL (4X)
- DAP MIC increased from 0.5 to 64 µg/mL (128X)
- VAN MIC increased from 1 to 16 µg/mL (16X)

CONCLUSIONS

In vitro, CF-301 is highly active against 250 contemporaneous clinical *S. aureus* isolates and strains, including antibiotic-resistant types and different genotypes/phenotypes.

On a molar basis, CF-301 activity is equivalent or superior to conventional antibiotics (daptomycin, vancomycin, and linezolid) in MIC assays.

Resistance of *S. aureus* to CF-301 is low relative to resistance to daptomycin or vancomycin

These in vitro data support clinical development of CF-301 for infections caused by *S. aureus* including antibiotic-resistant *S. aureus*.

REFERENCES

1. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Vol. 32. Wayne (PA): Clinical and Laboratory Standards Institute (US), 2012.

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