Poster # 2450

European Surveillance Study of CF-301 Activity Against Contemporary *Staphylococcus aureus* Isolates from Italy, Greece, and Hungary

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Abstract

Background: CF-301 is a novel, recombinantly-produced, bacteriophage-derived lysin (cell wall hydrolase) which is in Phase 2 of clinical development for the treatment of *S. aureus* bacteremia including endocarditis used in addition to standard of care antibiotics. Surveillance data from 3 European countries is presented here.

Materials and Methods: CF-301 activity was tested against 150 clinical isolates of methicillin-resistant (75) and methicillin susceptible (75) *S. aureus* (MRSA and MSSA, respectively) obtained from 4 sites in Italy, 3 sites in Greece and 2 sites in Hungary in 2015. The study was performed using a previously approved modification of the Clinical and Laboratory Standards Institute broth microdilution method (BMD) that uses CAMHB supplemented with 25% horse serum and 0.5 mM DL-Dithiothreitol solution for minimal inhibitory concentration (MIC) testing with CF-301.

Results:

CF-301 vs. *S. aureus* MICs and MIC_{50}/MIC_{90} by Country (µg/mL)

Country	Type	N	0.25	0.5	1	MIC_{50}	MIC_{90}	Range
Greece	MRSA	22	4	13	5	0.5	1.0	0.25 - 1
	MSSA	25	1	16	8	0.5	1	0.25 - 1
Italy	MRSA	28	6	17	5	0.5	1	0.25- 1
	MSSA	27	0	15	12	0.5	1	0.5 – 1
Hungry	MRSA	25	5	18	2	0.5	0.5	0.25 - 1
	MSSA	23	0	15	8	0.5	1	0.5 - 1

CF-301 MICs for contemporary clinical isolates from Greece, Italy and Hungary ranged from 0.25 – 1 µg/mL. The CF-301 MIC_{50} was 0.5µg/ml for isolates from Greece, Italy and Hungary. The MIC_{90} was 1 µg/mL, with the exception of MRSA from Hungary which had an MIC_{90} of 0.5 µg/ml.

Conclusions: All clinical *S. aureus* isolates collected in 2015 from three European countries had CF-301 MICs within a range of 0.25 to 1 µg/mL. MIC testing of CF-301 on *S. aureus* was reproducible using the CLSI approved method.

Introduction

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 Phase 2 clinical development for the treatment of bacteremia including endocarditis due to *S. aureus*.

Key Features of CF-301 lysin:

- Rapid, potent, and targeted activity against pathogens, including antibiotic resistant strains
- Novel mechanism of action: peptidoglycan hydrolysis and osmotic lysis
- Synergy with conventional antibiotics
- Clearance of biofilms
- Low propensity to develop resistance
- Suppression of antibiotic resistance

References

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2. Performance Standards for Antimicrobial Susceptibility Testing M100 26th Edition, Clinical and Laboratory Standards Institute, Wayne PA.
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4. PK-PD Driver of Efficacy for CF-301, a Novel Anti-Staphylococcal Lysin: Implications for Human Target., Rotolo, JA, Ramirez, RA, Schuch, R, Machacek, M, Ghahramani P, Wittekind M, Dose. Poster # LB-053, MICROBE 2016, Boston, MA June 2016.

Materials and Methods

- Clinical isolates were collected from Greece, Hungary and Italy in 2015 and frozen until used. Each was asked to collect 50 strains.
- Broth microdilution MIC panels were prepared according to CLSI M7-A10 standard[1]and frozen at -70 º C until use.
- CF-301 2-fold dilutions were prepared in cation-adjusted Mueller Hinton broth which was supplemented with supplemented with 25% horse serum and 0.5 mM DL-Dithiothreitol solution.
- Comparator agents 2-fold serial dilutions were prepared with CAMHB as per CLSI method and included oxacillin, vancomycin, daptomycin, linezolid, cefazolin, trimethoprim-sulfamethoxazole and clindamycin.
- In addition, cefoxitin disk and MIC screening tests were performed. PBP2a tests were done on all strains identified as oxacillin resistant.
- Each strain was subbed from the freezer followed with by second subculture performed from the 1st plate 18 -20 hours prior to inoculating MIC tests
- Inoculum was prepared using the direct inoculum method to match the density of a 0.5 McFarland standard.
- MIC panels were incubated in force air at 35 º C.
- MICs were read after 16 -20 hours incubation at 35 º C except for oxacillin and vancomycin which were read after 24 hours incubation as per CLSI standard.

Results

- CF-301 MICs ranged from 0.25 to 1 µg/ml for all strains of *S. aureus* tested.
- All MSSA and MRSA strains from all countries had MIC_{50s} of 0.5 µg/ml
- All MSSA and MRSA strains from all countries except Hungary (MSSA) had MIC_{90s} of 0.5 µg/ml.
- MSSA strains from Hungary had MIC_{90s} of 1 µg/ml.
- Tables 1 and 2 present the geomean, MIC_{50} and MIC_{90} and range of MICs for CF-301 and all comparator agents tested
- Tables 3 and 4 present the percent inhibited at each MIC endpoint for CF-301 and all comparator agents tested.

Conclusions

- The CF-301 susceptibility of clinical isolates from Europe (obtained from 2015) is nearly identical to that observed in the US isolates from 2011[3]
- The MIC50/90 values described here represent tentative baseline susceptibility values for CF-301
- We expect that strains with MIC90 ≤1 µg/ml will remain susceptible to the clinical CF-301 dose of 0.25 mg/kb based on previously presented exposure target attainment studies and PK modeling[4]
- Continued surveillance will be important to delineate the susceptibility profile of CF-301

Table 1.

S. aureus oxacillin susceptible	N	Drug	Geometric Mean	Mode	Minimum	Maximum	Range (Log2 Dilutions)	MIC50	MIC90
	75	Trimethoprim-Sulfamethoxazole MIC	0.102	0.06	0.06	4	7	0.06	0.50
	75	CF-301 MIC	0.642	0.5	0.25	1	3	0.5	1
	75	Vancomycin MIC	0.831	1	0.5	2	3	1	1
	75	Daptomycin MIC	0.477	0.5	0.25	1	3	0.5	0.5
	75	Oxacillin MIC	0.362	0.5	0.12	1	4	0.5	0.5
	75	Linezolid MIC	2.789	2	1	4	3	2	4
	75	Cefazolin MIC	0.435	0.5	0.12	4	6	0.5	0.5
	75	Clindamycin MIC	0.226	0.25	0.12	32	9	0.25	0.25

Table 2.

S. aureus oxacillin resistant	N	Drug	Geometric Mean	Mode	Minimum	Maximum	Range (Log2 Dilutions)	MIC50	MIC90
	75	Trimethoprim-Sulfamethoxazole MIC	0.081	0.06	0.06	2	6	0.06	0.12
	75	CF-301 MIC	0.486	0.5	0.25	1	3	0.5	1
	75	Vancomycin MIC	0.697	0.5	0.5	1	2	0.5	1
	75	Daptomycin MIC	0.519	0.5	0.25	1	3	0.5	0.5
	75	Oxacillin MIC	48.503	128	4	128	6	64	128
	75	Linezolid MIC	2.319	2	1	4	3	2	4
	75	Cefazolin MIC	23.371	64	1	128	8	64	128
	75	Clindamycin MIC	1.600	0.25	0.12	32	9	0.25	32

Table 3.

S. aureus oxacillin susceptible	MIC (µg/ml)	Trimethoprim-Sulfamethoxazole MIC		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
n = 75	0.03																
	0.06	49	65.3%														
	0.12	16	86.7%							1	1.3%			1	1.3%	17	22.7%
	0.25	2	89.3%	1	1.3%			6	8.0%	35	48.0%			19	26.7%	57	98.7%
	0.5	2	92.0%	46	62.7%	21	28.0%	68	98.7%	37	97.3%			52	96.0%		
	1	1	93.3%	28	100.0%	53	98.7%	1	100.0%	2	100.0%	1	1.3%	1	97.3%		
	2	3	97.3%			1	100.0%					37	50.7%	1	98.7%		
	4	2	100.0%									37	100.0%	1	100.0%		
	8																
	16																
	> 16 or 32															1	100.0%
	>32 or 64																
	>64 or 128																
	>128 or 256																
	Totals	75	100.0%	75	100.0%	75	100.0%	75	100.0%	75	100.0%	75	100.0%	75	100.0%	75	100.0%

Table 4.

S. aureus oxacillin resistant	MIC (µg/ml)	Trimethoprim-Sulfamethoxazole MIC		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
n = 75	0.03																
	0.06	52	69.3%														
	0.12	18	93.3%													3	4.0%
	0.25	3	97.3%	15	20.0%			3	4.0%							42	60.0%
	0.5	1	98.7%	48	84.0%	39	52.0%	65	90.7%							1	61.3%
	1			12	100.0%	36	100.0%	7	100.0%			3	4.0%	3	4.0%		
	2	1	100.0%									53	74.7%	11	18.7%		
	4									4	5.3%	19	100.0%	10	32.0%		
	8									10	18.7%			2	34.7%		
	16									8	29.3%			1	36.0%		
	> 16 or 32									4	34.7%			7	45.3%	29	100.0%
	>32 or 64									13	52.0%			22	74.7%		
	>64 or 128									36	100.0%			19	100.0%		
	>128 or 256																
	Totals	75	100.0%	75	100.0%	75	100.0%	75	100.0%	75	100.0%	75	100.0%	75	100.0%	75	100.0%

Acknowledgement

This study was sponsored by a grant from ContraFect Corporation