Influence of High-Level Gentamicin Resistance and Beta-Hemolysis on Susceptibility of Enterococci to the Bactericidal Activities of Ampicillin and Vancomycin

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The bactericidal activities of ampicillin and vancomycin against 40 recent isolates of Enterococcus faecalis were examined by kill-kinetic studies at concentrations of 4 × the MIC and 20 μg/ml. Greater killing was seen with ampicillin (3.57 ± 0.87 and 2.50 ± 1.09 log10 CFU/ml, respectively; mean ± standard deviation) than with vancomycin (1.25 ± 0.65 and 1.05 ± 0.57 log10 CFU/ml, respectively). Highly gentamicin-resistant strains showed a tendency toward reduced susceptibility to killing; beta-hemolytic strains were more susceptible than nonhemolytic strains when exposed to ampicillin at 20 μg/ml. Within each group, individual isolates demonstrated great variability in susceptibility to killing by the drugs.

In recent years, enterococcal isolates demonstrating high-level resistance to both streptomycin and gentamicin, conditions that preclude synergistic interactions between cell wall-active antibiotics and available aminoglycosides, have become common in many centers (5, 6). Against such isolates, there is no other combination of antimicrobial agents which reliably provides bactericidal activity. As a result, treatment with a cell wall-active agent alone, usually ampicillin or vancomycin, would emerge as the most logical alternative for antimicrobial therapy in serious infections caused by such organisms (1, 11). Although relative tolerance to killing by β-lactams is considered a characteristic of enterococci, it is clear that some variability exists among naturally occurring isolates of Enterococcus faecalis in their susceptibilities to the bactericidal effects of these agents (3, 7, 9, 15). Using time-kill curves, we examined the susceptibilities of 40 unique strains of E. faecalis to killing by ampicillin or vancomycin; half of the strains demonstrated high-level gentamicin resistance. Because of suggestions that patients with bloodstream infections caused by beta-hemolytic, gentamicin-resistant enterococci fare less well than do those with bloodstream infections caused by nonhemolytic strains (8), we also examined the influence of hemolysis properties on susceptibility to killing by the antimicrobial agents.

We selected 40 recently isolated, unique strains of E. faecalis, with 10 strains belonging to each of the following groups: non-beta-hemolytic and non-high-level gentamicin resistant, non-beta-hemolytic and high-level gentamicin resistant, beta-hemolytic and non-high-level gentamicin resistant, and beta-hemolytic and high-level gentamicin resistant. Thirty-four strains were chosen from 345 enterococci collected at our hospital in 1990 and 1991. Six strains referred from four other institutions between 1985 and 1990 were added to fill the complement of beta-hemolytic strains. One isolate was known to produce β-lactamase. Beta-hemolysis was determined on brucella agar plates with 5% horse blood (Northeast Laboratory, Waterville, Maine).

MICs were determined by a standard agar dilution tech-

ique (12) on Mueller-Hinton II agar (BBL Microbiological Systems, Cockeysville, Md.) and also on glucose phosphate broth medium (Scott Laboratories, Inc., West Warwick, R.I.) with 0.1% citrate sodium dihydrate solidified with 1.7% Bacto Agar (Difco Laboratories, Detroit, Mich.). Final inocula of 104 CFU were prepared from overnight cultures. The bactericidal activities of ampicillin and vancomycin were determined by previously described methods in glucose phosphate-citrate broth by using inocula of 106 CFU/ml (2, 14). For each drug, two concentrations were selected for testing on the basis of their achievable levels in serum: 20 μg/ml and 4 × the MIC for the strain (4 or 8 μg/ml); sulbactam was added to ampicillin (1:2) when testing the β-lactamase-producing strain. Colony counts as low as 1.3 log10 CFU/ml could be detected. Preliminary experiments excluded significant vancomycin carryover, because low-inoculum bacterial suspensions (10 to 1,000 CFU) prepared in the presence or absence of vancomycin at 4 and 8 μg/ml yielded identical growth (<5% difference) when plated onto antibiotic-free agar. Ampicillin was eliminated by the addition of excess penicillinase (BBL) at the moment of sampling. The magnitudes of bactericidal effects were compared by the Mann-Whitney U test.

The MICs of both ampicillin and vancomycin ranged from 1 to 2 μg/ml. No significant differences were observed in the MICs determined in the two media. Twenty isolates were highly resistant to gentamicin (MIC, >500 μg/ml). Figure 1 demonstrates the bactericidal activities of ampicillin at 4 × the MIC and 20 μg/ml against the 40 isolates. Results for vancomycin at these concentrations are shown in Fig. 2. The higher concentration of ampicillin (20 μg/ml) was less effective in killing than was the 4 × MIC at all sampling points from 8 to 48 h (0.001 < P < 0.037). Considering individual isolates, the bactericidal activity of the drug at the higher concentration at 24 h exceeded that of the drug at the lower concentration against only 7 of the 40 isolates, all of which were beta-hemolytic. The paradoxical bactericidal effect noted with ampicillin was not seen with vancomycin. The bactericidal activity of ampicillin exceeded that of vancomycin at either concentration of the drugs (Fig. 1 and 2). At 24 h, killing (mean ± standard deviation [SD] reduction in viable cells) by ampicillin at 4 × the MIC (3.57 ± 0.87 log10
CFU/ml) and 20 μg/ml (2.50 ± 1.09 log₁₀ CFU/ml) was significantly greater (P < 0.001) than that which occurred with the corresponding concentrations of vancomycin (1.23 ± 0.65 and 1.05 ± 0.57 log₁₀ CFU/ml, respectively).

For both ampicillin and vancomycin, the magnitude of bactericidal effects against individual strains varied over a wide range (Fig. 3). For two non-high-level gentamicin-resistant isolates which showed the greatest susceptibility to killing by ampicillin, when subinhibitory concentrations of gentamicin were combined with ampicillin, substantially greater rates of killing were noted over the first 8 h compared with the rates with ampicillin alone (data not shown).

To assess the reproducibility of killing as measured by this method, several strains were repeatedly tested with exposure to ampicillin (20 μg/ml). Two nontolerant isolates each yielded highly reproducible results, with mean ± SD killing at 24 h of 3.42 ± 0.46 and 3.97 ± 0.52 log₁₀ CFU/ml, respectively, in seven replicate tests. In contrast, replicate testing of a strain initially identified as a tolerant organism (0.9 log₁₀ CFU of killing per ml) yielded highly variable results, with a range of 0.51 to 4.46 log₁₀ CFU/ml reduction in viable cells at 24 h. Several possible explanations for this variability were investigated. Results were not affected by the inoculum used (10⁶ to 10⁷ CFU/ml), precise sampling time (23 to 28 h), commercial source of the broth medium, or rigor of agitation immediately prior to sampling of the flasks. We also examined two tolerant mutant strains of Enterococcus faecalis previously derived in our laboratory from a nontolerant stool isolate designated SI-E39 (7). In duplicate assays, the nontolerant parent strain gave identical results, with 3.46 and 3.53 log₁₀ CFU of killing per ml. On the other hand, the two tolerant mutants, designated SI-T5 and SI-T20, gave disparate results in the duplicate tests: 1.89 versus 3.25 log₁₀ CFU of killing per ml and 1.99 versus 2.88 log₁₀ CFU of killing per ml, respectively. In view of these results, we repeated the time-kill tests for all 40 recent isolates upon exposure to ampicillin at 20 μg/ml for 24 h. The mean ± SD reduction in viable cells observed (2.97 ± 0.77 log₁₀ CFU/ml) was within 0.5 log₁₀ unit of that demonstrated initially (2.50 ± 0.99 log₁₀ CFU/ml), but the difference was statistically significant (P = 0.013).

Highly gentamicin-resistant strains showed a tendency toward reduced susceptibility to killing by either drug, but differences were small and reached statistical significance only for ampicillin at 4× the MIC (Table 1). Even at this concentration of ampicillin, however, the range of killing observed at 24 h was quite broad for both gentamicin-susceptible (2.70 to 5.30 log₁₀ CFU/ml) and high-level gentamicin-resistant (1.37 to 5.10 log₁₀ CFU/ml) isolates. When exposed to ampicillin at 20 μg/ml, beta-hemolytic strains underwent 10-fold greater killing than did nonhemolytic strains (P = 0.01). The influence of hemolysis on vancomycin activity was small and unlikely to be of clinical significance. For highly gentamicin-resistant strains, the presence of beta-hemolysis tended to confer a small degree of enhanced susceptibility to killing by ampicillin (0.37 and 1.06 log₁₀ CFU/ml for 4× the MIC and 20 μg/ml, respectively).

In the early antibiotic era, penicillin alone effected cures in some patients with enterococcal endocarditis (4). Although there are as yet few detailed reports documenting endocarditis caused by high-level gentamicin-resistant Enterococcus faecalis, both cure and failure with ampicillin alone as the primary medical treatment have been described (10). Given the great variability among recent isolates in susceptibility to killing by either ampicillin or vancomycin, any analysis of new treatment regimens for endocarditis caused by highly aminoglycoside-resistant enterococci should take into account strain differences in susceptibility to killing in vitro. Unfortunately, it is apparent from these results that at least some tolerant strains will appear to be highly susceptible to killing by ampicillin on repeat testing. Thus, while time-kill studies as used in the study described here remain the most precise method of examining the bactericidal effects of antibiotics against enterococci, it is clear that a single test showing high degrees of killing by ampicillin does not reliably exclude the potential for tolerant responses on subsequent analyses.

While there was a consistent trend in decreased suscepti-
bility to killing among gentamicin-resistant isolates, differences in killing at 24 h compared with killing of non-high-level-resistant strains were generally small and therefore of uncertain clinical significance. The beta-hemolytic strains from diverse sources which we examined were not more resistant to killing than the nonhemolytic strains were. Any adverse effect of hemolysin production on the clinical response of enterococcal bacteremia (8) is thus more likely due to factors that influence virulence or other interactions with the host (13) rather than on resistance to killing by cell wall-active antimicrobial agents.

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