ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1996, p. 1148–1152 Vol. 40, No. 5
0066-4804/96/$04.00+0

Bactericidal Activities of Cefprozil, Penicillin, Cefaclor, Cefixime, and Loracarbef against Penicillin-Susceptible and -Resistant *Streptococcus pneumoniae* in an In Vitro Pharmacodynamic Infection Model

DIANE M. CAPPELLETTY[1] AND MICHAEL J. RYBAK[1,2]*

The Anti-Infective Research Laboratory, Department of Pharmacy Services, Detroit Receiving Hospital/University Health Center and College of Pharmacy and Allied Health Professions,[1] and Department of Internal Medicine, Division of Infectious Diseases, School of Medicine,[2] Wayne State University, Detroit, Michigan 48201

Received 3 July 1995/Returned for modification 1 December 1995/Accepted 1 March 1996

We examined the bactericidal activities of penicillin, cefprozil, cefixime, cefaclor, and loracarbef against three clinical isolates of *Streptococcus pneumoniae* which were susceptible, moderately susceptible, and resistant to penicillin. An in vitro two-compartment glass infection model was used to simulate human pharmacokinetics in the presence of bacteria. Also, changes in organism susceptibility and development of resistant subpopulations were evaluated. Simulated pediatric dosage regimens and target peak concentrations in the central compartment were as follows: penicillin V-potassium, 26 mg/kg of body weight every 6 h (q6h) and 14 μg/ml; cefaclor, 13.4 mg/kg q8h and 16 μg/ml; loracarbef, 15 mg/kg q12h and 19 μg/ml; cefprozil, 15 mg/kg q12h and 11 μg/ml; and cefixime, 8mg/kg q24h and 4 μg/ml. Targeted half-lives of each agent were 1 h for penicillin, cefaclor, and loracarbef; 1.3 h for cefprozil; and 3.5 h for cefixime. Growth controls were performed at two different pump rates, 0.8 and 2.0 ml/min (half-lives = 3.5 and 1 h, respectively). Each isolate demonstrated autolysis at the lower rate which was attributed to a decreased supply of fresh nutrients available to the organisms in the infection compartment. Against the susceptible isolate, the time to 99.9% killing was statistically significant between penicillin V-potassium and both cefaclor and cefixime ($P < 0.029$). Loracarbef never achieved a 99.9% reduction in the inoculum. At 48 h penicillin, cefprozil, and cefaclor were equivalent in extent of killing. Against the intermediately resistant isolate, cefprozil was superior to all other regimens with respect to rate of killing ($P < 0.013$) and extent of killing at 24 h ($P < 0.0003$). At 48 h penicillin, cefprozil, and cefaclor were equivalent in extent of killing. All of the regimens exhibited inferior activity against this penicillin-resistant isolate. A 99.9% kill was never obtained with any of the regimens, nor was there an appreciable decrease in the colony counts. In conclusion, it appears that cefprozil, penicillin, and cefaclor are effective therapies against sensitive and even intermediately sensitive isolates of *S. pneumoniae*. However, none of the oral therapies appear to be of any benefit against penicillin-resistant isolates. The in vitro model may be an effective tool in evaluating other multiple-dose therapies against this fastidious organism, since the continual supply of fresh medium maintains the viability of *S. pneumoniae* with minimal stationary-phase autolysis.

The development of resistance to commonly used antibiotics by *Streptococcus pneumoniae* has become a worldwide problem. Over the past 10 to 15 years *S. pneumoniae* has developed resistance to penicillin, macrolides, cephalosporins, trimethoprim-sulfamethoxazole, and several other antimicrobial agents. These agents are commonly utilized in the treatment of upper respiratory and middle ear infections. The incidence of drug-resistant *S. pneumoniae* in Europe varies from <1% in Northern Ireland and Greece to >30 to 40% in Spain, Hungary, and Iceland (2). In the United States the incidence of drug-resistant *S. pneumoniae* remains relatively low (5% as of 1987); however, there are regions in which the frequency is relatively high (southwestern states, Kentucky, and Tennessee [12 to 30%]) (1, 3, 16). It also appears that a higher percentage of resistant isolates are recovered from middle ear fluid than any other source of pneumococcal infections (16, 17).

* Corresponding author. Mailing address: The Anti-Infective Research Laboratory, Department of Pharmacy Services, Detroit Receiving Hospital, 4201 St. Antoine, Detroit, MI 48201. Phone: (313) 745-4554. Fax: (313) 993-2522.

Several factors may be limiting the ability to accurately identify drug-resistant isolates of *S. pneumoniae*. First, susceptibility testing of *S. pneumoniae* has not been routinely performed at most institutions, thereby limiting the ability to predict the actual incidence of penicillin-resistant isolates. Second, because of the fastidious nature of the organism it can be difficult to isolate it from a culture. Third, cultures may not be obtained from all patients, because of either the inability to obtain a specimen (i.e., middle ear fluid) or the lack of culturing and empiric treatment practices among primary-care physicians. As a result of these limitations the frequency of drug-resistant pneumococci may be higher than that currently estimated.

S. pneumoniae is the most common pathogen associated with acute otitis media (35 to 52%) and one of the top three isolates in chronic otitis media (6 to 16%) (2). *S. pneumoniae* is also one of the leading bacterial pathogens in meningitis. Because of the increasing frequency of isolation of drug-resistant *S. pneumoniae*, adequate and optimal treatment has become both a national and an international concern. The Centers for Disease Control and Prevention is suggesting that in areas with high rates of pneumococcal resistance to extended-spectrum cephalosporins, empiric treatment with vancomycin in addition

to extended-spectrum cephalosporins be considered for serious infections, such as meningitis, potentially caused by pneumococci (3).

The objectives of this study were to examine the bactericidal activities of penicillin, cefprozil, cefixime, cefaclor, and loracarbef against three clinical isolates of *S. pneumoniae* which were susceptible, moderately susceptible, and resistant to penicillin. Also changes in organism susceptibility and development of resistant subpopulations were evaluated.

MATERIALS AND METHODS

Bacterial strains. The following clinical strains of *S. pneumoniae* were studied: 237 (penicillin susceptible; serotype unknown), obtained from Detroit Receiving Hospital, and 276 (intermediately resistant to penicillin; serotype 23) and 263 (high-level resistance; serotype 6), both obtained from Baylor College of Medicine, Houston, Tex.

Medium. Cation-supplemented (25 mg of calcium and 12.5 mg of magnesium per ml) Mueller-Hinton broth with 3% lysed horse blood (MHB/LHB) was utilized for all model experiments and susceptibility testing. Colony counts were performed by utilizing tryptic soy agar (TSA) with 5% sheep blood, and cultures were incubated in candle jars (under approximately 3% CO_2) at 37°C.

Susceptibility testing. MIC determinations were performed by broth microdilution in cation-supplemented MHB/LHB according to National Committee for Clinical Laboratory Standards guidelines (12). MICs were also evaluated by utilizing an inoculum of 10^7 CFU/ml. MBCs were determined, and cultures were plated onto TSA with 5% sheep blood and incubated in candle jars at 37°C. Etests were performed for benzylpenicillin, cefaclor, and cefprozil by using a 0.5 McFarland suspension of organisms which was inoculated onto TSA with 5% sheep blood according to the manufacturer's guidelines.

Antibiotics. Penicillin V-potassium (penicillin VK; lot no. 2OH0290) supplied by Sigma, cefprozil (lot no. BMY-28100; Bristol-Myers Squibb), cefixime (lot no. 2M008; Lederle), and loracarbef (lot no. S102216) and cefaclor (lot no. S101411) supplied by Lilly were used.

In vitro model. An in vitro two-compartment glass infection model was used to simulate human pharmacokinetics in the presence of bacteria (4). Antibiotics were bolused into the central compartment represented by a 250-ml glass container with a peripheral compartment represented by a 10-ml hollow glass T tube fitted on the ends with a 0.45-μm-pore-size polysulfone membrane. The membrane allows the flow of broth and antibiotic through the peripheral compartment, provides a surface area-to-volume ratio of 0.18, and prevents migration of bacteria out of the compartment. Fresh MHB/LHB was pumped into the central compartment, displacing the antibiotic-containing medium at a rate equivalent to the elimination rate of each antibiotic. The models were placed in a 37°C waterbath for the duration of the experiments. Model experiments were carried out in duplicate for 48 h.

Simulated pediatric dosage regimens and target peak concentrations in the central compartment were as follows: penicillin VK, 26 mg/kg of body weight every 6 h (q6h) and 14 μg/ml; cefaclor, 13.4 mg/kg q8h and 16 μg/ml; loracarbef, 15 mg/kg q12h and 19 μg/ml; cefprozil, 15 mg/kg q12h and 11 μg/ml; and cefixime, 8 mg/kg q24h and 4 μg/ml (5, 7–9, 14). Targeted half-lives of each agent were 1 h for penicillin, cefaclor, and loracarbef; 1.3 h for cefprozil; and 3.5 h for cefixime.

Growth controls were performed at two different pump rates (0.8 and 2.0 ml/min) to ascertain the growth patterns at the faster and slower pump settings prior to drug experiments. Contamination of the central compartment was monitored by sampling media from this compartment during and at the end of each experiment. Organisms other than *S. pneumoniae* were considered contaminants, and when such organisms were present the results of the experiment were considered invalid.

Inoculum. A 1 McFarland suspension of organisms was prepared in 0.9% saline by using colonies from a 24-h TSA–5% sheep blood plate. One milliliter of this suspension was added to the peripheral compartment for an initial inoculum of 10^7 CFU/ml. The organisms were grown for 1 h in the model prior to dosing of the antibiotics in order to achieve an exponential growth phase.

Pharmacodynamic and pharmacokinetic analysis. Samples (0.1 ml) were taken from the central and peripheral compartments at the following times for pharmacokinetic and/or pharmacodynamic analysis of each dosing regimen: 0, 1, 2, 3, 4, 6, 8, 10, 12, 24, 26, 28, 30, 32, 34, 36, and 48 h. Suitable dilutions of the aliquots were made in 0.9% sodium chloride, and 20-μl portions were plated in triplicate on TSA containing 5% sheep blood. The plates were incubated at 37°C for 18 to 24 h in approximately 3% CO_2, and colony counts were performed. Time killing curves were constructed by plotting $\log_{10}$ CFU per milliliter against time over a 48-h period. The limit of detection for *S. pneumoniae* was determined to be 100 CFU/ml ($n = 20$), and the coefficient of variation was 7.8%. The time to 99.9% kill was ascertained by visual inspection of the graphs. Antibiotic carryover experiments were performed by utilizing 50% of anticipated central compartment peak concentrations for each antibiotic as the peripheral compartment peak concentrations against the susceptible strain of *S. pneumoniae*. The 10-fold serial dilutions performed for plating samples were adequate to prevent drug concentrations from impeding bacterial growth on the agar plates. Bacterial adherence to the inside surfaces of the filters at the end of each experiment was determined. The filters were aseptically removed from the glass T tube and placed in a sterile container with 0.9 ml of normal saline. The inner surfaces of the filters were then scraped with a sterile pipette tip, and 10-fold dilutions of the material were made in normal saline. The dilutions were plated (20 μl in triplicate) onto TSA with 5% sheep blood and incubated as for all other samples. Antibiotic concentrations within the model compartments were determined by agar diffusion microbioassay (15). The test organisms were *Bacillus subtilis* ATCC 6633 no. 2 spore suspension (Difco) for penicillin VK and *Micrococcus luteus* ATCC 9341 for cefprozil, cefaclor, and loracarbef, all in Mueller-Hinton agar. A clinical isolate of group G beta-streptococci (R135) was used in Mueller-Hinton agar for cefixime. All standards were prepared in MHB/LHB, and all samples were tested on the same day. The penicillin VK limit of detection was 0.0625 μg/ml, and intraday coefficients of variation were less than 6.25% over a range of 0.0625 to 10 μg/ml, and intraday coefficients of variation were less than 6.25% over a range of 0.0625 to 10 μg/ml. For cefprozil the limit of detection was 0.0625 and the intraday coefficients of variation were less than 4.1% over a range of 0.125 to 1 μg/ml. The limit of detection for loracarbef and cefaclor was 0.125 μg/ml, with intraday coefficients of variation of less than 8.5% over a range of 0.125 to 1 μg/ml. The cefixime limit of detection was 0.3 μg/ml, with intraday coefficients of variation of less than 7.9% over a range of 0.3 to 6 μg/ml. Correlation coefficients were ≥0.96 for all assays. Samples were stored at −70°C until analysis.

Detection of resistance. Emergence of resistance was determined by plating 20 μl of the 0-, 24-, and 48-h samples onto TSA containing 3% lysed horse blood and antibiotics at 4, 8, and 16 times the MIC for the organism. MICs were determined when organisms were isolated on the antibiotic-containing plates.

Statistics. Killing curves (residual CFU per milliliter at 12, 24, and 48 h) and the time to 99.9% reduction of the initial inoculum were compared by using a two-way analysis of variance and Tukey's test for multiple comparison of significance. A P value of ≤0.05 was considered significant.

TABLE 1. MICs and MBCs

S. pneumoniae strain	MIC/MBC (μg/ml) of:				
	Pen VK[a]	Cefprozil	Cefixime	Cefaclor	Loracarbef
237	0.064[b]/ND[c]	0.25/0.25	0.5/1	0.5/0.5	2/2
276	1/1	0.5/0.5	4/8	2/2	2/4
263	2/2	4/4	16/32	64/64	64/128

[a] Pen VK, penicillin VK.
[b] Etest result.
[c] ND, not done.

RESULTS

Susceptibility. The MICs and MBCs of the five antibiotics for each isolate are listed in Table 1. At the higher inoculum of 10^7 CFU/ml there was no change in MICs or MBCs. The results of the Etests were the same as the microtiter results for cefprozil and cefaclor with each isolate. The benzylpenicillin Etest MIC was identical to the microtiter MIC with phenoxymethylpenicillic acid for the intermediate and resistant isolates. However, with *S. pneumoniae* 237, microdilution testing resulted in a MIC of 0.125 μg/ml and the Etest MIC was 0.064 μg/ml. The clinical microbiology laboratory at our institute had reported a MIC of ≤0.03 μg/ml by their microtiter method. They also performed the oxacillin screening test and found that isolate 237 was susceptible. Because of the falsely elevated MIC obtained by the microdilution method, an accurate MBC could not be determined for the susceptible isolate.

Pharmacokinetics. Central- and peripheral-compartment pharmacokinetics are presented in Table 2. The mean elimination half-lives achieved for penicillin VK, cefaclor, and loracarbef were 0.78, 1.03, and 0.81 h, respectively. For cefprozil the mean half-life was 1.24 h, and for cefixime it was 2.8 h. The targeted central-compartment peak and trough concentrations were achieved with all regimens. A true peak concentration for the peripheral compartment is not available. This is due to variability in the rates of drug penetration across the membrane and the limited number of aliquots obtained so as not to

TABLE 2. Pharmacokinetic results of model experiments[a]

Antibiotic[b]	Half-life (h)	Concn (μg/ml) in:				
		Central compartment			Peripheral compartment	
		0 h	24 h	48 h	4 h	48 h
Pen VK	0.78 ± 0.01	16.31 ± 1.62	0.11 ± 0.04	0.11 ± 0.04	1.93 ± 0.69	1.72 ± 0.29
Cefpro	1.24 ± 0.16	12.13 ± 2.32	<0.06	<0.06	1.93 ± 0.54	0.55 ± 0.10
Cefix	2.8 ± 0.2	4.15 ± 0.63	<0.3	<0.3	1.8 ± 0.63	<0.3
Cefacl	1.03 ± 0.47	15.99 ± 2.99	<0.125	0.53 ± 0.41	1.27 ± 0.27	0.59 ± 0.16
Lora	0.81 ± 0.01	23.88 ± 0.32	<0.125	<0.125	2.86 ± 0.47	0.82 ± 0.33

[a] Data are means ± standard deviations.
[b] Pen VK, penicillin VK; Cefpro, cefprozil; Cefix, cefixime; Cefacl, cefaclor; Lora, loracarbef.

decrease the inoculum by oversampling. However, the highest observed concentrations ranged from approximately 2 to 3.5 μg/ml for penicillin VK and cefprozil, 2.7 to 3.7 μg/ml for loracarbef, 1 to 1.6 μg/ml for cefaclor, and 1 to 2.6 μg/ml for cefixime within 1 to 4 h after the initial dose.

Time-kill studies. (i) *S. pneumoniae* 237. The results of the *S. pneumoniae* 237 growth controls and time-kill studies with this isolate and each of the antibiotics are shown in Fig. 1. The growth controls at each of the pump settings were relatively similar for this isolate, with minimal variability over time. There was less a decrease of less than 1 $\log_{10}$ in the number of CFU per milliliter between the slower and faster settings at 24 h, and there was a difference of less than 2 $\log_{10}$ at 48 h. The time to 99.9% killing was 6 h for penicillin VK, 8 h for cefprozil, 11 h for cefaclor, and 9 h for cefixime. Loracarbef never achieved a 99.9% reduction in the inoculum. The time to 99.9% killing was statistically significant between penicillin VK and both cefaclor and cefixime ($P < 0.029$). Loracarbef exhibited poor killing activity against this susceptible isolate of *S. pneumoniae*. Residual colony counts, listed in Table 3, resulted in significant differences between loracarbef and all other regimens at the 12- and 48-h time points ($P < 0.0008$). At 24 h, in addition to loracarbef's demonstrating inferior activity, regrowth, which was also significant in comparison with penicillin VK, cefprozil, and cefaclor ($P < 0.04$), occurred with the cefixime simulation. With regard to the bacteria that adhered to the inner surface of the filter, the number varied for each of the antibiotics tested. Penicillin VK experiments produced the smallest quantity of bacteria attached to the filter, i.e., $\log_{10}$ 2.7 CFU/ml, and the quantities produced by the rest of the regimens ranged from approximately $\log_{10}$ 5 to $\log_{10}$ 8 CFU/ml. At all three evaluated time points cefprozil, cefaclor, and penicillin VK were fairly equivalent in the extent of killing activity. No resistance was detected for any of the regimens tested.

(ii) *S. pneumoniae* 276. The results of the *S. pneumoniae* 276 growth controls and time-kill studies are presented in Fig. 2. The growth control curves were distinctly different for each pump setting used. At 2.0 ml/min there was very little variability in the colony counts over 48 h. However, at the slower pump setting of 0.8 ml/min there was a considerable change in the counts (±1.5 $\log_{10}$ to 3 $\log_{10}$ CFU/ml) over the 48 h. Cefprozil was superior to penicillin VK, cefaclor, and cefixime with respect to rate of killing. The time to 99.9% kill was about 8 h for cefprozil, while penicillin, cefaclor, and cefixime required greater than 21 h to achieve the same kill ($P < 0.013$). Loracarbef never achieved a 99.9% reduction in the initial inoculum. The extents of killing compared at 12, 24, and 48 h (Table 3) varied in significance over the time interval. At 12 h cefprozil, cefaclor, and penicillin were significantly more active than cefixime or loracarbef ($P < 0.004$). At 24 h cefprozil was superior to all other regimens, resulting in a 2 $\log_{10}$ to 4.5 $\log_{10}$ decrease in colony count relative to the other agents ($P < 0.0003$). Cefaclor and penicillin VK were still significantly better than cefixime and loracarbef ($P < 0.0007$). At 48 h the extent of killing was similar to that at the 12-h time point, with cefprozil, cefaclor, and penicillin VK similar to each other in activity but all three superior to cefixime and loracarbef ($P < 0.015$). The number of bacteria adhering to the inner surface of the membrane at the end of the experiment was also smallest for the cefprozil therapy, as there was complete sterilization of the membranes. The rest of the regimens had colony counts of $\log_{10}$ 5 to $\log_{10}$ 8 CFU of bacteria per ml adhering to the membrane. The Pearson correlation coefficient between the colony count of bacteria in broth and that of bacteria adhering to the filters was 0.6 ($P < 0.01$). No resistance was detected for any of the regimens tested.

(iii) *S. pneumoniae* 263. The results of the growth controls and the time-kill curves for *S. pneumoniae* 263 are presented in Fig. 3. The growth controls demonstrated minimal variability in colony counts at the faster pump rate of 2.0 ml/min. However, at the slower setting (0.8 ml/min) there was a decrease in the count of approximately 2 $\log_{10}$ to 2.5 $\log_{10}$ between the first and second 12 h of the experiment. All of the regimens exhibited inferior activity against this penicillin-resistant isolate. A 99.9% kill was never obtained with any of the regimens, nor was there an appreciable decrease in the colony counts. The filters at the end of each experiment had bacteria adhering to the surface in the same quantity as that of the fluid within the

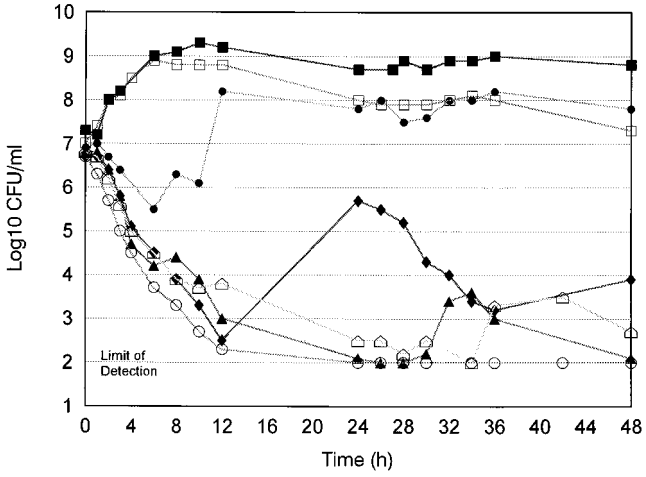

FIG. 1. Growth controls and killing curves for *S. pneumoniae* 237. ■, growth control at 2 ml/min; □, growth control at 0.8 ml/min; ○, penicillin VK; ⬠, cefprozil; ◆, cefixime; ▲, cefaclor; ●, loracarbef.

TABLE 3. Residual colony counts[a]

S. pneumoniae strain	Time (h)	Residual colony count (log_{10} CFU/ml) with:				
		Penicillin	Cefprozil	Cefaclor	Loracarbef	Cefixime
237	12	2.2 ± 0.3	3.8 ± 0.2	3.0 ± 0.1	7.7 ± 1.1	2.6 ± 0.3
	24	2.0 ± 0	2.5 ± 0	2.1 ± 0.1	7.7 ± 0.5	4.6 ± 1.2
	48	2.0 ± 0	2.7 ± 0	2.1 ± 0.1	7.4 ± 0.7	3.1 ± 1.2
276	12	5.0 ± 0.1	3.7 ± 0.5	4.9 ± 0.2	7.2 ± 0	7.1 ± 0.6
	24	4.4 ± 0.1	2.0 ± 0	3.6 ± 1.1	6.6 ± 0.2	6.0 ± 0.3
	48	2.8 ± 0.1	2.0 ± 0	2.2 ± 0.5	7.7 ± 0.4	6.0 ± 1.4
263	12	5.7 ± 0.2	7.4 ± 1.7	9.0 ± 0	9.2 ± 0.1	8.5 ± 0.6
	24	7.3 ± 0.4	7.9 ± 0.7	8.8 ± 0	8.8 ± 0.2	7.8 ± 0.6
	48	7.2 ± 0.6	7.2 ± 1.2	8.4 ± 0.2	8.4 ± 0.1	6.4 ± 0.9

[a] Data are means ± standard deviations.

chamber ranging from log_{10} 6.5 to log_{10} 8.4 CFU/ml. No growth was detected on any of the antibiotic-containing agar plates.

DISCUSSION

S. pneumoniae is a difficult organism to grow in broth media over periods longer than approximately 12 h because of the stationary-phase autolytic process that is common to this species. We had performed growth control experiments in test tubes with Todd-Hewitt broth, Todd-Hewitt broth plus yeast extract and MHB/LHB. At 8 h all isolates had increased in colony count by 2.5 log_{10} to 3 log_{10} CFU/ml; however, at 24 h the colony count decreased by 3 log_{10} to 6 log_{10} CFU/ml (data not shown). For this reason, in vitro test-tube killing curves are often carried out for only 6 to 12 h so that any organism death can be attributed to the antibiotic and not to the autolytic processes. Since fresh medium is not continually supplied for test-tube killing curves, required nutrients may be completely consumed by the bacteria present, resulting in a decrease in colony counts. The in vitro model continuously supplies fresh medium to the organisms in the infection compartment and continuously removes waste products produced by the bacteria. This elimination of waste and provision of a fresh supply of catalase from the lysed horse blood may be in part responsible for the minimal cell death that was observed during the stationary growth phase in our growth control experiments. This theory is supported by the differences in growth patterns that were demonstrated for all three isolates at the different pump rate settings. At 2 ml/min the colony counts decreased by only 0.4 log_{10} to 0.8 log_{10} CFU/ml during the stationary growth phase. At the slower setting of 0.8 ml/min, the amount of fresh medium provided was significantly smaller than that provided at the faster pump rate and the changes in colony counts were greater for each isolate. Between 12 and 24 h the inoculum decreased by 0.8 log_{10} to 2.7 log_{10} CFU/ml, and it decreased by 0.3 log_{10} to 3 log_{10} CFU/ml between 36 and 48 h. While these variations in growth patterns appear to be isolate dependent, they also seem to be related to the amount of fresh medium supplied.

The overall extents of killing observed for all of the oral beta-lactams, with the exception of loracarbef, with the susceptible isolate of *S. pneumoniae* were similar. While cefixime did result in regrowth at 24 h in the model experiments, extrapolation to an in vivo environment should be undertaken only with caution, since the model does not incorporate immune system components. Another phenomenon of in vitro killing curves is adherence of bacteria to surfaces, with the possibility that these bacteria could dislodge and unexpectedly increase colony counts during an experiment (6). This phe-

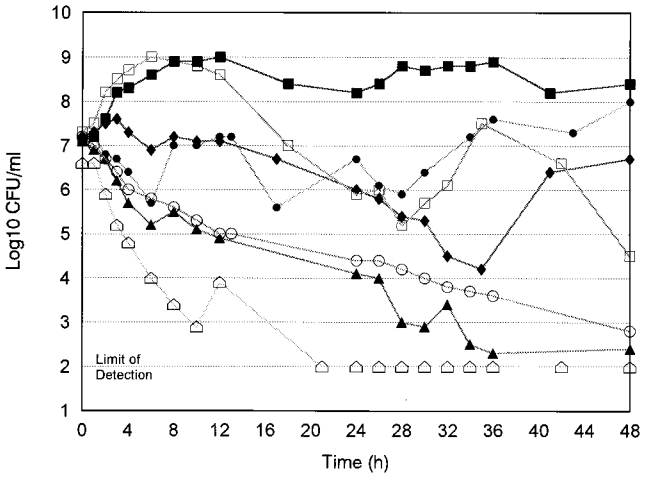

FIG. 2. Growth controls and killing curves for *S. pneumoniae* 276. ■, growth control at 2 ml/min; □, growth control at 0.8 ml/min; ○, penicillin VK; ⌓, cefprozil; ◆, cefixime; ▲, cefaclor; ●, loracarbef.

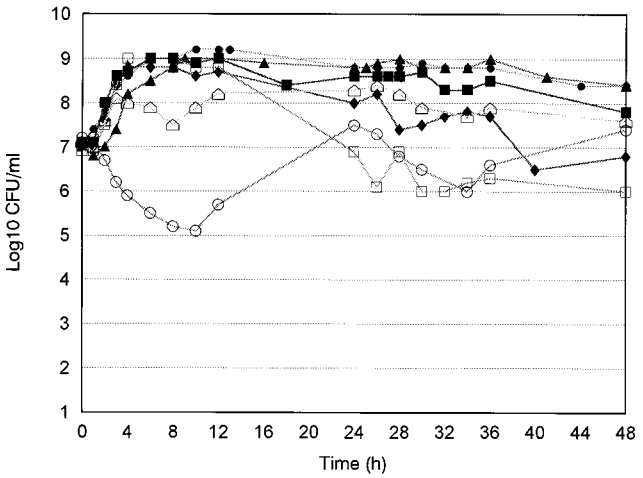

FIG. 3. Growth controls and killing curves for *S. pneumoniae* 263. ■, growth control at 2 ml/min; □, growth control at 0.8 ml/min; ○, penicillin VK; ⌓, cefprozil; ◆, cefixime; ▲, cefaclor; ●, loracarbef.

nomenon was examined in our experiments by determining the number of bacteria adhering to the inner surfaces of the membranes and comparing it with the number of organisms suspended in the fluid at the end of each experiment. There appeared to be a reasonable correlation ($r = 0.6$) between the organisms adhering to the membrane and the organisms suspended in the broth. Sterilization of the membranes was observed only for cefprozil therapy against the intermediately resistant isolate. We find this correlation interesting, although the clinical significance of the result is unknown. Cefprozil was superior in both the rate of killing and the extent of killing at 24 h to all other regimens with the intermediate isolate 276. By 48 h, however, cefaclor and penicillin VK were equivalent in extent of killing activity. Both cefixime and loracarbef were inferior therapies against this intermediate isolate. As anticipated, none of the oral therapies were effective against the penicillin-resistant isolate, which was also resistant to the cephalosporins. Amoxicillin is commonly used to treat infections caused by *S. pneumoniae* (especially otitis media, since middle ear concentrations relative to MICs for the organisms are good [8]). However, amoxicillin was not included in this study and it would be important to compare the killing activity of amoxicillin against several intermediate and resistant isolates with the killing achieved with cefprozil to help to define the role of cefprozil in the treatment of infections caused by these organisms.

The 10-ml peripheral compartment may represent a small microabscess or infected fluid space which could be consistent with infected middle ear fluid. The antibiotic concentrations obtained in the peripheral (infection) compartment were within the range of middle ear fluid concentrations reported in the literature (7–11, 13, 14). Even though approximately 50% of all otitis media cases spontaneously resolve, the data from this study suggest that cefprozil, penicillin, and cefaclor may help in resolution of infections caused by susceptible and intermediately susceptible isolates while the added benefit of cefixime or loracarbef is not as evident.

In conclusion, it appears that cefprozil, penicillin, and cefaclor are effective therapies against penicillin-susceptible and even intermediately susceptible isolates of *S. pneumoniae*. However, there is a need to test more intermediately susceptible isolates, since the MICs for this class of organisms can vary and approach breakpoints for resistance. None of the oral therapies appear to be of any benefit against penicillin-resistant isolates. To better elucidate the use of cefprozil against intermediately susceptible and penicillin-resistant isolates of *S. pneumoniae*, additional isolates should be tested and studies comparing the killing activity of cefprozil with that of amoxicillin should be performed. The in vitro model may be an effective tool for evaluating other multiple-dose therapies against this fastidious organism, since the continual supply of fresh medium maintains the viability of *S. pneumoniae* with minimal stationary-phase autolysis.

ACKNOWLEDGMENTS

This work was supported through a grant from Bristol-Myers Squibb.

We thank Edward O. Mason, Jr. (Baylor College of Medicine, Houston, Tex.), for providing the intermediately susceptible (276) and resistant (263) isolates of *S. pneumoniae* and the serotype information. We also thank Glenn Kaatz for his technical assistance.

REFERENCES

1. **Applebaum, P. C.** 1992. Antimicrobial resistance in *Streptococcus pneumoniae*: an overview. Clin. Infect. Dis. **15:**77–83.
2. **Baquero, F., E. Loza, C. Negri, F. Tubau, T. Alonso, and J. Linares.** 1993. Penicillin resistance in *S. pneumoniae* and in vitro activity of selected oral antibiotics in Europe. Infect. Med. **1993**(Suppl. D)**:**25–32.
3. **Block, S., J. Hedrick, P. Wright, R. Finger, R. Leggiadro, M. Appleton, S. Kahn, and R. Hutcheson.** 1994. Drug-resistant *Streptococcus pneumoniae*—Kentucky and Tennessee, 1993. Morbid. Mortal. Weekly Rep. **43:**23–25, 31.
4. **Cappelletty, D. M., M. J. Rybak, S. L. Kang, and S. M. Palmer.** 1995. Pharmacodynamics of ceftazidime administered as continuous infusion or intermittent bolus alone and in combination with single daily-dose amikacin against *Pseudomonas aeruginosa* in an in vitro infection model. Antimicrob. Agents Chemother. **39:**1797–1801.
5. **DeSante, K. A., M. L. Zeckel.** 1992. Pharmacokinetic profile of loracarbef. Am. J. Med. **92**(Suppl. 6A)**:**16s–19s.
6. **Haag, R., P. Lexa, and I. Werkhäuser.** 1986. Artifacts in dilution pharmacokinetic models caused by adherent bacteria. Antimicrob. Agents Chemother. **29:**765–768.
7. **Kamme, C., K. Lundgren, and H. Runderantz.** 1969. The concentration of penicillin V in serum and middle ear exudate in acute otitis media in children. Scand. J. Infect. Dis. **1:**77–83.
8. **Krause, P. J., N. J. Owens, C. H. Nightingale, J. J. Klimek, W. B. Lehmann, and R. Quintiliani.** 1982. Penetration of amoxicillin, cefaclor, erythromycin-sulfisoxazole, and trimethoprim-sulfamethoxazole into the middle ear fluid of patients with chronic serous otitis media. J. Infect. Dis. **145:**815–821.
9. **Kusmiesz, H., S. Shelton, O. Brown, S. Manning, and J. D. Nelson.** 1990. Loracarbef concentrations in middle ear fluid. Antimicrob. Agents Chemother. **34:**2030–2031.
10. **Lahikainen, E. A.** 1970. Penicillin concentration in middle ear secretion in otitis. Acta Oto-laryngol. **70:**358–362.
11. **Lundgren, K., L. Ingvarsson, and H. Rundcrantz.** 1979. The concentration of penicillin V in middle ear exudate. Int. J. Pediatr. Otorhinolaryngol. **1:**93–96.
12. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically, 3rd ed. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
13. **Nelson, J. D., C. M. Ginsburg, O. McLeland, J. Clahsen, M. C. Culbertson, Jr., and H. Carder.** 1981. Concentrations of antimicrobial agents in middle ear fluid, saliva and tears. Int. J. Pediatr. Otorhinolaryngol. **3:**327–334.
14. **Shyu, W. C., J. Haddad, J. Reilly, W. N. Kahn, D. A. Campbell, Y. Tsai, and R. H. Barbhaiya.** 1994. Penetration of cefprozil into middle ear fluid of patients with otitis media. Antimicrob. Agents Chemother. **38:**2210–2212.
15. **Simon, H. J., and E. J. Yin.** 1970. Microbioassay of antimicrobial agents. Appl. Microbiol. **19:**573–579.
16. **Spika, J. S., R. R. Facklam, B. D. Plikaytis, M. J. Oxtoby, and the Pneumococcal Surveillance Working Group.** 1991. Antimicrobial resistance of *Streptococcus pneumoniae* in the United States, 1979–1987. J. Infect. Dis. **163:** 1273–1278.
17. **Stutman, H. R.** 1993. Penicillin-resistant *Streptococcus pneumoniae* focus on otitis media. Infect. Med. **1993**(Suppl. D)**:**51–55.