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Relative Efficacies of Broad-Spectrum Cephalosporins for Treatment of Methicillin-Susceptible *Staphylococcus aureus* Experimental Infective Endocarditis

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The effects of treatment with broad-spectrum parenterally administered cephalosporins and cefuroxime, cefazolin, or nafcillin were compared in an experimental model of *Staphylococcus aureus* infective endocarditis, and the results in vivo were compared with the activities of the study drugs in vitro. After 3 days of treatment, all antimicrobial agents tested were more effective than no treatment in reducing the number of surviving bacteria in cardiac valve vegetations. Nafcillin was the most effective agent studied and was significantly more active than was ceftizoxime, ceftriaxone, cefotaxime, cefoperazone, cefuroxime, or cefazolin ($P \leq 0.05$). Cefpirome and ceftazidime were the most effective broad-spectrum cephalosporins. The outcome of treatment with cefpirome or ceftazidime was similar to that of treatment with nafcillin and significantly better than that of treatment with ceftizoxime or cefotaxime ($P \leq 0.05$). Treatment outcome correlated closely with the MICs of the antimicrobial agents for the study strain with the exception of ceftazidime, which was significantly more active in vivo in comparison with other agents than predicted by its MIC ($P \leq 0.0003$). When ceftazidime was excluded as an outlier, treatment outcome correlated with the MICs of the remaining study drugs (Spearman's correlation coefficient, 0.95; $P \leq 0.0004$), as well as with the estimated percentage of time during which the concentration of total drug (correlation coefficient, −0.85; $P \leq 0.007$) or free drug (correlation coefficient, −0.90; $P \leq 0.003$) exceeded the MIC. A consideration of total or free drug concentrations in relation to MICs did not significantly improve the correlation with outcome observed with the MICs alone.

Broad-spectrum cephalosporins are used widely in clinical practice. In general, these agents are more active in vitro than narrow-spectrum cephalosporins against gram-negative aerobic microorganisms, at the expense of variable decreases in antistaphylococcal activity (4). Broad-spectrum cephalosporins are not the antimicrobial agents of choice for known monobacterial staphylococcal infection, but they are frequently prescribed as empiric broad-spectrum antimicrobial therapy in situations in which *Staphylococcus aureus*, either alone or in combination with other microorganisms, may be among the significant pathogens. Controlled clinical data directly comparing the efficacies of these newer cephalosporins in patients who have staphylococcal bacteremia are unavailable. Previous animal model studies suggested that, for ceftazidime and staphylococci, in vitro data correlate poorly with in vivo efficacy (1, 9). The experimental endocarditis model of infection represents a stringent test of vivo antibacterial activity, is highly reproducible, and offers the advantages of quantitative endpoints under defined experimental conditions and the ability to simultaneously compare several treatments.

This study was designed to compare the relative antistaphylococcal activities in vivo of newer cephalosporins, including cefuroxime, cefoperazone, cefpirome, ceftazidime, ceftizoxime, cefotaxime, and ceftriaxone, in a rabbit model of experimental endocarditis. Cefazolin and nafcillin were included as indicators of the relative effectiveness of standard therapies in this model. We additionally sought to determine the relationship between in vivo outcome of treatment and in vitro antibacterial activity as assessed by standard laboratory methods, and to test the hypothesis that ceftazidime is an outlier in this relationship.

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MATERIALS AND METHODS

In vitro studies. Twenty-five isolates of *S. aureus* recovered from patients with infective endocarditis were stored in defibrinated sheep blood at −70°C until ready for use. Strains were thawed and subcultured on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) containing 5% sheep blood before testing. A macrodilution method (11) was used for susceptibility testing with standard (5×10^5 CFU/ml) and high (5×10^7 CFU/ml) inoculum sizes. Organisms were prepared from overnight broth cultures and inoculated into serial twofold dilutions of antimicrobial agents in tryptic soy broth. Subcultures were made to confirm inoculum sizes and purity. Tubes were incubated for 24 h at 35°C in room air. The MIC was defined as the lowest concentration of antimicrobial agent with no visible growth of staphylococci. The MBC was determined by subculturing 100 μl of broth from the control tube, from the first tube containing visible growth, and from all tubes not containing visible growth and was defined as the lowest concentration of antimicrobial agent that resulted in a 3-log (99.9%) decrease of the original inoculum at 24 h (10). For the strain chosen for in vivo experiments, the MIC of each of the study antimicrobial agents at the standard inoculum fell within the midrange of the 25 strains tested (data not shown). In vitro susceptibility testing of the study strain was performed as described above in fivefold replicates, and the average value was calculated as the geometric mean of the replicates. The

study strain produced a type C staphylococcal β-lactamase (6).

In vivo studies. For each antimicrobial agent, concentrations in the serum of five healthy animals were assayed at 30 min and 1, 2, 4, 8, 12, and 24 h after a single antimicrobial dose. The doses used in the in vivo experiments were chosen to approximate the usual peak concentrations of the respective antimicrobial agents in the serum of humans receiving recommended doses (12). A bioassay technique was used for all antimicrobial assays (15).

Experimental catheter-associated aortic valve infective endocarditis was established by a modification of the method described by Garrison and Freedman (5). In brief, New Zealand White rabbits (weight, >2 kg) were anesthetized with a mixture of ketamine and xylazine injected intramuscularly (i.m.). An incision was made in the neck and the right carotid artery was exposed. The artery was ligated distally and a sterile polyethylene catheter (PE 90; Clay Adams) was inserted into the artery through a small incision and advanced proximally across the aortic valve and into the left ventricle. The end of the catheter was sealed and the wound was closed over the catheter with surgical clips.

Staphylococci were inoculated into tryptic soy broth and incubated overnight. The broth cultures were then diluted to an inoculum size of 10^5 CFU of staphylococci per ml, and aliquots were frozen at −70°C for later use. After thawing, the inoculum size was confirmed by quantitative culturing and, 24 h after catheter placement, 1 ml of the inoculum was injected into a peripheral ear vein to seed the aortic valve vegetations. Blood cultures were done for all animals before antimicrobial treatment to confirm the presence of bacteremia.

Twenty-four hours after the intravenous injection of staphylococci, animals were placed in the following treatment groups: (i) 9 animals received no treatment; (ii) 15 animals received cefazolin (50 mg/kg of body weight) i.m. three times daily (t.i.d.); (iii) 15 animals received nafcillin (200 mg/kg of body weight) i.m. t.i.d.; (iv) 18 animals received cefuroxime (60 mg/kg of body weight) i.m. t.i.d.; (v) 17 animals received cefoperazone (50 mg/kg of body weight) i.m. t.i.d.; (vi) 12 animals received cefotaxime (100 mg/kg of body weight) intravenously t.i.d.; (vii) 17 animals received cefpirome (40 mg/kg of body weight) subcutaneously t.i.d.; (viii) 12 animals received ceftazidime (25 mg/kg of body weight) i.m. t.i.d.; (ix) 17 animals received ceftizoxime (50 mg/kg of body weight) i.m. t.i.d.; and (x) 17 animals received ceftriaxone (7.5 mg/kg of body weight) i.m. twice daily.

Antimicrobial therapy was administered for 3 days. Surviving treated animals were sacrificed by intravenous injection of sodium pentobarbital at least 12 h after administration of the last antimicrobial dose. Because untreated animals rarely survived 4 days after infection, these animals were sacrificed on day 2 postinfection. The chest was opened, the heart was excised and opened, and aortic valve vegetations were removed aseptically. The cardiac valve vegetations were weighed, homogenized with a stomacher (Seward Laboratories, London, England), and cultured quantitatively by a pour plate method. The results were expressed as $\log_{10}$ CFU of staphylococci per gram of valve vegetation. For purposes of quantitative analysis, samples with no growth at the lowest dilution ("sterile" vegetations) were uniformly assigned the value of the lowest detectable quantity of microorganisms among all such samples (approximately 0.6 $\log_{10}$ CFU per g of valve vegetation).

Analysis. The overall null hypothesis that no differences in mean $\log_{10}$ CFU of staphylococci per gram of valve vegetation existed among any of the treatment groups was analyzed statistically with the Kruskall-Wallis test to estimate the per-experiment type 1 error rate. The per-experiment error rate was controlled for multiple comparisons by pursuing individual pairwise comparisons only if the preliminary Kruskall-Wallis test indicated significant differences among treatments at the $\alpha = 0.05$ level (13). Pairwise comparisons between treatment groups were performed with the Wilcoxon rank sum test. The reported *P* values for individual treatment group comparisons therefore reflect the comparison-wise type 1 statistical error rate conditional on an experiment-wise error rate of ≤ 0.05.

Pharmacokinetic parameters were estimated by fitting a linear curve by the method of least squares to the logarithm of the observed antimicrobial concentrations in serum during the elimination phase. Estimates of the percentage of antimicrobial agent bound by protein in rabbit serum were obtained from published references (3, 7, 14). Correlation of treatment outcome with pharmacokinetic parameters and in vitro susceptibility was estimated by Spearman's rank correlation coefficient. To test the hypothesis that ceftazidime is an outlier in the relationship between in vitro susceptibility and treatment outcome, we examined the partial *t* statistic for the second variable in a bivariate linear regression model, where treatment outcome is the dependent variable, MIC is the first independent variable, and the second independent variable is 1 for ceftazidime and 0 otherwise. To test whether models including pharmacokinetic parameters improved the prediction of outcome over that obtained with the model using the MIC alone, we calculated the partial *t* statistic from

TABLE 1. In vitro susceptibilities of the *S. aureus* strain studied in vivo

Antimicrobial agent	In vitro susceptibility, mean mg/liter (range)[a] with a			
	Standard inoculum, 10^5 CFU/ml		High inoculum, 10^7 CFU/ml	
	MIC	MBC	MIC	MBC
Cefazolin	0.5 (0.5–0.5)	7.0 (0.5–64)	0.6 (0.5–1)	≥16 (1–>128)
Cefuroxime	1.7 (1–2)	13.9 (2–64)	1.1 (1–2)	≥16 (4–>128)
Cefoperazone	2.0 (1–8)	12.1 (1–64)	4.0 (4–4)	≥21 (8–>128)
Cefpirome	0.7 (0.5–1)	1.0 (0.5–4)	0.7 (0.5–1)	≥18 (1–>128)
Ceftazidime	10.6 (4–16)	≥42.2 (8–>128)	≥111 (32–>128)	>128 (>128)
Ceftizoxime	3.5 (1–8)	13.9 (8–32)	7.0 (4–8)	≥111 (32–>128)
Cefotaxime	2.3 (2–4)	≥32.0 (2–>128)	1.7 (1–2)	≥32 (4–>128)
Ceftriaxone	3.0 (2–4)	3.0 (2–4)	3.0 (2–4)	≥37 (8–>128)
Nafcillin	0.3 (0.125–0.5)	3.0 (0.5–128)	0.3 (0.25–0.5)	≥8 (0.5–>128)

[a] Five replicates.

TABLE 2. Concentrations of antimicrobial agents in rabbit serum measured from 0.5 to 12 h after administration

Antimicrobial agent (dose [mg/kg], route)	% Protein bound[a]	Total concn (mg/liter) in serum at h[b]:					
		0.5	1	2	4	8	12
Cefazolin (50, i.m.)	92	154	129	59	<6	<6	<6
Cefuroxime (60, i.m.)	43	115	48	13	<4	<4	<4
Cefoperazone (50, i.m.)	92	147	118	48	10	<3	<3
Cefpirome (40, subcutaneously)	4	59	54	34	10	<1	<1
Ceftazidime (25, i.m.)	14	109	86	27	<1	<1	<1
Ceftizoxime (50, i.m.)	28	77	31	10	<4	<4	<4
Cefotaxime (100, intravenously)	92	109	43	7	<4	<4	<4
Ceftriaxone (7.5, i.m.)	98	58	67	39	24	6	<4
Nafcillin (200, i.m.)	91	60	45	24	12	4	<0.2

[a] Estimated serum protein binding from Klesel and Seeger (7), Craig and Suh (3), and Takeda et al. (14).

[b] Mean concentration of antimicrobial agent in sera of five uninfected animals after a single dose.

bivariate linear regression using treatment outcome as the dependent variable and either the MIC alone or the MIC in combination with the pharmacokinetic parameter as the independent variables.

RESULTS

In vitro studies. The in vitro susceptibilities of the study strain are shown in Table 1. Repeated measurements of the MICs of the study drugs at either a standard or high inoculum demonstrated a difference of less than or equal to one dilution from the median in most cases. In contrast, measurements of the MBCs demonstrated a high degree of interassay variability and were poorly reproducible, as expected. MBC results were therefore not analyzed further. A comparison of the MICs at standard (10^5 CFU/ml) and high (10^7 CFU/ml) inocula demonstrated little inoculum effect with this strain, with the exception of ceftazidime. Because of the discordance between the activities of ceftazidime in vitro and in vivo (see below), the susceptibility of the study strain was also tested substituting 50% rabbit serum as the diluent. The MICs of ceftazidime and other study antimicrobial agents were unchanged in rabbit serum (data not shown), suggesting that a potential interaction with unrecognized factors in rabbit serum was not responsible for the superior activity of ceftazidime in vivo.

In vivo studies. The concentrations of antimicrobial agents observed in vivo at the doses given are shown in Table 2. Table 3 shows the results of treatment. Preliminary overall analysis revealed a highly significant difference among treatment groups ($P < 0.0004$). As assessed by the rate of clearance of bacteria from cardiac valve vegetations, all antimicrobial agents studied were more effective ($P < 0.05$) than no treatment. Nafcillin was the most effective agent and was significantly ($P < 0.05$) more effective than was ceftizoxime, cefotaxime, ceftriaxone, cefoperazone, cefuroxime, or cefazolin. Ceftazidime and cefpirome were similar in efficacy to nafcillin or cefazolin ($P > 0.10$) and were significantly more effective ($P < 0.05$) than was ceftizoxime, cefotaxime, or ceftriaxone. Differences in survival and in the proportion of valves sterilized after 3 days of treatment were not statistically significant between treatment groups.

A comparison of the rank order of the MICs of the study antimicrobial agents and treatment outcomes demonstrated

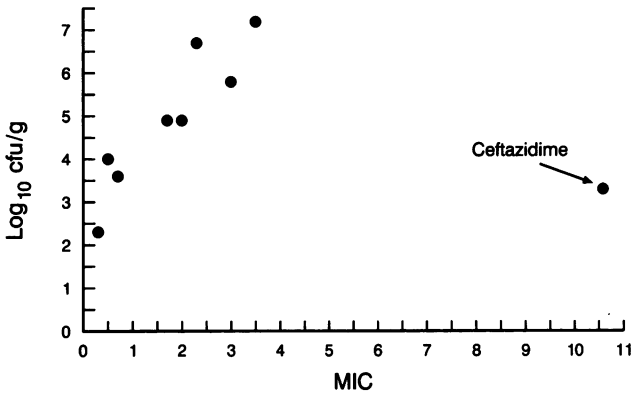

FIG. 1. Relationship between treatment outcome for *S. aureus* experimental endocarditis and the MICs of study antimicrobial agents at an inoculum of 10^5 CFU/ml.

a close correspondence between activities in vitro and in vivo, with the exception of ceftazidime, which was the least active drug in vitro but the second most effective drug in vivo (Table 1). Spearman's correlation coefficient comparing outcome with MIC for all study drugs was 0.49 (P not significant). As illustrated in Fig. 1, however, ceftazidime was a highly significant outlier ($P \leq 0.0003$) in the otherwise apparent correlation between treatment outcome and susceptibility in vitro. Ceftazidime was therefore excluded from further analysis of the relationship between treatment outcome and in vitro or pharmacokinetic parameters for the remaining eight drugs.

Excluding ceftazidime, outcome was highly correlated with susceptibility in vitro as assessed by MICs using standard inoculum (Spearman's correlation coefficient, 0.95; $P \leq 0.0004$). Treatment outcome was also significantly correlated with the estimated percentage of time during which either the total drug concentration or the free drug concentration exceeded the MIC using the standard inoculum (correlation coefficients, −0.85 and −0.90, respectively; $P \leq 0.007$ and $P < 0.003$, respectively). Bivariate linear regression analysis with the MIC as the first independent

TABLE 3. Results of β-lactam treatment of *S. aureus* experimental endocarditis in rabbits

Treatment	No. surviving/ no. treated	No. with sterile vegetations/no. surviving	Log_{10} CFU of staphylococci/ g of vegetation (mean ± SD)	Rank	
				In vivo	In vitro[a]
None		0/9	9.8 ± 2.2		
Ceftizoxime	15/15	0/15	7.2 ± 2.9[b]	9	8
Cefotaxime	12/12	1/12	6.7 ± 3.7[c]	8	6
Ceftriaxone	15/17	1/15	5.8 ± 3.0[c]	7	7
Cefoperazone	14/17	1/14	4.9 ± 3.3[d]	6	5
Cefuroxime	16/18	0/16	4.9 ± 3.5[d]	5	4
Cefazolin	15/15	0/15	4.0 ± 2.6[b,d]	4	2
Cefpirome	16/17	3/16	3.6 ± 2.3[b,c]	3	3
Ceftazidime	9/12	2/9	3.3 ± 3.0[b,c]	2	9
Nafcillin	14/15	2/14	2.3 ± 1.6[b,c,d]	1	1

[a] Based on the MIC at an inoculum of 10^5 CFU/ml.

[b] $P \leq 0.05$ for ceftizoxime compared with cefazolin, cefpirome, ceftazidime, or nafcillin.

[c] $P \leq 0.05$ for cefotaxime or ceftriaxone compared with cefpirome, ceftazidime, or nafcillin.

[d] $P \leq 0.05$ for cefoperazone, cefuroxime, or cefazolin compared with nafcillin.

variable and the estimated percentage of time that either the total drug concentration or the free drug concentration exceeded the MIC as the second independent variable revealed no significant improvement of prediction in the model by the addition of the pharmacokinetic term ($P \geq 0.20$).

DISCUSSION

We observed significant differences among cephalosporins in the outcome of treatment of experimental *S. aureus* endocarditis. The relative antistaphylococcal activities of the β-lactams studied in this model closely paralleled the activities in vitro as assessed by the MICs at the standard inoculum with the exception of ceftazidime, which was significantly more active in vivo than predicted by the susceptibility of the study strain. Assessment of the MICs at a higher inoculum did not enhance the ability to predict outcome compared with the results obtained by standard laboratory methods. Despite the recognized importance of bactericidal therapy in the treatment of infective endocarditis, the inherent variability in the measurement of the MBCs for staphylococci was expected and limits the precision and interpretability of this in vitro measurement of antibacterial activity. The technical difficulties in interpreting the results of MBC tests, especially for *S. aureus*, have been previously reviewed (16).

The observation of increased activity in vivo of ceftazidime extends the findings of Baker and Fass (1), who observed that therapy with ceftazidime or cephalothin was of equivalent efficacy in a rabbit model of experimental staphylococcal infective endocarditis, despite lesser activity in vitro of ceftazidime against the study strain as assessed by the MIC, MBC, serum inhibitory activity, and serum bactericidal activity. They concluded that, for cephalothin and ceftazidime, the results of laboratory tests that quantitated antimicrobial activity in vitro did not correlate with in vivo efficacy.

McColm et al. (9), using a rabbit model of experimental staphylococcal endocarditis, found treatment with ceftazidime or methicillin to be equally effective and concluded that different in vitro susceptibilities of the infecting strain were not paralleled by differential therapeutic responses in vivo. In a subsequent study of the single-dose pharmacokinetics of ceftazidime, cefuroxime, and methicillin in infected cardiac valve vegetations, McColm and Ryan (8) reported that, although ceftazidime showed a higher percentage of penetration into vegetations relative to concentrations in serum, this drug also had the shortest time above the MIC in infected vegetations. The agreement between the results of our study and those of previous studies (1, 9) suggests that the efficacy of ceftazidime in this model is not unique to the strain of *S. aureus* used in our study.

For drugs other than ceftazidime, treatment outcome as assessed by bacterial clearance from valve vegetations correlated closely with the MIC when a standard inoculum size and standard laboratory methods were used. Under experimental conditions, these data are consistent with the interpretation of the MIC performed in accordance with National Committee for Clinical Laboratory Standards standards as a predictor of in vivo antistaphylococcal efficacy for most but not all antimicrobial agents. We also found that the time during which either the total drug concentration or the free drug concentration exceeded the MIC correlated with treatment outcome. The lack of significant improvement in the prediction of outcome over that provided by the MIC alone is not surprising in view of the strong correlation of outcome with the MIC alone and the interrelationships of these in vitro and pharmacokinetic variables. Furthermore, doses were chosen in the present study to provide therapeutic concentrations of antimicrobial agents, rather than to compare the pharmacodynamic concentration-effect relationships of individual drugs at multiple doses below those required for a maximal effect. The relationships between the intrinsic activities in vitro of antimicrobial agents against specific microorganisms, the degree of protein binding, the concentrations of antimicrobial agents in serum, dose intervals, and the observed effects on the outcome of experimental endocarditis have recently been reviewed by Carbone (2).

The results of the current study are consistent with recommendations that β-lactamase-stable penicillins or narrow-spectrum cephalosporins be the drugs of choice for serious methicillin-susceptible *S. aureus* infections. Broad-spectrum cephalosporins were more effective than no treatment but, except for cefpirome and ceftazidime, were significantly less effective than nafcillin under these experimental conditions. The in vivo activity of cefpirome in this model paralleled its enhanced in vitro activity relative to those of other broad-spectrum cephalosporins against staphylococci. Ceftazidime was also highly effective in this model, but this observation should be extended with caution to staphylococcal infections in humans. The MIC of ceftazidime for *S. aureus* was not predictive of treatment outcome for this agent; treatment outcome for the study drugs other than ceftazidime correlated closely with the MIC.

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REFERENCES

1. **Baker, R. L., and R. J. Fass.** 1984. Correlation of in vitro activities of cephalothin and ceftazidime with their efficacies in the treatment of *Staphylococcus aureus* endocarditis in rabbits. Antimicrob. Agents Chemother. **26**:231–234.
2. **Carbone, C.** 1991. Impact of the antibiotic dosage schedule on efficacy in experimental endocarditis. Scand. J. Infect. Dis. **74**(Suppl.):163–172.
3. **Craig, W. A., and B. Suh.** 1986. Protein binding and the antimicrobial effects: methods for the determination of protein binding, p. 477–514. *In* V. Lorian (ed.), Antibiotics in clinical medicine, 2nd ed. The Williams & Wilkins Co., Baltimore.
4. **Donowitz, G. R., and G. L. Mandell.** 1990. Cephalosporins, p. 246–256. *In* G. L. Mandell, R. G. Douglas, Jr., and J. E. Bennett (ed.), Principles and practice of infectious diseases, 3rd ed. Churchill Livingstone, New York.
5. **Garrison, P., and L. Freedman.** 1970. Experimental endocarditis. Staphylococcal endocarditis in rabbits resulting from placement of a polyethylene catheter in the right side of heart. Yale J. Biol. Med. **42**:394–410.
6. **Kernodle, D. S., C. W. Stratton, L. W. McMurray, J. R. Chipley, and P. A. McGraw.** 1989. Differentiation of beta-lactamase variants in *Staphylococcus aureus* by substrate hydrolysis profiles. J. Infect. Dis. **159**:103–108.
7. **Klesel, N., and K. Seeger.** 1983. Pharmacokinetic properties of the new cephalosporin antibiotic HR810 in animals. Infection **11**:318–321.
8. **McColm, A. A., and D. M. Ryan.** 1985. Penetration of β-lactam antibiotics into cardiac vegetations, aorta and heart muscle in experimental *Staphylococcus aureus* endocarditis: comparison of ceftazidime, cefuroxime and methicillin. J. Antimicrob. Chemother. **16**:349–358.
9. **McColm, A. A., D. M. Ryan, and P. Acred.** 1984. Comparison of ceftazidime, cefuroxime and methicillin in the treatment of *Staphylococcus aureus* endocarditis in rabbits. J. Antimicrob.

Chemother. **14**:373–377.
10. **National Committee for Clinical Laboratory Standards.** 1987. Methods for determining bactericidal activity of antimicrobial agents. M26-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
11. **National Committee for Clinical Laboratory Standards.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2nd ed. M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
12. **Norris, S., C. H. Nightingale, and G. L. Mandell.** 1990. Tables of antimicrobial agent pharmacology, p. 443–444. *In* G. L. Mandell, R. G. Douglas, Jr., and J. E. Bennett (ed.), Principles and practice of infectious diseases, 3rd ed. Churchill Livingstone, New York.
13. **O'Brien, P. C., and M. A. Shampo.** 1988. Statistical considerations for performing multiple tests in a single experiment. 2. Comparisons among several therapies. Mayo Clin. Proc. **63:** 816–820.
14. **Takeda, K., H. Tsuji, I. Fukuda, T. Nagaki, T. Morita, Y. Watanabe, Y. Toshimitsu, J. Kinami, and K. Manbo.** 1986. Absorption, distribution, metabolism and excretion of cefuroxime axetil (CXM-AX) in rats and dogs. Chemotherapy (Tokyo) **34**(Suppl. 5):343–353.
15. **Washington, J. A.** 1985. Bactericidal tests, p. 691–735. *In* J. A. Washington and N. S. Brewer (ed.), Laboratory procedures in clinical microbiology, 2nd ed. Springer Verlag, New York.
16. **Washington, J. A.** 1989. In vitro testing of antimicrobial agents. Infect. Dis. Clin. North Am. **3**:375–387.