ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2005, p. 1932–1942 Vol. 49, No. 5
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.5.1932–1942.2005

Antipneumococcal Activity of Ceftobiprole, a Novel Broad-Spectrum Cephalosporin

Klaudia Kosowska,[1] Dianne B. Hoellman,[1] Gengrong Lin,[1] Catherine Clark,[1] Kim Credito,[1] Pamela McGhee,[1] Bonifacio Dewasse,[1] Bülent Bozdogan,[1] Stuart Shapiro,[2] and Peter C. Appelbaum[1]*

Department of Pathology, Hershey Medical Center, Hershey, Pennsylvania 17033,[1] and Basilea Pharmaceutica AG, CH-4005 Basel, Switzerland[2]

Received 11 November 2004/Returned for modification 9 January 2005/Accepted 10 January 2005

Ceftobiprole (previously known as BAL9141), an anti-methicillin-resistant *Staphylococcus aureus* cephalosporin, was very highly active against a panel of 299 drug-susceptible and -resistant pneumococci, with MIC_{50} and MIC_{90} values (μg/ml) of 0.016 and 0.016 (penicillin susceptible), 0.06 and 0.5 (penicillin intermediate), and 0.5 and 1.0 (penicillin resistant). Ceftobiprole, imipenem, and ertapenem had lower MICs against all pneumococcal strains than amoxicillin, cefepime, ceftriaxone, cefotaxime, cefuroxime, or cefdinir. Macrolide and penicillin G MICs generally varied in parallel, whereas fluoroquinolone MICs did not correlate with penicillin or macrolide susceptibility or resistance. All strains were susceptible to linezolid, quinupristin-dalfopristin, daptomycin, vancomycin, and teicoplanin. Time-kill analyses showed that at 1× and 2× the MIC, ceftobiprole was bactericidal against 10/12 and 11/12 strains, respectively. Levofloxacin, moxifloxacin, vancomycin, and teicoplanin were each bactericidal against 10 to 12 strains at 2× the MIC. Azithromycin and clarithromycin were slowly bactericidal, and telithromycin was bactericidal against only 5/12 strains at 2× the MIC. Linezolid was mainly bacteriostatic, whereas quinupristin-dalfopristin and daptomycin showed marked killing at early time periods. Prolonged serial passage in the presence of subinhibitory concentrations of ceftobiprole failed to yield mutants with high MICs towards this cephalosporin, and single-passage selection showed very low frequencies of spontaneous mutants with breakthrough MICs towards ceftobiprole.

The incidence of pneumococci resistant to penicillin G and other β-lactam antibiotics, as well as non-β-lactam antibiotics, has increased worldwide at an alarming rate. Major foci of infection include South Africa, Spain, and central and eastern Europe (1, 21, 22, 35, 48). A survey published in the mid-1990s showed an increase in resistance by pneumococci to penicillin from <5% before 1989 (including <0.02% of isolates with MICs of ≥2 μg/ml) to 6.6% in 1991 to 1992 (with 1.3% of isolates with MICs of ≥2 μg/ml) (5). A more recent survey (23) reported that 50.4% of 1,476 clinically significant pneumococcal isolates were not susceptible to penicillin and that high rates of macrolide-resistant pneumococci occurred in strains with elevated penicillin MICs, for an overall pneumococcal macrolide resistance rate of approximately 33%. Rates of macrolide resistance are even higher in Spain, France, central and eastern Europe, Korea, and Japan (1, 23, 24, 35). Although pneumococcal fluoroquinolone resistance is still uncommon, relatively high rates have been reported in Canada, Hong Kong, Spain, and Croatia (19, 29, 39, 45). Moreover, there is a high rate of isolation of penicillin-intermediate and -resistant pneumococci (approximately 30%) in middle ear fluids from patients with refractory otitis media, compared to rates from other isolation sites (3, 15, 16). The problem of drug-resistant pneumococci is compounded by the ability of resistant clones to spread rapidly over distant geographic regions (14, 32, 33).

Parenteral β-lactams active against pneumococci with elevated penicillin G MICs include carbapenems, such as imipenem and meropenem, and cephalosporins, such as cefotaxime, ceftriaxone, and cefepime (11, 40, 41, 43, 54). While these drugs are active against *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*, the principal bacterial causes of community-acquired respiratory tract infections, they are inactive towards methicillin-resistant staphylococci (18, 25). Moreover pathogenicity of novel community-acquired methicillin-resistant *Staphylococcus aureus* with increased virulence has become evident (P. J. Gavin, R. B. Thomson, Jr., A. D. Fisher, B. Kupfner, S. M. Paule, and L. R. Peterson, Abstr. 11th Int. Symp. Staphylococci and Staphylococcal Infect., abstr. CA-09, p. 25, 2004).

Ceftobiprole (previously known as BAL9141) is an experimental broad-spectrum parenteral cephalosporin with very good activity against gram-positive cocci, including penicillin-resistant pneumococci (2, 13, 18, 25). We compared the antipneumococcal activities of ceftobiprole to those of penicillin G, amoxicillin, cefepime, ceftriaxone, cefotaxime, cefuroxime, cefdinir, imipenem, ertapenem, levofloxacin, moxifloxacin, clarithromycin, azithromycin, telithromycin, linezolid, quinupristin-dalfopristin, daptomycin, vancomycin, and teicoplanin by (i) MIC testing of 299 pneumococcal clinical isolates by agar dilution; (ii) broth macrodilution and time-kill studies of the aforementioned drugs against 12 selected pneumococcal strains; and (iii) multi- and single-passage studies of the ability of ceftobiprole, ceftriaxone, moxifloxacin, telithromycin, linezolid, quinupristin-dalfopristin, and vancomycin to select for clones with elevated MICs of 10 pneumococcal strains with differing β-lactam, macrolide, and fluoroquinolone susceptibilities.

* Corresponding author. Mailing address: Department of Pathology, Hershey Medical Center, 500 University Dr., Hershey, PA 17033. Phone: (717) 531-5113. Fax: (717) 531-7953. E-mail: pappelbaum@psu.edu.

TABLE 1. MICs (μg/ml) of 299 pneumococcal strains by agar dilution

Drug	Selected strain	MIC		
		Range	50%	90%
Penicillin G	Penicillin S	0.016–0.06	0.016	0.03
	Penicillin I	0.12–1	0.25	1
	Penicillin R	2–16	2	4
	Macrolide S	0.016–4	2	2
	Macrolide R	0.016–16	2	4
	Quinolone S	0.016–16	2	4
	Quinolone R	0.016–16	2	4
Ceftobiprole	Penicillin S	0.008–0.03	0.016	0.016
	Penicillin I	0.008–1	0.06	0.5
	Penicillin R	0.016–4	0.5	1
	Macrolide S	0.008–1	0.25	0.5
	Macrolide R	0.008–4	0.5	1
	Quinolone S	0.008–4	0.5	0.5
	Quinolone R	0.008–2	0.5	1
Amoxicillin	Penicillin S	≤0.016–0.12	≤0.016	0.03
	Penicillin I	0.016–4	0.25	2
	Penicillin R	0.03–16	2	8
	Macrolide S	≤0.016–8	1	2
	Macrolide R	≤0.016–16	2	8
	Quinolone S	≤0.016–16	2	4
	Quinolone R	≤0.016–8	2	4
Imipenem	Penicillin S	≤0.004–0.03	0.008	0.016
	Penicillin I	0.008–0.5	0.03	0.12
	Penicillin R	0.03–2	0.25	0.5
	Macrolide S	≤0.004–0.5	0.12	0.25
	Macrolide R	≤0.004–2	0.25	0.5
	Quinolone S	≤0.004–2	0.25	0.5
	Quinolone R	≤0.004–0.5	0.25	0.25
Ertapenem	Penicillin S	≤0.004–0.12	0.016	0.03
	Penicillin I	0.016–1	0.12	0.5
	Penicillin R	0.06–4	0.5	1
	Macrolide S	≤0.004–1	0.5	0.5
	Macrolide R	≤0.004–4	0.5	1
	Quinolone S	≤0.004–4	0.5	1
	Quinolone R	≤0.004–2	0.5	1
Cefepime	Penicillin S	0.03–0.12	0.03	0.06
	Penicillin I	0.03–2	0.5	1
	Penicillin R	0.12–32	1	2
	Macrolide S	0.03–8	1	2
	Macrolide R	0.03–32	1	2
	Quinolone S	0.03–32	1	2
	Quinolone R	0.03–4	1	2
Ceftriaxone	Penicillin S	0.016–0.12	0.016	0.03
	Penicillin I	0.016–1	0.25	1
	Penicillin R	0.12–32	1	2
	Macrolide S	0.016–4	1	2
	Macrolide R	0.016–32	1	4
	Quinolone S	0.016–32	1	2
	Quinolone R	0.016–8	1	4
Cefotaxime	Penicillin S	0.016–0.12	0.016	0.03
	Penicillin I	0.016–1	0.25	1
	Penicillin R	0.12–32	1	2
	Macrolide S	0.016–4	1	2
	Macrolide R	0.016–32	1	2
	Quinolone S	0.016–32	1	2
	Quinolone R	0.016–8	1	2
Cefuroxime	Penicillin S	0.016–0.25	0.03	0.12
	Penicillin I	0.03–4	0.5	4
	Penicillin R	1 to >64	4	16
	Macrolide S	0.016–16	4	8
	Macrolide R	0.03 to >64	4	16
	Quinolone S	0.016 to >64	4	8
	Quinolone R	0.016–32	4	16
Cefdinir	Penicillin S	0.06–0.25	0.06	0.12
	Penicillin I	0.06–8	0.5	8
	Penicillin R	0.25 to >64	8	16
	Macrolide S	0.06–32	8	16
	Macrolide R	0.06 to >64	8	16
	Quinolone S	0.06 to >64	8	16
	Quinolone R	0.06–16	8	16
Levofloxacin	Penicillin S	0.5–32	2	16
	Penicillin I	0.5–16	1	2
	Penicillin R	0.5–32	1	4
	Macrolide S	0.5–32	1	16
	Macrolide R	0.5–32	1	2
	Quinolone S	0.5–2	1	2
	Quinolone R	4–32	16	32
Moxifloxacin	Penicillin S	0.06–4	0.25	4
	Penicillin I	≤0.03–4	0.12	0.25
	Penicillin R	≤0.03–8	0.12	0.5
	Macrolide S	0.06–8	0.25	2
	Macrolide R	≤0.03–8	0.12	0.5
	Quinolone S	≤0.03–1	0.12	0.25
	Quinolone R	0.25–8	2	8
Azithromycin	Penicillin S	0.06 to >64	0.12	>64
	Penicillin I	≤0.016 to >64	0.25	>64
	Penicillin R	≤0.016 to >64	4	>64
	Macrolide S	≤0.016–0.5	0.12	0.25
	Macrolide R	1 to >64	>64	>64
	Quinolone S	≤0.016 to >64	4	>64
	Quinolone R	≤0.016 to >64	0.12	>64
Clarithromycin	Penicillin S	≤0.016 to >64	0.06	>64
	Penicillin I	≤0.016 to >64	0.06	>64
	Penicillin R	≤0.016 to >64	1	>64
	Macrolide S	≤0.016–0.5	0.03	0.06
	Macrolide R	0.25 to >64	>64	>64
	Quinolone S	≤0.016 to >64	1	>64
	Quinolone R	≤0.016 to >64	0.06	>64
Linezolid	Penicillin S	0.25–2	1	2
	Penicillin I	0.25–4	1	2
	Penicillin R	0.25–4	1	2
	Macrolide S	0.25–4	1	2
	Macrolide R	0.25–2	1	2
	Quinolone S	0.25–4	1	2
	Quinolone R	0.5–2	1	2
Quinupristin-dalfopristin	Penicillin S	0.12–1	0.5	1
	Penicillin I	≤0.06–1	0.5	1
	Penicillin R	0.12–1	0.5	1
	Macrolide S	≤0.06–1	0.5	0.5
	Macrolide R	0.12–1	0.5	1
	Quinolone S	≤0.06–1	0.5	1
	Quinolone R	0.12–1	0.5	1
Daptomycin	Penicillin S	0.06–0.25	0.12	0.12
	Penicillin I	0.06–0.5	0.12	0.25
	Penicillin R	0.06–0.5	0.12	0.25
	Macrolide S	0.06–0.5	0.12	0.25
	Macrolide R	0.06–0.25	0.12	0.25
	Quinolone S	0.06–0.5	0.12	0.25
	Quinolone R	0.06–0.25	0.12	0.25
Vancomycin	Penicillin S	0.12–0.5	0.25	0.25
	Penicillin I	≤0.016–0.5	0.25	0.5
	Penicillin R	0.12–0.5	0.25	0.5
	Macrolide S	0.12–0.5	0.25	0.5
	Macrolide R	≤0.016–0.5	0.25	0.5
	Quinolone S	≤0.016–0.5	0.25	0.5
	Quinolone R	0.12–0.5	0.25	0.5
Teicoplanin	Penicillin S	≤0.016–0.12	0.03	0.06
	Penicillin I	≤0.016–0.12	0.03	0.06
	Penicillin R	≤0.016–0.25	0.06	0.12
	Macrolide S	≤0.016–0.12	0.06	0.12
	Macrolide R	≤0.016–0.25	0.06	0.12
	Quinolone S	≤0.016–0.25	0.06	0.12
	Quinolone R	≤0.016–0.12	0.06	0.12
Telithromycin	Penicillin S	0.008–1	0.016	0.5
	Penicillin I	≤0.004–1	0.03	0.12
	Penicillin R	≤0.004–4	0.03	0.5
	Macrolide S	≤0.004–0.12	0.016	0.03
	Macrolide R	0.008–4	0.12	1
	Quinolone S	≤0.004–4	0.03	0.5
	Quinolone R	0.008–1	0.016	0.25

TABLE 2. MICs (μg/ml) of 12 strains tested by time-kill

Drug	Strain identification no.											
	1072	3994	4945	5006	5048	3676	3258	3233	2527	1394	3791	6733[a]
Ceftobiprole	0.008	0.008	0.016	0.008	0.25	0.016	0.06	0.25	0.5	1	0.5	0.5
Amoxicillin	0.03	0.03	0.06	0.03	2	0.125	0.5	0.5	2	16	4	16
Imipenem	0.008	0.008	0.03	0.008	0.125	0.03	0.06	0.03	0.25	1	0.25	2
Ertapenem	0.03	0.03	0.125	0.03	0.5	0.125	0.25	0.125	0.5	4	0.5	4
Cefepime	0.03	0.03	0.03	0.03	1	0.125	0.5	0.5	2	2	2	2
Ceftriaxone	0.016	0.03	0.03	0.03	1	0.125	0.25	0.25	2	4	2	2
Cefotaxime	0.016	0.03	0.03	0.03	0.5	0.125	0.25	0.25	2	8	4	2
Cefuroxime	0.03	0.03	0.016	0.03	2	0.5	0.5	2	16	16	16	16
Cefdinir	0.06	0.125	0.06	0.125	0.5	0.125	1	1	8	8	8	16
Levofloxacin	16	1	2	1	1	1	1	1	16	1	1	0.5
Moxifloxacin	4	0.125	0.25	0.125	0.125	0.125	0.25	0.125	2	0.125	0.125	0.125
Azithromycin	>64	4	0.06	>64	8	4	>64	>64	0.016	>64	>64	8
Clarithromycin	>64	2	0.016	>64	2	2	>64	>64	0.016	>64	32	4
Telithromycin	0.03	0.125	0.016	0.5	0.125	0.125	0.016	0.03	0.004	0.125	0.06	0.25
Linezolid	1	1	2	1	0.5	0.5	0.5	2	0.5	2	2	2
Quinupristin-dalfopristin	1	0.5	1	1	0.5	0.5	1	0.5	0.5	1	1	1
Daptomycin	0.25	0.25	2	1	0.25	0.25	0.125	1	0.06	0.5	0.25	0.25
Vancomycin	0.25	0.5	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.25
Teicoplanin	0.06	0.06	0.125	0.25	0.06	0.06	0.06	0.03	0.06	0.06	0.06	0.125

[a] Tupelo strain (31).

MATERIALS AND METHODS

Bacteria. Strains for all studies were selected to present as large and varied a panel of drug-susceptible and drug-resistant pneumococci as possible. For agar dilution MIC determinations, clinical isolates comprising 30 penicillin-susceptible (MICs, ≤0.06 μg/ml), 60 penicillin-intermediate (MICs, 0.125 to 1 μg/ml), and 209 penicillin-resistant (MICs, ≥2 μg/ml) strains were selected from the culture collection at Hershey Medical Center isolated from 1994 to 2001; included among them was a vancomycin-tolerant pneumococcus (31). The test panel also contained 147 macrolide-susceptible strains (azithromycin MICs, ≤0.5 μg/ml) and 152 macrolide-resistant strains (azithromycin MICs, ≥1 μg/ml). The macrolide-resistant strains had defined resistance mechanisms [*erm*(B) and/or *mef*(E) genes, amino acid alterations in ribosomal proteins L4 and/or L22, or nucleotide alterations in 23S rRNA] (8, 9, 12, 35, 41, 46, 55). The test panel also contained 39 strains with levofloxacin MICs of ≥4 μg/ml, all with characterized mutations in one or more quinolone resistance determinant regions of type II topoisomerase (10, 11, 34); 11 of these also possessed a ciprofloxacin efflux mechanism, as determined by reserpine inhibition according to Brenwald et al. (6).

Antimicrobials and MIC testing. Ceftobiprole and telithromycin powders were provided by Basilea Pharmaceutica AG (Basel, Switzerland), whereas other drugs were obtained from their respective manufacturers. Agar dilution assays were performed using cation-adjusted Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% (vol/vol) sheep blood (41, 42). Quality control strains, including *S. pneumoniae* ATCC 49619, were included in each run of agar dilution assays (36). MICs of the 12 strains used for time-kill studies (see below) were determined by broth macrodilution (10, 44). Media were adjusted to contain 50 μg/ml of Ca^{2+} for testing daptomycin (28, 36).

Time-kill assays. From the panel of 299 clinical isolates, a subset comprising 12 strains was selected for time-kill analyses. The antibiotic resistance profile of this subset was as follows: 4 penicillin-susceptible, 4 penicillin-intermediate, and 4 penicillin-resistant strains; 2 macrolide-susceptible, 10 macrolide-resistant [4 strains with *erm*(B), 4 with *mef*(E), and 2 with modified L4 ribosomal proteins] strains; and 9 fluoroquinolone-susceptible and 3 fluoroquinolone-resistant (with defined mutations in type II topoisomerase) strains. Strains with macrolide MICs of >8 μg/ml, mediated by *erm*(B), were not tested due to solubilization difficulties at high drug concentrations, as well as lack of clinical significance of possible killing at very high macrolide concentrations. Antibiotic concentrations were chosen to range from 3 doubling dilutions above to 2 doubling dilutions below the broth macrodilution MIC.

Bacterial inocula were prepared by suspension of growth from an overnight blood agar plate in cation-adjusted Mueller-Hinton broth (CAMHB; BBL) and dilution of the suspension to 5×10^7 to 5×10^8 CFU/ml. Glass tubes containing antibiotic in 5 ml of CAMHB plus 5% lysed horse blood (10, 44) (and containing 50 μg/ml of Ca^{2+} for daptomycin testing) were inoculated by pipetting 0.05 ml of cell suspension beneath the surface of the broth; tubes were then vortexed and aliquots were plated on drug-free medium for viability counts within 10 min of inoculation. Tubes were incubated at 35°C in a shaking water bath. Growth controls using drug-free media were included in each experiment. Only tubes containing an initial inoculum of 5×10^5 to 5×10^6 CFU/ml were acceptable.

Viable counts for antibiotic-containing suspensions were obtained by plating 10-fold dilutions (CAMHB) of 0.1-ml aliquots from each tube onto plates of Trypticase soy agar plus 5% sheep blood (BBL), which were incubated for up to 72 h at 35°C in air enriched with 5% CO_2. Colony counts were performed on plates yielding 30 to 300 colonies, for which the lower limit of sensitivity was 300 CFU/ml (10, 44).

Time-kill assays were analyzed for each strain/antibiotic pair in terms of a $\Delta\log_{10}$ CFU/ml of −1, −2, and −3 at 3, 6, 12, and 24 h, compared to counts at 0 h. A given concentration of antimicrobial was considered bactericidal towards a particular strain if it reduced the original inoculum size by ≥3 $\log_{10}$ CFU/ml (≥99.9% killing) by 24 h or bacteriostatic if the inoculum size was reduced by <3 $\log_{10}$ CFU/ml during this same period. With the sensitivity threshold and inocula used in these studies, no problems were encountered delineating 99.9% killing. The problem of drug carryover was addressed by dilution as described previously (10, 44).

Multipassage resistance studies. Ten pneumococcal strains (1 penicillin susceptible, 2 penicillin intermediate, and 7 penicillin resistant; 2 macrolide susceptible and 8 macrolide resistant; and 7 fluoroquinolone susceptible and 3 fluoroquinolone resistant) were chosen from the panel of 299 clinical isolates for a multipassage study of emergence of endogenous resistance. Methods were based upon previous publications (7, 11, 34, 41) but with a higher initial inoculum (18). Each initial inoculum was prepared by suspending cells from an overnight Trypticase soy-blood agar plate (BBL) in CAMHB. Glass tubes containing 1 ml of CAMHB plus 5% lysed horse blood, supplemented with antibiotic ranging from 4 doubling dilutions above to 3 doubling dilutions below the broth macrodilution MIC of each agent for each strain, received 0.01 ml of culture containing ca. 10^6 CFU of 1 of the 10 strains. Tubes were incubated at 35°C for 24 h in ambient air. Cultures were passaged for up to 50 days using approximately 10^6 CFU (in a volume of 0.01 ml) inoculated from the tube nearest the MIC (1 or 2 dilutions below the MIC tube) that had the same turbidity as the antibiotic-free controls. Resistant mutants emerging during serial passage periodically were frozen at −70°C in double-strength skim milk for subsequent analysis. Optochin testing was performed every other day as a purity check of each strain. When an MIC for a given antibiotic towards a particular strain exceeded 64 μg/ml during four successive transfers, passaging was stopped and the last resistant clone was subcultured in antibiotic-free medium for 10 serial passages. Resistance properties of selected clones were determined as described below.

Single-passage resistance studies. This was performed as described previously (34). Bacterial cells were scraped from plates, washed once, and resuspended to

TABLE 3. Results of time-kill studies

Drug and concn	No. of strains with the indicated decrease[a] at:											
	3 h			6 h			12 h			24 h		
	−1	−2	−3	−1	−2	−3	−1	−2	−3	−1	−2	−3
Ceftobiprole												
4× MIC	12	4	0	12	9	3	12	12	10	12	12	12
2× MIC	11	4	0	12	9	2	12	12	10	12	12	11
MIC	9	1	0	12	6	1	12	11	9	11	11	10
Amoxicillin												
4× MIC	12	5	0	12	11	5	12	12	11	12	12	12
2× MIC	11	3	0	12	11	5	12	12	9	12	12	12
MIC	10	2	0	12	7	1	12	12	5	10	10	9
Imipenem												
4× MIC	12	7	3	12	11	4	12	12	10	12	12	12
2× MIC	12	5	2	12	10	4	12	12	10	12	11	11
MIC	11	3	2	12	10	4	12	12	8	12	11	10
Ertapenem												
4× MIC	12	3	0	12	10	3	12	12	11	12	12	12
2× MIC	12	4	0	12	9	3	12	12	12	12	12	12
MIC	9	3	0	12	8	2	12	11	9	11	10	9
Cefepime												
4× MIC	12	2	1	11	11	2	12	12	10	12	12	12
2× MIC	11	2	1	11	11	1	12	12	10	12	12	12
MIC	11	1	1	11	9	1	11	11	10	11	11	11
Ceftriaxone												
4× MIC	12	5	1	12	11	2	12	12	11	12	12	12
2× MIC	11	5	1	12	11	2	12	12	11	12	12	12
MIC	11	5	1	12	10	2	12	12	8	11	11	8
Cefotaxime												
4× MIC	12	2	0	12	7	1	12	12	9	12	12	12
2× MIC	11	2	0	12	7	1	12	11	9	12	12	12
MIC	9	2	0	12	6	1	11	11	9	12	11	9
Cefuroxime												
4× MIC	12	3	0	12	11	2	12	12	10	12	12	12
2× MIC	11	2	0	12	11	1	12	12	10	12	12	12
MIC	9	2	0	11	8	1	11	11	8	11	9	8
Cefdinir												
4× MIC	10	3	0	11	9	2	12	12	10	12	12	12
2× MIC	9	2	0	11	9	2	12	12	8	12	12	12
MIC	8	2	0	10	8	2	12	11	5	12	11	9
Levofloxacin												
4× MIC	11	6	1	11	9	3	12	12	10	12	12	12
2× MIC	9	6	1	11	7	2	12	12	10	12	12	12
MIC	6	1	0	0	5	0	12	11	7	12	9	9
Moxifloxacin												
4× MIC	11	3	0	12	9	2	12	12	10	12	12	12
2× MIC	10	1	0	12	9	1	12	12	9	12	12	11
MIC	7	0	0	12	2	0	12	9	4	12	10	6
Azithromycin[b]												
4× MIC	2	0	0	5	1	0	5	4	3	6	6	4
2× MIC	2	0	0	5	1	0	4	4	2	6	5	3
MIC	0	0	0	4	1	0	4	3	2	6	5	2
Clarithromycin[b]												
4× MIC	4	0	0	3	2	0	6	5	2	5	5	4
2× MIC	3	0	0	3	1	0	5	3	2	5	5	4
MIC	3	0	0	3	0	0	5	1	1	5	5	1
Linezolid												
4× MIC	2	0	0	9	0	0	11	6	1	12	11	3
2× MIC	1	0	0	6	0	0	11	3	0	12	10	2
MIC	0	0	0	3	0	0	6	0	0	10	4	0
Quinupristin-dalfopristin												
4× MIC	12	12	8	12	12	11	12	12	11	12	12	11
2× MIC	12	11	8	12	12	11	12	12	11	12	12	11
MIC	12	11	7	12	12	9	12	12	11	10	9	8
Daptomycin												
4× MIC	12	10	3	12	10	6	12	12	12	12	12	12
2× MIC	10	8	0	12	10	5	12	12	10	12	12	12
MIC	10	3	0	12	9	2	12	12	9	9	7	4
Vancomycin												
4× MIC	10	3	1	12	9	1	12	11	8	12	12	10
2× MIC	10	2	1	11	9	1	12	11	7	12	12	10
MIC	6	2	1	10	8	1	12	11	4	11	11	9
Teicoplanin												
4× MIC	4	0	0	7	1	0	11	10	3	12	12	10
2× MIC	3	0	0	6	1	0	11	9	1	12	12	10
MIC	2	0	0	6	1	0	11	9	1	11	11	8
Telithromycin												
4× MIC	7	3	0	9	4	1	12	8	3	12	11	8
2× MIC	4	2	0	9	4	1	12	5	2	11	11	5
MIC	4	1	0	6	3	1	9	2	1	10	7	2

[a] −1, 90% killing; −2, 99% killing; −3, 99.9% killing.
[b] Only eight strains with MICs of <8 μg/ml were tested.

a final concentration of ca. 10^{11} CFU/ml. An aliquot (0.1 ml) of bacterial suspension was spread onto brain heart infusion agar (BBL) plus 5% sheep blood supplemented with antibiotic at 1, 2, 4, and 8 times the agar dilution MIC. Plates were incubated at 35°C in air enriched with 5% CO_2 for 48 to 72 h, and resistance frequencies were calculated as the proportion of resistant colonies per inoculum (34). MICs for parent strains and resistant mutants were checked by agar dilution. Eleven randomly selected resistant mutants were picked and analyzed for their resistance mechanisms, as described below.

Pulsed-field gel electrophoresis. To confirm the identities of strains during prolonged serial passage, parental strains, resistant mutants, and all strains obtained following the final serial passages were examined by pulsed-field gel electrophoresis of SmaI-digested DNA using a CHEF DR III apparatus (Bio-Rad Laboratories, Inc., Hercules, Calif.), with a switch time of 5 to 20 s and a run time of 16 h (35).

Mechanisms of resistance. All macrolide-resistant parental strains and selected macrolide-resistant clones obtained by multi- or single-step passage were tested for the presence of *erm*(A), *erm*(B), and *mef*(E) genes by PCR amplification (55). Regulatory regions and the first 24 nucleotides of the *erm*(B) gene of all parental strains containing *erm*(B) and of selected resistant mutants emerging during serial passage were amplified using primers SR3 and SR5 (47). The ability to induce expression of *erm*(B) resistance was tested in all parents with this gene, as well as mutants selected by telithromycin from these parents, by the double-disk method. Mutations in ribosomal proteins L4 and L22 and in domains II and V of 23S rRNA were sought using primers and conditions described previously (8, 35); one fragment of 23S rRNA domain II spanning positions 389 to 1007 (*Escherichia coli* numbering) and two fragments of 23S rRNA domain V, the first spanning nucleotides 1904 to 2522 and the second spanning nucleotides 2314 to 2902 (*E. coli* numbering), were amplified. Nucleotide sequences of all amplified PCR products were obtained by direct sequencing using a CEQ8000 Genetic Analysis System (Beckman Coulter, Inc., Fullerton, Calif.).

Fluoroquinolone resistance mechanisms were examined for all parental strains and selected clones with elevated moxifloxacin MICs. Quinolone resistance determinant regions in the *gyrA*, *gyrB*, *parC*, and *parE* genes were amplified as described previously (34, 38).

TABLE 4. Results of multipassage resistance selection study with ceftobiprole and selected comparators

Parent strain	Initial strain susceptibility to group antibiotics			MICs (μg/ml)														
	Pen	FQ	Mac	Ceftobiprole					Ceftriaxone					Moxifloxacin				
				d 0	d 20	d 30	d 40	d 50	d 0	d 20	d 30	d 40	d 50	d 0	d 20	d 30	d 40	d 50
1059	I	R*	S	0.25	0.5	0.5	0.5	0.5	0.5	2	2	2	4	2	4	4	8	4
1076	S	R*	*erm*(B) + *mef*(E)	0.016	0.016	0.016	0.016	0.03	0.03	0.06	0.125	0.125	0.125	2	4	8	>32	32
1156	I	R*	S	0.06	0.125	0.125	0.125	0.06	0.125	0.5	0.25	0.5	0.25	4	8	4	8	4
1397	R	S	L4	0.5	0.5	0.5	0.5	0.5	4	8	8	8	8	0.125	0.25	0.25	0.25	0.5
2749	R	S	*erm*(B)	0.5	0.5	1	1	1	2	2	4	2	4	0.125	0.5	0.5	0.5	0.5
3260	R	S	*erm*(B)	0.5	0.5	0.5	0.5	0.5	1	1	2	2	2	0.125	0.125	0.125	0.125	0.25
3406	R	S	L4	1	1	1	1	1	8	8	8	8	8	0.5	4	2	4	4
3413	R	S	L4	0.5	0.25	1	0.5	0.5	2	2	4	2	4	0.125	0.5	1	1	1
3656	R	S	*mef*(E)	0.5	1	1	1	1	2	2	2	1	2	0.125	0.5	0.5	1	1
6733**	R	S	*mef*(E)	0.5	1	1	1	1	2	2	4	4	4	0.125	0.5	1	1	1

[a] Parental *S. pneumoniae* strain identification numbers and initial antibiotic susceptibility/resistance phenotypes are listed in the first four columns. MIC values at the start of serial passage (day 0) and after 20, 30, 40, and 50 days are shown. Pen, penicillin; FQ, fluoroquinolones; Mac, macrolides; d, day. R* (strain 1059), mutations GyrA (S81F), ParC (S79F, K137N), and ParE (I460V). R* (strain 1076), mutations GyrA (S81F), ParC (D83N), and ParE (I460V). R* (strain 1156), mutations GyrA (S81F) and ParC (S79F), 6733** (Tupelo strain), vancomycin-tolerant *S. pneumoniae* strain (31). —, a clone line had four successive serial passages with a MIC of >64 μg/ml, after which further passaging was discontinued.

RESULTS

MIC determinations. MIC results for the 299 strains, grouped according to penicillin G, macrolide, and fluoroquinolone susceptibilities, are compiled in Table 1. Ceftobiprole had the lowest MICs among the six cephalosporins tested, with MIC_{50} and MIC_{90} values (μg/ml) of, respectively, 0.016 and 0.016 (penicillin susceptible), 0.06 and 0.5 (penicillin intermediate), and 0.5 and 1 (penicillin resistant). Ceftobiprole MICs were similar to those of carbapenems against nearly all pneumococcal strains tested. The highest MIC encountered for ceftobiprole was 4 μg/ml, whereas the highest MIC encountered for imipenem was 2 μg/ml, and the highest MIC for ertapenem was 4 μg/ml. The single strain with an MIC towards ceftobiprole of 4 μg/ml had imipenem and ertapenem MICs of 0.25 μg/ml and cefepime, cefotaxime, and ceftriaxone MICs of 8, 32, and 32 μg/ml, respectively. This particular strain was fluoroquinolone and telithromycin susceptible but highly resistant to clarithromycin and azithromycin (MIC, >64 μg/ml), and it had amino acid alterations in the L4 ribosomal protein. Results from quality control strains were all as expected.

Time-kill analyses. Broth macrodilution MICs of the strains chosen for time-kill studies are listed in Table 2, and the time-kill data are shown in Table 3. At 4× the MIC, ceftobiprole was bactericidal against all 12 pneumococcal strains and bactericidal against 11/12 strains at 2× the MIC. Other β-lactams showed similar time-kill profiles. Levofloxacin and moxifloxacin were bactericidal against 11 to 12 strains at 2× the MIC, whereas azithromycin and clarithromycin at 4× the MIC were bactericidal towards only 4/8 strains, and telithromycin was bactericidal against only 5/12 strains at 2× the MIC. Despite claims that linezolid is bactericidal towards streptococci (58), the oxazolidinone proved to be largely bacteriostatic against the 12 pneumococci surveyed here; in contrast, quinupristin-dalfopristin, daptomycin, vancomycin, and teicoplanin were all bactericidal against 10 to 12 strains at 2× the MIC. The vancomycin-tolerant pneumococcus had a low MIC (0.25 μg/ml) towards this antibiotic, but the glycopeptide was only bacteriostatic towards this strain.

Multipassage selection studies. Serial passage for up to 50 days in the presence of subinhibitory concentrations of antibiotics yielded the following MIC ranges (μg/ml) for clones derived from the 10 parental strains: ceftobiprole, 0.03 to 1; ceftriaxone, 0.125 to 8; moxifloxacin, 0.25 to 32; telithromycin, 0.008 to >64; linezolid, 1 to >64; quinupristin-dalfopristin, 1 to 32; vancomycin, 0.5 (Table 4). Pulsed-field gel electrophoresis analysis performed at the end of passaging confirmed that all clones were identical to the parental strains from which they were derived.

During serial passage on moxifloxacin, one parent gave rise to a clone with an MIC of 32 μg/ml towards this antibiotic, whereas four others produced progeny with eightfold increases in MIC. Four of the five clones contained amino acid substitutions in topoisomerase II (7), and four had mutations in topoisomerase IV (Table 5). Most of the topoisomerase mutations detected in these mutants have been associated previously with reduced susceptibility to fluoroquinolones (7, 10, 11, 34, 38), though the GyrB G406V and ParE K458N substitutions have not been reported before.

Serial passage in the presence of telithromycin of parental strains containing either *erm*(B) or a mutation in ribosomal protein L4 yielded clones with MICs towards the ketolide of >64 μg/ml after 12 to 36 days, whereas macrolide-susceptible parents or parents harboring *mef*(E) as their only macrolide resistance determinant failed to develop telithromycin resistance. Parental strains containing *erm*(B) were constitutive methylase producers, as were their telithromycin-resistant progeny. Of the six clones with MICs of >64 μg/ml, three had no detectable changes in ribosomal proteins L4 or L22 or in 23S rRNA domains II and V, one had a C2611T substitution in 23S rRNA domain V, and two had deletions in ribosomal protein L22, as well as one or two nucleotide substitutions in 23S rRNA domain V (Table 6). The amino acid deletions in the L22 ribosomal protein may be associated with ≥250-fold increases in telithromycin MIC, although the two clones with amino acid deletions each also had a substitution (either G2133T plus C2611T or A2058T) in domain V of their 23S rRNA (8, 35, 46, 57). To our knowledge, this is the first

TABLE 4—*Continued*

MICs (μg/ml)																			
Telithromycin					Linezolid					Quinupristin-dalfopristin					Vancomycin				
d 0	d 20	d 30	d 40	d 50	d 0	d 20	d 30	d 40	d 50	d 0	d 20	d 30	d 40	d 50	d 0	d 20	d 30	d 40	d 50
0.008	0.004	0.03	0.016	0.03	1	8	16	16	16	0.25	0.5	1	1	1	0.5	0.5	0.5	0.5	0.5
1	—	—	—	—	1	8	32	64	>64	0.5	2	4	16	32	0.5	0.5	0.5	0.5	0.5
0.008	0.004	0.008	0.008	0.008	0.5	1	1	2	2	0.25	0.25	1	0.5	1	0.25	0.5	0.5	0.5	0.5
0.125	0.25	8	—	—	2	1	2	1	1	0.5	1	1	1	1	0.5	0.5	0.5	0.5	0.5
0.5	—	—	—	—	0.5	1	1	4	4	1	8	16	16	16	0.5	0.5	0.5	0.5	0.5
0.5	—	—	—	—	1	1	1	1	1	1	2	4	16	32	0.5	0.5	0.5	0.5	0.5
0.25	—	—	—	—	2	2	2	2	2	0.5	1	2	2	2	0.5	0.5	0.5	0.5	0.5
0.125	—	—	—	—	2	2	2	2	4	0.5	0.5	0.5	1	1	0.5	0.5	0.5	0.5	0.5
0.25	0.25	0.5	1	1	2	8	8	8	8	0.5	1	1	8	32	0.5	0.5	0.5	0.5	0.5
0.25	0.5	0.5	0.5	0.5	2	2	2	4	4	0.5	0.5	16	32	32	0.5	0.5	0.5	0.5	0.5

report of a G2133T substitution accompanying telithromycin resistance. Additionally, one clone had a deletion of two nucleotides (**AT**) within the −10 putative promoter region (underlined) **AT**AATA of *erm*(B).

Serial passage in the presence of quinupristin-dalfopristin produced clones with MICs towards the streptogramin pair of 16 to 32 μg/ml only if the parents harbored the *erm*(B) and/or *mef*(E) macrolide resistance determinant (Table 4). Three of these five highly quinupristin-dalfopristin-resistant clones were stable (within 1 $\log_2$ dilution step) after passage on drug-free medium, while two reverted to either quinupristin-dalfopristin-intermediate or quinupristin-dalfopristin-susceptible status (Table 7). Three of the five resistant clones had no detectable changes in ribosomal protein L4 or L22 or in 23S rRNA domain II or V (Table 6). Although the clone with an A2062C substitution in 23S rRNA domain V had an MIC towards quinupristin-dalfopristin of 32 μg/ml, this mutation was retained following its reversion to quinupristin-dalfopristin-susceptible status after 10 passages in antibiotic-free medium. This particular nucleotide substitution has been attributed responsibility for the elevated quinupristin-dalfopristin MIC (2 μg/ml) encountered in a pneumococcal clinical isolate (12). The clone derived from parent 1076 contained three amino acid substitutions (S20N, T93A, and S96T) in its L4 ribosomal protein. Substitutions S20N and T93A have previously been associated with macrolide resistance (46, 57) but not with streptogramin resistance.

Serial passage in the presence of linezolid produced one clone with an MIC towards the oxazolidinone of >64 μg/ml and three clones with MICs 4-, 8-, and 16-fold-higher than those of the parents (Table 4). There was no obvious correlation between any parental macrolide resistance determinant and a strain's ability to yield clones with reduced susceptibility to linezolid on the basis of the small size of the strain panel surveyed. Modification in either ribosomal protein L4 or 23S rRNA domain V was detected in all of the clones (Table 6). The G2576U substitution in 23S rRNA has been associated primarily with in vitro-selected linezolid resistance (4, 30), but the G71R and K68I substitutions in ribosomal protein L4 have not been associated hitherto with diminished linezolid susceptibility.

Twenty clones with elevated MICs, derived from serial passage, were analyzed for cross-resistance (Table 7). In two instances, an increase in MIC within the 1 $\log_2$-step margin of error (17) was encountered for an antibiotic of a class different from that on which it was passaged (parent 3260 passaged on telithromycin showed an increase in MIC towards quinupristin-dalfopristin of 1→2 μg/ml [susceptible→intermediate]; parent 3656 showed an increase in MIC towards linezolid of 2→4 μg/ml [susceptible →nonsusceptible]), whereas one strain showed a 2 $\log_2$-step increase in MIC for an antibiotic structurally unrelated to that on which it was passaged (parent 1397 passaged on telithromycin showed an increase in MIC towards quinupristin-dalfopristin of 0.5→2 μg/ml [susceptible→intermediate]). However, no cross-selection of resistance to one drug class by another drug class was observed. For two clones (derived from parents 3260 and 6733) selected with quinupristin-dalfopristin (MICs, 32 μg/ml after 50 serial passages), MICs dropped to 2 μg/ml (intermediate) or 4 μg/ml (resistant) after 10 passages on antibiotic-free medium (Table 7). Several other strains showed MIC drops of 1 $\log_2$ dilution step towards the

TABLE 5. Resistance mechanisms of selected clones obtained during serial passage; five mutants with elevated MICs towards moxifloxacin

Parental strain	Antibiotic	Initial MIC (μg/ml)	Increased MIC	No. of passages	Mutation in topoisomerases II and IV			
					GyrA	GyrB	ParC	ParE
1076	MXF[a]	2	32	50	E85K	G406V	—	K458N
3406	MXF	0.5	4	50	S81F	—	D83N	—
3413	MXF	0.125	1	50	E85Q	—	—	P454S
3656	MXF	0.125	1	50	—[b]	—	S79Y	—
6733	MXF	0.125	1	50	S81Y	—	—	—

[a] MXF, moxifloxacin.
[b] —, no mutation.

TABLE 6. Resistance mechanisms of selected clones obtained during serial passage; 15 mutants with elevated MICs towards telithromycin, linezolid, or quinupristin-dalfopristin

Parental strain	Antibiotic[a]	Initial MIC (μg/ml)	Increased MIC	No. of passages	Mutation in ribosomal proteins L4, L22, or 23S rRNA domain V		
					L4	L22	23S rRNA domain V
1059	LZD	1	16	50	—[b]	—	G2576U
1076	LZD	1	>64	50	—	—	G2576U
	QDA	0.5	32	50	S20N, T93A, S96T	—	—
	TEL[c]	1	>64	12	—	—	—
1397	TEL	0.125	>64	36	—	Δ(89FRPRAKG95)	G2133T, C2611T
2749	LZD	0.5	4	50	G71R	—	—
	QDA	1	16	50	—	—	—
	TEL	0.5	>64	19	—	—	—
3260	QDA	1	32	50	—	—	—
	TEL	0.5	>64	12	—	—	—
3406	TEL	0.25	>64	18	—	Δ(92RAKGSASPI100)	A2058T
3413	TEL	0.125	>64	19	—	—	C2611T
3656	LZD	2	8	50	K681	—	—
	QDA	0.5	32	50	—	—	—
6733	QDA	0.5	32	50	—	—	A2062C

[a] TEL, telithromycin; LZD, linezolid; QDA, quinupristin-dalfopristin.
[b] —, no mutation.
[c] Deletion in putative promoter region of *erm*(B).

antibiotic against which it was selected after passage on antibiotic-free medium.

Single-passage selection studies. Single-passage selection studies with 10 pneumococcal clinical isolates yielded the spontaneous mutants with antibiotic MIC breakthrough frequencies in Table 8. Single-step mutation frequencies for resistance to ceftobiprole for the 10 pneumococcal strains surveyed ranged from 1.7×10^{-3} to 1.2×10^{-8} (1× the MIC) to $<1.4 \times 10^{-8}$ to $<1.0 \times 10^{-9}$ (8× the MIC).

Eleven single-passage resistant clones (all from plates with pneumococci exposed to 1× the MIC) were tested for resistance mechanisms: two each selected with vancomycin, telithromycin, linezolid, or moxifloxacin and three selected with quinupristin-dalfopristin. One vancomycin-resistant clone (obtained from parent 1059) and one quinupristin-dalfopristin-resistant clone (obtained from parent 3406) had pairs of alterations in their L4 ribosomal protein (S77T and A137G and F162C and E203D, respectively) and single alterations in 23S rRNA domain V (G2239C and C2652G, respectively). A vancomycin-resistant clone (obtained from parent 2749) and a quinupristin-dalfopristin-resistant clone (obtained from parent 1059) contained single changes in 23S rRNA domain V (G2307T and G2239C, respectively). The clones selected by single passage for resistance to telithromycin or linezolid and

TABLE 7. Cross-resistance among selected multipassage mutants[a]

Parental strain	Antibiotic	Initial MIC (μg/ml)	Selected resistance		Retest MIC after 10 antibiotic-free subcultures						
			MIC	No. of passages	BPR	CRO	MXF	TEL	LZD	Q-D	VAN
1059	LZD	1	16	50	0.5	1	4	0.008	16	0.5	0.5
1076	MXF	2	32	50	0.008	0.03	>16	0.5	0.5	0.25	0.5
	TEL	1	>64	12	0.008	0.03	2	>64	1	1	0.5
	LZD	1	>64	50	0.016	0.03	2	1	64	0.5	0.5
	QDA	0.5	32	50	0.016	0.03	1	1	0.5	>16	0.5
1397	TEL	0.125	>64	36	0.5	4	0.125	>64	1	2	0.5
2749	TEL	0.5	>64	19	1	2	0.06	>64	1	1	0.25
	LZD	0.5	4	50	0.5	1	0.125	0.25	2	1	0.25
	QDA	1	16	50	0.5	1	0.125	0.5	0.5	2	0.25
3260	TEL	0.5	>64	12	0.25	0.5	0.06	>64	1	2	0.25
	QDA	1	32	50	0.25	0.5	0.125	0.5	0.5	2	0.25
3406	MXF	0.5	4	50	1	8	4	0.125	1	0.5	0.5
	TEL	0.25	>64	18	1	8	0.25	>64	1	1	0.5
3413	MXF	0.125	1	50	0.25	1	1	0.06	1	0.25	0.25
	TEL	0.125	>64	19	0.5	2	0.25	>64	1	1	0.5
3656	MXF	0.125	1	50	0.5	1	1	0.25	1	0.5	0.5
	LZD	2	8	50	0.25	1	0.125	0.25	4	0.25	0.5
	QDA	0.5	32	50	0.5	1	0.125	0.25	4	16	0.5
6733	MXF	0.125	1	50	0.25	1	0.5	0.125	1	0.25	0.5
	QDA	0.5	32	50	0.5	2	0.06	0.25	2	4	0.5

[a] Abbreviations: BPR, ceftobiprole; CRO, ceftriaxone; MXF, moxifloxacin; TEL, telithromycin; LZD, linezolid; Q-D, quinupristin-dalfopristin; VAN, vancomycin.

TABLE 8. Single-passage mutation frequencies of 10 pneumococcal strains

Strain	Selecting drug[a]	MIC	2× MIC	4× MIC	8× MIC	Strain	Selecting drug	MIC	2× MIC	4× MIC	8× MIC
1059	VAN[b]	3.3×10^{-7}	1.7×10^{-7}	$<8.3 \times 10^{-10}$	$<8.3 \times 10^{-10}$	3260	VAN	2.0×10^{-6}	$<5.0 \times 10^{-8}$	$<5.0 \times 10^{-8}$	$<5.0 \times 10^{-8}$
	Q-D[b]	2.0×10^{-6}	6.0×10^{-8}	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$		Q-D	2.5×10^{-3}	7.5×10^{-7}	$<5.0 \times 10^{-8}$	$<5.0 \times 10^{-8}$
	BPR	1.3×10^{-6}	2.7×10^{-8}	$<6.7 \times 10^{-9}$	$<6.7 \times 10^{-9}$		BPR	1.2×10^{-8}	$<1.2 \times 10^{-8}$	$<1.2 \times 10^{-8}$	$<1.2 \times 10^{-8}$
	LZD[b]	$>1.0 \times 10^{-2}$	$<3.3 \times 10^{-9}$	$<3.3 \times 10^{-9}$	$<3.3 \times 10^{-9}$		LZD	5.7×10^{-9}	$<1.4 \times 10^{-9}$	$<1.4 \times 10^{-9}$	$<1.4 \times 10^{-9}$
	CRO	$>3.0 \times 10^{-2}$	2.0×10^{-3}	$<1.0 \times 10^{-8}$	$<1.0 \times 10^{-8}$		CRO	3.8×10^{-9}	1.2×10^{-9}	$<1.2 \times 10^{-9}$	$<1.2 \times 10^{-9}$
	TEL	1.1×10^{-7}	$<2.9 \times 10^{-9}$	$<2.9 \times 10^{-9}$	$<2.9 \times 10^{-9}$		TEL	2.2×10^{-6}	5.0×10^{-7}	3.3×10^{-7}	1.7×10^{-7}
	MXF	6.0×10^{-4}	6.7×10^{-8}	$<6.7 \times 10^{-10}$	$<6.7 \times 10^{-10}$		MXF	4.0×10^{-4}	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$
1076	VAN	2.2×10^{-5}	$<3.3 \times 10^{-9}$	$<3.3 \times 10^{-9}$	$<3.3 \times 10^{-9}$	3406	VAN	1.7×10^{-3}	3.3×10^{-8}	$<1.7 \times 10^{-9}$	$<1.7 \times 10^{-9}$
	Q-D	1.0×10^{-5}	1.0×10^{-8}	$<3.3 \times 10^{-9}$	$<3.3 \times 10^{-9}$		Q-D[b]	2.1×10^{-3}	1.1×10^{-7}	3.6×10^{-8}	$<7.1 \times 10^{-10}$
	BPR	5.0×10^{-8}	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$		BPR	1.1×10^{-5}	$<1.4 \times 10^{-8}$	$<1.4 \times 10^{-8}$	$<1.4 \times 10^{-8}$
	LZD	3.2×10^{-5}	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$		LZD[b]	$>5.0 \times 10^{-3}$	3.3×10^{-8}	$<1.7 \times 10^{-9}$	$<1.7 \times 10^{-9}$
	CRO	8.8×10^{-8}	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$		CRO	1.3×10^{-5}	$<6.7 \times 10^{-9}$	$<6.7 \times 10^{-9}$	$<6.7 \times 10^{-9}$
	TEL	1.0×10^{-2}	2.5×10^{-6}	1.9×10^{-6}	1.0×10^{-6}		TEL[b]	4.3×10^{-5}	5.7×10^{-5}	8.6×10^{-7}	2.9×10^{-9}
	MXF	$>1.5 \times 10^{-3}$	3.2×10^{-6}	$<5.0 \times 10^{-10}$	$<5.0 \times 10^{-10}$		MXF[b]	1.7×10^{-4}	8.3×10^{-8}	$<1.7 \times 10^{-9}$	$<1.7 \times 10^{-9}$
1156	VAN	$<3.3 \times 10^{-9}$	$<3.3 \times 10^{-9}$	$<3.3 \times 10^{-9}$	$<3.3 \times 10^{-9}$	3413	VAN	6.0×10^{-4}	4.0×10^{-9}	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$
	Q-D	2.0×10^{-5}	2.0×10^{-8}	$<1.0 \times 10^{-8}$	$<1.0 \times 10^{-8}$		Q-D	2.1×10^{-7}	$<8.3 \times 10^{-10}$	$<8.3 \times 10^{-10}$	$<8.3 \times 10^{-10}$
	BPR	1.7×10^{-7}	$<3.3 \times 10^{-9}$	$<3.3 \times 10^{-9}$	$<3.3 \times 10^{-9}$		BPR	1.7×10^{-3}	2.0×10^{-7}	$<3.3 \times 10^{-9}$	$<3.3 \times 10^{-9}$
	LZD	2.1×10^{-5}	$<5.3 \times 10^{-10}$	$<5.3 \times 10^{-10}$	$<5.3 \times 10^{-10}$		LZD	1.0×10^{-8}	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$
	CRO	5.0×10^{-7}	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$		CRO	2.0×10^{-2}	$<2.0 \times 10^{-8}$	$<2.0 \times 10^{-8}$	$<2.0 \times 10^{-8}$
	TEL	4.0×10^{-6}	$<2.0 \times 10^{-9}$	$<2.0 \times 10^{-9}$	$<2.0 \times 10^{-9}$		TEL	3.8×10^{-7}	$<2.5 \times 10^{-8}$	$<2.5 \times 10^{-8}$	$<2.5 \times 10^{-8}$
	MXF	4.0×10^{-7}	$<1.0 \times 10^{-8}$	$<1.0 \times 10^{-8}$	$<1.0 \times 10^{-8}$		MXF	2.1×10^{-5}	$<1.4 \times 10^{-8}$	$<1.4 \times 10^{-8}$	$<1.4 \times 10^{-8}$
1397	VAN	3.2×10^{-6}	5.0×10^{-9}	$<5.0 \times 10^{-10}$	$<5.0 \times 10^{-10}$	3656	VAN	1.9×10^{-8}	$<4.0 \times 10^{-10}$	$<4.0 \times 10^{-10}$	$<4.0 \times 10^{-10}$
	Q-D	1.1×10^{-7}	$<5.6 \times 10^{-10}$	$<5.6 \times 10^{-10}$	$<5.6 \times 10^{-10}$		Q-D	9.3×10^{-7}	6.7×10^{-7}	$<3.3 \times 10^{-9}$	$<3.3 \times 10^{-9}$
	BPR	3.3×10^{-6}	3.3×10^{-7}	$<3.3 \times 10^{-9}$	$<3.3 \times 10^{-9}$		BPR	4.5×10^{-4}	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$
	LZD	$<1.4 \times 10^{-9}$	$<1.4 \times 10^{-9}$	$<1.4 \times 10^{-9}$	$<1.4 \times 10^{-9}$		LZD	8.0×10^{-8}	$<1.0 \times 10^{-8}$	$<1.0 \times 10^{-8}$	$<1.0 \times 10^{-8}$
	CRO	8.0×10^{-3}	$<1.0 \times 10^{-8}$	$<1.0 \times 10^{-8}$	$<1.0 \times 10^{-8}$		CRO	2.5×10^{-4}	$<5.0 \times 10^{-7}$	$<5.0 \times 10^{-7}$	$<5.0 \times 10^{-7}$
	TEL	7.5×10^{-7}	1.5×10^{-7}	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$		TEL	3.2×10^{-3}	1.0×10^{-5}	$<5.0 \times 10^{-7}$	$<5.0 \times 10^{-7}$
	MXF	3.0×10^{-6}	1.8×10^{-7}	$<4.0 \times 10^{-9}$	$<4.0 \times 10^{-9}$		MXF	2.8×10^{-4}	2.8×10^{-6}	$<1.4 \times 10^{-8}$	$<1.4 \times 10^{-8}$
2749	VAN[b]	9.3×10^{-6}	2.2×10^{-7}	$<1.4 \times 10^{-9}$	$<1.4 \times 10^{-9}$	6733	VAN	4.2×10^{-7}	1.4×10^{-8}	$<2.8 \times 10^{-10}$	$<2.8 \times 10^{-10}$
	Q-D[b]	7.1×10^{-3}	4.3×10^{-5}	1.4×10^{-8}	$<1.4 \times 10^{-8}$		Q-D	5.0×10^{-6}	3.0×10^{-8}	3.0×10^{-9}	$<5.0 \times 10^{-10}$
	BPR	1.7×10^{-6}	3.3×10^{-7}	$<1.1 \times 10^{-8}$	$<1.1 \times 10^{-8}$		BPR	1.0×10^{-6}	8.0×10^{-8}	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$
	LZD	1.6×10^{-5}	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$		LZD	3.0×10^{-9}	$<5.0 \times 10^{-10}$	$<5.0 \times 10^{-10}$	$<5.0 \times 10^{-10}$
	CRO	$>3.0 \times 10^{-3}$	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$		CRO	2.5×10^{-5}	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$	$<2.5 \times 10^{-9}$
	TEL[b]	3.0×10^{-3}	7.0×10^{-4}	1.5×10^{-6}	1.5×10^{-6}		TEL	1.5×10^{-5}	1.0×10^{-8}	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$
	MXF[b]	$>5.0 \times 10^{-3}$	1.7×10^{-6}	$<1.7 \times 10^{-9}$	$<1.7 \times 10^{-9}$		MXF	5.0×10^{-8}	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$

[a] VAN, vancomycin; Q-D, quinupristin-dalfopristin; BPR, ceftobiprole; LZD, linezolid; CRO, ceftriaxone; TEL, telithromycin; MXF, moxifloxacin.
[b] Colony picked for analysis of resistance mechanisms.

the quinupristin-dalfopristin-resistant clone derived from parent 3406 had no detectable changes in L4, L22, or 23S rRNA. Of two clones selected for resistance to moxifloxacin, one (derived from parent 3406) had an S81Y alteration in GyrA, whereas the other (derived from parent 2749) contained no changes in topoisomerase II or topoisomerase IV. The S81Y substitution, which has been described previously (7, 38), was also identified in one of the clones with reduced susceptibility to moxifloxacin selected during serial passage (Table 5).

DISCUSSION

Ceftobiprole, the active component of prodrug ceftobiprole medocaril (formerly BAL5788), is a broad-spectrum pyrrolidinone-3-ylidenemethyl cephem with well-documented activity against methicillin-resistant staphylococci, penicillin-resistant pneumococci, and ampicillin-susceptible enterococci, while preserving the anti-gram-negative activity of existing broad-spectrum cephalosporins (13, 18, 20, 25–27). The activity of ceftobiprole against methicillin-resistant staphylococci is attributable to its very high affinity for penicillin-binding protein 2a (as well as the normal complement of staphylococcal penicillin-binding proteins), leading to formation of a stable acyl-enzyme complex, and to its remarkable resistance to class A PC1-type β-lactamases (18). Ceftobiprole also binds to and inhibits PBP2x, the enzyme principally responsible for penicillin resistance in pneumococci (18). Ceftobiprole exhibits a modest postantibiotic effect (~0.5 h) for methicillin-resistant *Staphylococcus aureus* and a more prolonged postantibiotic effect (~2 h) for penicillin-resistant pneumococci (Basilea Pharmaceutica AG, unpublished data).

The present work shows that, while MICs towards pneumococci of all β-lactams rose in parallel with that of penicillin G, ceftobiprole had the lowest MICs of all six cephalosporins surveyed. These MICs were similar to those of carbapenems. Assuming NCCLS intravenous cephalosporin nonmeningeal pneumococcal breakpoints (37), 98.3% of 299 pneumococcal strains were susceptible to ceftobiprole, 1.3% were intermediate, and 0.3% were resistant, compared to 73.2% of strains that were susceptible, 20.1% that were intermediate, and 6.7% that were resistant to ceftriaxone. A total of 70.9% of strains were susceptible, 24.0% were intermediate, and 5.1% were resistant to cefepime.

All pneumococci examined were susceptible to linezolid, quinupristin-dalfopristin, daptomycin, vancomycin, and teicoplanin, and 99.3% of these strains were susceptible to telithromycin at a susceptibility breakpoint of ≤1 μg/ml. Results for other agents are in agreement with previous findings (2, 18, 25, 54, 56). Macrolide MICs generally rose in parallel with those of penicillin G, whereas fluoroquinolone MICs did not vary in a coordinated fashion with either β-lactam or macrolide susceptibility.

During 50 serial passages in the presence of subinhibitory concentrations of antibiotics, the highest MIC achieved for ceftobiprole by a panel of 10 pneumococcal isolates was 1 μg/ml. Under these experimental conditions, resistance (or nonsusceptibility) occurred most noticeably for telithromycin, linezolid, and quinupristin-dalfopristin (Table 4), three antibiotics targeting the ribosome in the vicinity of the peptidyl transferase center. Macrolide resistant parental strains harboring *erm*(B) [with or without *mef*(E)] appear to be particularly prone to produce clones with elevated telithromycin, linezolid, or quinupristin-dalfopristin MICs; whereas parental strains harboring *mef*(E) as their sole macrolide resistance determinant generated clones with elevated linezolid or quinupristin-dalfopristin MICs but without MICs significantly elevated towards telithromycin. The macrolide-susceptible parental strains yielded no quinupristin-dalfopristin-resistant or telithromycin-resistant clones, though one macrolide-susceptible parent gave rise to non-linezolid-susceptible progeny by 30 days of serial passage (Table 4). The occurrence of a double deletion within the putative promoter region of *erm*(B), found in a single telithromycin-resistant clone, did not prevent RNA polymerase binding, as shown by the fact that the clone remained a constitutive methylase producer.

In summary, ceftobiprole proved to be a potent, bactericidal agent towards pneumococci, irrespective of their susceptibilities to other β-lactams or other classes of antibiotics. Ceftobiprole did not select for clones with MICs exceeding 1 μg/ml during prolonged serial passage in the presence of subinhibitory concentrations of this cephalosporin, and single-passage selection experiments showed very low frequencies of endogenous resistance emergence to ceftobiprole. The excellent in vitro activities of ceftobiprole against multiresistant pneumococci and staphylococci, combined with a gram-negative spectrum of broad-spectrum or fourth-generation cephalosporins and the excellent pharmacokinetic and safety profiles of prodrug ceftobiprole medocaril (49–53), make it a very promising candidate for empirical parenteral treatment of early-onset nosocomial infections where community and hospital pathogens must be suspected.

ACKNOWLEDGMENTS

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

We thank J. McCullers (Memphis, Tenn.) for providing the Tupelo strain of *S. pneumoniae*.

REFERENCES

1. **Appelbaum, P. C.** 1992. Antimicrobial resistance in *Streptococcus pneumoniae*——an overview. Clin. Infect. Dis. **15:**77–83.
2. **Azoulay-Dupuis, E., J. P. Bédos, J. Mohler, A. Schmitt-Hoffmann, M. Schleimer, and S. Shapiro.** 2004. Efficacy of BAL5788, a prodrug of cephalosporin BAL9141, in a mouse model of acute pneumococcal pneumonia. Antimicrob. Agents Chemother. **48:**1105–1111.
3. **Block, S., C. J. Harrison, J. A. Hedrick, R. D. Tyler, R. A. Smith, E. Keegan, and S. A. Chartrand.** 1995. Penicillin-resistant *Streptococcus pneumoniae* in acute otitis media: risk factors, susceptibility patterns and antimicrobial management. Pediatr. Infect. Dis. J. **14:**751–759.
4. **Bozdogan, B., and P. C. Appelbaum.** 2004. Oxazolidinones: activity, mode of action, and mechanism of resistance. Int. J. Antimicrob. Agents **23:**113–119.
5. **Breiman, R. F., J. C. Butler, F. C. Tenover, J. A. Elliott, and R. R. Facklam.** 1994. Emergence of drug-resistant pneumococcal infections in the United States. JAMA **271:**1831–1835.
6. **Brenwald, N. P., M. J. Gill, and R. Wise.** 1998. Prevalence of a putative efflux mechanism among fluoroquinolone-resistant clinical isolates of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **42:**2032–2035.
7. **Browne, F. A., B. Bozdogan, C. Clark, L. M. Kelly, L. Ednie, K. Kosowska, B. Dewasse, M. R. Jacobs, and P. C. Appelbaum.** 2003. Antipneumococcal activity of DK-507k, a new quinolone, compared with the activities of 10 other agents. Antimicrob. Agents Chemother. **47:**3815–3824.
8. **Canu, A., B. Malbruny, M. Coquemont, T. A. Davies, P. C. Appelbaum, and R. Leclercq.** 2002. Diversity of ribosomal mutations conferring resistance to macrolides, clindamycin, streptogramin, and telithromycin in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **46:**125–131.
9. **Davies, T. A., B. E. Dewasse, M. R. Jacobs, and P. C. Appelbaum.** 2000. In vitro development of resistance to telithromycin (HMR 3647), four macro-

lides, clindamycin, and pristinamycin in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **44:**414–417.
10. **Davies, T. A., L. M. Kelly, G. A. Pankuch, K. L. Credito, M. R. Jacobs, and P. C. Appelbaum.** 2000. Antipneumococcal activities of gemifloxacin compared to those of nine other agents. Antimicrob. Agents Chemother. **44:** 304–310.
11. **Davies, T. A., G. A. Pankuch, B. E. Dewasse, M. R. Jacobs, and P. C. Appelbaum.** 1999. In vitro development of resistance to five quinolones and amoxicillin/clavulanate in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **43:**1177–1182.
12. **Depardieu, F., and P. Courvalin.** 2001. Mutation in 23S rRNA responsible for resistance to 16-membered macrolides and streptogramins in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **45:**319–323.
13. **Deshpande, L. M., and R. N. Jones.** 2003. Bactericidal activity and synergy studies of BAL9141, a novel pyrrolidinone-3-ylidenemethyl cephem, tested against streptococci, enterococci and methicillin-resistant staphylococci. Clin. Microbiol. Infect. **9:**1120–1124.
14. **Farrell, D. J., S. G. Jenkins, and R. R. Reinert.** 2004. Global distribution of *Streptococcus pneumoniae* serotypes isolated from paediatric patients during 1999–2000 and the in vitro efficacy of telithromycin and comparators. J. Med. Microbiol. **53:**1109–1117.
15. **Friedland, I. R., and G. S. Istre.** 1992. Management of penicillin-resistant pneumococcal infections. Pediatr. Infect. Dis. J. **11:**433–435.
16. **Friedland, I. R., and G. H. McCracken, Jr.** 1994. Management of infections caused by antibiotic-resistant *Streptococcus pneumoniae*. N. Engl. J. Med. **331:**377–382.
17. **Haight, T. H., and M. Finland.** 1952. Resistance of bacteria to erythromycin. Proc. Soc. Exp. Biol. Med. **81:**183–188.
18. **Hebeisen, P., I. Heinze-Krauss, P. Angehrn, P. Hohl, M. G. P. Page, and R. L. Then.** 2001. In vitro and in vivo properties of Ro 63–9141, a novel broad-spectrum cephalosporin with activity against methicillin-resistant staphylococci. Antimicrob. Agents Chemother. **45:**825–836.
19. **Ho, P.-L., T.-L. Que, S. S. Chiu, R. W. H. Yung, T.-K. Ng, D. N. C. Tsang, W.-H. Seto, and Y.-L. Lau.** 2004. Fluoroquinolone and other antimicrobial resistance in invasive pneumococci, Hong Kong, 1995–2001. Emerg. Infect. Dis. **10:**1250–1257.
20. **Issa, N. C., M. S. Rouse, K. E. Piper, W. R. Wilson, J. M. Steckelberg, and R. Patel.** 2004. In vitro activity of BAL9141 against clinical isolates of gram-negative bacteria. Diagn. Microbiol. Infect. Dis. **48:**73–75.
21. **Jacobs, M. R.** 1992. Treatment and diagnosis of infections caused by drug-resistant *Streptococcus pneumoniae*. Clin. Infect. Dis. **15:**119–127.
22. **Jacobs, M. R., and P. C. Appelbaum.** 1995. Antibiotic-resistant pneumococci. Rev. Med. Microbiol. **6:**77–93.
23. **Jacobs, M. R., S. Bajaksouzian, A. Zilles, G. Lin, G. A. Pankuch, and P. C. Appelbaum.** 1999. Susceptibilities of *Streptococcus pneumoniae* and *Haemophilus influenzae* to 10 oral antimicrobial agents based on pharmacodynamic parameters: 1997 U.S. surveillance study. Antimicrob. Agents Chemother. **43:**1901–1908.
24. **Jacobs, M. R., S. Bajaksouzian, and P. C. Appelbaum.** 2003. Telithromycin post-antibiotic and post-antibiotic sub-MIC effects for 10 gram-positive cocci. J. Antimicrob. Chemother. **52:**809–812.
25. **Jones, R. N., L. M. Deshpande, A. H. Mutnick, and D. J. Biedenbach.** 2002. In vitro evaluation of BAL9141, a novel parenteral cephalosporin active against oxacillin-resistant staphylococci. J. Antimicrob. Chemother. **50:**915–932.
26. **Kresken, M., M. Heep, and I. Wiegand.** 2004. Gram-negative bacteria producing beta-lactamases: in vitro activities of BAL 9141 and comparators. Clin. Microbiol. Infect. **10**(Suppl. 3)**:**120–121.
27. **Kresken, M., and M. Heep.** 2004. In vitro activities of BAL9141 and seven other beta-lactam antimicrobial agents towards clinical isolates of 12 members of the Enterobacteriaceae. Clin. Microbiol. Infect. **10**(Suppl. 3)**:**334.
28. **Laganas, V., J. Alder, and J. A. Silverman.** 2003. In vitro bactericidal activities of daptomycin against *Staphylococcus aureus* and *Enterococcus faecalis* are not mediated by inhibition of lipoteichoic acid biosynthesis. Antimicrob. Agents Chemother. **47:**2682–2684.
29. **Liñares, J., A. G. Campa, and R. Pallares.** 1999. Fluoroquinolone-resistance in *Streptococcus pneumoniae*. N. Engl. J. Med. **20:**1546–1548.
30. **Livermore, D. M.** 2003. Linezolid in vitro: mechanism and antibacterial spectrum. J. Antimicrob. Chemother. **51**(Suppl. 2)**:**ii9–ii16.
31. **McCullers, J. A., B. K. English, and R. Novak.** 2000. Isolation and characterization of vancomycin-tolerant *Streptococcus pneumoniae* from the cerebrospinal fluid of a patient who developed recrudescent meningitis. J. Infect. Dis. **181:**369–373.
32. **McDougal, L. K., R. Facklam, M. Reeves, S. Hunter, J. M. Swenson, B. C. Hill, and F. C. Tenover.** 1992. Analysis of multiply antimicrobial-resistant isolates of *Streptococcus pneumoniae* from the United States. Antimicrob. Agents Chemother. **36:**2176–2184.
33. **Muñoz, R., J. M. Musser, M. Crain, D. E. Briles, A. Marton, A. J. Parkinson, U. Sorensen, and A. Tomasz.** 1992. Geographic distribution of penicillin-resistant clones of *Streptococcus pneumoniae*: characterization by penicillin-binding protein profile, surface protein A typing, and multilocus enzyme analysis. Clin. Infect. Dis. **15:**112–118.
34. **Nagai, K., T. A. Davies, G. A. Pankuch, B. E. Dewasse, M. R. Jacobs, and P. C. Appelbaum.** 2000. In vitro selection of resistance to clinafloxacin, ciprofloxacin, and trovafloxacin in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **44:**2740–2746.
35. **Nagai, K., P. C. Appelbaum, T. A. Davies, L. M. Kelly, D. B. Hoellman, A. T. Andrasevic, L. Drukalska, W. Hryniewicz, M. R. Jacobs, J. Kolman, J. Miculeviciene, M. Pana, L. Setchanova, M. K. Thege, H. Hupkova, J. Trupl, and P. Urbaskova.** 2002. Susceptibilities to telithromycin and six other agents and prevalence of macrolide resistance due to L4 ribosomal protein mutation among 992 pneumococci from 10 Central and Eastern European countries. Antimicrob. Agents Chemother. **46:**371–377.
36. **National Committee for Clinical Laboratory Standards.** 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th ed. Approved standard M7-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
37. **National Committee for Clinical Laboratory Standards.** 2004. Performance standards for antimicrobial susceptibility testing—13th informational supplement. M100-S14. National Committee for Clinical Laboratory Standards, Wayne, Pa.
38. **Pan, X. S., J. Ambler, S. Mehtar, and L. M. Fisher.** 1996. Involvement of topoisomerase IV and DNA gyrase as ciprofloxacin targets in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **40:**2321–2326.
39. **Pankuch, G. A., B. Bozdogan, K. Nagai, A. Tambić-Andrašević, S. Schoenwald, T. Tambić, S. Kalenić, S. Pleško, N. K. Tepeš, Z. Kotarski, M. Payerl-Pal, and P. C. Appelbaum.** 2002. Incidence, epidemiology, and characteristics of quinolone-nonsusceptible *Streptococcus pneumoniae* in Croatia. Antimicrob. Agents Chemother. **46:**2671–2675.
40. **Pankuch, G. A., T. A. Davies, M. R. Jacobs, and P. C. Appelbaum.** 2002. Antipneumococcal activity of ertapenem (MK-0826) compared to those of other agents. Antimicrob. Agents Chemother. **46:**42–46.
41. **Pankuch, G. A., S. A. Juenemann, T. A. Davies, M. R. Jacobs, and P. C. Appelbaum.** 1998. In vitro selection of resistance to four β-lactams and azithromycin in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **42:**2914–2918.
42. **Pankuch, G. A., M. A. Visalli, M. R. Jacobs, and P. C. Appelbaum.** 1998. Susceptibilities of penicillin- and erythromycin-susceptible and -resistant pneumococci to HMR 3647 (RU 66647), a new ketolide, compared with susceptibilities to 17 other agents. Antimicrob. Agents Chemother. **42:**624–630.
43. **Pankuch, G. A., M. R. Jacobs, and P. C. Appelbaum.** 1995. Comparative activity of ampicillin, amoxycillin, amoxycillin/clavulanate and cefotaxime against 189 penicillin-susceptible and -resistant pneumococci. J. Antimicrob. Chemother. **35:**883–888.
44. **Pankuch, G. A., M. R. Jacobs, and P. C. Appelbaum.** 1994. Study of comparative antipneumococcal activities of penicillin G, RP 59500, erythromycin, sparfloxacin, ciprofloxacin, and vancomycin by using time-kill methodology. Antimicrob. Agents Chemother. **38:**2065–2072.
45. **Powis, J., A. McGeer, K. Green, O. Vanderkooi, K. Weiss, G. Zhanel, T. Mazzulli, M. Kuhn, D. Church, R. Davidson, K. Forward, D. Hoban, A. Simor, D. E. Low, and the Canadian Bacterial Surveillance Network.** 2004. In vitro antimicrobial susceptibilities of *Streptococcus pneumoniae* clinical isolates obtained in Canada in 2002. Antimicrob. Agents Chemother. **48:** 3305–3311.
46. **Reinert, R. R., A. Wild, P. Appelbaum, R. Lütticken, M. Y. Cil, and A. Al-Lahham.** 2003. Ribosomal mutations conferring resistance to macrolides in *Streptococcus pneumoniae* clinical strains isolated in Germany. Antimicrob. Agents Chemother. **47:**2319–2322.
47. **Rosato, A., H. Vicarini, and R. Leclercq.** 1999. Inducible or constitutive expression of resistance in clinical isolates of streptococci and enterococci cross-resistant to erythromycin and lincomycin. J. Antimicrob. Chemother. **43:**559–562.
48. **Schito, A. M., G. C. Schito, E. Debbia, G. Russo, J. Linares, E. Cercenado, and E. J. Bouza.** 2003. Antibacterial resistance in *Streptococcus pneumoniae* and *Haemophilus influenzae* from Italy and Spain: data from the PROTEKT surveillance study, 1999–2000. J. Chemother. **15:**226–234.
49. **Schmitt-Hoffmann, A., B. Roos, M. Schleimer, J. Sauer, A. Man, N. Nashed, T. Brown, A. Perez, E. Weidekamm, and P. Kovács.** 2004. Single-dose pharmacokinetcis and safety of a novel broad-spectrum cephalosporin (BAL5788) in healthy volunteers. Antimicrob. Agents Chemother. **48:**2570–2575.
50. **Schmitt-Hoffmann, A., L. Nyman, B. Roos, M. Schleimer, J. Sauer, N. Nashed, T. Brown, A. Man, and E. Weidekamm.** 2004. Multiple-dose pharmacokinetics and safety of a broad-spectrum cephalosporin (BAL5788) on healthy volunteers. Antimicrob. Agents Chemother. **48:**2576–2580.
51. **Schmitt-Hoffmann, A. H., B. Roos, M. Heep, M. Schleimer, E. Weidekamm, A. Man, and N. Abdou.** 2004. Influence of gender on the pharmacokinetics of BAL9141 after intravenous infusion of prodrug BAL5788. Clin. Microbiol. Infect. **10**(Suppl. 3)**:**276–277.
52. **Schmitt-Hoffmann, A. H., M. Harsch, M. Heep, M. Schleimer, T. Brown, A. Man, and W. O'Riordan.** 2004. BAL5788 in patients with complicated skin and skin structure infections caused by gram-positive pathogens including methicillin-resistant *Staphylococcus* species. Interim pharmacokinetic results from 20 patients. Clin. Microbiol. Infect. **10**(Suppl. 3)**:**277.

53. **Schmitt-Hoffmann, A. H., B. Roos, M. Schleimer, E. Weidekamm, T. Brown, M. Heep, A. Man, and L. G. Nilsson.** 2004. Dose adjustment in subjects with normal and impaired renal function based on the pharmacokinetics of BAL5788. Clin. Microbiol. Infect. **10**(Suppl. 3)**:**277.
54. **Spangler, S. K., M. R. Jacobs, G. A. Pankuch, and P. C. Appelbaum.** 1993. Susceptibility of 170 penicillin-susceptible and -resistant pneumococci to six oral cephalosporins, four quinolones, desacetylcefotaxime, Ro 23–9424 and RP 67829. J. Antimicrob. Chemother. **31:**273–280.
55. **Sutcliffe, J., T. Grebe, A. Tait-Kamradt, and L. Wondrack.** 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agents Chemother. **40:**2562–2566.
56. **Visalli, M. A., M. R. Jacobs, and P. C. Appelbaum.** 1996. MIC and time-kill study of DU-6859a, ciprofloxacin, levofloxacin, sparfloxacin, cefotaxime, imipenem, and vancomycin against nine penicillin-susceptible and -resistant pneumococci. Antimicrob. Agents Chemother. **40:**362–366.
57. **Walsh, F., J. Willcock, and S. Amyes.** 2003. High-level resistance in laboratory-generated mutants of *Streptococcus pneumoniae*. J. Antimicrob. Chemother. **52:**345–353.
58. **Zurenko, G. E., B. H. Yagi, R. D. Schaadt, J. W. Allison, J. O. Kilburn, S. E. Glickman, D. K. Hutchinson, M. R. Barbachyn, and S. J. Brickner.** 1996. In vitro activities of U-100592 and U-100766, novel oxazolidinone antibacterial agents. Antimicrob. Agents Chemother. **40:**839–845.