

# Activity of Antistaphylococcal Lysin CF-301 against Contemporary *Staphylococcus aureus* Clinical Isolates from the USA, Europe and South America

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## ABSTRACT

**Background:** CF-301 is a novel, recombinantly-produced bacteriophage-derived lysin (cell wall hydrolase) and is the first agent of this class in the US to enter into Phase 2 of clinical development for the treatment of bacteremia including endocarditis due to *S. aureus*. Hallmark features of CF-301 include rapid and pathogen-specific bacteriolytic activity, synergy with antibiotics, an extended postantibiotic effect, biofilm-disrupting activity, a low propensity for resistance, and the capacity to suppress antibiotic resistance. This is the first report of an international surveillance study for CF-301.

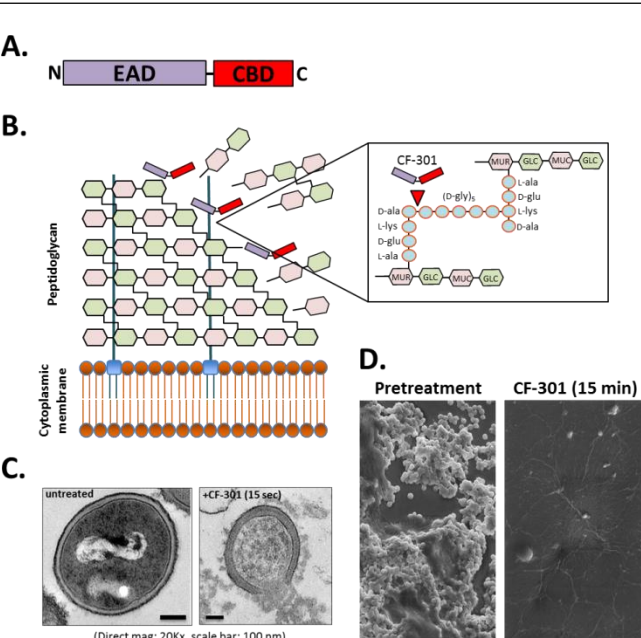
**Methods:** 556 methicillin-sensitive and -resistant *S. aureus* (MSSA and MRSA, respectively) isolates were collected from infection sources at multiple hospitals from 2015-2017 in the US, Greece, Italy, Chile and Columbia. In addition to the contemporary isolates, data is also presented for 149 clinical MSSA and MRSA isolates from US hospital sources obtained in 2011. A set of laboratory stock MSSA and MRSA strains from both CMI and ContraFect were also examined. MICs for CF-301 were determined using a new antimicrobial susceptibility testing (AST) medium for broth microdilution recently endorsed by the CLSI for use with CF-301. The testing medium consists of cation-adjusted Muller Hinton Broth supplemented with 25% horse serum and 0.5 mM DTT (caMHB-HSD). Susceptibility to conventional antibiotics was examined using standard methodology (CLSI document M07-A10).

**Results:** CF-301 had MIC<sub>50</sub>, MIC<sub>90</sub>, and MIC<sub>100</sub> values of 0.5, 1, and 2 µg/mL, respectively, against each set of contemporary MSSA (n=274) and MRSA (n=282) clinical isolates. There were no notable differences with respect to the geographic source of isolates. Furthermore, the CF-301 MICs reported here for 2015-2017 isolates were identical to that observed for MSSA and MRSA isolates from 2011 and lab stock strains.

**Conclusion:** CF-301 demonstrated potent in vitro activity against a total of 556 clinical *S. aureus* isolates from a range of human infections (including bacteremia) and different geographies. Contemporary clinical isolates did not demonstrate reduced susceptibility to CF-301 compared to either 2011 isolates or laboratory stock strains.

## INTRODUCTION

With the increasing worldwide prevalence of antibiotic resistant bacteria, lysins are a promising novel alternative to small molecular antibiotics. Lysins are phage-encoded, recombinantly produced peptidoglycan hydrolases which are rapidly bacteriolytic when applied to target Gram-positive bacteria. Lysin CF-301 is an antistaphylococcal lysin in Phase 2 of clinical development.



Characteristics of lysins including CF-301. **(A)** Lysins have N-terminal enzymatically active domain (EAD) fused to a C-terminal cell wall binding domain (CBD). **(B)** Lysins rapidly degrade cell wall peptidoglycan to trigger lysis. The CF-301-sensitive bond is indicated. **(C)** Bacteriolytic effect of CF-301. **(D)** Removal of catheter biofilm by CF-301.

The major objective of this work was to determine the MIC distribution of CF-301 against contemporary clinical isolates and stock strains of *S. aureus*. The data was collected using a modification of the CLSI method for broth microdilution susceptibility testing (M07-A10). The modification was based on the use of an AST medium, caMHB-HSD, developed specifically for CF-301 and approved for use by the CLSI.

A total of 556 isolates were collected from each of 6 clinical sites in the US, 3 in the EU and 2 in South America. A roughly equal mix of MSSA and MRSA strains were obtained and analyzed. The PBP2a latex test was used to confirm the presence of *mecA* in all MRSA isolates. The MIC distributions of 7 comparator antibiotics were also determined for each isolate. **Table 1** shows CF-301 susceptibility test results the contemporary clinical isolates. **Table 2** shows the MIC frequency distributions for contemporary isolates from different geographical locations (and different resistance types) and provides a comparison to surveillance data obtained in a previous analysis of North American clinical isolates from 2011.

**Table 1.** CF-301 susceptibility test of contemporary clinical *S. aureus* isolates (2015-2017)<sup>1</sup>

Country	State	Type	N	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	
USA	California	MSSA	25	0.5	1	0.5-1	
		MRSA	25	0.5	1	0.25-1	
	New York	MSSA	24	0.5	1	0.5-1	
		MRSA	26	0.5	1	0.5-1	
	Minnesota	MSSA	25	0.5	1	0.5-1	
		MRSA	25	0.5	1	0.5-1	
	Oregon	MSSA	28	0.5	1	0.5-1	
		MRSA	22	0.5	1	0.25-1	
	Mass.	MSSA	25	0.5	1	0.25-1	
		MRSA	26	0.5	1	0.25-1	
	Texas	MSSA	24	0.5	1	0.5-1	
		MRSA	26	1	1	0.5-1	
	Greece	n.a.	MSSA	26	0.5	1	0.25-1
			MRSA	21	0.5	1	0.25-1
Italy	n.a.	MSSA	27	0.5	1	0.5-1	
		MRSA	28	0.5	1	0.25-1	
Hungary	n.a.	MSSA	23	0.5	1	0.5-1	
		MRSA	25	0.5	0.5	0.25-1	
Columbia	n.a.	MSSA	28	0.5	1	0.25-1	
		MRSA	22	1	1	0.5-1	
Chile	n.a.	MSSA	25	0.5	1	0.25-2	
		MRSA	30	1	2	0.5-2	

### Result Summary: Table 1 and 2

- During the surveillance period of 2015-2017, all MSSA and MRSA clinical isolates from the US, Europe and South America exhibited similar MIC<sub>50</sub>, MIC<sub>90</sub> and MIC<sub>100</sub> values
- MIC values of contemporary isolates were similar to that of clinical isolates from 2011
- Only 3 contemporary isolates with CF-301 MIC values of 2 µg/mL were identified (2 of these isolates were Dap<sup>R</sup>)
- MIC values of 2 µg/mL were previously observed in 2 isolates from the surveillance study of 2011 strains
- For contemporary clinical isolates (2015-2017), the staphylococcal MIC<sub>50/90</sub> of CF-301 is 0.5/1 µg/mL with a range of 0.125-2 µg/mL

<sup>1</sup>MIC values are reported in µg/mL. Abbreviations: n.a., not applicable; MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

**Table 2.** Cumulative frequency distribution of CF-301 MIC results for *S. aureus* in the USA, Europe and South America

Organism (no. of isolates) <sup>1</sup>	Year <sup>2</sup>	No. of isolates inhibited by CF-301 MIC (µg/mL) of:					MIC <sub>50</sub> (µg/mL)	MIC <sub>90</sub> (µg/mL)
		0.125	0.25	0.5	1	2		
<i>S. aureus</i>								
NA (301)	2016-2017		7	199	95		0.5	1
EU (150)	2015		16	94	40		0.5	1
SA (105)	2017		3	51	46	5	0.5	1
NA (149)	2011	1	5	125	16	2	0.5	1
MSSA								
NA (145)	2016-2017		5	102	38		0.5	1
EU (76)	2015		1	46	29		0.5	1
SA (53)	2017		3	30	19	1	0.5	1
NA (74)	2011	1	4	63	6		0.5	1
MRSA								
NA (156)	2016-2017		2	97	57		0.5	1
EU (74)	2015		15	48	11		0.5	1
SA (52)	2017			21	27	4	1	1
NA (75)	2011		1	62	10	2	0.5	1

<sup>1</sup>Abbreviations: NA, North America; EU, Europe; and SA, South America.

<sup>2</sup>Surveillance study results from the analysis of clinical isolates from 2011 are included for comparison.

## METHODS and RESULTS

A group of 61 laboratory stock MSSA and MRSA strains from Clinical Microbiology Institute (CMI) were tested and results were compared to that of 94 stock strains from ContraFect (**Table 3** and **Table 4**, respectively). **Table 5** provides a comparison of all stock strains (from CMI and ContraFect) and clinical isolates (from a 2011 study and from the current 2015-2017 study).

**Table 3.** CF-301 susceptibility test of CMI stock *S. aureus* strains<sup>1</sup>

Type	N	MIC <sub>50</sub>	MIC <sub>90</sub>	Range
MSSA	19	0.5	1	0.125-1
MRSA	42	0.5	1	0.5-1

<sup>1</sup>MIC values are reported in µg/mL.

**Table 4.** CF-301 susceptibility test of ContraFect stock *S. aureus* strains<sup>1</sup>

Type	N	MIC <sub>50</sub>	MIC <sub>90</sub>	Range
MSSA	45	0.5	1	0.25-1
MRSA	49	0.5	1	0.5-2

<sup>1</sup>MIC values are reported in µg/mL.

**Table 5.** CF-301 susceptibility test of all stock *S. aureus* isolates

Type	N	MIC <sub>50</sub>	MIC <sub>90</sub>	Range
Stock strains	155	0.5	1	0.125-2
Clinical isolates (2015-2017)	556	0.5	1	0.25-2
Clinical isolates (2011)	149	0.5	1	0.125-2

- The MIC<sub>50/90</sub> and range values for all stock MSSA and MRSA and all clinical isolates (from both 2011 and 2015-2017 periods) were similar and consistently 0.5/1 µg/mL and 0.125-2 µg/mL, respectively. Thus, these MIC and range values represent tentative baseline susceptibility values for CF-301.
- Isolates with MIC values ≤2 µg/mL will be susceptible to the clinical CF-301 dose (0.25 mg/kg), based on previous exposure target attainment studies and PK modeling

**Table 6.** Antibiotic susceptibility profiles for the contemporary clinical MRSA isolates obtained from 2015-2017 and CMI lab stock MRSA strains are described.

Species	MIC (µg/ml)	Trimethoprim-Sulfamethoxazole		CF-301 MIC		Vancomycin MIC		Daptomycin MIC		Oxacillin MIC		Linezolid MIC		Cefazolin MIC		Clindamycin MIC		
		MIC Frequency	Cumulative % Inhibited	MIC Frequency	Cumulative % Inhibited	MIC Frequency	Cumulative % Inhibited	MIC Frequency	Cumulative % Inhibited	MIC Frequency	Cumulative % Inhibited	MIC Frequency	Cumulative % Inhibited	MIC Frequency	Cumulative % Inhibited	MIC Frequency	Cumulative % Inhibited	
MRSA (n=324)	0.015																	
	0.03																	
	0.06	228	71.5%															
	0.12	59	90.0%													38	11.9%	
	0.25	9	92.8%	23	7.2%			6	1.9%					1	0.3%	176	67.1%	
	0.5	2	93.4%	192	67.4%	89	27.9%	282	90.3%	2	0.6%			2	0.9%	4	68.3%	
	1	2	94.0%	104	100.0%	220	96.9%	29	99.4%	1	1.3%	11	3.4%	11	4.4%	5	69.9%	
	2	3	95.0%			3	97.8%	2	100.0%			1	1.6%	249	81.5%	46	18.8%	
	4										5	3.1%	49	96.9%	88	46.4%		
	8	2	95.6%								26	11.3%	1	97.2%	52	62.7%		
	16	4	96.9%								72	33.9%			14	67.1%		
	32	10	100.0%								80	58.9%	3	98.1%	18	72.7%	96	100.0%
	64										47	73.7%	6	100.0%	30	82.1%		
	128						7	100.0%			84	100.0%			57	100.0%		
	256																	
	512																	
Totals	319	100.0%	319	100.0%	319	100.0%	319	100.0%	319	100.0%	319	100.0%	319	100.0%	319	100.0%		

## Conclusions

- Contemporary clinical isolates did not demonstrate reduced susceptibility to CF-301 compared to either 2011 isolates or laboratory stock strains.
- Continued surveillance is planned to further delineate the susceptibility profile of CF-301