Vancomycin has significant inhibitory activity against enterococci, but it is not bactericidal (4, 6). Vancomycin in combination with an aminoglycoside has been shown to be synergistic against enterococci (2, 4, 6). However, the combination of vancomycin and an aminoglycoside may be potentially nephrotoxic. Rifampin, which has no nephrotoxic potential, has significant activity against enterococci (5). In this investigation, we studied the effect of a vancomycin-rifampin combination against enterococci by the time-kill curve method.

Forty-eight strains of enterococci were used in this study. All strains grew in 6.5% NaCl brain heart infusion broth as colonies surrounded by black zones on bile-esculin agar. Vancomycin was obtained from Eli Lilly & Co. and rifampin from Dow Chemical Co. A standard stock solution of each antibiotic was prepared according to the manufacturer's instructions, stored at −80°C, and thawed immediately before use.

The minimal inhibitory concentration (MIC) and minimal bacterecidal concentration (MBC) of each antibiotic were determined by the World Health Organization-International Collaborative Study broth dilution method (1). Serial twofold dilutions of the antibiotic were made in Mueller-Hinton broth of from 32 to 0.3 μg/ml. The inoculum was 1 ml of 10^5 to 10^6 organisms diluted from an 18-h culture. The MIC was the lowest concentration of the antibiotic that allowed no visible growth after incubation at 37°C for 18 to 24 h. The MBC was the lowest concentration of an antibiotic that allowed no growth (or one colony) from a 0.01-ml subculture from each clear tube on agar plates after incubation at 37°C for 18 to 24 h.

The standard time-kill curve method was used to study the interaction between vancomycin and rifampin. Mueller-Hinton broth was used. The antibiotic concentrations were vancomycin, 10 μg/ml, and rifampin, 10 μg/ml, and the combination was vancomycin, 10 μg/ml, with rifampin, 10 μg/ml. A broth culture with no antibiotic was set up as a control. The inoculum was between 10^9 and 10^8 organisms per ml, made from an 18- to 24-h culture. All tubes were incubated in a dry bath (Fisher Scientific Co.) at 37°C. At 0, 6, 24, and 48 h, the viable numbers of organisms were enumerated by serial 10-fold dilutions plated on Mueller-Hinton agar.

When the result of the combination was at least log_{10} less than that from both drugs alone at a given time, it was defined as synergism. When the result of the combination was at least log_{10} more than that from either drug alone, it was defined as antagonism.

For the 48 strains of enterococci, the MIC of vancomycin ranged from 0.25 to 4.0 μg/ml (median, 2.0 μg/ml), and the MBC was >32 μg/ml. The MIC of rifampin ranged from 0.25 to 32 μg/ml (median, 8.0 μg/ml), and the MBC was also >32 μg/ml for all strains. There was no synergism demonstrated for any strain. Antagonism was shown at 6 h against one strain and at 48 h against four other strains (there was no antagonism if the more rigid criterion of at least a 2 log_{10} increase in colony count was used). For 43 of the 48 strains, neither synergism nor antagonism was demonstrated. With rifampin alone, there was regrowth of enterococci at 48 h and sometimes as early as 24 h. In the presence of vancomycin and rifampin, the regrowth phenomenon did not occur. The time-kill curves of vancomycin alone and vancomycin-rifampin were virtually identical (Fig. 1).

The results of this study confirm the lack of bactericidal activity of vancomycin against enterococci (4, 6). The study also demonstrated the regrowth phenomenon of enterococci in the presence of rifampin, which is also encountered in Staphylococcus aureus (7). The lack of synergism between rifampin and vancomycin for enterococci is not surprising. Iannini and colleagues reported antagonism between ampicillin
and rifampin against three strains of enterococci (3). It appears that rifampin may not be useful as an adjunct in the therapy of serious enterococcal infections, unless animal and clinical studies indicate otherwise.

LITERATURE CITED

FIG. 1. Time-kill curves of a strain of enterococci showing neither synergism nor antagonism between vancomycin and rifampin.