

## Antibiotic Activity In Vitro Against Methicillin-Resistant *Staphylococcus epidermidis* and Therapy of an Experimental Infection

FRANKLIN D. LOWY,\* JULIA A. WALSH, MARGUERITE M. MAYERS, ROBERT S. KLEIN, AND NEAL H. STEIGBIGEL

*Division of Infectious Diseases, Department of Medicine, Montefiore Hospital and Medical Center, and Department of Medicine, The Albert Einstein College of Medicine, Bronx, New York 10467*

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*Staphylococcus epidermidis* is a major pathogen in early prosthetic valve endocarditis and cerebrospinal fluid shunt infections. Approximately 10 to 15% of hospital isolates are methicillin resistant. Ten clinically significant isolates of the latter were collected for antibiotic studies in vitro and in an experimental infection in animals. Time-kill studies of five strains showed gentamicin to be the single most effective antibiotic; however, dwarf colony variants emerged as survivors with two of these strains when challenged with gentamicin alone. The addition of a second antibiotic to gentamicin did not significantly improve the bactericidal rate but prevented the emergence of variant strains. A blood culture isolate of methicillin-resistant *S. epidermidis* combined with 5% hog gastric mucin was used to establish an experimental intraperitoneal infection in mice. Neither methicillin nor nafcillin treatment reduced mortality below that of untreated animals. Cephalothin treatment delayed early mortality but did not diminish overall mortality. Gentamicin was the most effective single antibiotic, and gentamicin in combination with vancomycin was the most effective regimen overall. The combination of rifampin plus vancomycin was as effective as gentamicin alone. The combinations of cephalothin or nafcillin with gentamicin and cephalothin with vancomycin demonstrated antagonism. The antagonism was not due to multiple injections or drug-drug inactivation.

*Staphylococcus epidermidis*, in addition to being a common blood culture contaminant, may be an important cause of life-threatening infections. It is the predominant pathogen in meningitis associated with intraventricular shunts (30, 31) and early prosthetic valve endocarditis (14, 32) and accounts for 2 to 5% of reported cases of subacute bacterial endocarditis (9, 18, 21). Despite this clinical importance, appropriate antimicrobial therapy for *S. epidermidis* infections remains uncertain. Approximately 10 to 15% of *S. epidermidis* isolates are methicillin resistant (15, 25). Susceptibilities of hospital isolates of this organism to other antibiotics are variable (1, 10), but routine antibiotic susceptibility studies usually suggest that strains of methicillin-resistant *S. epidermidis* (MRSE) are susceptible to gentamicin, cephalothin, vancomycin, and rifampin (1, 10, 28). This study investigates the activity of these drugs, singly and in combination, against clinical isolates of MRSE in time-kill experiments in vitro and in a mouse peritonitis model.

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### MATERIALS AND METHODS

**Bacterial strains.** Ten clinically significant isolates of MRSE were collected from three hospitals. Isolates came from patients with the following infections: prosthetic valve endocarditis, five patients; intravenous catheter septicemia, two; septicemia from arteriovenous fistula, atrioventricular shunt infection, and recurrent infection of lacrimal duct, one each. All isolates were methicillin and oxacillin resistant by standard Kirby-Bauer disk susceptibility testing (3), and the methicillin minimal inhibitory concentration (MIC) for the isolates by tube dilution studies was 6.25 µg/ml or greater. The strains identified as *S. epidermidis* had positive catalase and anaerobic glucose fermentation tests and negative coagulase and mannitol fermentation tests and were novobiocin susceptible. The strain (Sut) of MRSE used in both in vitro studies and the mouse peritonitis model was isolated from the blood of a renal transplant patient with intravenous catheter sepsis.

**Susceptibility testing.** Disk diffusion testing was performed by the Kirby-Bauer method (3). Tube dilution testing was performed in duplicate in Mueller-Hinton broth, using an inoculum of  $2.5 \times 10^5$  colony-forming units (CFU)/ml obtained from a 1:1,000 dilution of an overnight culture. The MIC was interpreted by visual inspection after 24 h of incubation at 35°C. Using a 0.01-ml calibrated loop, subcultures of each tube were plated on sheep blood agar and incubated overnight. The minimal bactericidal concentration was defined by 99.9% killing and was recorded as that antibiotic concentration allowing growth of fewer than three colonies. Similar tube dilution studies were also performed with higher inocula obtained from 1:10 and 1:100 dilutions of an overnight specimen.

**Time-kill studies.** Time-kill studies were performed at 37°C, using an overnight suspension of the organism diluted 1/10 or 1/5,000 in Mueller-Hinton broth and incubated for 0.5 h before the addition of antibiotics. Aliquots from the growth flasks were removed for bacterial colony counting at 0, 1, 2, 4, 6, and 24 h after the addition of antibiotics and were treated with *Bacillus cereus* beta-lactamase to minimize carry-over of beta-lactam antibiotic activity (26). Duplicate plating of serial tenfold dilutions was performed using heart infusion agar at pH 5.5. The lowered pH was used to diminish carry-over of aminoglycoside activity (26). Statistical analysis was done by using Student's *t* test.

**Mouse peritonitis model.** Newly weaned, 15-g Swiss albino mice (Camm Research Laboratories, Wayne, N.J.) were injected intraperitoneally with a 1-ml suspension consisting of 0.5 ml of 5% hog gastric mucin and 0.5 ml of the Sut strain of *S. epidermidis* (20). The hog gastric mucin (American Laboratory, Inc., Omaha, Neb.) was prepared with distilled water and then autoclaved. *S. epidermidis* Sut was stored at -70°C at a density of  $10^7$  CFU/ml in 1-ml aliquots consisting of 0.5 ml of Mueller-Hinton broth and 0.5 ml of defibrinated sheep blood (BBL Microbiology Systems, Cockeysville, Md.). Before an experiment, 0.5 ml of the thawed suspension of the organisms was inoculated into 1,000 ml of Mueller-Hinton broth, incubated overnight in a shaker bath at 37°C, and then centrifuged, washed, and resuspended in 100 ml of sterile 0.9% saline. The average bacterial density of the suspension to be mixed with hog gastric mucin for injection was  $3.5 (\pm 2.5) \times 10^9$  CFU/ml.

There was no mortality in 41 mice given a 1-ml intraperitoneal injection of 0.5 ml of 5% hog gastric mucin mixed with 0.5 ml of 0.9% sterile saline. Mortality in 141 untreated animals was 96% by 44 h after intraperitoneal inoculation of the MRSE. Peritoneal fluid was purulent at autopsy. Ten infected mice were sacrificed 6 h after bacterial inoculation, and peritoneal fluid bacterial colony counts were made. The average count was  $3.2 \times 10^9$  CFU/ml. Metastatic infection was documented in liver, kidney, and spleen by culture. Portions of the liver, kidney, and spleen were excised, immersed in alcohol, and then flamed to remove surface contamination. They were then homogenized (A. H. Thomas, 3431-E04AA) in 0.5 ml of 0.9% NaCl and plated on heart infusion agar after serial dilution.

Pharmaceutical preparations of the antibiotics were

administered subcutaneously at 2 and 4 h after the intraperitoneal bacterial injection, in the following doses: nafcillin, 8 mg; methicillin, 8 mg; cephalothin, 8 mg; vancomycin, 0.4 mg; gentamicin, 0.4 mg; and rifampin, 0.6 mg. Antibiotic dosages were originally chosen to be comparable to doses given to patients on the basis of body surface area, assuming the mice to have a surface area of 0.005 m<sup>2</sup> (34). These doses were then further adjusted to produce serum antibiotic levels roughly comparable to achievable human antibiotic levels. Antibiotic regimens were evaluated in multiple experiments, with untreated controls included with each treatment group. Except for the vancomycin-plus-gentamicin treatment group, all regimens using more than one antibiotic were given in separate injections at different sites. All animals received the same total amount of fluid (0.8 ml) for each treatment regimen. Cumulative mouse mortality was noted in each treatment group at 5, 20, 24, 44, and 72 h. Mortality which occurred before the completion of both antibiotic injections was excluded from statistical evaluation, but very few animals died between the first and second injections. Chi-square analysis with Yates correction was used for statistical evaluation.

A search for potential gentamicin-resistant variants was made in 12 infected mice treated with gentamicin alone. These animals were sacrificed at 5 and 25 h. Peritoneal swabs were used to streak Mueller-Hinton agar plates on which standard 10- $\mu$ g gentamicin disks were placed. In addition, a 1:10 dilution of peritoneal fluid was incorporated into plates of heart infusion agar at pH 7, with and without 1  $\mu$ g of gentamicin per ml.

**Antibiotic assay.** Mouse serum antibiotic levels were determined in a group of five uninfected mice, using a modification of the microbiological disk assay described by Sabath et al. (26). These animals received a single subcutaneous dose of antibiotic. Blood was taken for assay at 0.5 and 3 h. Depending on the antibiotic being measured, either an *S. epidermidis* or a *Sarcina* strain was incorporated into 1% streptomycin assay agar. All measurements of zones of inhibition were made in quadruplicate, and each drug level represents an average of serum levels in five mice.

**Study of possible drug-drug inactivation.** Drug-drug inactivation experiments were undertaken to study the increase in mouse mortality which occurred when cephalothin was given in combination with gentamicin or vancomycin.

(i) **In vitro.** Pharmaceutical antibiotic preparations of cephalothin (50  $\mu$ g/ml) and gentamicin (10  $\mu$ g/ml) were incubated at 37°C over 24 h in Mueller-Hinton broth separately and in combination. A microbiological antibiotic disk assay (26) was used to determine if there was any loss of activity when the two drugs were incubated together. This experiment was also performed in the presence of  $10^9$  CFU of strain Sut MRSE per ml. Cephalothin in the presence of gentamicin was assayed using a cephalothin-susceptible *S. epidermidis* strain with sodium polyanethol sulfonate (Hoffman-LaRoche, Nutley, N.J.) incorporated into the agar to inactivate gentamicin (12). Gentamicin in the presence of cephalothin was assayed with a cephalothin-resistant *Enterobacter aerogenes* strain.

(ii) **In vivo.** Two groups of six mice each were given

subcutaneous injections of either gentamicin (0.4 mg) alone or gentamicin (0.4 mg) and cephalothin (8 mg) (in separate sites). Serum levels were taken at 0.5 and 1.5 h. The gentamicin level was measured in both groups, using the *E. aerogenes* strain, by the microbiological disk assay described above.

**Antibiotics.** Antibiotics were obtained from the pharmaceutical suppliers of these drugs: vancomycin (Lilly), cephalothin (Lilly), methicillin (Bristol), nafcillin (Wyeth), gentamicin (Schering), and rifampin (Ciba).

## RESULTS

**Susceptibility testing.** The results of standard Kirby-Bauer disk susceptibility tests of ten clinical isolates of MRSE indicated that all were resistant to methicillin and oxacillin and susceptible to gentamicin, cephalothin, vancomycin, and novobiocin. Three isolates were susceptible to nafcillin, four were resistant, and three were of intermediate susceptibility. Eight of the isolates were resistant to clindamycin. Tube dilution susceptibility studies using an inoculum of  $2.5 \times 10^5$  CFU of MRSE per ml showed the MIC of methicillin for the ten isolates to have a median of 25  $\mu\text{g/ml}$ , with a range of 6.2 to 50  $\mu\text{g/ml}$ , and a median minimal bactericidal concentration of  $>100$   $\mu\text{g/ml}$ , with a range of 25 to  $>100$   $\mu\text{g/ml}$ . The median and range of MICs of nafcillin were 0.8 and 0.4 to 1.6  $\mu\text{g/ml}$ , respectively, and for minimal bactericidal concentrations they were  $>100$  and 6.2 to  $>100$   $\mu\text{g/ml}$ , respectively.

**Time-kill studies.** Time-kill studies performed using the first five clinical isolates of MRSE collected at an initial concentration of  $5 \times 10^5$  CFU/ml are summarized in Fig. 1. Gentamicin, 0.5  $\mu\text{g/ml}$ , was the most effective single drug and demonstrated the most rapid rate of early killing. It was significantly ( $P < 0.01$ ) more bactericidal than cephalothin or vancomycin alone at the 1- and 2-h points. However, with two of the five strains, dwarf colony variants emerged with exposure to gentamicin alone. These variants did not emerge when gentamicin was used in combination with other drugs. However, no combination improved on the early bactericidal activity shown by gentamicin alone.

Figures 2 and 3 show time-kill experiments with the Sut strain of *S. epidermidis* subsequently used in the mouse peritonitis model. These studies were performed at a higher bacterial inoculum,  $5 \times 10^7$  CFU/ml, to more closely parallel the higher bacterial densities found in infections. The results again show gentamicin to be the most effective antibiotic. The addition of cephalothin, vancomycin, or rifampin to gentamicin did not significantly change the killing rates. Rifampin alone exhibited a rapid early bactericidal killing rate, similar to gentamicin, but this rate was not maintained, partly due to the emergence of organisms shown to be rifampin resistant. Rifampin in combination with vancomycin was as effective as gentamicin alone. The killing rates of cephalothin and vancomycin

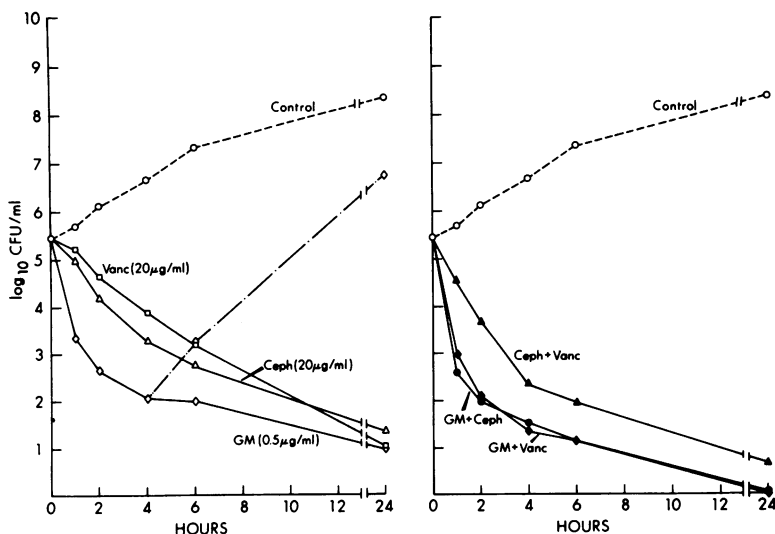


FIG. 1. Time-kill studies with five methicillin-resistant *S. epidermidis* clinical isolates. Each point represents the mean of the five studies. Antibiotics: gentamicin (GM), vancomycin (Vanc), and cephalothin (Ceph). The antibiotic concentrations of drugs used in combination (right panel) were the same as those used when single antibiotics were employed (left panel). Emergence of surviving small colony variants were noted with two of the strains exposed to gentamicin (---).

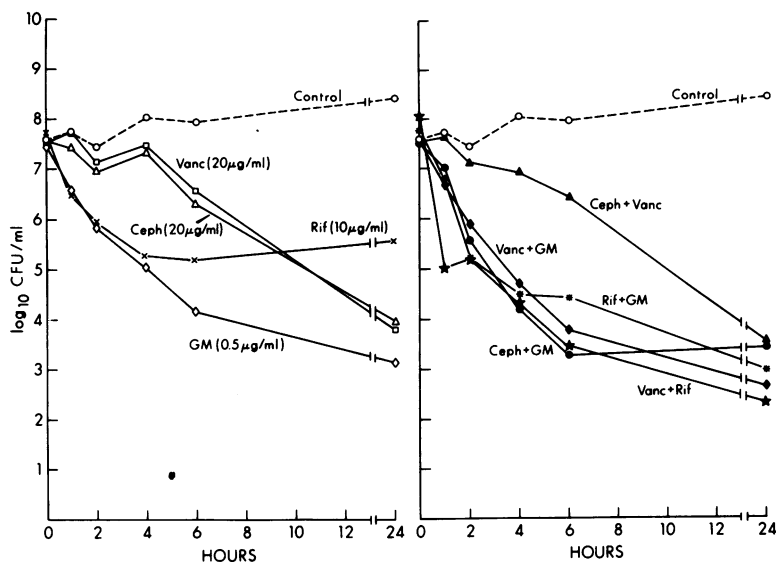


FIG. 2. Time-kill studies using the *Sut* strain of MRSE. Antibiotics: gentamicin (GM), vancomycin (Vanc), rifampin (Rif), and cephalothin (Ceph). The antibiotic concentrations of drugs used in combination (right panel) were the same as those used when single antibiotics were employed (left panel).

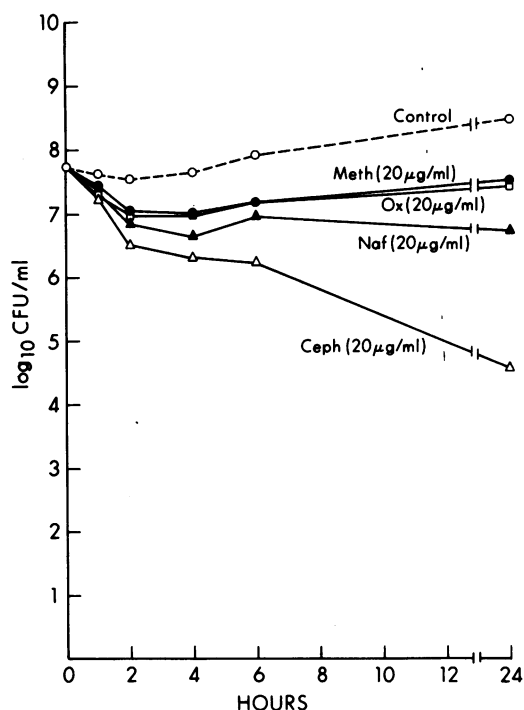


FIG. 3. Time-kill studies using the *Sut* strain of MRSE. Antibiotics: methicillin (Meth), oxacillin (Ox), nafcillin (Naf), and cephalothin (Ceph).

were approximately equal. Figure 3 demonstrates little difference in the bactericidal activities of nafcillin, methicillin, and oxacillin at the higher inoculum. Although cephalothin was

somewhat more effective at 24 h, there was no appreciable difference between cephalothin and the semisynthetic penicillins over the first 6 h.

**Mouse mortality experiments.** The tube dilution susceptibilities of *S. epidermidis Sut* used in these studies and the average serum levels achieved in uninfected mice at 0.5 and 3 h after injection are recorded in Table 1. Infected mice received the same doses at 2 h and again at 4 h after bacterial inoculation. The cumulative mortality in the untreated controls was not significantly different from that for the methicillin or nafcillin treatment groups. Cephalothin treatment results significantly differed from those of the control and the semisynthetic penicillin treatment groups only at 20 and 24 h ( $P < 0.05$ ).

TABLE 1. Tube dilution susceptibilities of methicillin-resistant *S. epidermidis* strain *Sut*<sup>a</sup> and average serum antibiotic concentrations achieved in mice 0.5 and 3 h after subcutaneous injection

Antibiotic	MIC ( $\mu\text{g}/\text{ml}$ )	MBC <sup>b</sup> ( $\mu\text{g}/\text{ml}$ )	Serum concn ( $\mu\text{g}/\text{ml}$ )	
			0.5 h	3 h
Nafcillin	1.6	>100	56.3	2.9
Cephalothin	0.8	6.3	130	4
Vancomycin	1.6	12.5	30.3	2.7
Gentamicin	0.05	0.4	19.0	0.4
Rifampin	0.03	0.03	9.8	0.4
Methicillin	12.5	>100		
Oxacillin	25	>100		

<sup>a</sup> Inoculum,  $2.5 \times 10^5$  CFU/ml.

<sup>b</sup> MBC, Minimal bactericidal concentration.

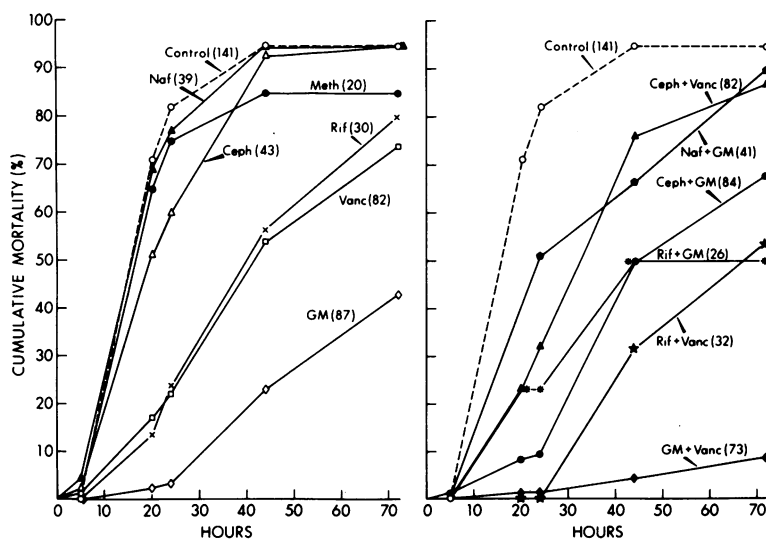


FIG. 4. Cumulative mortality in mouse peritonitis experiments. Time indicates the number of hours after inoculation of bacteria into the peritoneal cavity. Antibiotics (methicillin, Meth; nafcillin, Naf; cephalothin, Ceph; rifampin, Rif; vancomycin, Vanc; and gentamicin, GM) were given 2 and 4 h after the bacterial challenge. Numbers of mice used are indicated in parentheses.

Gentamicin was the most effective single drug. Rifampin or vancomycin was less effective than gentamicin alone ( $P < 0.05$ ), but when combined the two drugs provided lower mortality ( $P < 0.05$ ) than either of the single drugs. The combination of vancomycin and gentamicin was the most effective regimen overall. Although the vancomycin-plus-gentamicin treatment group was the only group to receive combination antibiotics in the same syringe, a subsequent experiment using separate antibiotic injections did not significantly change the results.

An unexpected finding was that combining cephalothin with either gentamicin or vancomycin resulted in a significantly ( $P < 0.05$ ) increased mortality after 24 h compared with the use of either drug alone. The combination of nafcillin with gentamicin produced similar antagonistic results.

Gentamicin-resistant variants were not recovered from positive peritoneal cultures taken from mice treated with gentamicin and sacrificed 5 and 25 h after infection. Peritoneal cultures taken from mice sacrificed at 72 h were sterile.

**Studies of possible drug-drug interaction.** Studies were undertaken to evaluate the increased mouse mortality observed when the combination of cephalothin with either gentamicin or vancomycin was used.

(i) **In vitro studies.** Time-kill studies with the Sut strain of *S. epidermidis* (Fig. 2) failed to show any antagonism of bactericidal killing when cephalothin was combined with either

vancomycin or gentamicin. No significant difference in loss of antibiotic activities as determined by microbiological assay was noted over 24 h between cephalothin and gentamicin when incubated separately or in combination (Table 2). The addition of *S. epidermidis* Sut did not modify the results. In these experiments, pharmaceutical preparations of antibiotics were used to be consistent with the animal studies.

(ii) **In vivo studies.** In studies with two groups of six uninfected mice, each given gentamicin alone or gentamicin plus cephalothin, microbiological assay of serum for gentamicin activity showed no evidence of inactivation of gentamicin by cephalothin over 1.5 h (Table 3).

**In vitro studies of inoculum effect.** In view of the different mortality results obtained with cephalothin and vancomycin, in contrast to their similar in vitro activities against strain Sut MRSE at  $10^5$  CFU/ml, tube dilution susceptibility studies were performed at varying inocula with these antibiotics. This is of interest considering the high bacterial densities ( $10^9$  CFU/ml) of the mouse peritonitis model. MICs of cephalothin at inocula of  $10^5$ ,  $10^6$ , and  $10^7$  CFU/ml were 0.8, 1.5, and 12.5  $\mu\text{g/ml}$ , respectively, whereas those of vancomycin were 1.6, 3.1, and 6.3  $\mu\text{g/ml}$ , respectively. Minimal bactericidal concentrations of cephalothin at the same inocula were 6.3, 25, and 400  $\mu\text{g/ml}$ , respectively, whereas those of vancomycin were 12.5, 12.5, and 800  $\mu\text{g/ml}$ , respectively. Therefore, the effect of increasing the bacterial inoculum 100-fold

TABLE 2. Antibiotic activity in vitro in Mueller-Hinton broth over time at 37°C

Antibiotic	Antibiotic concn (µg/ml)		
	1 h	4 h	24 h
Incubated without added bacteria			
Cephalothin	24.5	21	14.5
Gentamicin	15.8	14.6	16.5
Mixture of cephalothin and gentamicin	24.5	20.5	14.3
	16.1	14.6	16.5
Incubated in presence of <i>S. epidermidis</i> Sut <sup>a</sup>			
Cephalothin	25	21.5	12
Gentamicin	17	14	16.5
Mixture of cephalothin and gentamicin	25	20.5	12.5
	17	14.5	15.1

<sup>a</sup> Inoculum, 10<sup>8</sup> CFU/ml.

TABLE 3. Average serum concentrations of gentamicin in two groups of six mice each given gentamicin alone or in combination with cephalothin

Antibiotic	Dose (mg/kg)	Serum concn (µg/ml)	
		0.5 h <sup>a</sup>	1.5 h <sup>a</sup>
Gentamicin	25	26.4 ± 5.9	7.7 ± 1.8
Gentamicin plus Cephalothin	25	27.0 ± 3.3	10.1 ± 3.7
	500		

<sup>a</sup> Time after injection.

in tube dilution studies resulted in a 16-fold increase in the MIC of cephalothin and only a 4-fold increase in that of vancomycin.

## DISCUSSION

The studies described here compare the activities of several antibiotic regimens against MRSE in vitro and in an animal model. Previous in vitro antibiotic susceptibility studies have demonstrated a striking variation in susceptibility patterns for these organisms, especially when compared with the more uniform susceptibility patterns of *Staphylococcus aureus* (1, 4, 10, 15, 28). *S. epidermidis* isolates associated with clinical infections have been shown to be more resistant to antibiotics in general and, specifically, are more likely to be resistant to methicillin than isolates of questionable clinical significance (1, 10). Despite this pattern, most clinical isolates are susceptible to gentamicin, vancomycin, and rifampin (1, 10, 28).

The specific mechanism of methicillin resistance in *S. epidermidis* is unknown but is likely to be similar to methicillin resistance found in *S. aureus* (22, 25, 29). Both organisms display a heterogeneous pattern of resistance, with only a very small proportion of a strain's population

being truly resistant at 37°C (1, 2, 5, 25). One notable difference between the two species is that most methicillin-resistant strains of *S. aureus* are also cephalothin resistant, whereas most methicillin-resistant strains of *S. epidermidis* are susceptible when tested by the Kirby-Bauer disk method (25). Some authors have used this reported in vitro susceptibility to cephalothin as a basis for recommending its use as therapy for MRSE infections (1, 14).

Our results suggest that cephalothin would not be the optimal antimicrobial agent for treatment of life-threatening *S. epidermidis* infections. Whereas Kirby-Bauer disk testing, as well as tube dilution studies, performed at routine inocula suggest susceptibility to cephalothin, our animal studies did not support this. This pattern may also be found with nafcillin. It should be noted that the animal studies reported here represent treatment results with only a single strain of MRSE. This strain was, however, typical of our clinical isolates of MRSE in its in vitro antibiotic susceptibilities to the drugs studied. In time-kill studies performed at a high inoculum, cephalothin activity was not different from methicillin activity over the first 6 h. Cephalothin MICs determined at high inocula showed an appreciable increase over those determined at the usual inoculum. This inoculum effect with cephalothin has been noted by others (17, 27). In the mouse peritonitis model, cephalothin treatment was only slightly more effective than no treatment. These results suggest that standard Kirby-Bauer disk susceptibility testing of MRSE with respect to cephalothin, as well as to nafcillin, may be misleading and that in serious MRSE infections these antibiotics may not be optimally effective.

The antagonism observed between the combination of cephalothin with either gentamicin or vancomycin and between nafcillin and gentamicin in the animal model is unexplained. Previously reported in vitro studies with *S. aureus* (4, 6, 16) and our own in vitro studies with *S. epidermidis* have not demonstrated antagonism. Our experiments failed to demonstrate any mutual drug inactivation either in vitro or in vivo. In addition, the increase in mortality did not appear to be a consequence of multiple injections. The possibility remains that the antagonism is an artifact of the animal model itself, which uses hog gastric mucin to impair host immune responses and a high bacterial inoculum and which limits therapy to two doses. However, the peritonitis model does allow for a general comparison of therapeutic regimens within one system.

Other investigators have used animal models to investigate antimicrobial regimens for staph-

ylcoccal infections (7, 8, 35), but there have been no studies using methicillin-resistant *S. epidermidis*. Bulger et al. found enhanced therapeutic efficacy when two marginally effective drugs, cephalothin and kanamycin, were combined as therapy for experimental methicillin-resistant *S. aureus* renal infections (7). In another mouse peritonitis study, using fecal flora, Smith and Hazard found what appeared to be an optimal dosage of antimicrobial therapy. Not only was mortality increased in those mice receiving too little antibiotic, but it was also increased in animals given a relatively high dose (33). These results suggest the possibility that high concentrations of antibiotic may have paradoxical effects on bactericidal activity, a phenomenon previously described *in vitro* (11). It is of note that the peak serum concentrations of cephalothin achieved in the mice treated in our study were higher than those of the other antibiotics.

Gentamicin was the most effective single antibiotic in both the *in vitro* studies and the mouse experiments. Gentamicin has been used alone in the treatment of *S. epidermidis* genitourinary infections with satisfactory results (24). Caution has been advised in using single-drug aminoglycoside therapy for deep-seated *S. aureus* infections because of the potential for the emergence of aminoglycoside-resistant mutants (19). Whereas gentamicin-resistant variants were not detected in our mouse experiments, small strain variants were detected in the time-kill studies with gentamicin alone. The latter did not appear when a second drug was used in combination.

Vancomycin alone was an effective agent in the peritonitis model; however, vancomycin plus gentamicin was the most effective regimen overall. It is of interest that in several *in vitro* studies with methicillin-resistant *S. aureus*, gentamicin and vancomycin were consistently the most effective single drugs (4, 16). The reasons for the greater effectiveness of vancomycin over cephalothin in our animal studies are unknown. However, vancomycin showed a somewhat smaller inoculum effect than cephalothin in our *in vitro* studies. In addition, vancomycin, in contrast to cephalothin, would not likely be subject to the "intrinsic" resistance to beta-lactam antibiotics that characterizes methicillin-resistant staphylococci (29). The combination of rifampin plus vancomycin, although not as effective as vancomycin plus gentamicin in the mouse peritonitis model, may represent a less toxic alternative in the therapy of patients. Therefore, both of these combination regimens, vancomycin with either gentamicin or rifampin, warrant careful comparative studies with vancomycin alone in the treatment of serious MRSE infections.

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