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In vitro Activity of Exebacase (CF-301) against *Staphylococcus aureus* Causing Bacteremia in the United States, Including Multidrug-Resistant Subsets

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Introduction

- Bloodstream infections (BSI) and infective endocarditis (IE) continue to be characterized by increased morbidity and mortality and are among the most common life-threatening infection syndromes.
- *Staphylococcus aureus*, including difficult to treat methicillin-resistant *S. aureus* (MRSA), remains the predominant causative bacterial species in BSI and IE in the USA.
- Exebacase (CF-301) is a lysin (peptidoglycan hydrolase enzyme) with anti-staphylococcal bacteriolytic activity currently in Phase 3 of clinical development for the treatment of patients with bacteremia, including right-sided IE caused by *S. aureus*.
- *S. aureus* causing bacteremia (SAB), including IE, among hospitalized patients in United States medical centers were collected during 2020 as part of the SENTRY longitudinal and prevalence-based surveillance study. This collection of *S. aureus* isolates reflects the current exebacase Phase 3 trial target patient population.
- This study evaluated the *in vitro* activity of exebacase and comparator agents, including those commonly used to treat BSI against contemporary *S. aureus* clinical isolates collected during the year of the global COVID-19 pandemic.

Materials and Methods

Bacterial isolates

- A total of 2,849 pathogens (1 per patient infection episode) were consecutively recovered from blood cultures of patients hospitalized in 29 US medical centers (20 states) during 2020 as part of the SENTRY Antimicrobial Surveillance Program. Among these 2,849 pathogens, 666 (23.4%) *S. aureus* were identified and included in this study (Figure 1).
- Overall, MRSA represented 38.6% of the *S. aureus* isolate collection (Figure 2). 20 *S. aureus* were documented as causative pathogens in patients with IE (40% of these were MRSA).
- Bacterial identification was confirmed by standard biochemical algorithms supported by matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany).

Susceptibility testing

- Isolates were tested for susceptibility by broth microdilution following the Clinical and Laboratory Standards Institute (CLSI) M07 (2018) guidelines.
- Frozen-form broth microdilution panels were prepared by JMI Laboratories (North Liberty, IA, USA) and contained cation-adjusted Mueller-Hinton broth (CA-MHB) for 11 comparators. Exebacase utilized CA-MHB supplemented with 25% donor herd horse serum and 0.5mM DL-dithiothreitol, as per CLSI recommendations (CLSI M100, 2021).
- Quality assurance was performed by sterility checks, colony counts, and testing CLSI-recommended quality control reference strains. Categorical MIC interpretations for comparator agents used CLSI M100 (2021) criteria.
- MRSA isolates were defined as methicillin-resistant based on an oxacillin resistance phenotype. These isolates are usually defined as multidrug-resistant (MDR) by standard phenotypic classifications. Here, these isolates were further categorized as MDR, when in addition to oxacillin, non-susceptible phenotypes were observed for 2 or more of the following agents: ceftaroline, erythromycin, clindamycin, doxycycline, levofloxacin, gentamicin, linezolid, trimethoprim-sulfamethoxazole, daptomycin and vancomycin.

Results

- *S. aureus* (24.2% overall) remained the most predominant causative pathogen of BSI in US hospitals during the last 5 years of surveillance (Figure 1).
  - The second most common species was *Escherichia coli* (20.8%), followed by *Klebsiella pneumoniae* (8.7%), coagulase-negative staphylococci (6.6%), and other pathogens represented 5.5% or less (data not shown).
  - The annual prevalence of MRSA among all organisms responsible for BSI remained between 9.0% and 10.6% during the last 5 years of surveillance (Figure 1).
- The annual occurrence of a methicillin resistance phenotype among *S. aureus* was observed between 43.1% and 38.6% (Figure 2).
- Exebacase inhibited all *S. aureus* isolates at MIC values of ≤1 µg/mL (MIC range, 0.06–1 µg/mL), with MIC_{50}, MIC_{90} and modal MIC values of 0.5 µg/mL (Table 1).
- Exebacase showed equivalent MIC results for the methicillin-susceptible (MSSA) and MRSA subsets (Table 1).
- In general, of the comparator agents tested, most were active (91.7%–100% susceptible) against the MSSA population, except for erythromycin (67.2% susceptible) (Table 2).
- In contrast, many comparators exhibited reduced susceptibility against MRSA, including ceftaroline (88.3% susceptible); however, among drugs indicated for treating SAB caused by MRSA, daptomycin and vancomycin were active (100% susceptible) against all isolates (Table 2).
- A total of 62.3% of MRSA isolates were categorized as MDR by the definition used in this study. Exebacase showed equal MIC_{50} and MIC_{90} results against the MDR ($MIC_{50/90}$, 0.5/0.5 µg/mL) and non-MDR ($MIC_{50/90}$, 0.5/0.5 µg/mL) populations (Table 1).
- Daptomycin and vancomycin were also active (100% susceptible) against the subset of MRSA isolates displaying a MDR phenotype (Table 2).

Conclusions

- COVID-19 appeared to have had a minimal impact on the etiology of BSI in the US, as *S. aureus* continued to represent the main pathogen responsible for BSI during the SENTRY Antimicrobial Surveillance Program for 2020.
  - Similar to the previous 4 years, *S. aureus* accounted for approximately 25% of all pathogens recovered from blood specimen.
  - The occurrence of a methicillin-resistant phenotype within *S. aureus* causing BSI in the USA in 2020 were slightly lower (38.6%) compared to the previous 4 years (39.5%–43.1%).
- The exebacase *in vitro* activity was uniform (MIC range, MIC_{50} and MIC_{90}) when tested against *S. aureus* clinical isolates responsible for BSI, including IE, isolated from patients in the US in 2020.
- In addition, the exebacase activity was consistent, regardless of resistance phenotype (MSSA, MRSA, including MDR isolates).
- These MIC data against contemporary *S. aureus* isolates from US BSI are in good agreement with exebacase MIC data generated by Traczewski et al. (2019) against worldwide *S. aureus* isolates sourced from various infection types.
- The surveillance isolates included in this study reflect those recovered from patients enrolled in the on-going Phase 3 clinical trial for exebacase. The data presented here further support the clinical development of exebacase as a promising option for treatment of SAB, including those caused by MDR MRSA isolates.

Acknowledgements

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Table 1. MIC distribution of exebacase against *S. aureus* isolated from patients with BSI, including IE, in US hospitals in 2020.

S. aureus/ Subset (no. of isolates)	No. and cumulative % of isolates inhibited at MIC (µg/mL) of: ≤0.03	0.06	0.12	0.25	0.5	1	2	MIC_{50}	MIC_{90}
All[a] (666)		1 0.2	0 0.2	43 6.6	577 93.2	45 100.0		0.5	0.5
Methicillin-susceptible (409)			0 0.0	29 7.1	352 93.2	28 100.0		0.5	0.5
Methicillin-resistant (257)		1 0.4	0 0.4	14 5.8	225 93.4	17 100.0		0.5	0.5
MDR (160)		1 (0.6)	0 (0.6)	10 (6.9)	137 (92.5)	12 (100.0)		0.5	0.5
Non-MDR (97)				4 (4.1)	88 (94.8)	5 (100.0)		0.5	0.5

[a] Isolates were defined as methicillin-resistant based on an oxacillin resistance phenotype. A multidrug resistance (MDR) phenotype was defined among MRSA isolates when non-susceptible phenotypes were observed for oxacillin and 2 or more of the following agents: ceftaroline, erythromycin, clindamycin, doxycycline, levofloxacin, gentamicin, linezolid, trimethoprim-sulfamethoxazole, daptomycin and vancomycin.

Figure 1. Prevalence of *S. aureus* and MRSA among all causative pathogens of BSI in US hospitals over a 5-year period

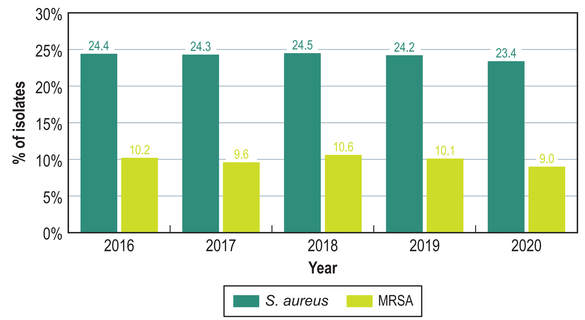

Figure 2. Proportion of a methicillin-resistance phenotype among *S. aureus* isolated from patients with BSI, including IE, in US hospitals over a 5-year period

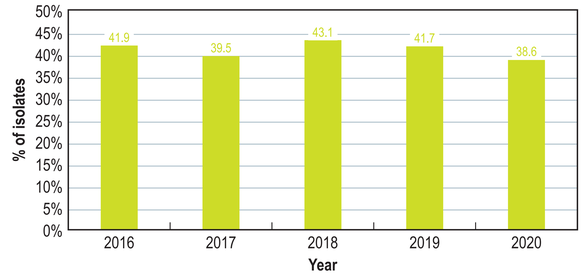

Table 2. Antimicrobial activity of exebacase and comparator agents against *S. aureus* isolated from patients with BSI, including IE, in US hospitals in 2020.

Antimicrobial agent	MIC (µg/mL) MIC_{50}	MIC_{90}	MIC range	CLSI[a] %S	%I	%R
All (666)						
Exebacase	0.5	0.5	0.06 to 1	—	—	—
Erythromycin	8	>8	≤0.06 to >8	46.8	2.3	50.9
Levofloxacin	0.25	>4	≤0.06 to >4	69.5	0.6	29.9
Oxacillin	0.5	>8	0.12 to >8	61.4	—	38.6
Clindamycin	0.06	>2	≤0.03 to >2	87.4	0.2	12.5
Ceftaroline	0.25	1	≤0.06 to 2	95.5	4.5 [b]	0.0
Daptomycin	0.25	0.5	≤0.12 to 1	100.0	—	—
Doxycycline	≤0.06	0.5	≤0.06 to >8	98.2	1.5	0.3
Gentamicin	≤1	≤1	≤1 to >8	98.0	0.2	1.8
Linezolid	1	2	≤0.12 to 4	100.0	—	0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5 to >16	97.9	—	2.1
Vancomycin	1	1	0.25 to 2	100.0	0.0	0.0
MSSA[c] (409)						
Exebacase	0.5	0.5	0.25 to 1	—	—	—
Erythromycin	0.25	>8	≤0.06 to >8	67.2	3.2	29.6
Levofloxacin	0.25	0.5	≤0.06 to >4	91.7	0.2	8.1
Oxacillin	0.5	1	0.12 to 2	100.0	—	0.0
Clindamycin	0.06	0.12	≤0.03 to >2	95.8	0.0	4.2
Ceftaroline	0.25	0.5	≤0.06 to 1	100.0	0.0	0.0
Daptomycin	0.25	0.5	≤0.12 to 1	100.0	—	—
Doxycycline	≤0.06	0.12	≤0.06 to >8	99.5	0.2	0.2
Gentamicin	≤1	≤1	≤1 to >8	98.5	0.0	1.5
Linezolid	1	2	≤0.12 to 4	100.0	—	0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5 to >16	99.8	—	0.2
Vancomycin	1	1	0.25 to 2	100.0	0.0	0.0
MRSA[c] (257)						
Exebacase	0.5	0.5	0.06 to 1	—	—	—
Erythromycin	>8	>8	≤0.06 to >8	14.4	0.8	84.8
Levofloxacin	4	>4	≤0.06 to >4	34.2	1.2	64.6
Oxacillin	>8	>8	4 to >8	0.0	—	100.0
Clindamycin	0.06	>2	≤0.03 to >2	73.9	0.4	25.7
Ceftaroline	1	2	0.12 to 2	88.3	11.7 [b]	0.0
Daptomycin	0.25	0.5	≤0.12 to 1	100.0	—	—
Doxycycline	≤0.06	1	≤0.06 to >8	96.1	3.5	0.4
Gentamicin	≤1	≤1	≤1 to >8	97.3	0.4	2.3
Linezolid	1	2	≤0.12 to 2	100.0	—	0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5 to >16	94.9	—	5.1
Vancomycin	1	1	0.5 to 2	100.0	0.0	0.0
MDR[c] (160)						
Exebacase	0.5	0.5	0.06 to 1	—	—	—
Erythromycin	>8	>8	0.25 to >8	1.9	0.6	97.5
Levofloxacin	>4	>4	0.25 to >4	3.1	1.9	95.0
Oxacillin	>8	>8	8 to >8	0.0	—	100.0
Clindamycin	0.06	>2	≤0.03 to >2	58.1	0.6	41.2
Ceftaroline	1	2	0.25 to 2	81.2	18.8 [b]	0.0
Daptomycin	0.25	0.5	≤0.12 to 0.5	100.0	—	—
Doxycycline	≤0.06	1	≤0.06 to >8	94.4	5.0	0.6
Gentamicin	≤1	≤1	≤1 to >8	96.2	0.6	3.1
Linezolid	1	2	0.25 to 2	100.0	—	0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5 to >16	91.9	—	8.1
Vancomycin	1	1	0.5 to 2	100.0	0.0	0.0

[a] Criteria as published by CLSI (2021). "—", breakpoint not available.
[b] Intermediate may be interpreted as susceptible-dose dependent.
[c] Isolates were defined as methicillin-resistant based on an oxacillin resistance phenotype. A multidrug resistance (MDR) phenotype was defined among MRSA isolates when non-susceptible phenotypes were observed for oxacillin and 2 or more of the following agents: ceftaroline, erythromycin, clindamycin, doxycycline, levofloxacin, gentamicin, linezolid, trimethoprim-sulfamethoxazole, daptomycin and vancomycin.

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