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In Vitro Activity of Ceftaroline against Community-Associated Methicillin-Resistant, Vancomycin-Intermediate, Vancomycin-Resistant, and Daptomycin-Nonsusceptible *Staphylococcus aureus* Isolates[▽]

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This study assessed the *in vitro* activities of ceftaroline and five comparator agents against a collection of *Staphylococcus aureus* isolates. Ceftaroline demonstrated potent activity against community-associated methicillin-resistant *S. aureus* (CA-MRSA) isolates and showed bactericidal activity against vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *S. aureus* (VRSA), heteroresistant VISA (hVISA), and daptomycin-nonsusceptible *S. aureus* (DNSSA) isolates. Ceftaroline may represent a bactericidal treatment option for infections caused by these pathogens.

The increasing prevalence of resistant *Staphylococcus aureus* strains, including methicillin-resistant *S. aureus* (MRSA), community-associated MRSA (CA-MRSA), and *S. aureus* strains with reduced susceptibility to vancomycin, emphasizes the need for innovative antimicrobials with activity against these pathogens (19, 24). Although the prevalence of *S. aureus* strains with reduced vancomycin susceptibility remains low, such strains have been associated with vancomycin treatment failure, limiting the treatment options for patients with such infections (3, 8). Ceftaroline is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against Gram-positive organisms, including MRSA and multidrug-resistant *Streptococcus pneumoniae* (MDRSP), as well as common Gram-negative pathogens (6, 9, 21). Ceftaroline is currently in phase 3 development for the treatment of complicated skin and skin structure infections and community-acquired bacterial pneumonia. The study described here evaluated the *in vitro* activities of ceftaroline and five comparator agents against CA-MRSA, vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *S. aureus* (VRSA), heteroresistant VISA (hVISA), and daptomycin-nonsusceptible *S. aureus* (DNSSA) isolates.

(A preliminary report of these results was presented at the 48th Interscience Conference on Antimicrobial Agents and Chemotherapy-Infectious Diseases Society of America Annual Meeting, Washington, DC, 25 to 28 October 2008.)

A total of 132 MRSA strains were selected for evaluation. CA-MRSA strains ($n = 92$) were isolated from patients admitted to St. John Hospital and Medical Center in Detroit, MI. These patients had positive MRSA cultures within 48 h of admission, in accordance with the definition of CA-MRSA described by the Centers for Disease Control and Prevention (2). DNSSA strains ($n = 7$) and hVISA strains ($n = 3$) were obtained from blood collected from patients at the same hospital. The hVISA isolates were identified by a modified population analysis profile method (12). VISA isolates ($n = 20$) and VRSA isolates ($n = 10$) were obtained via the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) program, supported under NIAID/NIH contract HHSN 272200700055C.

All CA-MRSA isolates were typed by pulsed-field gel electrophoresis (PFGE) with the restriction enzyme SmaI, followed by visual interpretation and categorization. PCR methods were used to determine the staphylococcal cassette chromosome *mec* (SCC*mec*) type, the presence of Panton-Valentine leukocidin (PVL) genes *lukS-PV* and *lukF-PV*, and the presence of the arginine catabolic mobile element (ACME) via detection of the *arcA* locus.

In vitro susceptibility tests were performed with the antimicrobials ceftaroline (lot CI 170-07; Forest Laboratories, Inc., New York, NY), vancomycin-HCl (Sigma), daptomycin (Cubist), clindamycin-HCl (Sigma), linezolid (Pfizer), and trimethoprim-sulfamethoxazole (Sigma), which were obtained individually and reconstituted to a 1:19 ratio. All antibiotics were received in powder form and were reconstituted according to guidelines of the Clinical and Laboratory Standards Institute (CLSI). MICs and minimum bactericidal concentrations (MBCs) were determined according to the guidelines of the CLSI (4, 5). Microdilution tests with cation-adjusted Mueller-Hinton broth were used to identify the MICs of all antimicrobial agents tested. The percentages of susceptible isolates were determined by using the CLSI breakpoints. For the testing of daptomycin, additional calcium was added to the broth for a final concentration of 50 mg/liter. The MICs were read visually and corresponded to the concentration in the well with the lowest drug concentration with no visible bacterial growth. The MBC was defined as the antibiotic concentration that reduced the number of viable cells by ≥99.9%. This was based on colony counts from the growth control well and rejection values determined by tables provided by Pearson (18).

S. aureus ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as the control strains for the MIC determinations, and *S. aureus* ATCC 25923 was used as the control strain for the MBC determinations.

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TABLE 1. SCC *mec* elements, PVL and ACME genes, and ceftaroline MIC_{90}s and MBC_{90}s of CA-MRSA isolates[a]

SCC*mec* type	PVL	ACME	No. of isolates	Ceftaroline MIC_{90} (μg/ml)	Ceftaroline MBC_{90} (μg/ml)	PFGE strain(s)
IVa	+	+	40	0.5	0.5	USA 300 (n = 40)
IV	−	−	13	0.25	0.5	USA 100 (n = 4), 9 genetically unrelated isolates
II	−	−	38	1	1	USA 100 (n = 15), USA 600 (n = 2), 21 genetically unrelated isolates
Not typeable	−	−	1	NA[b]	NA	Genetically unrelated to the other isolates

[a] A total of 92 CA-MRSA isolates were tested: 26 from blood, 33 from the respiratory tract, and 33 from wounds or tissues.
[b] NA, not applicable.

Approximately 58% of the CA-MRSA isolates were SCC*mec* type IV/IVa, whereas the majority of the other isolates were SCC*mec* type II (Table 1). All SCC*mec* type IVa isolates were positive for the PVL and ACME genes, whereas isolates of all other SCC*mec* types were negative for these genes. Of all the other *S. aureus* strains, only two DNSSA isolates were SCC*mec* type IVa and positive for the PVL and ACME genes.

The highest ceftaroline MIC observed for the CA-MRSA, VISA, VRSA, and DNSSA isolates was 1 μg/ml (Table 2). The MICs of ceftaroline were not influenced by traits conferring resistance to other classes of antimicrobials. All isolates susceptible to the drugs ceftaroline, vancomycin, daptomycin, and trimethoprim-sulfamethoxazole demonstrated bactericidal activity, with the MIC_{90}/MBC_{90} ratio being less than or equal to 1:2. The MBC_{90} of 1 μg/ml for ceftaroline, vancomycin, and daptomycin against CA-MRSA isolates was equal to the MIC_{90}, indicative of bactericidal activity, which was not observed for the bacteriostatic agents linezolid and clindamycin.

Previous studies have associated CA-MRSA isolates containing SCC*mec* type I through III genes with multidrug-resistant nosocomial infections, whereas the SCC*mec* type IV gene is typically found in CA-MRSA strains susceptible to various antibiotics (7, 13, 16, 22). In our study, approximately 58% of the CA-MRSA isolates were characterized as SCC*mec* type IV or IVa, and 41% were characterized as SCC*mec* type II. The MICs of ceftaroline against CA-MRSA observed in this study

TABLE 2. MIC_{50}/MIC_{90} and MBC_{50}/MIC_{90} values for all antimicrobials tested for their activities against CA-MRSA, VISA, hVISA, VRSA, and DNSSA isolates[a]

Isolate and antimicrobial	MIC (μg/ml)			% S	% R	MBC (μg/ml)		
	Range	50%	90%[b]			Range	50%	90%[b]
CA-MRSA (n = 92)								
CPT	0.25–1	0.5	1	NA	NA	0.25–1	0.5	1
VAN	0.5–2	1	1	100	0	0.5–2	1	1
DAP	0.25–1	0.5	1	100		0.25–2	0.5	1
LZD	1–4	2	2	100		4–>8	>8	>8
CLI	0.06–>64	0.12	>64	64	36	1–>64	8	>64
SXT	1.2/0.06–>76/4	2.4/0.12	9.5/0.5	98	2	1.2/0.06–>76/4	2.4/0.12	19/1
VISA (n = 20) and hVISA (n = 3)								
CPT	0.25–1	0.5	1	NA	NA	0.5–2	1	1
VAN	1–8	4	8	13	0	1–8	4	8
DAP	0.5–8	2	4	35		0.5–8	2	4
LZD	0.5–2	2	2	100		1–>8	4	>8
CLI	0.06–>64	>64	>64	17	83	0.12–>64	>64	>64
SXT	1.2/0.06–>76/4	4.8/0.25	>76/4	78	22	2.4/0.12–>76/4	9.5/0.5	>76/4
VRSA (n = 10)								
CPT	0.12–1	0.5	0.5	NA	NA	0.12–1	0.5	1
VAN	32–>64	>64	>64	0	100	64–>64	>64	>64
DAP	0.5–1	0.5	1	100		0.5–1	1	1
LZD	1–4	2	2	100		8–>8	>8	>8
CLI	>64	>64	>64	0	100	>64	>64	>64
SXT	1.2/0.06–>76/4	2.4/0.12	38/2	90	10	2.4/0.12–>76/4	2.4/0.12	>76/4
DNSSA (n = 7)								
CPT	0.25–1	0.5	0.55	NA	NA	0.25–1	1	0.74
VAN	1–2	2	1.64	100	0	2	2	2
DAP	4	4	4	0		4–8	8	5.94
LZD	1–2	2	1.48	100		2–>8	>8	10.7
CLI	<0.03–>64	>64	35	14	86	2–>64	>64	70.66
SXT	1.2/0.06–>76/4	2.4/0.12	5.6/0.40	71	29	2.4/0.12–>76/4	4.8/0.25	11.65/0.60

[a] Abbreviations: CPT, ceftaroline; VAN, vancomycin; DAP, daptomycin; LZD, linezolid; CLI, clindamycin; SXT, trimethoprim-sulfamethoxazole; NA, not available; S, susceptible; R, resistant.
[b] For DNSSA, geometric mean MICs and MBCs are used in place of MIC_{90}s and MBC_{90}s, respectively, because less than 10 isolates were studied.

TABLE 3. SCC*mec* elements, PVL and ACME genes, and MICs of VISA and VRSA isolates[a]

Isolate	SCC*mec* type	MIC (μg/ml)					
		CPT	VAN	DAP	LZD	SXT	CLI
VISA							
NRS-3	II	1	8	4	2	19/1	>64
NRS-4	II	0.5	4	1	2	2.4/0.12	>64
NRS-17	II	0.5	8	4	1	2.4/0.12	0.06
NRS-18	II	0.5	4	1	2	2.4/0.12	>64
NRS-19	II	0.5	4	2	1	9.5/0.5	>64
NRS-22	II	0.5	4	2	1	>76/4	>64
NRS-23	II	0.5	4	2	2	>76/4	>64
NRS-24	II	0.5	4	2	2	9.5/0.5	>64
NRS-26	II	0.25	4	4	1	>76/4	>64
NRS-27	II	0.5	4	1	1	>76/4	>64
NRS-51	II	1	4	1	2	2.4/0.12	>64
NRS-68	II	0.5	4	1	1	4.8/0.25	>64
NRS-73	IVd	0.5	4	2	1	>76/4	0.06
NRS-74	II	0.25	4	4	2	2.4/0.12	>64
NRS-76	II	0.25	4	2	2	2.4/0.12	0.06
NRS-118	I	1	8	4	1	19/1	>64
NRS-126	II	1	4	2	2	2.4/0.12	>64
NRS-402	II	0.5	8	8	0.5	1.2/0.06	>64
NRS-403	II	0.5	4	2	1	2.4/0.12	>64
NRS-404	II	0.5	8	2	2	9.5/0.5	0.06
VRSA							
VRS-1	II	1	>64	1	2	38/2	>64
VRS-2	II	0.5	32	0.5	2	19/1	>64
VRS-3	IV	0.5	32	0.5	2	1.2/0.06	>64
VRS-4	IV	0.5	>64	0.5	2	2.4/0.12	>64
VRS-5	II	0.5	>64	0.5	2	2.4/0.12	>64
VRS-6	II	0.5	>64	1	4	1.2/0.06	>64
VRS-7	NT	0.12	>64	1	1	1.2/0.06	>64
VRS-8	NT	0.12	>64	0.5	1	1.2/0.06	>64
VRS-9	II	0.25	>64	0.5	1	>76/4	>64
VRS-10	II	0.5	>64	0.5	2	38/2	>64

[a] Abbreviations: NT, not typeable; CPT, ceftaroline; VAN, vancomycin; DAP, daptomycin; LZD, linezolid; SXT, trimethoprim-sulfamethoxazole; CLI, clindamycin.

correlate well with the activity of ceftaroline previously reported against this pathogen (20). Ceftaroline demonstrated *in vitro* activity against all CA-MRSA isolates, regardless of their SCC*mec* type, with MICs ranging from 0.25 to 1 μg/ml and with the MIC_{90} being 1 μg/ml. These findings are supported by those of earlier studies, in which the ceftaroline MICs ranged from 0.25 to 1 μg/ml for CA-MRSA and from 0.12 to 2 μg/ml for MRSA (1, 6, 10, 14, 15, 20, 21).

Daptomycin nonsusceptibility among VISA isolates has been reported (11, 17), potentially limiting the treatment options for patients infected with these pathogens. In the present study, ceftaroline demonstrated activity against all VISA isolates, including those not susceptible to daptomycin (Table 3). Recent work by Vidaillac et al. has evaluated the *in vitro* activity of ceftaroline against MRSA and hVISA strains by using an experimental pharmacokinetic/pharmacodynamic model and has shown that ceftaroline not only demonstrated activity equal to or greater than that of vancomycin but also had a lower potential to select for resistant mutants (23).

This study assessed the *in vitro* activities of ceftaroline and five comparator agents against a collection of *S. aureus* isolates characterized into USA types by PFGE. The SCC*mec* elements were characterized, and the presence of PVL and ACME genes was determined. Ceftaroline demonstrated bactericidal activity against CA-MRSA, VISA/hVISA, VRSA, and DNSSA isolates. Ceftaroline represents a bactericidal option for the treatment of MRSA infections, including those caused by isolates with reduced susceptibilities to vancomycin and daptomycin, and should undergo further clinical studies.

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