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In vitro activity of Exebacase (CF-301) against clinical *Staphylococcus aureus* surveillance isolates from the United States, Europe, and Latin America, 2015–2017

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ABSTRACT

Lysins are direct lytic agents which act through enzymatic cell-wall-hydrolysis and represent a potential new class of antimicrobial agents in development to treat antibiotic-resistant bacterial infections. Exebacase (CF-301) is a first-in-class lysin now in clinical development for the treatment of *Staphylococcus aureus* (*S. aureus*) bacteremia and infective endocarditis (IE) when used in addition to conventional antibiotics. Exebacase and comparator antibiotics were tested by broth microdilution against a set of 535 clinical MSSA and MRSA isolates collected from 2015 to 2017 throughout the United States, Europe and South America. All *S. aureus* isolates were inhibited by ≤1 mg/L exebacase ($MIC_{50/90}$, 0.5/1 mg/L) with a range of 0.25–1 mg/L. No difference in susceptibility was observed between the MSSA and MRSA isolates. Exebacase was uniformly and equivalently active against all recent clinical MSSA and MRSA surveillance isolates from a broad survey across 3 continents.

The emergence and spread of drug- and multidrug-resistant bacteria which are not effectively treated by antibiotics have created an urgent need for new antimicrobials with differentiated mechanisms of action compared to conventional agents. A promising new therapeutic approach to targeting resistant bacterial pathogens is the use of direct lytic agents (DLAs), including recombinantly-produced, bacteriophage-derived lysins which kill bacteria via the hydrolysis of peptidoglycan (Nelson et al. 2012; Wittekind and Schuch 2016; Love et al. 2018). When applied exogenously as purified enzymes to Gram-positive bacteria, lysins elicit rapid osmotic lysis and bacterial cell death. The potent ability to rapidly kill pathogenic bacteria is the foundation for the advancement of lysins as powerful new therapeutic agents with direct lytic activities (Schuch et al. 2014).

Exebacase (lysin CF-301) is a potent antistaphylococcal lysin, with distinguishing features that include a low propensity for resistance, synergy with conventional antibiotics, activity against antibiotic-resistant strains, an extended post-antibiotic effect and, importantly, potent activity against biofilm bacteria (Schuch et al. 2014; Schuch et al. 2017; Oh et al. 2018a; Oh et al. 2018b; Indiani et al. 2019; Oh et al. 2019). Exebacase is, furthermore, the first lysin to report results from a Phase 2 (Ph2) clinical trial, which demonstrated 42.8% higher clinical responder rates with a single dose of exebacase used in addition to standard of care antibiotics (SOC) vs SOC alone for the treatment of methicillin-resistant *S. aureus* (MRSA) bacteremia including endocarditis (ClinicalTrials.gov 2017; Fowler et al. 2019).

To further define the scope of exebacase activity and to understand susceptibility patterns in wild-type populations of clinical *S. aureus* isolates, a surveillance study was performed to establish baseline activity against 535 nonduplicative clinical MSSA and MRSA isolates (277 and 258 each, respectively) collected from 2015 to 2017 from medical centers in the US (six sites in New York, Massachusetts, California, Minnesota, Oregon, and Texas), the EU (three sites in Italy, Greece, and Hungary), and South America (two sites in Chile and Columbia). The isolates were obtained from a variety of infection sites, including blood, bone, wound, nasal, urinary tract, abscesses, tracheal, deep tissue, and conjunctival.

Exebacase MICs were determined by broth microdilution (BMD) following Clinical and Laboratory Standards Institute (CLSI) guidelines in document M07-A10 (CLSI 2015), using a nonstandard antimicrobial susceptibility testing (AST) medium comprised of cation-adjusted Mueller Hinton broth (CAMHB) supplemented with horse serum (Sigma Aldrich; catalog number H1270) and dithiothreitol (Sigma Aldrich; catalog number 646563) to final concentrations of 25% and 0.5 mM, respectively. This medium, referred to as CAMHB-HSD, is approved for use in exebacase AST by the CLSI AST Subcommittee (CLSI, 2017a). Comparator antibiotics were tested exactly according to CLSI guidelines M7-A10 (CLSI 2015) and M100-S27 (CLSI 2017b). Broth

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Table 1
In vitro activities of exebacase tested against MSSA and MRSA isolates.

S. aureus group	Isolate source (N)[a]	Number (cumulative % inhibited) of isolates at MIC (mg/L) of:					MIC_{50}	MIC_{90}
		0.12	0.25	0.5	1	2		
MSSA	All surveillance (277)		9 (3.2)	184 (66.4)	84 (100)		0.5	1
	US (150)		5 (3.3)	108 (72)	37 (100)		0.5	1
	Europe (75)		1 (1.3)	46 (61.3)	28 (100)		0.5	1
	South America (52)		3 (5.7)	30 (57.7)	19 (100)		0.5	1
MRSA	All surveillance (258)		17 (6.6)	152 (58.9)	89 (100)		0.5	1
	US (148)		2 (1.4)	89 (60.1)	57 (100)		0.5	1
	Europe (75)		15 (20)	48 (64)	12 (100)		0.5	1
	South America (35)			15 (42.8)	20 (100)		1	1

[a] All surveillance groups represent the sum of isolates from the US, Europe, and South America.

microdilution MIC test panels were prepared as described in CLSI document M07-A10 and stored frozen at −70 °C until use (CLSI 2015). Exebacase frozen stock solution (Lot #NB0020-11-13) was obtained from ContraFect Corporation (Yonkers, NY). Concurrent testing of CLSI quality control organisms, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212, was performed to confirm in-range results for exebacase (Traczewski et al. 2017; CLSI 2017a). Comparator antibiotics were purchased from standard sources.

All MRSA isolates were tested at the monitoring laboratory (CMI, Wilsonville, OR) by disk diffusion using a cefoxitin disk test and with a MRSA Latex Test for PBP2a (Denka Seiken Co., LTD) to confirm the presence of *mecA* as described (Lee et al. 2004; Swenson and Tenover 2005). For cases in which an isolate was oxacillin (or cefazolin) susceptible by MIC and the cefoxitin broth screen test was positive, the isolate was also tested for PBP2a and the cefoxitin disk test to determine if it was *mecA* positive. The broth test was performed in a single well of the broth microdilution panel containing 4 mg/L of cefoxitin; growth in this well indicated a positive test and no growth indicated a negative test.

Exebacase MIC distributions for the surveillance isolates tested are shown in Table 1. The MIC values were consistent for the combined MSSA and MRSA groups (277 and 258 total isolates tested, respectively), with a very tight range of 0.25–1 mg/L. There was a strong mode for the MSSA and MRSA groups (0.5 mg/L), with $MIC_{50/90}$ values of 0.5/1 mg/L for each. Identical $MIC_{50/90}$ values of 0.5/1 mg/L were observed for the MSSA and MRSA isolates from the US and Europe, as well as the MSSA isolates from South America. In contrast, the $MIC_{50/90}$ of MRSA isolates collected in surveillance from South America (N = 35) was slightly higher, with $MIC_{50/90}$ values of 1/1 mg/L (range of 0.5–1 mg/L). The analysis of comparator agents against MSSA and MRSA isolates indicated most isolates were susceptible to the antibiotics tested, including trimethoprim-sulfamethoxazole, daptomycin, vancomycin, linezolid and clindamycin (Table 2). The comparator antibiotic activity mirrored the activity seen in other published studies, and, furthermore, confirmed that the *S. aureus* isolates collected in this study were typical (Pfaller et al. 2018; Pfaller et al. 2019; Sader et al. 2019).

Surveillance data from the present investigation demonstrated the potent in vitro activity of exebacase against all MSSA and MRSA surveillance isolates collected between 2015 and 2017 from the US, EU and South America. The MSSA and MRSA groups were equivalently susceptible to exebacase, with $MIC_{50/90}$ values of 0.5/1 mg/L and/or a range of 0.25–1 mg/L. A potent (and equivalent) activity against both MSSA and MRSA groups was also recently reported in a preliminary analysis of isolates from the exebacase Ph2 clinical trial (Anastasiou et al. 2018).

Importantly, an exebacase-resistant subpopulation, outside the wildtype distribution, was not observed in the current study. Overall, the findings reported here demonstrate the potent activity of exebacase against the target pathogen *S. aureus.*

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Declaration of Competing Interest

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Table 2
In vitro activities of comparator antibiotics and exebacase tested against all *S. aureus* isolates in this study.

Organism (N)	Antibiotic	MIC (mg/L)			
		Range	MIC_{50}	MIC_{90}	% Susceptible[a]
MSSA (277)	Trimethoprim-sulfamethoxazole	0.06–4	0.06	0.12	98.9
	Vancomycin	0.5–2	1	1	100
	Daptomycin	0.06–1	0.5	0.5	100
	Oxacillin	0.06–2	0.25	0.5	100
	Linezolid	1–4	2	4	100
	Cefazolin	0.12–4	0.5	0.5	99.6
	Clindamycin	0.12–32	0.25	0.25	97.5
	Exebacase	0.25–1	0.5	1	n.a.[b]
MRSA (258)	Trimethoprim-sulfamethoxazole	0.06–32	0.06	0.25	96.5
	Vancomycin	0.5–2	1	1	100
	Daptomycin	0.25–1	0.5	0.5	100
	Oxacillin	0.5–128	32	128	1.8[c]
	Linezolid	1-4	2	4	100
	Cefazolin	0.5–128	8	128	20
	Clindamycin	0.12–32	0.25	32	71
	Exebacase	0.25–1	0.5	1	n.a.[b]

[a] Susceptibility is based on MIC breakpoint values in CLSI document M100-S27 (CLSI 2017a).
[b] Data not available (n.a.) as breakpoints have not yet been determined.
[c] Isolates were oxacillin susceptible by MIC, but grew in the cefoxitin broth screen test and were positive in both the cefoxitin disk.

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