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Antimicrobial Activities of Ceftaroline and ME1036 Tested against Clinical Strains of Community-Acquired Methicillin-Resistant *Staphylococcus aureus*▽

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Two investigational anti-methicillin-resistant *Staphylococcus aureus* (anti-MRSA) β-lactams, ceftaroline (a cephalosporin) and ME1036 (a carbapenem), were subjected to susceptibility testing by reference broth microdilution methods using 152 strains of community-acquired MRSA from the United States (47 medical centers). Ceftaroline and ME1036 were 64- and >128-fold more potent than ceftriaxone, respectively. All isolates had the Panton-Valentine leukocidin genes and staphylococcal cassette chromosome *mec* type IV, while 67.8% of isolates displayed pulsed-field gel electrophoresis clonal type USA300-0114.

Ceftaroline fosamil (formerly known as PPI-0903 and TAK-599) is an N-phosphono-type prodrug cephalosporin (15). Its active form, ceftaroline, is released in vivo upon the hydrolysis of the phosphonate group (5). This cephem has documented high affinity for penicillin binding protein 2a (PBP2a) and potent in vitro activity against oxacillin (methicillin)-resistant *Staphylococcus aureus* (MRSA) and many other gram-positive organisms, while retaining activity against gram-negative bacilli (6, 14, 15).

Staphylococcus spp., including MRSA and oxacillin-resistant coagulase-negative staphylococci, appear to be particularly susceptible to ceftaroline (MIC_{90}, 0.25 to 2 μg/ml) (5, 15). Ceftaroline has also shown good activity against vancomycin-nonsusceptible MRSA, a pathogen of concern that has been increasingly documented in recent years (9, 16), and key respiratory pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. Ceftaroline has demonstrated bactericidal activity and increased in vitro potency compared with currently available cephalosporins against drug-resistant *S. pneumoniae* strains (16).

The spectrum of activity of ceftaroline against gram-negative bacteria is similar to those of the broad-spectrum cephalosporins. Among the *Enterobacteriaceae*, the vast majority of *Citrobacter freundii* (MIC_{90}, 2 μg/ml), non-extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (MIC_{90}, 0.12 μg/ml) and *Klebsiella pneumoniae* (MIC_{90}, 0.5 μg/ml), *Morganella morganii* (MIC_{90}, 0.12 μg/ml), *Proteus mirabilis* (MIC_{90}, 0.12 μg/ml), and *Serratia marcescens* (MIC_{90}, 2 μg/ml) strains are inhibited at ≤2 μg of ceftaroline/ml (15). However, like the MICs of other broad-spectrum cephalosporins, ceftaroline MICs for some *Enterobacter cloacae*, *Proteus vulgaris*, and *Providencia* spp. strains and ESBL-producing strains regardless of species were previously observed to be elevated (15).

ME1036 (formerly CP5609) is a novel parenteral carbapenem with a 7-acylated imidazo[5,1-*b*]thiazole-2-yl group attached directly to the carbapenem moiety of the C-2 position (9). ME1036 has demonstrated high affinity for altered staphylococcal PBP2′ and potent in vitro activity against MRSA. This compound has also shown potent in vitro activities against penicillin-resistant *S. pneumoniae*, *H. influenzae*, and *Enterococcus faecalis*. When tested against members of the *Enterobacteriaceae*, ME1036 is more active than ceftriaxone and other broad-spectrum cephalosporins (including ceftaroline) due to a higher level of resistance to hydrolysis by ESBLs and AmpC β-lactamases produced by these organisms (8, 9, 12).

The objective of the present study was to evaluate the in vitro activities of these two novel β-lactam compounds against community-acquired MRSA (CA-MRSA) strains isolated throughout the United States.

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A collection of 152 strains from geographically diverse locations (47 medical centers) were selected from surveillance programs in the United States to include well-characterized CA-MRSA strains isolated in cases of human infection. The isolates were collected in the period from 2000 to 2005. Reagent-grade ceftaroline and ME1036 compounds were provided by Cerexa, Inc. (Alameda, CA). Comparator agents were purchased from Sigma Chemical Co. (St. Louis, MO) or obtained from their respective manufacturers in the United States. MICs were determined and interpreted by reference broth microdilution methods according to procedures established by the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (2, 3).

PCR amplification of Panton-Valentine leukocidin (PVL) genes (*lukF-PV* and *lukS-PV*) was performed by previously described procedures (10) with the following primers: luk-PV-F, ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A, and luk-PV-R, GCA TCA AST GTA TTG GAT AGC AAA AGC. All PVL gene-positive isolates were characterized by the type of staphylococcal cassette chromosome *mec* (SCC*mec*) element by

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TABLE 1. In vitro activity of ceftaroline and ME1036 tested against a selected collection of 152 well-characterized CA-MRSA strains

Antimicrobial agent	MIC (μg/ml)			% Of susceptible strains	% Of resistant strains
	50%	90%	Range		
ME1036	0.12	0.25	0.06–0.5	–[a]	—
Ceftaroline	0.5	0.5	0.25–1	–	—
Ceftriaxone	32	>32	16–>32	0.0 (0.0)[b]	98.7
Imipenem	≤0.5	≤0.5	≤0.5–2	100.0 (0.0)[b]	0.0
Meropenem	2	2	≤0.5–8	98.0 (0.0)[b]	0.0
Amoxicillin-clavulanate	8	>8	2–>8	1.3 (0.0)[b]	98.7
Erythromycin	>8	>8	2–>8	1.3	98.0
Clindamycin	≤0.25	≤0.25	≤0.25	100.0	0.0
Levofloxacin	0.25	0.5	≤0.12–>4	92.8	7.2
Tetracycline	≤0.5	≤0.5	≤0.5–>16	94.7	4.6
Trimethoprim-sulfamethoxazole	≤0.25	≤0.25	≤0.25	100.0	0.0
Linezolid	2	2	1–2	100.0	—
Daptomycin	0.25	0.5	0.25–1	100.0	0.0
Vancomycin	1	1	0.5–2	100.0	0.0

[a] –, no breakpoint has been established by the CLSI (2) for this category.
[b] The number in parentheses indicates the percentage of strains considered to be susceptible according to CLSI M100-S17 criteria.

using a multiplex PCR strategy (13). The primers amplified various DNA segments within the SCC*mec* elements that were characteristic of each of the types I, II, III, and IV. The *mecA* gene was amplified as part of the multiplex PCR to serve as an internal control. PCR products were separated on 2% agarose gel in Tris-acetate-EDTA buffer on a Criterion Sub-cell GT system (Bio-Rad, Hercules, CA) and stained with ethidium bromide. SCC*mec* types were assigned based on the numbers and sizes of the amplicons obtained.

PVL gene-positive CA-MRSA isolates were subjected to pulsed-field gel electrophoresis (PFGE) according to the procedure described by Tenover et al. (17). PFGE patterns were compared to those of CA-MRSA clones prevalent in the United States (17). The PFGE patterns were designated by a capital letter (e.g., C, F, G, and K). Strains were assigned to the same PFGE pattern only when all bands matched. When there was a difference of one or two bands, the strains were classified as a subtype or variant of the major type, and the subtype was designated with the same capital letter used for the major type, followed by an Arabic numeral (e.g., C1, C2, C3, etc.).

The in vitro activities of ceftaroline, ME1036, and comparator agents are summarized in Table 1. Both ceftaroline and ME1036 were highly active against the collection of CA-MRSA strains evaluated. Ceftaroline showed an MIC_{50} and an MIC_{90} of 0.5 μg/ml, and the highest MIC was 1 μg/ml; ME1036 showed an MIC_{50} of 0.12 μg/ml, an MIC_{90} of 0.25 μg/ml, and a highest MIC of only 0.5 μg/ml.

All isolates appeared to be susceptible to imipenem (the highest MIC was 2 μg/ml), clindamycin (all MICs were ≤0.25 μg/ml), trimethoprim-sulfamethoxazole (all MICs were ≤0.25 μg/ml), linezolid (the highest MIC was 2 μg/ml), daptomycin (the highest MIC was 1 μg/ml), and vancomycin (the highest MIC was 2 μg/ml). Furthermore, the vast majority (>90%) of strains tested were susceptible to meropenem (98.0% of strains were susceptible), levofloxacin (92.8% of strains were susceptible), and tetracycline (94.7% of strains were susceptible). In contrast, the vast majority of strains were resistant to ceftriaxone, amoxicillin-clavulanate, and erythromycin (Table 1).

Table 2 shows the MIC distributions for the β-lactam compounds evaluated in the study. The ceftaroline MIC range was very narrow (0.25 to 1 μg/ml), and the MIC for 148 strains (97.4%) was 0.5 μg/ml. ME1036 MICs ranged from 0.06 to only 0.5 μg/ml, with an MIC of 0.12 μg/ml for 69.7% of strains. Ceftriaxone and amoxicillin-clavulanate showed limited activity against these CA-MRSA strains, with MICs for 98.7% of strains being greater than or equal to the respective resistance breakpoint. On the other hand, the carbapenems imipenem (MIC_{90}, ≤0.5 μg/ml) and meropenem (MIC_{90}, 2 μg/ml) were very active in vitro against this collection of strains, even though MRSA isolates are considered to be resistant to all β-lactams according to CLSI criteria (2).

All isolates showed positive PCR results for PVL genes and SCC*mec* type IV. PCR screening for *agr* type was performed with a selected group of 23 strains, and 22 of those exhibited

TABLE 2. MIC distributions for ceftaroline, ME1036, and four other β-lactams tested against a collection of 152 CA-MRSA strains isolated in the United States

Antimicrobial agent	No. of strains (cumulative %) inhibited at a MIC (μg/ml) of:								
	≤0.06	0.12	0.25	0.5	1	2	4	8	16
ME1036	16 (10.5)	106 (80.3)	26 (97.4)	4 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)
Ceftaroline	0 (0.0)	0 (0.0)	1 (0.7)	148 (98.0)	3 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)
Ceftriaxone	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.3)
Imipenem	–[a]	–	–	139 (91.4)	9 (97.4)	4 (100.0)	0 (100.0)	0 (100.0)	0 (100.0)
Meropenem	–	–	–	9 (5.9)	22 (20.4)	106 (90.1)	12 (98.0)	3 (100.0)	0 (100.0)
Amoxicillin-clavulanate	–	–	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	2 (1.3)	121 (80.9)	–

[a] –, untested dilution concentration.

agr type I, while the remaining strain exhibited *agr* type III. The vast majority of strains (94.1%) showed the major PFGE type C, which is equivalent to that presented by clone USA300 and variants (17). USA300 is the most common CA-MRSA clone reported in the United States. One hundred three strains (67.8%) showed identical PFGE patterns that were equivalent to that of the USA300-0114 clone. Forty other strains showed recognized variations of the major PFGE pattern (one or two bands different), comprising 14 subtypes. One of these subtypes was observed in 19 strains, while the others were observed in one (10 subtypes) to five isolates. Furthermore, seven strains (4.6%) showed a major pattern (with three subtypes) identical to that presented by clone USA400 (17).

Before the emergence and rapid dissemination of MRSA, β-lactams were the standard treatment for staphylococcal infections (11). The safety and clinical efficacy of these antimicrobials were well recognized by physicians, but the perception of the clinical utility of this class diminished with the increasing prevalence of MRSA infections. Furthermore, due to some reports of the clinical failure of treatment of MRSA infections with β-lactam antibiotics to which the infecting strain was categorized as susceptible, MRSA has been considered resistant to all β-lactam antibiotics independent of the susceptibility testing result (2). However, a series of cephems and carbapenems, including ceftaroline and ME1036, with anti-MRSA activity due to potent binding to PBP2a are currently under investigation (1). The anti-MRSA β-lactams have demonstrated excellent in vitro and in vivo bactericidal activity against MRSA (including both CA-MRSA and hospital-associated MRSA). These agents have the potential to restore physicians' confidence in using β-lactam antibiotics for the treatment of MRSA infections.

CA-MRSA expressing the PVL has emerged as a serious problem in the United States. CA-MRSA infections are increasing in incidence in both urban and rural settings and commonly affect the young and the healthy. Most infections present as skin and soft-tissue infections, especially cellulitis, abscesses, and folliculitis. However, a severe syndrome of lung and septic joint involvement often affects children and young adults and may be fatal (4). Thus, empirical antimicrobial treatment of seriously ill patients with community- or hospital-acquired infections that may be caused by *S. aureus* should include a drug with anti-MRSA activity (7).

The results of this study showed that ceftaroline and ME1036 were very active against a well-characterized collection of CA-MRSA isolates obtained from patients throughout the United States. The collection of CA-MRSA strains evaluated was dominated by strains with PFGE patterns identical or similar to that of the USA300 strain (94.1% of strains), which is the predominant endemic/epidemic CA-MRSA clone on the North American continent. These compounds may be an important addition to the antimicrobial armamentarium for the treatment of both community- and hospital-acquired infections.

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