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Single and Combination Antibiotic Therapy of *Staphylococcus aureus* Experimental Endocarditis: Emergence of Gentamicin-Resistant Mutants

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The efficacy of nafcillin and gentamicin used alone and in combination at doses giving serum concentrations comparable to those achieved in patients was studied in rabbits with experimental *Staphylococcus aureus* endocarditis. The organism used was a penicillinase-producing, methicillin-susceptible, clinical isolate. The addition of gentamicin to nafcillin significantly increased the rate of killing of organisms in valvular vegetations, compared to the effect of nafcillin alone. Gentamicin alone delayed mortality but was not effective in reducing the bacterial populations of the vegetations. Bacteremia persisted in the animals treated with gentamicin alone, in contrast to the groups treated with nafcillin or the combination. Selection of a subpopulation of aminoglycoside-resistant small-colony variants occurred in animals treated with gentamicin alone. This variant was subsequently employed in the rabbit model and produced endocarditis, metastatic infection, and bacteremia comparable to those caused by the parent strain. Animals with infection produced by the variant died later than animals infected by the parent strain. Nafcillin was equally effective in reducing the population of both parent and variant strains in vitro and in therapy of the infected animals. Population studies showed the variant to be a mutant emerging at a rate of 1.9 $\times 10^{-7}$. It was shown to differ from the parent strain in coagulase and hemolysin production, colonial morphology, and aminoglycoside susceptibility, but was similar by light and electron microscopy and in phage type, pigmentation of colonies, deoxyribonuclease production, mannitol fermentation, and growth rate.

In vitro time-kill studies with blood culture isolates of penicillinase-producing, methicillin-susceptible *S. aureus* have demonstrated that gentamicin at a concentration of 5 μg/ml is rapidly bactericidal and reduces the bacterial population at a rate greater than that achieved by nafcillin at 20 μg/ml (N. H. Steigbigel, J. I. Casey, and B. J. Heeter, Clin. Res. **21**:976, 1973). The initial rate of killing at a lower concentration of gentamicin (0.5 μg/ml) was similar to that obtained with 5 μg/ml but was associated with the later overgrowth, in each of 24 strains tested, of a small-colony variant which showed increased resistance when rechallenged with aminoglycosides. The combination of nafcillin and gentamicin (0.5 μg/ml) demonstrated the initial rapid killing without the subsequent emergence of the variant strain. Studies using a high inoculum of organisms, 10^9 colony-forming units (CFU) per ml, demonstrated that gentamicin alone or in combination with nafcillin was significantly active, whereas nafcillin alone did not decrease the population. The relevance of these in vitro observations was studied in a model of *S. aureus* endocarditis in rabbits.

This study was presented in part at the 16th Interscience Conference on Antimicrobial Agents and Chemotherapy, October, 1976.

MATERIALS AND METHODS

Organism. The *S. aureus* strain used in these studies was a penicillinase-producing blood culture isolate susceptible to methicillin, nafcillin, and gentamicin by the Kirby-Bauer technique. The strain was coagulase and deoxyribonuclease positive, fermented mannitol, and produced hemolysis on sheep and rabbit blood agar. Coagulase assays were performed using both the tube and slide tests. For the former, equal volumes of an overnight culture grown in Mueller-Hinton broth were added to both human plasma (General Diagnostics) and rabbit plasma (BBL) and allowed to incubate at 37°C. The tubes were examined for clotting at 4, 6, and 24 h. The slide test used an emulsion of a colony of the test organism to which rabbit or human plasma was added. Agglutination occurring in 10 s after mixing was considered a positive assay. Mannitol fermentation and deoxyribonuclease production were determined using cystine Trypticase

soy medium with mannitol (BBL) and deoxyribonuclease plates (Scott Laboratories). Hemolysin production was evaluated by the detection of hemolysis around colonies on sheep and rabbit blood agar plates (21). The phage type was 29/52 and was determined by the City of New York Department of Health, Public Health Laboratory. For electron microscopic examination, cells were fixed in suspension in 2.5% glutaraldehyde treated with uranyl acetate, and then embedded in Epon. Thin sections were prepared and stained in uranyl and lead salts for electron microscopy.

In vitro studies. An inoculum of 5×10^5 CFU of this organism per ml, obtained from a 1:1,000 dilution of an overnight growth, was used to determine the minimal inhibitory concentration (MIC) of antibiotics in Mueller-Hinton broth. Minimal bactericidal concentrations were determined by streaking 0.01-ml samples from the inhibition tests on sheep blood agar plates (BBL); fewer than six colonies after incubation for 24 h was considered as 99.9% killing. The MICs of nafcillin and gentamicin were 0.5 μg/ml. The minimal bactericidal concentration of nafcillin was 1 μg/ml, and that of gentamicin was 2 μg/ml.

Experimental endocarditis model. Endocarditis was produced using a modification (5) of the method described by Perlman and Freedman (19). A plastic catheter (Deseret Intercath, 19 gauge) was placed across the aortic valve cusps by cannulating the right carotid artery in 3- to 5-pound (ca. 1.4- to 2.3-kg) New Zealand white rabbits. Seventy-two hours after catheter placement, 2×10^5 to 5×10^5 CFU of the organism in 1 ml was injected into the marginal ear vein. The infecting organism had been grown overnight in Mueller-Hinton broth, washed twice, and diluted 1:1,000 in normal saline. Animals demonstrating bacteremia and a temperature of 104.5°F (ca. 40.3°C) or greater at 40 h after inoculation were evaluated in the treatment studies. Animals were sacrificed at regular intervals, and the presence of endocarditis in each was confirmed by autopsy. Aortic vegetations were removed aseptically, weighed, and homogenized in 0.5 ml of sterile distilled water in a tissue grinder (A.H. Thomas, 3431-E04 AA) at approximately 1,800 rpm until uniform suspensions were observed. Homogenization time was 0.5 to 3 min, and control samples showed no change in bacterial density with up to 10 min of homogenization. Homogenized samples were diluted in normal saline, and duplicate pour plates were made with 1.0-ml portions of diluted sample in heart infusion agar (Difco) for colony counting. To determine the potential effect of antibiotic carry-over to pour plates, some valve homogenates were also treated with *Bacillus cereus* beta-lactamase (ATCC 27348) to inactivate the nafcillin, and diluted samples were plated on heart infusion agar with pH adjusted to 5.5 to inactivate the aminoglycosides (22). Controls showed that these methods inactivated the respective antibiotics. No differences in counts were noted between samples treated to inactivate antibiotic carry-over and samples not so treated. Blood cultures prior to the institution of therapy were obtained aseptically from the marginal ear vein by adding 0.5 ml of blood to 4.5 ml of Mueller-Hinton broth and incubating at 37°C for 24 h. In addition, blood samples from animals receiving 6 days of nafcillin, gentamicin, or both were incubated in Mueller-Hinton broth with and without gentamicin (1 μg/ml).

Administration of antibiotics. Antimicrobial therapy was begun 42 h after infection and continued until sacrifice 2, 4, or 6 days later. Antibiotics were given every 8 h intramuscularly. Six animals treated with gentamicin, which died between days 5 and 6 of gentamicin therapy, were also included in the 6-day results.

Pharmacokinetic studies. Infected animals were treated intramuscularly every 8 h with either nafcillin (180 mg/kg) gentamicin (3.5 mg/kg), or both. On the first day of therapy, serum concentrations of antibiotics were assayed from 29 animals receiving nafcillin and 29 animals receiving gentamicin. On the day prior to sacrifice, serum for antibiotic assay was taken from nine animals treated with nafcillin and eight with gentamicin. At least three blood samples were collected from each animal at intervals during the 8 h following drug administration. A blood sample was obtained from all animals at the time of sacrifice, which occurred 8 to 10 h after the last antibiotic dose. Antibiotic serum levels were assayed in duplicate by the microbiological disk plate method. Standards of nafcillin and gentamicin were prepared in pooled rabbit sera. Assay organisms were *Sarcina lutea* (ATCC 3941) to determine nafcillin and an isolate of *Klebsiella pneumoniae* (6) to determine gentamicin in the presence or absence of nafcillin. Nafcillin in the presence of gentamicin was measured after inactivation of gentamicin by pH adjustment of the media to 5.5 (22). The first-order elimination rate constants and the half-life of each drug were calculated using standard methods (9). The mean serum concentration for each drug was derived from the area under the curve using the trapezoid approximation (18).

Statistical analysis was performed using Student's *t* test, chi-square, or Fischer's exact test.

Isolation and characterization of the aminoglycoside-resistant variant. Valve homogenates were screened for the presence of aminoglycoside-resistant variant organisms from all 6-day treated animals and untreated controls. Undiluted homogenates were swabbed directly on Mueller-Hinton agar (Difco) plates onto which were placed standard Kirby-Bauer antibiotic disks (BBL). The plates were incubated for 18 to 24 h at 37°C and then inspected for zones of inhibition. To obtain MICs from organisms surviving in valve homogenates, the latter were plated directly on sheep blood agar, and small-colony variants and typical colonies noted after 24 h of incubation at 37°C were selected. MICs and minimal bactericidal concentrations were determined in Mueller-Hinton broth as noted above. Time-kill studies with antibiotics were performed on the variant strain and parent strain using an inoculum of 5×10^5 CFU/ml at 37°C in a shaker bath, with survival determined by counting colonies in duplicate pour plates of heart infusion agar. To determine the approximate frequency of the aminoglycoside-resistant variant, serial 10-fold dilutions of an overnight culture of the organisms were inoculated into two sets of tubes containing Mueller-Hinton broth with a gentamicin concentration of 1 and 3 μg/ml. The tubes were incubated for 18 h, checked for turbidity, and subcultured on sheep blood agar. Rep-

lica plating (14) on antibiotic assay medium no. 5 (Difco) with and without gentamicin (1 μg/ml) and fluctuation analysis (16) were used to further characterize the aminoglycoside-resistant variant. For the latter, Mueller-Hinton broth was inoculated and incubated for 5 h to achieve a bacterial density approximately equal to the reciprocal of the mutation frequency. One milliliter of broth was plated on Mueller-Hinton agar containing 1 μg of gentamicin per ml. The mutation rate was calculated from the fluctuation analysis experiments (13). For these calculations 1 CFU was considered to equal 2.1 organisms. This approximation was made by determining the mean number of organisms per cluster during log-phase growth using light microscopy.

The pathogenicity of the gentamicin-resistant variant that appeared in the rabbits treated with gentamicin alone was evaluated by its ability to produce endocarditis in rabbits as described for the parent strain. The challenging inoculum was obtained from a single colony of the aminoglycoside-resistant small-colony variant. The same inoculum was used as for the parent strain. To screen for revertants to the aminoglycoside-susceptible parent strain in vivo, valve homogenates from rabbits infected with the variant were plated on rabbit blood agar to observe hemolysis and on Mueller-Hinton agar onto which antibiotic disks were placed. In vitro reversion of the aminoglycoside-resistant variant to the parent strain was evaluated after 18 h of growth in drug-free Trypticase soy broth (BBL). Serial dilutions were made, and samples were plated on Trypticase soy agar with 0.4% yeast extract containing 4% rabbit erythrocytes, and then incubated at 37°C in a 10% carbon dioxide environment for 24 h. Isolated colonies were scored for hemolysis and colonial morphology and compared with the parent strain. Samples were also plated on Mueller-Hinton agar without gentamicin and with gentamicin at concentrations of 1, 2, and 4 μg/ml and incubated overnight without added CO_2. Twenty-one serial transfers of the variant strain were also made according to the method of Stocker (28). One hundred colonies from the latter experiment were subcultured on Mueller-Hinton agar containing 4% rabbit erythrocytes and gentamicin at concentrations of 1 and 4 μg/ml. Tube coagulase production was tested from 10 of the colonies.

Antibiotics. In vitro studies used antibiotic susceptibility testing powder obtained from the drug manufacturer: nafcillin (Wyeth Laboratories) and gentamicin (Schering Corp.). Studies in animals used the regular pharmaceutical preparations obtained from the same suppliers.

RESULTS

Pharmacokinetic studies. Serum concentrations of antibiotics in infected animals are shown in Fig. 1. The mean 1-h nafcillin peak concentration was 58 μg/ml, and the trough was 8 μg/ml at 7 h. Half-life for nafcillin was 122 min. The mean concentration calculated from the area under the curve was 22 μg/ml. The mean concentration at the time of sacrifice was 7.4 μg/ml. Gentamicin showed a 1-h peak concentration of 11 μg/ml, a trough of 0.9 μg/ml, and a half-life of 98 min. The mean serum concentration was 3.7 μg/ml, and the mean concentration at time of sacrifice was 0.5 μg/ml. Serum concentrations of nafcillin and gentamicin determined in 17 animals over 7 h on the day prior to sacrifice showed no significant differences from concentrations determined on day 1 of therapy.

Antibiotic treatment studies. (i) Changes in bacterial densities in valvular vegetations, mortality, and bacteremia. In 15 untreated animals, valvular vegetations contained a mean bacterial density of 9.8 ± 1.1 (log_{10}) CFU/g (Fig. 2). There was no significant reduction in bacterial density at day 2, 4, or 6 in those animals treated with gentamicin alone. Although at day 2 there were no significant differences among the three treatment groups, by day 4 the differences were highly significant ($P < 0.001$). Animals treated with nafcillin plus gentamicin showed bacterial counts significantly lower than those treated with nafcillin alone,

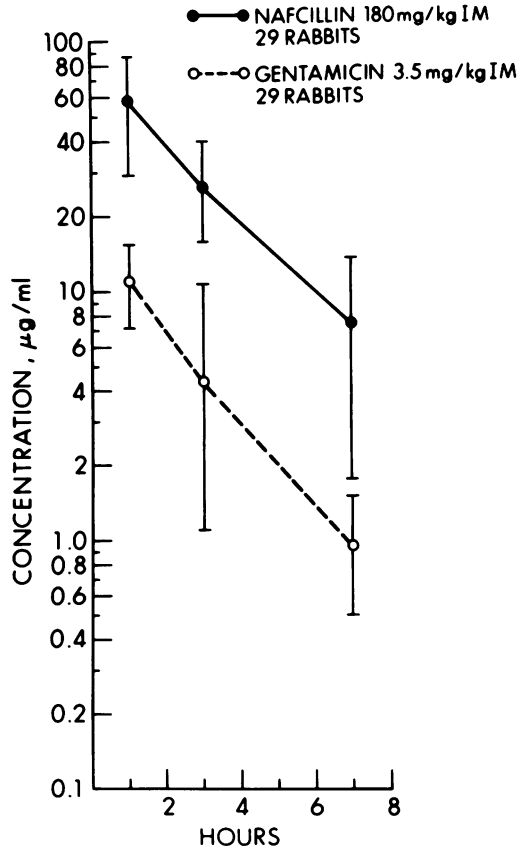

FIG. 1. *Mean serum concentrations (± standard deviation) of drug at 1, 3, and 7 h after intramuscular (IM) injection in rabbits with S. aureus endocarditis.*

and both of these groups showed significant reductions of counts compared to animals treated with gentamicin alone. At day 6 the animals treated with the combination showed lower bacterial counts than those with nafcillin alone, but the difference was no longer significant. The ability of the antibiotic regimens to sterilize the valvular vegetations is shown in Table 1. None of the 29 animals receiving gentamicin alone had sterile valves. At day 4 the proportion of animals with sterile valves was significantly greater in the group treated with nafcillin and gentamicin than in the group treated with nafcillin alone ($P < 0.001$). However, by day 6 the differences between these two groups were no longer significant.

Untreated animals showed a 95% mortality by day 4 of infection, which corresponds to day 2 of therapy for animals receiving antibiotics. The spontaneous mortality in treated animals prior to day 4 of therapy was low in all treatment groups (15%), with most deaths occurring in moribund animals on the first day of treatment. Autopsies in this group of animals suggested death was due to infection with high densities of organisms in multiple organs. Between days 4 and 6 of therapy, the spontaneous mortality in the group treated with gentamicin alone was 31% (6/16), compared to 7% (1/14) in the group treated with nafcillin. Blood cultures were positive at day 5 in 10 of 11 animals treated with gentamicin alone, as compared to 0 of 9 treated with nafcillin ($P < 0.001$).

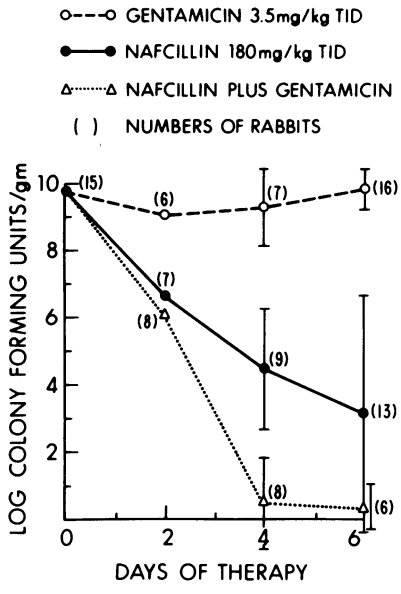

FIG. 2. *Rate of killing of S. aureus in valvular vegetations in rabbits receiving intramuscular nafcillin, gentamicin, or the combination, three times daily, and in untreated controls. Results express the mean ± standard deviation.*

TABLE 1. *Time for sterilization of infected valves*

Antibiotic	No. of rabbits with sterile valves/total at:		
	2 days	4 days	6 days
Gentamicin	0/6	0/7	0/16
Nafcillin	1/7	0/9	6/13
Nafcillin + gentamicin	1/8	7/8	5/6

(ii) Emergence of aminoglycoside-resistant variants. Direct plating of valve homogenates from 12 of 13 animals treated with gentamicin alone showed double zones of inhibition around aminoglycoside disks, suggesting the presence of both resistant and susceptible subpopulations of *S. aureus*. The more resistant subpopulation appeared to represent a minority of the total surviving population (Fig. 3). Single zones of inhibition were seen around other antibiotics. Valve homogenates from animals treated with gentamicin alone yielded both small colonies and typical colonies of *S. aureus* when plated on sheep blood agar and observed at 24 h of incubation. Standard Kirby-Bauer disk diffusion susceptibility tests showed the small-colony variants to be resistant to gentamicin, kanamycin, streptomycin, and tobramycin. Results of broth dilution susceptibility testing with nafcillin, gentamicin, and amikacin against a typical small-colony variant and a normal-sized colony isolate from an animal treated with gentamicin alone are shown in Table 2. The MIC of the aminoglycosides against the small-colony variant was increased 32-fold over that against the normal-sized colony isolate. There was no change in the MIC of nafcillin. Double zones of inhibition surrounding gentamicin disks were not demonstrated on valve homogenates from each of five animals treated with nafcillin in whom organisms had not been eradicated, or from six controls.

In addition, all of the positive blood cultures taken at day 5 of therapy from animals treated with gentamicin alone demonstrated growth in media with gentamicin at 1 μg/ml, suggesting the presence of the aminoglycoside-resistant variant.

(iii) Pathogenicity of the aminoglycoside-resistant variant. Endocarditis was produced in 29 of 30 rabbits challenged with the aminoglycoside-resistant small-colony variant that had emerged from an animal treated with gentamicin alone. No differences were noted between animals infected with the parent strain and those infected with the aminoglycoside-resistant var-

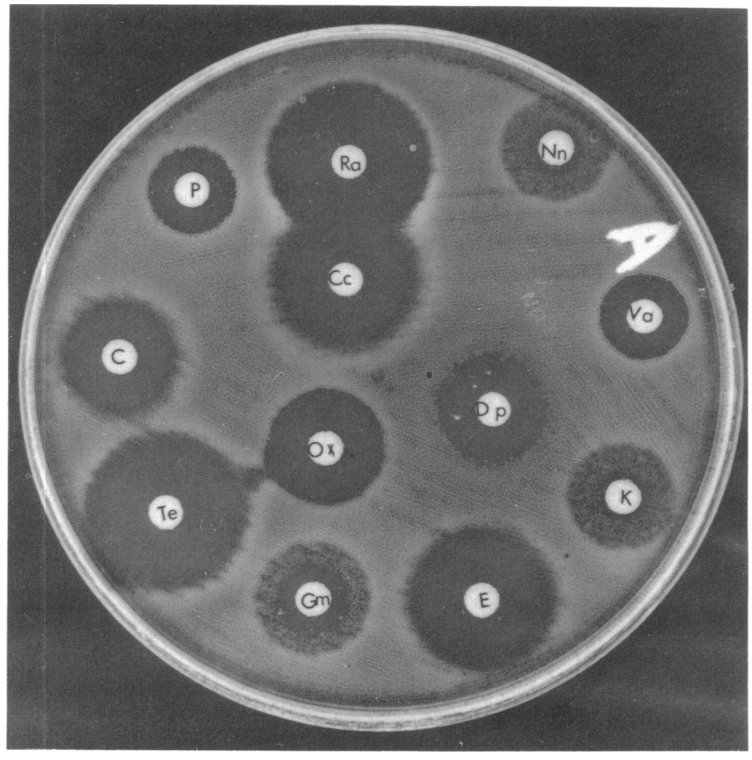

FIG. 3. *Photogram of a Mueller-Hinton agar plate seeded with an undiluted homogenate from a vegetation of an animal receiving 6 days of intramuscular gentamicin. Standard Kirby-Bauer antibiotic disks placed on the agar include penicillin (P), oxacillin (Ox), methicillin (Dp), chloramphenicol (C), tetracycline (Te), erythromycin (E), clindamycin (Cc), vancomycin (Va), rifampin (Ra), and the aminoglycosides, tobramycin (Nn), gentamicin (Gm), and kanamycin (K).*

TABLE 2. *Aminoglycosides and nafcillin against S. aureus survivors from infected valvular vegetations of rabbits treated with gentamicin alone*

Inoculum	MIC (μg/ml)		
	Genta-micin	Ami-kacin	Naf-cillin
Normal-size colony	0.5	2	0.5
Small-colony variant	16	64	0.5

iant in terms of the bacterial densities present in blood, heart valve vegetations, spleen, kidney, and liver (Table 3). Untreated animals with endocarditis produced by the variant strain had a mean survival of 6.6 days, and none of them died within 72 h of inoculation. In contrast, 19% (21/112) of animals with endocarditis produced by the parent strain died within 72 h of inoculation, despite treatment of 8 of these 21 with up to 20 h of antibiotics ($P = 0.01$).

(iv) Further characterization of the aminoglycoside-resistant variant. The aminoglycoside variant had the same phage type as the parent strain, and both strains had a similar appearance on light and electron microscopy. Both strains were pigmented, demonstrated coagulase by the slide test, produced deoxyribonuclease, and fermented mannitol. The aminoglycoside-resistant variant, however, did not produce coagulase in the tube test and did not produce hemolysis on sheep or rabbit blood agar. The diameters of typical colonies of the aminoglycoside-resistant variant were approximately 1/4 of those of the parent strain after 24 h of incubation at 37°C on sheep blood agar plates. The growth rate in Mueller-Hinton broth in a 37°C shaker bath was the same for both strains, with doubling times at log-phase growth of 27.5 min (Fig. 4). Growth of the variant in broth for 18 h failed to demonstrate revertants in 10^4 colonies screened for gentamicin susceptibility and 3×10^3 screened for hemolysin production. After serial passage of the variant for 21 days, 61% of colonies tested showed no growth on Mueller-Hinton agar containing 1 and 4 μg of gentamicin per ml; 89% of these were hemolytic for rabbit erythrocytes. In none of 10 revertants tested, however, was tube coagulase positive as compared to a parent strain control. There was

TABLE 3. *S. aureus in rabbits with endocarditis infected with parent or aminoglycoside-resistant variant strains*[a]

Infecting organism	Mean bacterial density[b]				
	Blood	Valve	Spleen	Kidney	Liver
Parent	3.58 ± 0.44	10.03 ± 0.72	5.43 ± 0.86	6.38 ± 0.69	4.96 ± 0.93
Variant	3.35 ± 0.26	10.81 ± 0.18	5.12 ± 1.01	5.67 ± 0.90	4.64 ± 0.77

[a] Groups of six rabbits each. Animals were sacrificed 2 days after infection.
[b] Log_{10} CFU/ml (or CFU/g) ± standard deviation.

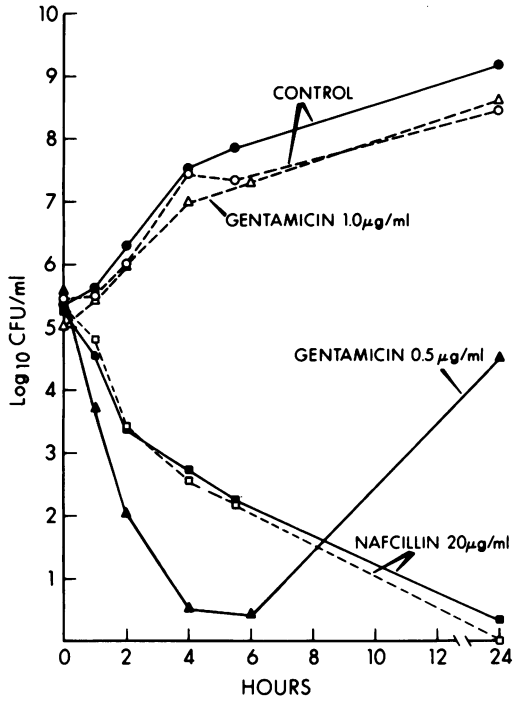

FIG. 4. *Survival in Mueller-Hinton broth, with and without nafcillin or gentamicin, of parent S. aureus (solid line) and a small-colony variant (dashed line).*

TABLE 4. *Distribution of the numbers of gentamicin-resistant colonies in a series of similar cultures*

Expt 1[a]		Expt 2[b]	
Resistant bacteria	No. of cultures[c]	Resistant bacteria	No. of cultures[c]
0	58	0	59
1	18	1	3
2	2	2	9
3	4	3	0
4	1	4	2
5	1	5	3
6-10	3	6-10	2
11-20	5	11-20	4
21-50	4	21-50	8
51-100	3	51-100	2
101-200	1	101-200	1
201-500	0	201-500	5
501-1,000	0	501-1,000	2

[a] Final CFU per culture, 1.4×10^6; mutation rate, 1.8×10^{-7}.
[b] Final CFU per culture, 1.3×10^6; mutation rate, 1.9×10^{-7}.
[c] Number of cultures containing the specified number or range of resistant mutants.

no evidence of reversion to the parent form in animals infected with the aminoglycoside-resistant variant strain, as demonstrated by the failure of valve homogenates to show either double zones around aminoglycoside disks or hemolytic colonies on rabbit blood agar plates. The variant was shown to be a mutant by replica plating on gentamicin-containing agar. Fluctuation analysis (Table 4) demonstrates marked variation in the number of resistant organisms per culture, consistent with the presence of a mutational event. The mutation rate was 1.9×10^{-7}. Time-kill studies with 20 μg of nafcillin per ml showed no differences in the activity of this antibiotic against either strain (Fig. 4). The addition of gentamicin (1 μg/ml) to nafcillin (20 μg/ml) did not increase the killing rate achieved by nafcillin alone against the variant strain. Ten animals infected with the aminoglycoside-resistant mutant and treated with nafcillin (180 mg/kg intramuscularly, three times daily) for a period of 6 days had a mean bacterial density in valve homogenates of heart valve vegetation of 3.5 ± 3.4 (log_{10}) CFU/g. Thirteen animals infected with the parent strain and similarly treated had a mean of 3.11 ± 3.5 (log_{10}) CFU/g in valve homogenates at 6 days of therapy.

DISCUSSION

In vitro studies have demonstrated that the addition of gentamicin to nafcillin increases the rate of killing of penicillinase-producing *S. aureus* compared to that achieved by nafcillin alone (30, 23; Steigbigel et al., Clin. Res. 21:976, 1973). These in vitro studies and those reported by Wilson and Sanders (31) show that gentamicin in low concentrations is associated with the selection of small-colony aminoglycoside-resistant variants. The results of the studies in rabbits with endocarditis reported here are consistent

with those in vitro observations. The addition of gentamicin to nafcillin significantly increased the rate of sterilization of valvular vegetations, similar to the observations reported by Sande and Courtney (23). Gentamicin alone was not effective in reducing the bacterial density in valvular vegetations, but this regimen delayed mortality. Bacteremia and spontaneous mortality after day 4 of therapy were frequent with gentamicin therapy, in contrast to animals treated with nafcillin alone or with the combination.

Two populations of *S. aureus* survivors were demonstrated in animals treated with gentamicin alone: a population with increased resistance to aminoglycosides, and a population of the parent strain. The aminoglycoside-resistant variant selected by gentamicin therapy was shown to be a mutant. The selection of the aminoglycoside-resistant mutant, however, does not fully explain the ineffectiveness of gentamicin when used alone in reducing the bacterial population. The predominant organism in all gentamicin-treated animals was the aminoglycoside-susceptible parent strain, and one animal had no resistant variants demonstrated. The aminoglycoside-resistant variant showed limited reversion in vitro to the susceptible strain, which could be demonstrated only after prolonged serial passage. No reversion was demonstrated in animals specifically inoculated with the resistant strain, despite the development of a high population density in vegetations. Therefore, the presence of a large population of the parent strain in gentamicin-treated animals cannot be explained by a reverse mutation of the variant. The continued presence of the gentamicin-susceptible strain in the vegetations of animals having gentamicin serum levels more than adequate to kill this organism suggests limited aminoglycoside penetration or activity within vegetations.

The aminoglycoside-resistant mutant was shown to be pathogenic, as indicated by its ability to produce endocarditis, bacteremia, and metastatic infection similar to those produced by the parent strain. The parent strain demonstrated greater virulence, as indicated by its production of higher early spontaneous mortality; it was not associated with a bacterial density in infected lesions larger than that of the mutant. The lack of production of hemolysin on rabbit blood agar by the mutant suggests the possibility that differences in toxin production may explain the difference in virulence between the two strains (8). The pathogenicity in rodents of a similar aminoglycoside-resistant *S. aureus* isolated in vitro has recently been reported (17).

Most clinical isolates of *S. aureus* resistant to particular aminoglycosides have normal colonial morphology and possess plasmids directing the production of specific aminoglycoside-inactivating enzymes (4, 15, 20, 25, 26, 33). The aminoglycoside-resistant strain described in this study is not of this type. It is a mutant forming small colonies and is resistant to aminoglycosides in general. Examination of this strain has failed to show the presence of aminoglycoside-inactivating enzymes (J. Davies, personal communication) or a diminished rate of growth in Mueller-Hinton broth. The mechanism of resistance to aminoglycosides in these small-colony variants of *S. aureus* is uncertain. Defects in oxidative metabolism in small-colony variants of *S. aureus* have been suggested (10, 11). Oxidative metabolism is required for aminoglycoside uptake (1, 2), and therefore an impairment in uptake is a likely mechanism for the general resistance to aminoglycosides associated with the small-colony mutant described in this study.

Aminoglycoside-resistant mutants emerging in vitro are generally considered of little potential clinical significance, since they occur infrequently and often possess properties which place them at a selective disadvantage (3). However, there have been several reports of small-colony aminoglycoside-resistant variants of *S. aureus* isolated from patients (7, 12). It has been suggested that the presence of these mutants in human infection is underestimated (32). It is of interest that the mutant characterized in this study was shown to be pathogenic in the endocarditis model and occurs at a rate consistent with its potential presence in clinical infection.

The significance of this and other studies (23, 24, 27) in relationship to the therapy of staphylococcal endocarditis in humans is uncertain. However, the results suggest that the use of aminoglycosides alone for the treatment of serious, deep-seated staphylococcal infections be avoided. Combination therapy with a penicillinase-resistant penicillin, such as nafcillin, and gentamicin may lead to more rapid reduction of the bacterial population and thereby to decreased morbidity and mortality from sepsis and cardiac valve destruction. However, the potential increased toxicity of the combined therapy is of concern. A recent retrospective report of the use of single-agent and combination therapy with gentamicin in patients with staphylococcal endocarditis suggests no benefit from use of the combination (29). A controlled prospective study of these antimicrobial agents alone and in combination in the treatment of patients is required to clarify this important question.

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