Antistaphylococcal Activity of Ceftobiprole, a New Broad-Spectrum Cephalosporin

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Received 16 May 2005/Returned for modification 28 June 2005/Accepted 7 July 2005

Ceftobiprole (formerly BAL9141), the active component of the prodrug BAL5788 (ceftobiprole medocaril), is a novel cephalosporin with expanded activity against gram-positive bacteria. Among 152 *Staphylococcus aureus* isolates, including 5 vancomycin-intermediate and 2 vancomycin-resistant strains, MIC₅₀ and MIC₉₀ values for ceftobiprole were each 0.5 µg/ml against methicillin-susceptible strains and 2 µg/ml against methicillin-resistant strains. Against 151 coagulase-negative staphylococci (including 4 vancomycin-intermediate strains), MIC₅₀ and MIC₉₀ values were, respectively, 0.125 µg/ml and 1 µg/ml against methicillin-susceptible and 1 µg/ml and 2 µg/ml against methicillin-resistant strains. Teicoplanin was less active than vancomycin against coagulase-negative strains. Linezolid, quinupristin-dalfopristin, and daptomycin were active against all strains, whereas increased MICs for amoxicillin-clavulanate, cefazolin, minocycline, gentamicin, trimethoprim-sulfamethoxazole, levofloxacin, rifampin, mupirocin, fusidic acid, and fosfomycin were sometimes observed. At 2× MIC, ceftobiprole was bactericidal against 11 of 12 test strains by 24 h. Prolonged serial passage in the presence of subinhibitory concentrations of ceftobiprole failed to select for clones with MICs >4 times those of the parents; the maximum MIC achieved for ceftobiprole after 50 passages (in 1 of 10 strains) was 8 µg/ml. Single-passage selections showed very low frequencies of resistance to ceftobiprole irrespective of genotype or phenotype; the maximal ceftobiprole MIC of recovered clones was 8 µg/ml.

The emergence of methicillin- and fluoroquinolone-resistant staphylococci and, more recently, glycopeptide-intermediate staphylococci (6, 17, 33, 46) and the risk of serious infections posed by these strains (10, 42) necessitate alternative therapeutic modalities (9, 31, 32, 44). During 2002, two clinical specimens of *Staphylococcus aureus* carrying *vanA*, one in Michigan and the other in Pennsylvania, were identified (5, 57), and in 2004, the isolation in New York of a third vancomycin-resistant *S. aureus* (VRSA) strain was reported (12). A fourth VRSA was isolated in Michigan in early 2005 (J. T. Rudrick, personal communication).

Ceftobiprole (formerly BAL9141), the active component of the prodrug ceftobiprole medocaril (formerly BAL5788), is a novel parenteral cephalosporin whose broad spectrum of activity includes most clinically important gram-positive and gram-negative bacteria (23, 29). Preliminary surveys have indicated that ceftobiprole has excellent in vitro activity against methicillin-resistant staphylococci, vancomycin-intermediate *S. aureus* (VISA), VRSA, and coagulase-negative staphylococci (8, 14, 20, 23, 29). The present study sought to determine (i) the MICs of ceftobiprole and 15 comparators against 303 staphylococci, (ii) the bacteriostatic or bactericidal activities of ceftobiprole and comparators against 12 selected staphylococcal strains, and (iii) the proclivity of ceftobiprole and some comparators to select for endogenous resistance from among 10 staphylococci with diverse resistotypes.

MATERIALS AND METHODS

Bacteria. The complete strain panel was comprised of 126 methicillin-resistant *S. aureus* (MRSA) and 26 methicillin-susceptible *S. aureus* (MSSA) strains and 125 methicillin-resistant coagulase-negative staphylococci (MRCoNS) and 26 methicillin-susceptible coagulase-negative staphylococci (MSCoNS); no species identification of coagulase-negative staphylococci was attempted. The complete panel included the Michigan and Pennsylvania VRSA strains and five VISA strains, plus four vancomycin-intermediate coagulase-negative staphylococci (VICO NS). The Michigan VRSA strain and all vancomycin-intermediate staphylococci were obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* via Focus Technologies, Inc. (Herndon, Va.). Most strains studied, including MRSA, were isolated within the 5 years prior to the study. No information on whether strains were community acquired or nosocomial is available.

Antimicrobial agents and MIC testing. Ceftobiprole powder was obtained from Basilea Pharmaceutica AG (Basel, Switzerland); other antimicrobial agents (cefazolin, amoxicillin-clavulanate, linezolid, quinupristin-dalfopristin, minocycline, gentamicin, trimethoprim-sulfamethoxazole, vancomycin, teicoplanin, daptomycin, levofloxacin, rifampin, mupirocin, fusidic acid, and fosfomycin) were obtained from their respective manufacturers. Cefazolin was the only selected comparator from the β-lactam group because this parenteral cephalosporin is highly effective for treatment of skin and soft tissue infections caused by MSSA (and beta-hemolytic streptococci). No currently available β-lactams are effective against MRSA.

Agar dilution MICs were determined according to CLSI (formerly NCCLS) guidelines (39) using cation-adjusted Mueller-Hinton agar (BBL). For fosfomycin susceptibility testing, the agar was supplemented with glucose-6-phosphate (final concentration, 25 µg/ml) (1, 39), whereas for daptomycin susceptibility testing, the agar was supplemented with calcium chloride (final Ca²⁺ concentration, 50 µg/ml) (22, 39). Vancomycin MICs were read after a full 24 h of incubation (40). *S. aureus* ATCC 29213 (40) was included in each run for quality control; MICs for test strains were recorded only if quality control strain MICs were within acceptable ranges.

Time-kill studies. For time-kill testing, two MSSA (including *S. aureus* ATCC 29213), four MRSA, two MSCoNS, and four MRCoNS strains were selected; this panel also included the Michigan and Pennsylvania VRSA strains, two VISA strains, and two VICO NS strains. Methods were based upon those described previously by our group (30, 43). Briefly, glass tubes containing doubling antibi-

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TABLE 1. Summary of MICs ($\mu\text{g/ml}$) for staphylococcal strains

Drug ^a	<i>Staphylococcus aureus</i>				Coagulase-negative staphylococci			
	Methicillin resistant (<i>n</i> = 126)		Methicillin susceptible (<i>n</i> = 26)		Methicillin resistant (<i>n</i> = 125)		Methicillin susceptible (<i>n</i> = 26)	
	MIC range	MIC ₅₀ /MIC ₉₀	MIC range	MIC ₅₀ /MIC ₉₀	MIC range	MIC ₅₀ /MIC ₉₀	MIC range	MIC ₅₀ /MIC ₉₀
Ceftobiprole	0.25–2	2/2	0.25–0.5	0.5/0.5	0.125–4	1/2	0.03–1	0.125/1
Cefazolin	1–>32	>32/>32	0.5–1	0.5/1	0.5–>32	4/32	0.125–2	0.25/1
AMC	1–>16	>16/>16	0.125–1	0.5/1	0.25–>16	2/8	0.125–1	0.25/1
Linezolid	≤0.5–4	2/2	1–2	2/2	≤0.5–2	1/2	≤0.5–2	1/2
Q-D	≤0.06–0.5	0.5/0.5	≤0.06–0.5	0.25/0.5	0.125–1	0.125/0.5	≤0.06–0.5	0.125/0.25
Minocycline	0.016–8	0.125/2	≤0.008–4	0.125/0.125	0.03–8	0.125/0.5	0.06–0.5	0.125/0.25
Gentamicin	0.25–>16	1/>16	0.5–>16	1/>16	0.125–>16	>16/>16	0.06–4	0.25/0.25
Trimethoprim-sulfamethoxazole	≤0.125–>8	≤0.125/>8	≤0.125–>8	≤0.125/8	≤0.125–>8	4/>8	≤0.125–8	0.25/8
Vancomycin	0.5–>256	1/1	0.5–4	1/1	0.25–8	2/2	0.25–2	1/2
Teicoplanin	0.125–128	0.5/2	0.125–16	0.5/1	0.125–64	8/16	0.125–4	2/4
Daptomycin	0.125–2	0.25/0.5	0.125–2	0.25/0.5	≤0.06–1	0.5/0.5	≤0.06–0.5	0.25/0.5
Levofloxacin	0.25–>16	16/>16	0.12–>16	0.5/>16	0.125–>16	4/16	0.125–16	0.25/4
Rifampin	≤0.004–>4	0.008/0.016	≤0.004–0.016	0.008/0.016	≤0.004–>4	0.008/>4	≤0.004–0.016	≤0.004/0.008
Mupirocin	≤0.06–>32	0.25/16	0.125–0.25	0.25/0.25	0.125–>32	0.5/>32	0.125–>32	0.125/>32
Fusidic acid	≤0.06–0.5	0.125/0.25	≤0.06–2	0.125/0.25	≤0.06–8	0.125/0.25	0.125–16	0.125/2
Fosfomycin	1–>32	8/16	1–32	8/16	0.25–>32	4/>32	0.25–>32	4/>32

^a AMC, amoxicillin-clavulanate; Q-D, quinupristin-dalfopristin; trimethoprim-sulfamethoxazole; 1:19 (wt/wt).

otic concentrations in 5 ml of cation-adjusted Mueller-Hinton broth (CAMHB; BBL) (with added calcium for daptomycin testing) were inoculated to obtain a final concentration of 5×10^5 to 5×10^6 CFU/ml and incubated at 35°C in a shaking water bath. Viable counts were obtained by plating 10-fold dilutions (CAMHB) of 0.1-ml aliquots from each tube onto plates of Trypticase Soy Agar plus 5% (vol/vol) defibrinated sheep blood (BBL), which were incubated for 24 to 48 h at 35°C in air enriched with 5% CO₂.

Kill kinetics were monitored by determining the fraction of strains yielding a $\Delta(\log_{10}$ CFU/ml) of -1 (corresponding to 90% killing), -2 (99% killing), and -3 (99.9% killing) at 0, 3, 6, 12, and 24 h. A given concentration of antimicrobial (expressed as a multiple of the MIC) was considered bactericidal if it reduced the inoculum size by $\geq 3 \log_{10}$ CFU/ml by 24 h or bacteriostatic if the inoculum size was reduced by $< 3 \log_{10}$ CFU/ml during the same period. With the sensitivity threshold and inocula employed in this study, no problems were encountered in delineating 99.9% killing. Issues of antibiotic carryover were addressed by dilution, as described previously (43).

The complete time-kill test panel was comprised of 12 staphylococcal strains, but due to innate resistance by some strains to particular antibiotics, time-kills were not performed for five strains with gentamicin; four strains with trimethoprim-sulfamethoxazole or amoxicillin/clavulanate; three strains with teicoplanin, cefazolin, or mupirocin; and two strains with vancomycin, levofloxacin, or rifampin. Since the CLSI (40) advises against broth dilution for determining fosfomycin MICs, time-kill studies were not performed with that antibiotic.

Multipassage resistance selection studies. Multipassage resistance selection studies using ceftobiprole, linezolid, quinupristin-dalfopristin, vancomycin, daptomycin, minocycline, and mupirocin were performed with two MSSA and three MRSA (including two VRSA and one VISA) strains and two MSCoNS and three MRCoNS (including one VICO NS) strains. Initial inocula (ca. 10^8 to 10^9 CFU/ml) were prepared by suspending growth from an overnight plate of Trypticase Soy Agar plus 5% (vol/vol) defibrinated sheep blood in CAMHB. Glass tubes containing 1 ml of antibiotic-free or antibiotic-supplemented CAMHB (antibiotic concentrations ranging from 4 doubling dilutions above to 3 doubling dilutions below the MIC of each drug for each strain) were inoculated beneath the surface of the broth with 10 μl of cell suspension. The tubes were incubated at 35°C for 24 h prior to each serial passage; passages were performed at 24-h intervals for up to 50 days by transferring a 10- μl aliquot of culture containing ca. 10^6 CFU from the tube nearest the MIC (usually 1 to 2 dilutions below) which had the same turbidity as antibiotic-free controls. Resistant or nonsusceptible mutants emerging during serial passage were frozen at -70°C in double-strength skim milk for subsequent analysis. When an MIC for a strain stabilized at $> 64 \mu\text{g/ml}$ during four successive passages, serial transfer in the presence of antibiotic was discontinued, and the strains were subjected to 10 passages in antibiotic-free medium. To confirm that clones obtained during and at the end of serial passage derived from their parental strains, the parental strains, intermediary clones with

elevated MICs, and clones obtained following final passage were examined by pulsed-field gel electrophoresis using a CHEF DR III apparatus (Bio-Rad, Hercules, Calif.), as previously described (4, 36).

For clones resistant to quinupristin-dalfopristin or nonsusceptible to linezolid, portions of genes encoding domains II and V of 23S rRNA, as well as genes encoding ribosomal proteins L4 and L22, were amplified and sequenced (34), and the sequences were compared to those of the parental strains from which each clone was derived. For mupirocin-resistant *S. aureus* clones and their respective parents, amplification and sequencing of the KMSKS region of the isoleucyl-tRNA synthetase (*ileS*) gene was performed. Mutations in the latter are known to be associated with low-level mupirocin resistance (2). The following primers were used: ileS1473F (5'-ACC GTT ACC AGT ATT TTA TGC-3') and ileS2307R (5'-ACG ACG GAT ATG AGA ATC AC-3').

Single-passage resistance selection studies. Bacterial cells were scraped from overnight plates, washed once with physiological saline, and resuspended in saline to a final concentration of ca. 10^{10} to 10^{11} CFU/ml. An aliquot (50 μl) of bacterial suspension was spread onto plates of cation-adjusted Mueller-Hinton agar containing the same antibiotics used for multipassage resistance selection at two, four, and eight times the agar dilution MIC. The plates were incubated aerobically at 35°C for 48 h. Randomly selected colonies growing on antibiotic-containing media were retested using agar dilution methodology. Resistance in single-passage studies was defined as an MIC ≥ 4 times higher than that of the parent. Resistance frequencies for each MIC for each strain/antibiotic pair were calculated as the proportion of resistant colonies per inoculum (38).

RESULTS

MIC determinations. Ceftobiprole MICs are listed in Table 1. Among 152 *S. aureus* (including 5 VISA and 2 VRSA) strains, MIC₅₀ and MIC₉₀ values for ceftobiprole were each 0.5 $\mu\text{g/ml}$ against MSSA and 2 $\mu\text{g/ml}$ against MRSA. Among 151 coagulase-negative staphylococci (including 4 VICO NS strains), MIC₅₀ and MIC₉₀ values were 0.125 $\mu\text{g/ml}$ and 1 $\mu\text{g/ml}$, respectively, against MSCoNS strains and 1 $\mu\text{g/ml}$ and 2 $\mu\text{g/ml}$, respectively, against MRCoNS strains. Vancomycin was active against all staphylococci surveyed except the 11 strains recognized as having elevated MICs for this glycopeptide; in contrast, teicoplanin proved less potent against coagulase-negative strains than against *S. aureus*. Linezolid, quinupristin-dalfopristin, and daptomycin were active against all

TABLE 2. MICs of strains tested by time-kills^a

Drug	MIC ($\mu\text{g/ml}$)											
	SA1 ^a	SA2	SA3 ^b	SA4 ^b	SA5 ^c	SA6 ^c	CN1	CN2	CN3	CN4	CN5 ^b	CN6 ^b
Ceftobiprole	0.5	0.5	2	2	2	1	0.125	0.25	2	1	1	1
Cefazolin	0.5	0.5	8	>32	>32	>32	0.25	0.5	8	4	2	8
AMC	0.5	0.25	16	16	>16	>16	0.25	0.5	8	4	2	8
Linezolid	4	4	4	2	4	4	4	2	4	2	4	4
Q-D	0.25	0.25	0.25	0.5	0.5	0.5	0.125	0.5	0.125	0.125	0.5	0.5
Minocycline	0.25	0.25	0.25	4	1	0.25	0.25	0.25	0.25	0.125	0.125	0.25
Gentamicin	1	2	1	>16	>16	>16	0.5	0.25	0.5	0.25	>16	>16
Trimethoprim-sulfamethoxazole	0.125	0.125	0.25	>8	>8	>8	0.5	1	4	8	>8	8
Vancomycin	1	1	4	4	>256	128	4	1	4	2	16	8
Teicoplanin	0.5	0.5	8	4	128	16	8	0.5	16	8	32	32
Daptomycin	0.5	1	4	2	1	0.5	0.5	0.25	0.5	0.5	2	2
Levofloxacin	0.25	0.5	16	8	>16	>16	0.25	0.25	16	4	4	16
Rifampin	0.008	0.008	>4	4	>4	0.004	0.016	0.016	0.016	0.03	0.03	0.016
Mupirocin	0.25	0.25	0.5	0.5	32	0.25	0.5	1	0.5	0.25	>32	>32
Fusidic acid	0.5	0.5	0.25	0.125	1	0.5	1	0.5	1	1	1	0.5

^a SA, *Staphylococcus aureus*; CN, coagulase-negative staphylococci. Among the strains tested, SA 1 and 2 and CN 1 and 2 were methicillin susceptible and SA 3 to 6 and CN 3 to 6 were methicillin resistant.

^b Vancomycin intermediate.

^c Vancomycin resistant.

strains examined, whereas increased MICs to cefazolin, amoxicillin-clavulanate, minocycline, gentamicin, trimethoprim-sulfamethoxazole, levofloxacin, rifampin, mupirocin, fusidic acid, and fosfomycin were observed for some staphylococcal strains.

Time-kill studies. MICs for the 12 staphylococcal strains tested by time-kill are presented in Table 2, and the results of the time-kill studies are summarized in Table 3. Ceftobiprole was bactericidal at $2\times$ MIC after 24 h against 11 of the 12 strains; the one strain for which ceftobiprole was bacteriostatic by 24 h was a VISA for which ceftobiprole was bactericidal at the MIC but not at higher drug concentrations [$\Delta(\log_{10}$ CFU/ml) of >3 at the MIC compared to $\Delta(\log_{10}$ CFU/ml) of 1.4 at $4\times$ MIC]. Linezolid, quinupristin-dalfopristin, teicoplanin, minocycline, rifampin, mupirocin, and fusidic acid were bacteriostatic against all or most of the strains examined by time-kill. Vancomycin and teicoplanin were bactericidal at $2\times$ MIC by 24 h for 8/10 and 3/9 strains, respectively, and gentamicin was bactericidal at $2\times$ MIC by 24 h for 6/7 strains. Daptomycin and gentamicin were the most rapidly bactericidal drugs among those tested.

Multipassage resistance selection studies. Data relating to emergence of elevated MICs for selected members of the staphylococcal panel are shown in Table 4. After 50 serial passages in the presence of subinhibitory concentrations of ceftobiprole, MICs for this cephalosporin immediately after passage ranged from 1 $\mu\text{g/ml}$ to 8 $\mu\text{g/ml}$; in 7/10 cases, the elevated MICs represented increases of $2\log_2$ dilution steps, and in 3/10 cases the MICs were raised by $\leq 1\log_2$ dilution step. Mupirocin MICs rose to $>64\mu\text{g/ml}$ for 9/10 strains. Linezolid MICs reached 64 $\mu\text{g/ml}$ in two clones, and in five others, the MICs increased ≥ 8 -fold (up to 16 $\mu\text{g/ml}$). For quinupristin-dalfopristin, ≥ 8 -fold increases in the MIC were recorded for all clones, two of which achieved MICs of 64 $\mu\text{g/ml}$. By 50 serial passages, daptomycin MICs reached a value of $\geq 64\mu\text{g/ml}$ in four clones whose parental MICs were in the 0.5- to 2- $\mu\text{g/ml}$ range; for other clones, daptomycin MICs were in the 8- to 32- $\mu\text{g/ml}$ range. Following six serial passages in the

presence of vancomycin, parental strain SA510 (VRSA) gave rise to a clone with an MIC of $>64\mu\text{g/ml}$; three vancomycin-susceptible parental strains gave rise to vancomycin-resistant clones (MICs of 16 to 32 $\mu\text{g/ml}$) by 50 passages.

Stability of some clones displaying elevated MICs, as well as possible cross-resistance between antibiotics of diverse structural families, were determined after 10 serial passages of clones in antibiotic-free medium (Table 4). The only selected clone with a ceftobiprole MIC of 8 $\mu\text{g/ml}$ (parental MIC, 2 $\mu\text{g/ml}$) was stable after being subcultured without antibiotic. From among six clones selected for having MICs for mupirocin of $>64\mu\text{g/ml}$, three clones retained mupirocin MICs of $>64\mu\text{g/ml}$ in the absence of antibiotic selection pressure, two clones had mupirocin MICs of 64 $\mu\text{g/ml}$, and one clone (derived from parent CN137) had a mupirocin MIC of 32 $\mu\text{g/ml}$. From among four clones selected for having elevated vancomycin MICs, all retained their high MICs for vancomycin in the absence of antibiotic selection pressure. The one daptomycin-nonsusceptible clone (originating from parent SA079) passaged in the absence of antibiotic went from an MIC of $>64\mu\text{g/ml}$ to 64 $\mu\text{g/ml}$, whereas the two linezolid-nonsusceptible clones (derived from parents CN137 and CN031) experienced a drop in MIC from 64 $\mu\text{g/ml}$ to 32 $\mu\text{g/ml}$ during subculture without antibiotic.

Several instances of increases in MIC of $\geq 2\log_2$ dilution steps for structurally unrelated antibiotics were noted; in some cases, these changes in MIC would lead to a reclassification of a clone's susceptibility to a given drug. The vancomycin-resistant clones derived from parents SA079, SA099, and CN137 also had four- to eightfold increases in the MIC for daptomycin, so that the clones would be regarded as daptomycin nonsusceptible. This may be related to changes in the composition of the cell envelope, which is the target of both vancomycin and daptomycin. The mupirocin-resistant clones originating from parents CN225, CN226, and CN137 had four- to eightfold increases in the MIC for vancomycin, resulting in reclassifications from vancomycin susceptible to vancomycin intermediate

TABLE 3. Time-kill analyses for 12 staphylococci

Drug and dose	No. of strains											
	3 h			6 h			12 h			24 h		
	-1 ^a	-2 ^a	-3 ^a	-1	-2	-3	-1	-2	-3	-1	-2	-3
Ceftobiprole												
4× MIC	6	1	0	11	7	4	11	11	9	12	11	11
2× MIC	6	0	0	11	6	5	12	11	9	12	12	11
MIC	4	0	0	10	5	2	11	11	5	10	7	6
Cefazolin ^b												
4× MIC	4	1	0	8	5	3	9	8	6	9	8	8
2× MIC	4	1	0	7	4	2	9	7	4	8	6	3
MIC	2	0	0	7	2	0	8	3	2	4	2	0
Amoxicillin-clavulanate ^b												
4× MIC	7	2	0	8	7	5	8	8	7	8	8	7
2× MIC	6	2	0	8	6	3	8	8	7	8	7	6
MIC	5	0	0	8	5	1	8	8	4	5	3	2
Linezolid												
4× MIC	0	0	0	0	0	0	0	0	0	5	0	0
2× MIC	0	0	0	0	0	0	0	0	0	3	0	0
MIC	0	0	0	0	0	0	0	0	0	2	0	0
Quinupristin-dalfopristin												
4× MIC	2	0	0	4	1	0	5	2	0	9	4	2
2× MIC	1	0	0	2	0	0	5	1	0	8	2	1
MIC	0	0	0	0	0	0	3	0	0	2	1	0
Minocycline												
4× MIC	0	0	0	0	0	0	1	0	0	6	0	0
2× MIC	0	0	0	0	0	0	1	0	0	4	0	0
MIC	0	0	0	0	0	0	0	0	0	1	0	0
Gentamicin ^b												
4× MIC	7	7	5	7	7	7	7	7	7	7	7	6
2× MIC	6	6	1	7	7	7	7	7	7	7	6	6
MIC	5	3	0	6	6	4	7	7	4	2	1	1
Trimethoprim-sulfamethoxazole ^b												
4× MIC	0	0	0	3	1	0	6	4	3	7	6	4
2× MIC	0	0	0	2	0	0	5	4	3	6	5	4
MIC	0	0	0	2	0	0	4	3	1	5	3	2
Vancomycin ^b												
4× MIC	2	0	0	6	3	0	9	7	4	10	9	8
2× MIC	1	0	0	5	2	0	9	6	4	10	9	8
MIC	0	0	0	5	1	0	8	6	3	7	6	5
Teicoplanin ^b												
4× MIC	1	0	0	6	1	0	7	5	3	9	7	5
2× MIC	1	0	0	4	1	0	7	5	2	8	6	3
MIC	1	0	0	2	1	0	6	4	0	5	3	0
Daptomycin												
4× MIC	9	6	3	12	12	10	12	12	12	12	12	12
2× MIC	7	4	1	12	10	6	12	12	12	12	12	12
MIC	4	1	0	10	6	3	12	11	7	8	6	5
Levofloxacin ^b												
4× MIC	6	4	1	10	7	3	10	10	8	10	10	10
2× MIC	5	2	0	10	7	1	10	9	8	10	10	9
MIC	3	0	0	7	3	0	10	5	3	8	3	2
Rifampin ^b												
4× MIC	2	0	0	2	0	0	5	0	0	7	3	0
2× MIC	2	0	0	4	0	0	5	0	0	8	2	0
MIC	2	0	0	2	1	0	4	1	0	3	0	0
Mupirocin ^b												
4× MIC	0	0	0	0	0	0	1	0	0	3	1	0
2× MIC	0	0	0	0	0	0	0	0	0	2	1	0
MIC	0	0	0	0	0	0	0	0	0	0	0	0
Fusidic acid												
4× MIC	0	0	0	0	0	0	0	0	0	3	0	0
2× MIC	0	0	0	0	0	0	0	0	0	2	0	0
MIC	0	0	0	0	0	0	0	0	0	0	0	0

^a -1, 90% killing; -2, 99% killing; -3, 99.9% killing.^b Resistant strains were not tested (see Materials and Methods).

TABLE 4. Results of multipassage resistance selection by ceftobiprole, vancomycin, daptomycin, linezolid, quinupristin-dalfopristin, mupirocin, and minocycline

Strain	Initial MIC (μg/ml)							Selected resistance			Cross-resistance (MICs; μg/ml)						
	BPR ^a	VAN	DAP	LZD	Q-D	MUP	MIN	Selecting agent	No. of passages	MIC (μg/ml)	BPR	VAN	DAP	LZD	Q-D	MUP	MIN
SA510-VRSA-Pennsylvania MRSA	2	32	0.25	2	0.5	0.125	0.25	BPR	50	2	1	>64	0.5	4	0.5	0.125	0.25
								VAN	6	>64							
								DAP	50	64							
								LIN	50	16							
								Q-D	50	16							
								MUP	23	>64							
SA509 VRSA-Michigan MRSA	2	>128 ^b	0.5	2	0.5	16	0.5	MIN	50	8	1	>64	0.5	4	1	>64	0.25
								BPR	50	2							
								VAN	50	2							
								DAP	50	32							
								LIN	50	8							
								Q-D	50	64							
SA504 VISA MSSA	2	4	2	1	0.5	0.25	16	MUP	10	>64	0.5	4	1	4	0.25	64	0.25
								MIN	50	2							
								BPR	50	8							
								VAN	50	16							
								DAP	50	64							
								LIN	50	8							
SA079 VSSA MSSA	1	1	0.5	2	0.5	0.25	0.125	Q-D	50	4	2	16	2	4	1	0.125	0.125
								MUP	36	>64							
								MIN	50	64							
								BPR	50	4							
								VAN	50	16							
								DAP	48	>64							
SA099 VSSA MSSA	0.25	1	1	2	0.5	0.25	0.125	LIN	50	16	1	4	0.5	2	2	64	0.125
								Q-D	50	64							
								MUP	30	>64							
								MIN	50	4							
								BPR	50	1							
								VAN	50	16							
CN225 VCoNS MRCoNS	1	8	1	1	0.5	16	0.125	DAP	50	64	0.5	16	4	4	0.5	0.125	0.25
								LIN	50	4							
								Q-D	50	8							
								MUP	24	>64							
								MIN	50	1							
								BPR	50	4							
CN226 VCoNS MRCoNS	1	8	2	1	0.25	32	0.125	VAN	50	32	0.5	4	0.5	32	1	0.125	0.25
								DAP	50	16							
								LIN	50	16							
								Q-D	50	8							
								MUP	11	>64							
								MIN	50	1							
CN137 VSCoNS MRCoNS	1	1	0.5	1	0.5	0.25	1	BPR	50	4	2	32	4	2	0.5	0.5	1
								VAN	50	16							
								DAP	50	32							
								LIN	50	64							
								Q-D	50	8							
								MUP	40	>64							
CN031 VSCoNS MSCoNS	0.5	1	0.5	2	0.5	0.125	0.125	MIN	50	8	0.5	4	0.5	32	1	0.125	0.25
								BPR	50	2							
								VAN	50	8							
								DAP	50	16							
								LIN	50	64							
								Q-D	50	8							
CN154 VSCoNS MSCoNS	0.25	1	0.25	2	0.25	0.5	0.125	MUP	50	8	0.25	4	0.5	2	0.25	>64	0.25
								MIN	50	2							
								BPR	50	1							
								VAN	50	8							
								DAP	50	8							
								LIN	50	8							

^a BPR, ceftobiprole; VAN, vancomycin; DAP, daptomycin; LZD, linezolid; Q-D, quinupristin-dalfopristin; MUP, mupirocin; MIN, minocycline.

^b This strain had a VAN MIC of 1024 $\mu\text{g/ml}$ and was not passaged in VAN-containing media.

or from vancomycin intermediate to vancomycin resistant. However, the mupirocin-resistant clone stemming from parent SA510 had an MIC for vancomycin of >64 $\mu\text{g/ml}$, compared to 32 $\mu\text{g/ml}$ for the parental strain. Additionally, the mupirocin-resistant strain derived from parent SA079 had an MIC for quinupristin-dalfopristin of 2 $\mu\text{g/ml}$ (intermediate), compared to 0.5 $\mu\text{g/ml}$ (susceptible) for the parental strain. Several other increases in the MIC of >1 $\log_2$ dilution step were observed, but none of these would lead to a reclassification of the clone from susceptible to intermediate or intermediate to resistant.

Sequencing of the *ileS* gene of mupirocin-resistant clones of *S. aureus* revealed multiple mutations in each strain: SA510, Phe587Leu and Gly591Ser; SA509, Leu582Tyr and Val588Phe (this mutation was present in the parent strain with a mupirocin MIC of 16 $\mu\text{g/ml}$); SA504, Gly593Asp and Val631Phe; SA079, Gly591Asp, Asn602Asp, and Ser634Phe; SA099, Gly593Asp and Val631Phe.

No differences in 23S rRNA domain II or V, or in ribosomal proteins L4 and L22, were observed between parental strains and quinupristin-dalfopristin-intermediate or -resistant clones. Sequence analysis of the 23S rRNAs of linezolid-nonsusceptible clones derived from parental strains CN137 and CN031 revealed a G2576U (*Escherichia coli* numbering) substitution in the former and a G2447U (*E. coli* numbering) substitution in the latter. Only clones with linezolid MICs of 64 $\mu\text{g/ml}$ harbored changes in 23S rRNA; clones with linezolid MICs of 8 to 16 $\mu\text{g/ml}$, although classified as linezolid nonsusceptible (40), did not contain altered L4 or L22 ribosomal proteins or altered 23S rRNA domains II or V, so far as could be determined.

Single-passage resistance selection studies. Frequencies of single-step mutations for ceftobiprole, linezolid, quinupristin-dalfopristin, daptomycin, minocycline, and mupirocin were obtained for all 10 parental strains used in multipassage selection studies and for vancomycin for 8 vancomycin-intermediate and vancomycin-susceptible staphylococcal strains. Mutation frequencies for each strain-drug-concentration combination are summarized in Table 5. Mutation frequencies for ceftobiprole ranged from 9.6×10^{-8} to 9.2×10^{-10} (at $2\times$ MIC) to $<7.7 \times 10^{-10}$ to $<0.9 \times 10^{-10}$ (at $8\times$ MIC); the highest MIC for ceftobiprole found in any single-passage clone was 8 $\mu\text{g/ml}$. Agar dilution retesting of clones designated "resistant" showed four- to eightfold increases in the MIC for vancomycin and up to fourfold increases for daptomycin and minocycline MICs compared to parental MICs. For linezolid, no clones examined had more than a twofold increase in the MIC compared to parental MICs. The greatest increases in MIC for single-passage clones were seen for quinupristin-dalfopristin (4- to 32-fold increases in the MIC) and mupirocin (8- to 64-fold increases in the MIC). Analysis of sequenced fragments of the *ileS* gene in mupirocin-resistant *S. aureus* mutants (mupirocin MICs, 16 to >64 $\mu\text{g/ml}$) revealed a Val588Phe mutation in all strains and a Phe563Val mutation in a mutant derived from SA509 (this strain had an initial mupirocin MIC of 16 $\mu\text{g/ml}$ and had Val588Phe mutation).

DISCUSSION

Ceftobiprole is a pyrrolidinone-3-ylidenemethyl cephem with well-documented activity against methicillin-resistant

staphylococci, *Enterococcus faecalis*, and penicillin-resistant pneumococci, while preserving the anti-gram-negative activity of broad-spectrum and "fourth-generation" cephalosporins (e.g., cefepime and ceftipime) (23, 28, 29). The activity of ceftobiprole for methicillin-resistant staphylococci results from the potent, long-lasting inhibition by ceftobiprole of PBP2a (as well as the normal sensitive penicillin binding proteins) and the refractoriness of ceftobiprole to hydrolysis by the class A β -lactamases found in most staphylococcal clinical isolates (23).

Ceftobiprole proved to be very active against all staphylococcal strains surveyed irrespective of susceptibilities to other antibiotics. The MIC₉₀ values for MRSA/VRSA and for MRCoNS strains were each 2 $\mu\text{g/ml}$, consistent with previously reported MIC₉₀ values (≤ 4 $\mu\text{g/ml}$) for ceftobiprole obtained using diverse panels of methicillin-resistant staphylococcal clinical isolates (23, 29; R. Reynolds, D. M. Livermore, and the BSAC Working Party on Bacteraemia Resistance Surveillance, 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E-2035 and E-2036, 2004). Kill kinetics showed that ceftobiprole was bactericidal by 24 h at $2\times$ MIC for 11/12 staphylococcal strains examined; the single exception was VISA strain SA507, for which ceftobiprole was bactericidal at the MIC but not at higher drug concentrations (data not reported). Such an "Eagle effect" (19) for staphylococci has been reported previously for ceftobiprole (16). With respect to the comparators, the MICs and kill kinetics are in accord with data reported for these compounds (13, 15, 16, 18, 24, 26, 27, 31, 52, 55, 58).

The results of experiments involving prolonged serial transfer of staphylococci in the presence of subinhibitory concentrations of ceftobiprole or comparators and determinations of single-step mutation frequencies confirm previous reports that staphylococci (14, 20, 25) and other pathogens (3, 30; M. Rouse, P. Anguita-Alonso, M. H. Hein, J. M. Steckelberg, and R. Patel, Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. B-1177, 2004) are refractory to development of endogenous resistance to ceftobiprole. Ceftobiprole failed to select for clones with MICs exceeding by 2 $\log_2$ dilution steps those of parental strains, the largest observed change in the MIC being from 2 $\mu\text{g/ml}$ to 8 $\mu\text{g/ml}$ for the clone derived from VISA strain SA504 after 50 serial passages.

Daptomycin was the most rapidly bactericidal among the antibiotics surveyed. Though it has been claimed that daptomycin resistance in clinical and laboratory strains of gram-positive bacteria is very rare (35, 54; K. Rezai, J. P. Quinn, R. Hayes, K. Lolans, R. A. Weinstein, and M. K. Hayden, Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. K97-a, 2004), emergence of strains with MICs for daptomycin in the 32- to >64 - $\mu\text{g/ml}$ range occurred in 6/10 of the staphylococcal strains examined here (Table 4). The mechanism of resistance in these daptomycin-nonsusceptible clones is under investigation. Parental strains with vancomycin MICs of 1 $\mu\text{g/ml}$ yielded clones with vancomycin MICs of 16 $\mu\text{g/ml}$ (*S. aureus*) and 8 to 16 $\mu\text{g/ml}$ (coagulase-negative staphylococci); the mechanism of vancomycin nonsusceptibility in these clones is being explored. The cause of loss of minocycline resistance in the mupirocin-resistant clone derived from VISA strain SA504 is unknown but may be related to loss of a plasmid-mediated resistance determinant, such as *tetK* (53).

In the present study, mupirocin selected for the most mutants in both multipassage and single-passage resistance selec-

TABLE 5. Frequencies of single-step resistance selection in staphylococci

Strain ^a	Frequency at:			
	Drug	2× MIC	4× MIC	8× MIC
SA510	Ceftobiprole	$<1.5 \times 10^{-10}$	$<1.5 \times 10^{-10}$	$<1.5 \times 10^{-10}$
	Vancomycin	Not tested	Not tested	Not tested
	Linezolid	$<3.3 \times 10^{-10}$	$<3.3 \times 10^{-10}$	$<3.3 \times 10^{-10}$
	Quinupristin/dalfopristin	$\sim 3.3 \times 10^{-6}$	$\sim 3.3 \times 10^{-8}$	$<3.3 \times 10^{-10}$
	Daptomycin	$\sim 2.6 \times 10^{-7}$	$<2.6 \times 10^{-10}$	$<2.6 \times 10^{-10}$
	Minocycline	$<2.5 \times 10^{-10}$	$<2.5 \times 10^{-10}$	$<2.5 \times 10^{-10}$
	Mupirocin	$\sim 6.1 \times 10^{-8}$	$\sim 2.2 \times 10^{-8}$	$\sim 6.1 \times 10^{-9}$
SA509	Ceftobiprole	$<1.2 \times 10^{-10}$	$<1.2 \times 10^{-10}$	$<1.2 \times 10^{-10}$
	Vancomycin	Not tested	Not tested	Not tested
	Linezolid	$<2.6 \times 10^{-10}$	$<2.6 \times 10^{-10}$	$<2.6 \times 10^{-10}$
	Quinupristin/dalfopristin	$\sim 3.9 \times 10^{-9}$	$<2.6 \times 10^{-10}$	$<2.6 \times 10^{-10}$
	Daptomycin	$<1.7 \times 10^{-10}$	$<1.7 \times 10^{-10}$	$<1.7 \times 10^{-10}$
	Minocycline	$\sim 2.3 \times 10^{-10}$	$<2.3 \times 10^{-10}$	$<2.3 \times 10^{-10}$
	Mupirocin	$\sim 4.8 \times 10^{-8}$	$\sim 4.8 \times 10^{-10}$	$\sim 9.5 \times 10^{-10}$
SA504	Ceftobiprole	$\sim 9.2 \times 10^{-10}$	$<0.9 \times 10^{-10}$	$<0.9 \times 10^{-10}$
	Vancomycin	$<0.9 \times 10^{-10}$	$<0.9 \times 10^{-10}$	$<0.9 \times 10^{-10}$
	Linezolid	$<2.6 \times 10^{-10}$	$<2.6 \times 10^{-10}$	$<2.6 \times 10^{-10}$
	Quinupristin/dalfopristin	$\sim 3.0 \times 10^{-7}$	$\sim 1.3 \times 10^{-9}$	$<2.6 \times 10^{-10}$
	Daptomycin	$\sim 2.0 \times 10^{-9}$	$<1.4 \times 10^{-10}$	$<1.4 \times 10^{-10}$
	Minocycline	$<1.2 \times 10^{-10}$	$<1.2 \times 10^{-10}$	$<1.2 \times 10^{-10}$
	Mupirocin	$\sim 4.4 \times 10^{-8}$	$\sim 1.3 \times 10^{-8}$	$\sim 1.4 \times 10^{-9}$
SA079	Ceftobiprole	$\sim 1.4 \times 10^{-9}$	$\sim 2.8 \times 10^{-10}$	$<1.4 \times 10^{-10}$
	Vancomycin	$\sim 2.8 \times 10^{-8}$	$\sim 2.8 \times 10^{-9}$	$<1.4 \times 10^{-10}$
	Linezolid	$<3.9 \times 10^{-10}$	$<3.9 \times 10^{-10}$	$<3.9 \times 10^{-10}$
	Quinupristin/dalfopristin	$\sim 2.4 \times 10^{-8}$	$\sim 3.9 \times 10^{-9}$	$<3.9 \times 10^{-10}$
	Daptomycin	$<0.8 \times 10^{-10}$	$<0.8 \times 10^{-10}$	$<0.8 \times 10^{-10}$
	Minocycline	$<2.4 \times 10^{-10}$	$<2.4 \times 10^{-10}$	$<2.4 \times 10^{-10}$
	Mupirocin	$\sim 1.7 \times 10^{-7}$	$\sim 3.4 \times 10^{-9}$	$<3.4 \times 10^{-10}$
SA099	Ceftobiprole	$\sim 9.6 \times 10^{-8}$	$<0.9 \times 10^{-10}$	$<0.9 \times 10^{-10}$
	Vancomycin	$\sim 3.6 \times 10^{-8}$	$<0.9 \times 10^{-10}$	$<0.9 \times 10^{-10}$
	Linezolid	$<1.6 \times 10^{-10}$	$<1.6 \times 10^{-10}$	$<1.6 \times 10^{-10}$
	Quinupristin/dalfopristin	$\sim 1.4 \times 10^{-9}$	$\sim 4.8 \times 10^{-10}$	$<1.6 \times 10^{-10}$
	Daptomycin	$\sim 1.0 \times 10^{-7}$	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$
	Minocycline	$<2.7 \times 10^{-10}$	$<2.7 \times 10^{-10}$	$<2.7 \times 10^{-10}$
	Mupirocin	$\sim 6.2 \times 10^{-7}$	$\sim 9.2 \times 10^{-9}$	$\sim 3.1 \times 10^{-9}$
CN225	Ceftobiprole	$<1.6 \times 10^{-10}$	$<1.6 \times 10^{-10}$	$<1.6 \times 10^{-10}$
	Vancomycin	$<1.6 \times 10^{-10}$	$<1.6 \times 10^{-10}$	$<1.6 \times 10^{-10}$
	Linezolid	$<8.3 \times 10^{-10}$	$<8.3 \times 10^{-10}$	$<8.3 \times 10^{-10}$
	Quinupristin/dalfopristin	$\sim 2.5 \times 10^{-3}$	$\sim 5.4 \times 10^{-8}$	$<8.3 \times 10^{-10}$
	Daptomycin	$<4.4 \times 10^{-10}$	$<4.4 \times 10^{-10}$	$<4.4 \times 10^{-10}$
	Minocycline	$\sim 7.7 \times 10^{-8}$	$<7.7 \times 10^{-10}$	$<7.7 \times 10^{-10}$
	Mupirocin	$\sim 1.2 \times 10^{-3}$	$<1.2 \times 10^{-9}$	$<1.2 \times 10^{-9}$
CN226	Ceftobiprole	$<2.7 \times 10^{-10}$	$<2.7 \times 10^{-10}$	$<2.7 \times 10^{-10}$
	Vancomycin	$<2.7 \times 10^{-10}$	$<2.7 \times 10^{-10}$	$<2.7 \times 10^{-10}$
	Linezolid	$<8.7 \times 10^{-10}$	$<8.7 \times 10^{-10}$	$<8.7 \times 10^{-10}$
	Quinupristin/dalfopristin	$\sim 6.1 \times 10^{-7}$	$\sim 8.7 \times 10^{-9}$	$<8.7 \times 10^{-10}$
	Daptomycin	$<3.3 \times 10^{-10}$	$<3.3 \times 10^{-10}$	$<3.3 \times 10^{-10}$
	Minocycline	$<6.1 \times 10^{-10}$	$<6.1 \times 10^{-10}$	$<6.1 \times 10^{-10}$
	Mupirocin	$\sim 2.2 \times 10^{-3}$	$<2.2 \times 10^{-9}$	$<2.2 \times 10^{-9}$
CN137	Ceftobiprole	$\sim 1.1 \times 10^{-8}$	$\sim 1.1 \times 10^{-9}$	$<5.3 \times 10^{-10}$
	Vancomycin	$\sim 5.3 \times 10^{-8}$	$\sim 5.3 \times 10^{-10}$	$<5.3 \times 10^{-10}$
	Linezolid	$<8.3 \times 10^{-10}$	$<8.3 \times 10^{-10}$	$<8.3 \times 10^{-10}$
	Quinupristin/dalfopristin	$\sim 3.3 \times 10^{-5}$	$\sim 5.8 \times 10^{-6}$	$\sim 3.3 \times 10^{-9}$
	Daptomycin	$\sim 4.4 \times 10^{-5}$	$<7.4 \times 10^{-10}$	$<7.4 \times 10^{-10}$
	Minocycline	$\sim 9.5 \times 10^{-8}$	$<9.5 \times 10^{-10}$	$<9.5 \times 10^{-10}$
	Mupirocin	$\sim 2.0 \times 10^{-6}$	$\sim 1.0 \times 10^{-7}$	$\sim 1.5 \times 10^{-8}$
CN031	Ceftobiprole	$<2.3 \times 10^{-10}$	$<2.3 \times 10^{-10}$	$<2.3 \times 10^{-10}$
	Vancomycin	$\sim 1.6 \times 10^{-8}$	$\sim 2.3 \times 10^{-10}$	$<2.3 \times 10^{-10}$

Continued on following page

TABLE 5—Continued

Strain ^a	Drug	Frequency at:		
		2× MIC	4× MIC	8× MIC
	Linezolid	$<6.7 \times 10^{-10}$	$<6.7 \times 10^{-10}$	$<6.7 \times 10^{-10}$
	Quinupristin/dalfopristin	$\sim 6.7 \times 10^{-6}$	$\sim 8.7 \times 10^{-9}$	$\sim 4.7 \times 10^{-9}$
	Daptomycin	$\sim 2.9 \times 10^{-8}$	$<2.4 \times 10^{-10}$	$<2.4 \times 10^{-10}$
	Minocycline	$<4.8 \times 10^{-10}$	$<4.8 \times 10^{-10}$	$<4.8 \times 10^{-10}$
	Mupirocin	$\sim 7.5 \times 10^{-8}$	$\sim 6.3 \times 10^{-10}$	$<6.3 \times 10^{-10}$
CN154	Ceftobiprole	$<7.7 \times 10^{-10}$	$<7.7 \times 10^{-10}$	$<7.7 \times 10^{-10}$
	Vancomycin	$\sim 1.5 \times 10^{-7}$	$\sim 4.6 \times 10^{-8}$	$<7.7 \times 10^{-10}$
	Linezolid	$<1.3 \times 10^{-9}$	$<1.3 \times 10^{-9}$	$<1.3 \times 10^{-9}$
	Quinupristin/dalfopristin	$\sim 1.3 \times 10^{-6}$	$\sim 1.3 \times 10^{-8}$	$\sim 1.3 \times 10^{-9}$
	Daptomycin	$<4.8 \times 10^{-10}$	$<4.8 \times 10^{-10}$	$<4.8 \times 10^{-10}$
	Minocycline	$<6.7 \times 10^{-9}$	$<6.7 \times 10^{-9}$	$<6.7 \times 10^{-9}$
	Mupirocin	$\sim 7.1 \times 10^{-8}$	$\sim 2.9 \times 10^{-8}$	$<2.9 \times 10^{-9}$

^a For strain descriptions, see Table 4.

tion studies. It is interesting that all *S. aureus* mutants obtained by single passage had one common mutation, Val588Phe (except for a mutant derived from strain SA509 that originally had this mutation and developed a second mutation, Phe563Val). However mutation Val588Phe was not detected in mutants obtained after multiple passages. Instead, these mutants had double mutations in the vicinity of the evolutionarily conserved KMSKS motif. It has been shown previously that mutations affecting this region are responsible for low-level mupirocin resistance in *S. aureus* (2).

Mutations in ribosomal protein L4 or L22, which form part of the peptide exit tunnel in the large ribosomal subunit, as well as mutations in domain II or V of 23S rRNA, can lead to bacterial resistance to some antibiotics targeting the ribosome (7, 11, 21, 34, 41, 56, 59, 60). No mutations in genes encoding riboproteins or 23S rRNA were identified in staphylococcal clones that became intermediate or resistant to quinupristin-dalfopristin during serial passage in the presence of subinhibitory concentrations of the antibiotic. Two clones with high-level linezolid resistance contained mutations in domain V of 23S rRNA. One clone, derived from parental strain CN137, contained a G2576U transition close to the peptidyl binding site, consistent with the proposed mechanism of action of oxazolidinones (7); such mutations are known to give rise to linezolid resistance in *S. aureus* during therapy (37, 45). The other clone, derived from parental strain CN031, contained a G2447U transition, known only in laboratory mutants with decreased ribosomal binding of oxazolidinones (7). The high-level linezolid nonsusceptibility of both clones diminished by 1 log₂ dilution step, from 64 µg/ml to 32 µg/ml, after 10 serial passages in antibiotic-free medium. The mechanism of this decrease in MIC remains to be clarified but may reflect a gene dosage effect. Meka et al. (37) reported a linezolid-nonsusceptible (MIC, 16 µg/ml) clinical isolate of *S. aureus* with G2576U mutations in four of five copies of its 23S rRNA gene, which, after 30 passages in antibiotic-free medium, had an MIC for linezolid of 8 µg/ml and G2576U mutations in only two copies of its 23S rRNA gene.

The results of this study clearly point to a potential role for ceftobiprole in the treatment of staphylococcal infections, including those caused by methicillin- and vancomycin-resistant

strains. This cephalosporin is bactericidal at low concentrations and highly refractory to development of endogenous resistance. The excellent in vitro activities of ceftobiprole against staphylococci, as well as against multiresistant pneumococci (30), combined with a gram-negative spectrum of extended-spectrum cephalosporins (23, 29), and the excellent pharmacokinetic and safety profiles of the prodrug ceftobiprole medocaryl (48–51), make it a very attractive candidate for empirical parenteral treatment of early-onset nosocomial infections when community (47) as well as hospital pathogens must be suspected.

ACKNOWLEDGMENT

This study was supported by a grant from Basilea Pharmaceutica AG, Basel, Switzerland.

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