Absence of Synergistic Activity between Ampicillin and Vancomycin against Highly Vancomycin-Resistant Enterococci

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The emergence of clinical enterococcal isolates resistant to both ampicillin and vancomycin is a cause of great concern, as there are few therapeutic alternatives for treatment of infections caused by such organisms. We evaluated the effects of the combination of ampicillin with vancomycin against vancomycin-resistant clinical enterococcal isolates. Using both the checkerboard technique and time-kill curves, we examined 28 strains of enterococci (17 Enterococcus faecalis and 11 Enterococcus faecium strains) with different levels of resistance to vancomycin. Of these, 15 strains were also highly gentamicin resistant, and 9 demonstrated resistance to ampicillin. Only seven strains of E. faecalis were inhibited synergistically by the combination of vancomycin with ampicillin, and even then, the concentrations of vancomycin at which synergism was demonstrated were above levels achievable in serum. None of the ampicillin-resistant isolates (all E. faecium) were inhibited synergistically at any concentration of the drugs. In no instance was bactericidal synergism observed, and in most cases the combination resulted in less killing than with ampicillin alone. Antagonism was not observed at clinically relevant concentrations. The results of this study suggest that the combination of vancomycin with ampicillin has little to offer against these emerging pathogens.

Treatment of serious enterococcal infections is undertaken with combinations of penicillin, ampicillin, and/or vancomycin with an aminoglycoside in order to achieve bactericidal synergism. However, the presence of enterococci with high-level resistance to aminoglycosides, which in consequence are also resistant to the synergistic bactericidal actions of these combinations, is becoming a prevalent situation in many centers (4, 6). We recently have demonstrated among clinical isolates of Enterococcus faecalis great variability in their susceptibility to killing by ampicillin or vancomycin as single agents, which suggests that the likelihood of successful treatment with a single agent when bactericidal therapy is required will prove strain dependent (1). A more recent problem has been the appearance of enterococcal strains that are resistant to vancomycin, some of which are also resistant to ampicillin and penicillin (5, 10). The emergence of such isolates is a cause of concern and leaves few therapeutic alternatives. However, it has been suggested that combinations of vancomycin with ampicillin may be synergistic against vancomycin-resistant enterococci (7, 13). Therefore, we undertook the present study in order to evaluate the effects of the combination of ampicillin with vancomycin against vancomycin-resistant clinical enterococcal isolates. Using both the checkerboard technique and time-kill curves, we examined 28 strains of enterococci with different levels of resistance to vancomycin. Of these, 15 strains were also highly gentamicin resistant, and 9 demonstrated resistance to ampicillin.

**MATERIALS AND METHODS**

**Bacterial strains.** We studied a total of 28 vancomycin-resistant clinical isolates of enterococci (17 E. faecalis and 11 Enterococcus faecium isolates). Twenty-five of these demonstrated high-level resistance to vancomycin (≥256 µg/ml), and the others were inhibited at concentrations of 8 to 64 µg of the drug per ml. Fifteen strains also presented high-level resistance to gentamicin. Of these, 9 were also resistant to ampicillin, with MICs of ≥128 µg/ml. None of the isolates produced β-lactamase by nitrocefin disk testing (Cefinase; BBL Microbiology Systems, Cockeysville, Md.). The strains were referred to our laboratory from seven different institutions in the United States and from one hospital in Madrid (Spain). All organisms were identified by the API Rapid Strep System (Analytab Products, Plainview, N.Y.).

**Antimicrobial agents.** Ampicillin was obtained from Bristol Laboratories, Bristol-Meyers Company, Evansville, Ind., and vancomycin was from Abbott Laboratories, North Chicago, Ill. Teicoplanin was a gift of Marion Merrell Dow, Cincinnati, Ohio.

**Susceptibility tests.** MICs were initially determined by a standard agar dilution technique (8) with Mueller-Hinton II agar (BBL). Bacterial inocula were prepared by appropriate dilutions of overnight broth cultures of organisms in fresh Mueller-Hinton broth (BBL) and applied to antibiotic-containing plates with a 32-prong inoculating device, yielding final inocula of approximately 10⁶ CFU per spot. The plates were examined for growth after 18 to 20 h of incubation at 35°C. Staphylococcus aureus ATCC 29213 and Streptococcus faecalis ATCC 29212 were used as control strains.

**Study of combined antimicrobial activity.** Inhibitory activities of ampicillin and vancomycin in combination were studied by the microdilution checkerboard technique. Plastic microdilution trays (Dynatech Laboratories, Inc., Alexandria, Va.) contained vancomycin in serial twofold dilutions from 2,048 to 0.2 µg/ml alone and in combination with ampicillin in serial twofold dilutions from 1,024 to 0.12 µg/ml. The inoculum was adjusted to approximately 10⁶ CFU/ml, and after incubation for 24 h, the MICs of each antibiotic alone and in combination were noted; fractional inhibitory concentration (FIC) indexes were calculated. Syn-
ergism was defined as a FIC index of ≤0.5, and antagonism was defined as a FIC index of >4.

**Time-kill kinetic studies.** Bactericidal activities of ampicillin and vancomycin alone and in combination were determined by previously described time-kill methods with inocula of 10^5 CFU/ml in Mueller-Hinton II broth and in dextrose phosphate broth (Scott Laboratories, West Warwick, R.I.) with 0.1% sodium citrate (3, 9). Samples were withdrawn for colony counts at 0, 4, 8, and 24 h. The antimicrobial agent concentrations selected for testing based on clinically achievable levels in blood were 10 and 20 μg of ampicillin and vancomycin, respectively, per ml. For ampicillin-resistant strains, 500 μg of ampicillin per ml with either 20 or 200 μg of vancomycin per ml was examined. With the method used, colony counts as low as 1.3 log_{10} CFU/ml could be detected. Preliminary experiments excluded significant vancomycin carryover effect; samples of low-inoculum bacterial suspensions (approximately 10^1 to 10^3 CFU) prepared in the presence or absence of vancomycin (20 and 200 μg/ml) alone or in combination with ampicillin were plated onto antibiotic-free plates (with addition of excess penicillinase [BBL] when ampicillin was added). Colony counts in the presence or absence of antibiotic differed by less than 5%. Ampicillin carryover effect was excluded by the addition of excess penicillinase at the moment of each sampling. Bactericidal activity was defined as a >2 log_{10} decrease in cell density at 24 h of incubation. Synergism was defined as a >2 log_{10} decrease in CFU/ml between the combination and its most active component after 24 h of incubation.

### RESULTS

**Susceptibility tests.** Nineteen strains were susceptible to ampicillin (<2 μg/ml), and MICs of ampicillin against the remaining 9 were ≥128 μg/ml. Twenty-five strains demonstrated high-level resistance to vancomycin (≥256 μg/ml), and of these two were highly susceptible to teicoplanin (MICs, 0.12 and 0.5 μg/ml), and the remainder were inhibited by teicoplanin at concentrations of between 8 and 128 μg/ml. MICs of vancomycin against three strains were 8, 32, and 64 μg/ml, and these were all susceptible to teicoplanin at ≤1 μg/ml. Nine strains were highly resistant both to ampicillin and vancomycin.

**Inhibitory activity of the ampicillin-vancomycin combinations.** Table 1 shows the characteristics of seven strains inhibited synergistically by the combination of vancomycin with ampicillin. These were all isolates of *E. faecalis* and all were resistant to teicoplanin (MICs, 16 to 64 μg/ml) and thus represented the VanA phenotype. The concentrations of vancomycin at which synergism was demonstrated were above concentrations achievable in serum with one excep-

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<th>Conc (μg/ml) at lowest FIC index</th>
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**DISCUSSION**

Resistance of enterococci to vancomycin precludes useful synergism between this antimicrobial agent and aminoglycosides which require inhibitory activity of the cell wall-active drug (7, 11). The results of this study demonstrate that the combination of ampicillin with vancomycin is also not synergistic against vancomycin-resistant enterococci whether or not they are resistant to ampicillin. Our results differ from those reported by Shlaes et al. (13) and Leclercq et al. (7), who found synergy between penicillins and vancomycin against vancomycin-resistant enterococci, but are in accordance with those recently reported by Handwerger et al. (5), who found no synergy between penicillin and vancomycin against vancomycin-resistant enterococci. However, although results obtained by Leclercq et al. (7) indicate the presence of synergy, they found that the combinations were not bactericidal as tested by time-killing experiments and in some cases tended to be antagonistic.

Among the isolates we have studied, strains presenting the VanA and VanB phenotypes of resistance were included and
a lack of synergistic effect of ampicillin and vancomycin was observed against both types. Furthermore, three of our strains presented a novel type of vancomycin resistance described only recently in the United States (2, 12), which consists of high-level resistance to vancomycin but full susceptibility to teicoplanin. No synergism was observed against these either. We demonstrated a trend towards decreased killing by this combination relative to ampicillin alone, but the clinical significance of the differences observed is not obvious. The increasing emergence of strains of enterococci with concomitant resistance to the antibiotics used as standard therapies for serious enterococcal infections, as well as the absence of synergistic activity with combinations of these antimicrobial agents, is disquieting. The results of this study suggest that the combination of vancomycin with ampicillin has little to offer against these emerging nosocomial pathogens.

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REFERENCES


