# In Vitro Activity of Ampicillin or Vancomycin Combined with Gentamicin or Streptomycin Against Enterococci

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Minimum inhibitory and bactericidal concentrations of four antibiotics and their combinations were determined for 38 strains of enterococci by a microtitration tube dilution technique. The drugs were ampicillin, vancomycin, gentamicin, and streptomycin; the combinations were ampicillin-gentamicin, ampicillin-streptomycin, vancomycin-gentamicin, and vancomycin-streptomycin. At achievable serum concentrations, ampicillin alone killed 60% of strains, whereas combination with streptomycin increased this to 90% and with gentamicin to 100%. Vancomycin alone showed striking inhibitory activity, but very poor bactericidal activity at achievable concentrations. Combination with one of the aminoglycosides increased the bactericidal activity substantially. When combined with ampicillin, gentamicin was both more active and showed synergistic bactericidal activity significantly more often ($P < 0.01$) than streptomycin.

Despite the advent of new antibiotics, endocarditis due to the group D *Streptococcus* (enterococcus) has remained more difficult to treat successfully than endocarditis due to other streptococci (9). This difficulty is likely because of the organism's relative resistance to antibiotics. Early studies demonstrating synergistic activity between penicillin and streptomycin (7) led to the establishment of this combination as standard therapy for enterococcal endocarditis. Mortality rates for this disease have remained relatively high, however (8), and a significant number of strains do not respond to this combination (15). Other regimens, including penicillin plus kanamycin (6), ampicillin alone (1), and ampicillin plus streptomycin (12) have been proposed. More recently, very low concentrations of gentamicin have been reported to act synergistically with penicillin (13). Because previous experiments from this laboratory (14) have suggested that both ampicillin alone and ampicillin plus streptomycin were more active than penicillin plus streptomycin, we compared the in vitro effectiveness of the ampicillin-gentamicin combination with that of ampicillin-streptomycin to see if this activity of ampicillin could be enhanced by use of another aminoglycoside.

Alternate therapy for enterococcal endocarditis in patients who cannot tolerate penicillin or its derivatives remains an open question. Vancomycin alone has been suggested (4, 12), but the wide gap between concentrations of vancomycin which inhibit and those which kill the enterococcus (3) has encouraged search for ways to increase the effectiveness of this drug. Streptomycin has been found to act synergistically with vancomycin in vitro (11), and at least one patient has been treated successfully with this combination (17). To confirm and extend these studies to the use of another aminoglycoside, we compared the activity of vancomycin plus streptomycin with that of vancomycin plus gentamicin.

## MATERIALS AND METHODS

Thirty-eight strains of enterococci were studied. Of these, 31 were isolated in the clinical laboratory: 17 from urine, 10 from local infections, 3 from sputum, 2 from cerebrospinal fluid, and 1 from ascitic fluid. The remainder consisted of four strains from the American Type Culture Collection and the GK strain, whose antibiotic sensitivities have been previously described (14). Identification as an enterococcus was established by Gram-stain morphology, negative catalase reaction, growth in 6.5% salt medium at pH 9.4, and

growth in 0.05% sodium azide with acid production (SF medium, Difco).

Antibiotics were prepared in frozen stock solutions at the following concentrations ($\mu$g/ml): sodium ampicillin, 800 and 100; vancomycin hydrochloride, 800 and 100; gentamicin sulfate, 1,600 and 800; and streptomycin sulfate, 12,800 and 6,400.

For single antibiotic sensitivity determinations, the test was performed by using a microtitration technique previously outlined (5), except that "prepared" disposable styrene 96 well microtitration plates (Linbro Chemical Co., New Haven, Conn.) were used. Organisms were grown overnight in Trypticase-soybroth, but were diluted and tested in Mueller-Hinton broth. Additional microtitration plates were employed when very high concentrations of antibiotics were required to reach an endpoint. To perform the combination antibiotic sensitivities, two sets of dilutions were made in the same microtitration plate, one vertically and one horizontally. The procedure was as follows: 50 $\mu$liters of broth was dropped into each well. Fifty microliters of the higher concentration of the aminoglycoside antibiotic was added to the top well in the left-most column. Fifty microliters of the lower concentration of the aminoglycoside (one-half the higher concentration) was then added to the remaining seven wells in this column; 11 serial dilutions were made from left to right by using 50-$\mu$liter microdiluters (Linbro Chemical Co.); 50 $\mu$liters of the 100 $\mu$g/ml concentration of ampicillin or vancomycin was added to all 12 wells in the top row; seven serial dilutions from top to bottom were made with the 50-$\mu$liter loops in each column. Finally, 100 $\mu$liters of Mueller-Hinton broth and 50 $\mu$liters of a 1:2,000 dilution of the overnight culture of the test organism were added to each well. The final titer of the test organism was approximately $10^5$ per ml.

Concentrations of aminoglycosides increased in twofold steps from 0.098 to 100 $\mu$g/ml for gentamicin and from 0.78 to 800 $\mu$g/ml for streptomycin. Each of these concentrations was tested against eight concentrations of ampicillin or vancomycin from 0.098 to 12.5 $\mu$g/ml. The minimum inhibitory concentration (MIC) was the first well with no visible turbidity after 24 h of incubation; the minimum bactericidal concentration (MBC) was the first well from which zero or one colony was recovered after subculture to a blood agar plate with a standard 3-mm loop. Synergistic bactericidal activity was defined as a reduction in the MBC of two or more dilutions of both drugs in the combination. For example, the bactericidal levels of vancomycin alone and gentamicin alone for *Streptococcus faecalis* strain *GK* were 200 and 25 $\mu$g/ml, respectively, whereas the combination of 1.56 $\mu$g of vancomycin with 0.19 $\mu$g of gentamicin per ml was bactericidal. This represented a reduction of six dilutions for vancomycin and seven for gentamicin when the drugs were combined. The total concentration of antibiotics was well below that of either drug acting singly. We have assumed the following concentrations to be acceptably safe, achievable blood levels ($\mu$g/ml): streptomycin, 25; gentamicin, 12; ampicillin, 25; and vancomycin, 6 (3; 16).

## RESULTS

Antibiotic sensitivities of the enterococci to the four individual drugs are shown in Fig. 1–4. At achievable blood levels few isolates were inhibited or killed by gentamicin (Fig. 1) or streptomycin (Fig. 2). Ampicillin (Fig. 3) showed inhibitory activity at achievable concentrations, but 200 $\mu$g/ml was required for bactericidal activity against all 38 strains. Vancomycin (Fig. 4) was extremely active, inhibiting 96% of strains at 3.125 $\mu$g/ml. However, concentrations required for bactericidal activity were 4 to 500 times greater.

Results of antibiotic combination studies are shown in Fig. 5–8. The effect of adding gentamicin or streptomycin to vancomycin was not striking when only the inhibitory concentrations were examined (Fig. 5A and 6A), but both streptomycin and gentamicin dramatically enhanced bactericidal activity (Fig. 5B and 6B). Streptomycin, however, required concentrations very difficult to achieve clinically (100 $\mu$g/ml with 12.5 $\mu$g of vancomycin per ml) to kill 90% of strains, whereas achievable concentrations of gentamicin (12.5 $\mu$g/ml) and vancomycin (6.25 $\mu$g/ml) showed sufficient activity in combination to kill all 38 strains.

Tests of the combinations with ampicillin showed similar results. Although the difference between gentamicin (Fig. 7A and 7B) and streptomycin (Fig. 8A and 8B) was less striking than with the vancomycin combinations, gentamicin plus ampicillin was the more active pair. The frequency of occurrence of synergistic bactericidal activity for the four drug combinations is shown in Table 1. When combined with ampicillin, gentamicin showed synergistic activity significantly more often than did streptomycin ($P < 0.01$). The same trend was seen with vancomycin. When synergism was demonstrated, it occurred at or below achievable levels in every case. No evidence of antagonism was seen.

In testing penicillin-streptomycin combinations, Standiford et al. (15) observed that synergism was not likely to occur when the MIC for streptomycin of a given enterococcus was greater than 250 $\mu$g/ml.

Our studies suggest that no such relationship exists between the activity of streptomycin and likelihood of synergistic activity between streptomycin and ampicillin. Table 2 shows that whatever dividing line was chosen for the MIC of streptomycin, the likelihood of synergistic bactericidal activity occurring was essentially the same at higher or lower concentrations.

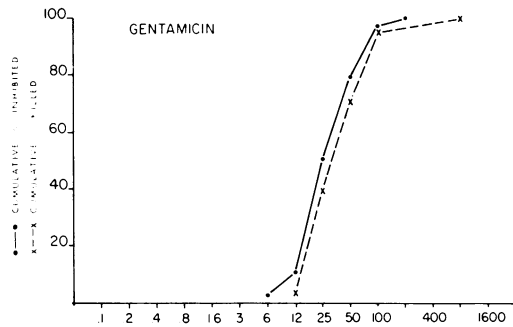The gentamicin MICs or MBCs also had no

FIG. 1. *Cumulative percent (ordinate) of 38 strains of enterococci strains inhibited* ●——● *or killed* ×— —× *by increasing concentrations of gentamicin (abscissa).*
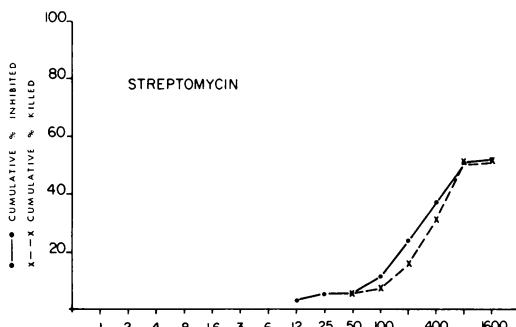


FIG. 2. *Cumulative percent (ordinate) of 38 strains of enterococci strains inhibited* ●——● *or killed* ×— —× *by increasing concentrations of streptomycin (abscissa).*
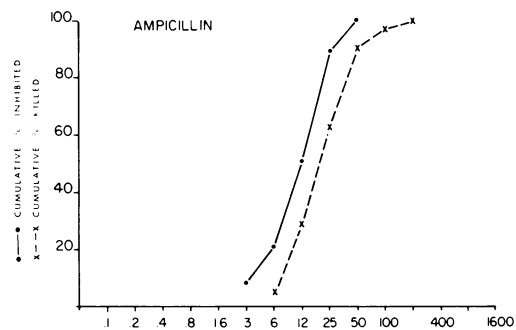


FIG. 3. *Cumulative percent (ordinate) of 38 strains of enterococci strains inhibited* ●——● *or killed* ×— —× *by increasing concentrations of ampicillin (abscissa).*

relationship to the likelihood of synergism with either ampicillin or vancomycin, but the strains in which synergism did not occur were very few.

In the case of vancomycin plus streptomycin all 20 organisms with MICs for streptomycin of

800 µg/ml or less exhibited synergism, whereas 5 of 18 (28%) with MICs above 800 µg/ml did not. A single test of streptomycin inhibitory activity at 1,600 µg/ml might be of some predictive value.

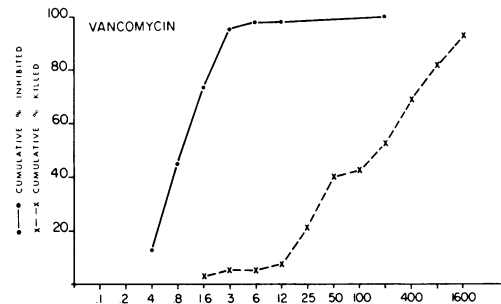These studies have demonstrated synergistic



FIG. 4. *Cumulative percent (ordinate) of 38 strains of enterococci strains inhibited* ●——● *or killed* ×— —× *by increasing concentrations of vancomycin (abscissa).*

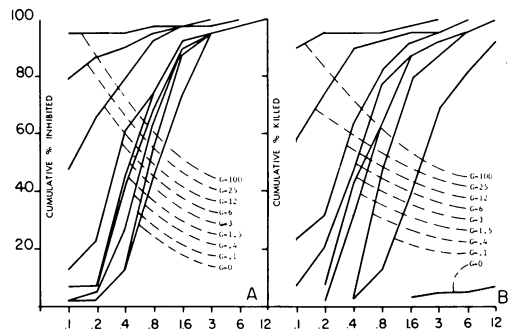

FIG. 5. *Cumulative percent (ordinate) of 38 strains of enterocci (A) inhibited and (B) killed by various concentrations of gentamicin combined with increasing concentrations of vancomycin (abscissa).*
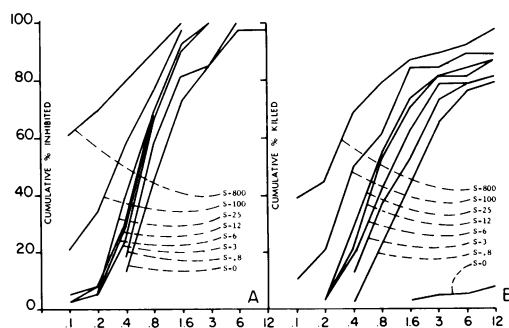


FIG. 6. *Cumulative percent (ordinate) of 38 strains of enterococci (A) inhibited and (B) killed by various concentrations of streptomycin combined with increasing concentrations of vancomycin (abscissa).*

antimicrobial activity against a variety of strains of enterococci by using combinations of either ampicillin or vancomycin with gentamicin or streptomycin. In each case the combination with gentamicin was more active at a lower concentration and was more likely to show synergistic bactericidal activity than was streptomycin.

## DISCUSSION

Concern over achieving bactericidal rather than bacteriostatic activity in patients with endocarditis is based on experience that bacteriostatic antibiotics often fail to cure the disease (2). The ready synergism of vancomycin and gentamicin, despite the obvious toxicity of both drugs, would seem to make this combination very effective alternative therapy to penicillin or ampicillin in combination and should allow the achievement of serum bactericidal activity in the patient with serious penicillin allergy.

These data suggest the use of gentamicin as the aminoglycoside of choice in the combination antibiotic therapy of enterococcal infection

TABLE 1. Frequency of synergistic bactericidal action with four antibiotic combinations against 38 strains of enterococci[a]

| Drug | Synergistic activity |
|------|----------------------|
| Ampicillin-Gentamicin | 34[b]/38(90%) |
| Ampicillin-Streptomycin | 21[b]/38(55%) |
| Vancomycin-Gentamicin | 37/38(97%) |
| Vancomycin-Streptomycin | 33/38(87%) |

[a] Synergistic activity is defined as a reduction by at least two twofold dilutions in the concentration of each antibiotic required for bactericidal action at attainable serum levels. Antagonistic activity is an increase of more than one twofold dilution in the concentration of either antibiotic required for bactericidal action; in all drug combinations antagonistic activity was 0/38.
[b] $P < 0.01$.

TABLE 2. Likelihood of synergistic bactericidal activity between ampicillin and streptomycin for 38 strains of enterococci based on MIC of streptomycin

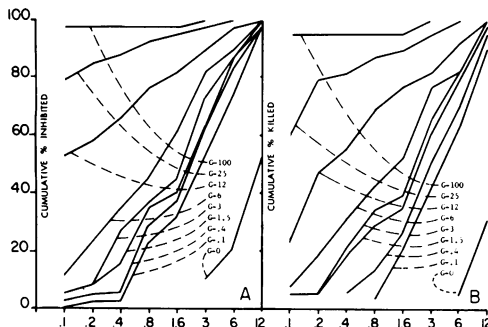| Streptomycin MIC ($\mu$g/ml) | Synergistic activity (%) | No synergistic activity (%) | No. of strains in group |
|------|------|------|------|
| ≤200 | 57 | 43 | 7 |
| >200 | 55 | 45 | 31 |
| ≤400 | 55 | 45 | 11 |
| >400 | 56 | 44 | 27 |
| ≤800 | 50 | 50 | 18 |
| >800 | 60 | 40 | 20 |
| ≤1,600 | 50 | 50 | 22 |
| >1,600 | 62 | 38 | 16 |



FIG. 7. Cumulative percent (ordinate) of 38 strains of enterococci (A) inhibited and (B) killed by various concentrations of gentamicin combined with increasing concentrations of ampicillin (abscissa).
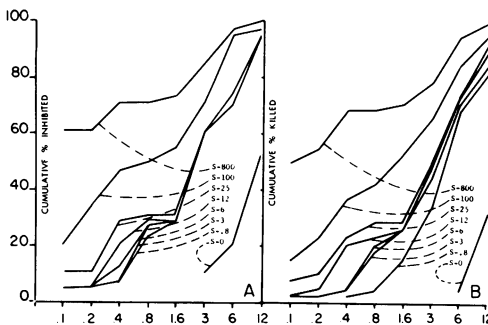


FIG. 8. Cumulative percent (ordinate) of 38 strains of enterococci (A) inhibited and (B) killed by various concentrations of streptomycin combined with increasing concentrations of ampicillin (abscissa).

based on in vitro evidence of its greater activity over streptomycin. Unfortunately, gentamicin is also a more toxic drug, and little clinical data is available at this time to support its use in endocarditis. However, in the patient not responding well to conventional penicillin-streptomycin therapy, and in the situation where laboratory facilities for determination of serum bactericidal activity are not available, the use of ampicillin plus gentamicin or, if necessary, vancomycin plus gentamicin should be considered. Recent experiments treating enterococcal pyelonephritis in the rat (Harwick, Kalmanson, and Guze, J. Infect. Dis., in press) have confirmed the in vitro data regarding the antimicrobial activity of the vancomycin-gentamicin combination.

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