Effects of Ampicillin-Amikacin and Ampicillin-Rifampin on Enterococci

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Fifty-seven clinical isolates of enterococcus were tested for susceptibility to 10 antibiotics in a microtiter broth dilution system. Amoxicillin, ampicillin, vancomycin, and rifampin inhibited all strains at concentrations easily achievable in blood. Resistance to rifampin developed rapidly. Of the aminoglycosides, gentamicin was most active, followed in decreasing order by tobramycin, amikacin, kanamycin, and streptomycin. High-level resistance to streptomycin was present in 26% of the strains and to kanamycin in 23% of the strains. Growth curve studies of selected strains revealed synergy with ampicillin-amikacin and antagonism with ampicillin-rifampin. It is suggested that ampicillin-gentamicin constitutes adequate initial therapy for enterococcal infections until the results of tests for high-level resistance to kanamycin and streptomycin are known and that clinical trails of ampicillin-amikacin are warranted.

Most enterococci are resistant to clinically achievable blood levels of aminoglycoside antibiotics (5). Strains resistant to streptomycin demonstrate either low-level resistance to less than 1,000 µg/ml or high-level resistance to 6,000 µg/ml or more (6). Strains demonstrating high-level resistance to streptomycin are rarely synergistically affected by the addition of penicillin, whereas the majority of strains with low-level resistance act synergistically with penicillin (3, 6). Similar effects have been reported for penicillin-kanamycin (9). High-level resistance to gentamicin has not been demonstrated and penicillin and gentamicin have acted synergistically against all strains tested (6, 7, 10). Tobramycin high-level resistance has similarly not been demonstrated, and penicillin and tobramycin have been synergistic against all strains of *Streptococcus faecalis* tested (7). The activity of the new aminoglycoside amikacin alone and in combination with ampicillin against enterococci has not been reported.

In the following study, the percentage of clinical enterococcal isolates showing high-level resistance to streptomycin, kanamycin, gentamicin, tobramycin, and amikacin was studied, as was the activity of ampicillin-amikacin. Finally the activities of amoxicillin, rifampin, ampicillin, cephalothin, and vancomycin were determined, and the effect of ampicillin-rifampin was investigated.

MATERIALS AND METHODS

Fifty-seven clinical isolates of enterococcus were obtained from the diagnostic microbiology laboratories of the Denver General (24 isolates), Denver Veterans Administration (18 isolates), and Colorado General (15 isolates) hospitals. Enterococci were identified as streptococci capable of growth in 6.5% NaCl and able to hydrolyze bile esculin.

A single colony from a pure culture was inoculated into 2 ml of Trypticase soy broth (TSB; Becton, Dickinson, & Co.) in a screw-cap vial and incubated at 37 C for 18 h. The vials were then frozen at −70 C until used.

Laboratory standard powders of ampicillin, amoxicillin, kanamycin, amikacin (Bristol Laboratories, Syracuse, N.Y.), tobramycin, streptomycin, cephalothin, vancomycin (Eli Lilly and Co., Indianapolis, Ind.), and gentamicin (Schering Corp., Kenilworth, N.J.) were dissolved in appropriate volumes of TSB to yield a final concentration of 200 µg/ml. Rifampin (Dow Chemical Corp., Indianapolis, Ind.) was dissolved in TSB with 10% dimethyl sulfoxide (Sargent Welch Corp., Denver, Col.) to a concentration of 200 µg/ml. Serial twofold dilutions were then made in TSB to a final concentration of 0.1 µg/ml. Higher concentrations of antibiotics were prepared in a similar manner. A 0.05-ml aliquot of each antibiotic concentration was placed in a microtiter well. To this was added 0.05 ml of a 10^{-5} dilution of an 18-h TSB culture of test organism. The trays were sealed, mixed, and incubated for 18 h at 37 C before being examined. Quantitative pour-plate colony counts were performed on each inoculum to ensure that it contained 10^4 to 10^5 organisms.

The minimal inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic preventing visible growth. Minimal bactericidal concentrations (MBC) were performed using a modified Steers replicator to sample 0.001 to 0.003 ml from the 0.1 ml in the microtiter plate wells, plated on Trypticase soy agar containing 5% defibrinated sheep blood, and incubated for 18 h at 37 C. The MBC was

defined as the lowest concentration of antibiotic completely inhibiting bacterial growth. *Staphylococcus aureus* (ATCC 29923) and *Escherichia coli* (ATCC 29922) with known MICs and MBCs to the antibiotics tested were included as controls.

Growth curves were determined using an inoculum calculated to yield a final concentration in the test flask of 10^4 to 10^5 organisms/ml. A quantity of antibiotic calculated to result in a final concentration of 5 µg of ampicillin and rifampin and 12 µg of amikacin per ml was added to the test flasks. The final volume was 20 ml. The flasks were then incubated at 37 C with constant mixing, and 2-ml aliquots for quantitative culture were removed at 0, 1, 2, 4, 8, 12, and 24 h. Colony counts were carried out in duplicate and enumerated after overnight incubation at 37 C.

Ampicillin and rifampin levels were determined by a modified agar-well diffusion method (1). The assay organism for ampicillin levels was *S. aureus* 209P, which had been rendered resistant to >100 µg of rifampin per ml by multiple passages on Mueller-Hinton agar to which 10 µg of rifampin per ml had been added. *Bacillus subtilis* was the assay organism for rifampin levels. To determine rifampin concentrations in the presence of ampicillin, 0.5 ml of each sample was first mixed with 0.1 ml of a beta-lactamase derived from *Bacillus cereus* 569 by the method of Sabath et al. (8).

RESULTS

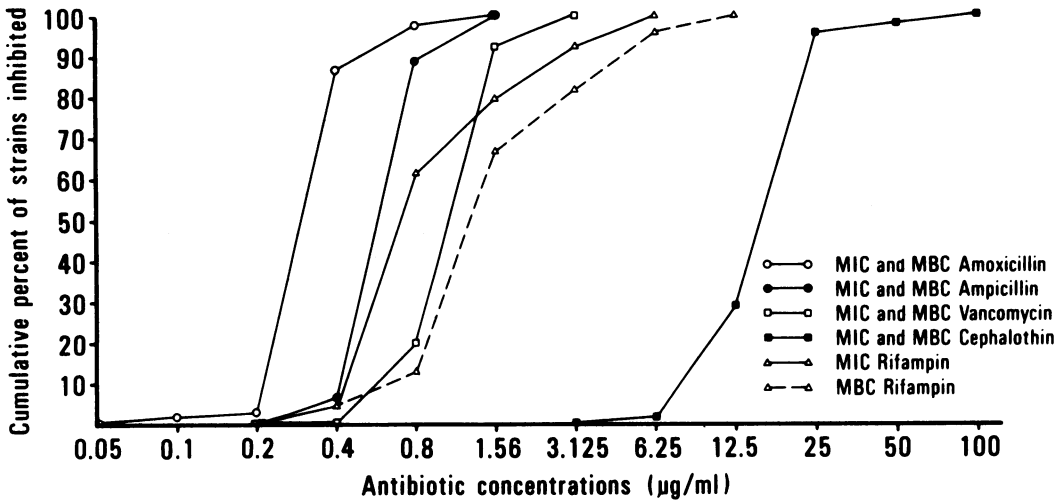Frequency distribution curves of the MICs and MBCs of the 57 enterococcal isolates stud-

FIG. 1. *MICs and MBCs for 57 isolates of enterococcus in TSB against amoxicillin, ampicillin, vancomycin, rifampin, and cephalothin.*
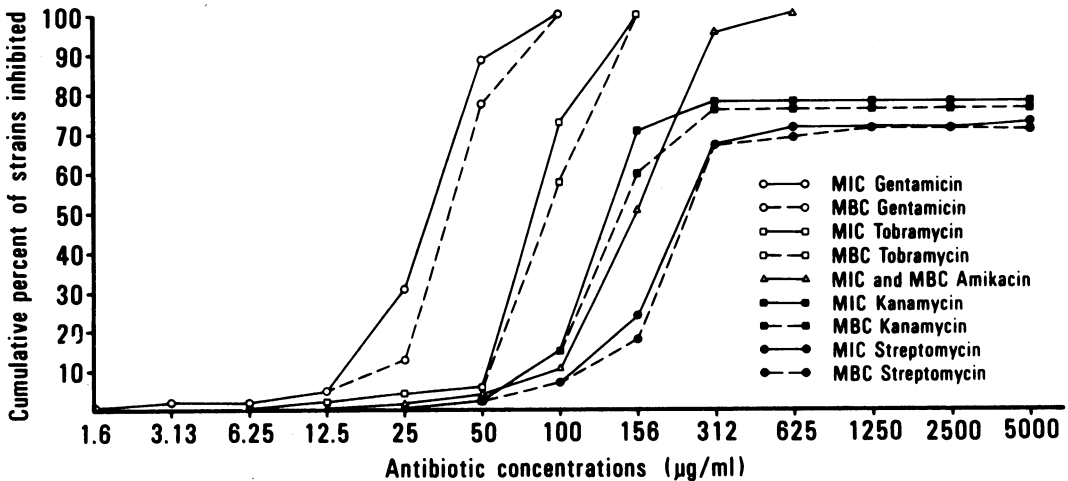

FIG. 2. *MICs and MBCs for 57 isolates of enterococcus in TSB against gentamicin, tobramycin, kanamycin, amikacin, and streptomycin.*

ied are presented in Fig. 1 and 2. Amoxicillin was more active than ampicillin, but all isolates were inhibited by 1.56 μg of either antibiotic per ml. Vancomycin and rifampin inhibited all isolates at 3.125 and 6.25 μg/ml, respectively. Rifampin inhibited more strains at lower concentrations; 62% of isolates were inhibited by 0.78 μg of rifampin per ml, whereas 19% were inhibited by vancomycin in the same concentration. Cephalothin in a concentration of 25 μg/ml inhibited 96% of isolates.

Gentamicin was the most active of the aminoglycosides; in a concentration of 100 μg/ml it inhibited all isolates. Tobramycin and amikacin in concentrations of 156 and 625 μg/ml, respectively, inhibited all isolates. Amikacin, however, inhibited 96% of isolates at 312 μg/ml. Kanamycin inhibited 78% of isolates at 312 μg/ml and streptomycin inhibited 71% at 625 μg/ml. Neither kanamycin nor streptomycin showed any significant increase in activity at 5,000 μg/ml. The MBCs for these isolates were not different from the MICs, except for rifampin. With this antibiotic the MBC was generally twice the MIC.
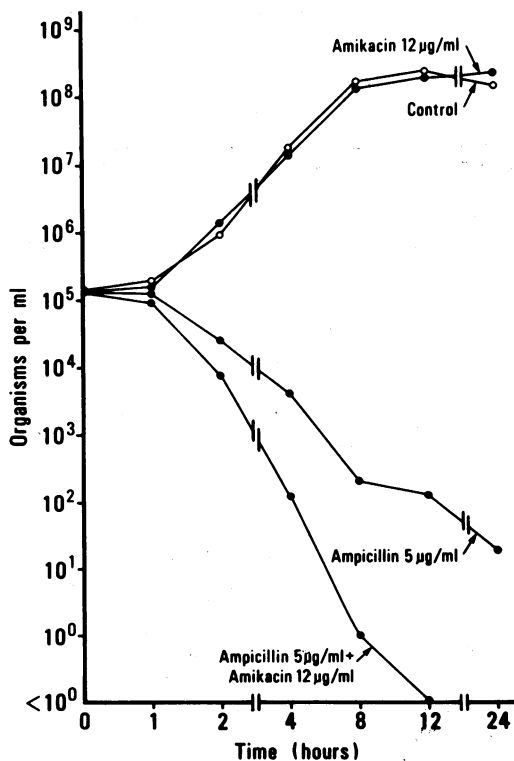
Growth curves of three randomly selected

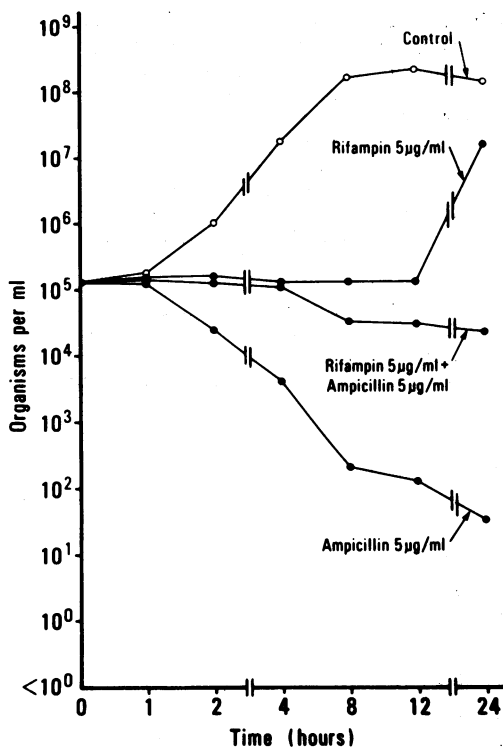FIG. 4. *Antagonistic effects of ampicillin-rifampin against three strains of enterococcus.*

FIG. 3. *Synergistic effects of ampicillin-amikacin against three strains of enterococcus.*

isolates cultivated in the presence of 5 μg of ampicillin per ml, 12 μg of amikacin per ml, and the two drugs in combination are depicted in Fig. 3. These two agents showed a synergistic action and completely sterilized the culture at 24 h of incubation.

Similar studies using ampicillin (5 μg/ml), rifampin (5 μg/ml), and a combination of both drugs are depicted in Fig. 4. Rifampin was bacteriostatic, but resistant strains emerged at 24 h. The MICs of rifampin for the isolates recovered at 24 h of incubation were greater than 100 μg/ml, as compared to 0.8 μg/ml for the original strains. The combination of ampicillin and rifampin showed antagonism but prevented the emergence of rifampin-resistant strains.

Assays for ampicillin and rifampin both alone and in combination showed no evidence of physcial incompatability or inactivation (Table 1). In addition, it was shown that the presence of Me$_2$SO, used as a solvent for rifampin, did not inactive the ampicillin.

High-level resistance to streptomycin occurred in 26% of isolates, as compared to 23% being resistant to kanamycin. All strains showing high-level resistance to kanamycin simi-

TABLE 1. *Antibiotic concentrations of ampicillin and rifampin in combination and alone*

Flask	Contents (5 μg/ml each)	Mean antibiotic concn (μg/ml)			
		0 h	4 h	8 h	24 h
A	Ampicillin	5.0	3.6	3.4	2.5
B	Ampicillin (in Me₂SO)	5.0	3.5	3.3	2.6
C	Rifampin (in Me₂SO)	5.0	3.5	3.5	3.4
D	Ampicillin	5.0	3.4	3.4	2.2
	Rifampin[a]	5.0	3.7	3.2	3.2

[a] Rifampin was dissolved in Me₂SO prior to dilution.

larly showed high-level resistance to streptomycin. Two of the strains with high-level resistance to streptomycin were inhibited by 156 μg of kanamycin per ml. High-level resistance to gentamicin, tobramycin, and amikacin was not detected. There was no significant variation in the percentage of strains showing high-level resistance when the isolates from the three hospitals were compared.

DISCUSSION

Ampicillin or penicillin G, plus streptomycin or kanamycin, is currently recommended for therapy of enterococcal infections (4). For enterococcal endocarditis, penicillin or ampicillin plus streptomycin is recommended (2). These combinations, however, are not synergistic for approximately one-quarter of the isolates from our institutions, as predicted by the presence of high-level resistance to these aminoglycosides (6, 9). Similar observations have been reported by Moellering et al. (5). A combination of ampicillin and gentamicin would appear to be optimal therapy for serious enterococcal infections until the results of tests for high-level resistance to streptomycin and kanamycin are available. In hospitals where these tests are not performed, therapy with ampicillin-gentamicin would appear to be indicated.

Although tobramycin and amikacin in combination with ampicillin are synergistic against enterococci, clinical experience with these combinations is lacking. Their place in the therapy of enterococcal infections is yet to be determined.

The activity of rifampin, at levels easily achievable in serum, might suggest that this drug would be a useful agent in the therapy of serious enterococcal infections in patients allergic to penicillin. Unfortunately, however, as with other organisms, resistance rapidly

emerges. In our experience, the emergence of resistance strains was prevented by the addition of ampicillin, but the resultant combination was antagonistic. Physical incompatability and inactivation did not occur. The mechanism of this antagonism is unknown but may relate to the bacteriostatic effect of rifampin by interference with deoxyribonucleic acid-dependent ribonucleic acid polymerase, thus diminishing cell division and cell wall synthesis and precluding the bactericidal action of ampicillin at this site.

An evaluation of rifampin in combination with other antibiotics not dependent on cell wall synthesis for activity seems indicated. It is as active as vancomycin, the currently recommended drug for enterococcal infections in patients allergic to penicillin, but far less toxic.

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