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Inconsistent Bactericidal Activity of Triple-Combination Therapy with Vancomycin, Ampicillin, and Gentamicin against Vancomycin-Resistant, Highly Ampicillin-Resistant *Enterococcus faecium*

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Combination therapy with ampicillin, vancomycin, and gentamicin in vitro against several clinical isolates of vancomycin-resistant, highly ampicillin-resistant *Enterococcus faecium*, including VanA and VanB strains, was evaluated. The MICs of ampicillin were not significantly decreased by induction with vancomycin, and the combination of ampicillin and vancomycin was not inhibitory for any strain. Triple-combination therapy was least active against highly resistant VanA isolates, achieving a reduction of less than 1 log CFU at 24 h, but demonstrated slightly more activity against VanB strains.

Since 1986, clinical isolates of enterococci with inducible resistance to the glycopeptide antibiotic vancomycin have emerged as a major clinical problem in Europe and the United States (6, 7, 13). During 1991, several hospitals in the Philadelphia, Pa., area experienced outbreaks of colonization and infection due to vancomycin-resistant *Enterococcus faecium* (11). These isolates demonstrate high-level inducible resistance to vancomycin and other glycopeptides (VanA phenotype), as well as high-level resistance to ampicillin (MIC, 256 μg/ml). Currently available alternatives for therapy of infections caused by these strains are limited, particularly for bloodstream and deep-seated tissue infections requiring bactericidal therapy.

Several investigators have reported that antibiotic combinations employing concentrations of vancomycin and penicillin achievable in serum are inhibitory in vitro for enterococcal strains exhibiting both glycopeptide and beta-lactam resistance (3, 10, 12) and that the combination of vancomycin, penicillin, and an aminoglycoside could be bactericidal in the absence of high-level aminoglycoside resistance (10, 12). These studies have recently been verified in a rabbit model of enterococcal endocarditis (2). However, few of these strains demonstrated high-level resistance to beta-lactams (2, 10, 12), and more recent data by Handwerger et al. have suggested that triple therapy may be ineffective against some isolates (5). Thus, we wished to assess the in vitro efficacy of combination therapy for a variety of vancomycin-resistant, highly ampicillin-resistant clinical isolates. These included strains obtained from the recent Philadelphia outbreak as well as other phenotypically distinct vancomycin-resistant *E. faecium* isolates.

The following strains were utilized in these studies: *E. faecium* TJ100, a bloodstream isolate from Thomas Jefferson University Hospital; *Enterococcus faecalis* TJ153, a urinary tract isolate also from Thomas Jefferson University Hospital; *E. faecium* MCD1644, a urinary tract isolate from Wilmington, Del.; *E. faecium* OBO, a bloodstream isolate from New York City, N.Y.; and *E. faecium* WC100, a bloodstream isolate from Westchester County, N.Y. Additional characteristics of these strains are shown in Table 1.

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MICs were determined by broth macrodilution in cation-adjusted Mueller-Hinton broth (BBL) with an initial inoculum of 10^6 organisms. MICs were determined during vancomycin induction by preincubation of the test strain overnight in the constant presence of 8 μg of vancomycin per ml or by addition of vancomycin simultaneously with the other antibiotics. There were no differences in the results with either method of vancomycin induction. MBCs, defined as killing of 99% of the initial inoculum, were determined from an initial inoculum of 10^6 organisms with subculture volumes of 0.010 ml. Vancomycin, ampicillin, and gentamicin powder were purchased from Sigma. Killing curves were determined for overnight cultures which had been diluted 1/100 and allowed to regrow to the logarithmic phase of growth. Inocula were adjusted to approximately 5×10^5 and 5×10^7 organisms per ml for low- and high-inoculum experiments, respectively. Killing curves were determined for cultures grown in Mueller-Hinton broth. When tested in brain heart infusion (BBL), antibiotic combinations demonstrated activity equal to or less than that in Mueller-Hinton broth for all strains (data not shown). For antibiotic combination killing curves, vancomycin was added simultaneously with the other antibiotics. Preinduction of strains with 8 μg of vancomycin per ml did not significantly affect 24-h killing curves, though some minor differences were observed at the 4-h time point. Colony counts were measured by standard pour plate techniques, with a limit of detection of 10^2 organisms per ml. Pour plates prepared from uninoculated media at the maximal antibiotic concentrations used did not affect the growth of a fixed inoculum of control organisms for any of the strains studied.

Results. Effects of induction with 8 μg of vancomycin per ml on the MIC and MBC of ampicillin for 99.9% of strains are shown in Table 1. It is notable that there was at most a one- to two- tube (two- to fourfold) decrease in the MIC of ampicillin with vancomycin induction for all the highly ampicillin-resistant strains tested; in no case did the MIC fall into a clinically useful range. This is markedly different from what has been previously described for moderately ampicillin-resistant strains, which demonstrated 16-fold or greater decreases in the penicillin MIC after vancomycin induction (12). Interestingly, the addition of vancomycin to ampicillin-

TABLE 1. Sources, characteristics, and MICs for strains used

Strain[a]	Source	Species	Phenotype	Vancomycin MIC (μg/ml)	Ampicillin MIC (μg/ml)	Ampicillin MBC (μg/ml)	Gentamicin MIC (μg/ml)
TJ100	Blood	*E. faecium*	VanA	1,024	256	>256	16
TJ100 (I)					128	>256	
TJ153	Urine	*E. faecalis*	VanA	1,024	4	>32	>2,000
TJ153 (I)					1	8	
MCD1644	Urine	*E. faecium*	VanA	1,024	512	>512	16
MCD1644 (I)					512	>512	
WC100	Blood	*E. faecium*	VanB	16–32	256	>256	16
WC100 (I)					128	>256	
OBO	Blood	*E. faecium*	VanB	128	128	>256	4–8
OBO (I)					32–64	>256	

[a] I, induced with 8 μg of vancomycin per ml.

susceptible *E. faecalis* TJ153 did not significantly decrease the MIC of ampicillin; however, the MBC of ampicillin markedly decreased, consistent with other reports (12).

Killing-curve data for several strains are summarized in Table 2. Data shown are for low-inoculum studies only; all of the combinations were significantly less active against high inocula. For each strain and antibiotic combination, the data shown represent the mean change in the log CFU from the time zero point for at least two separate experiments. In TJ100, the most ampicillin- and vancomycin-resistant isolate, no lethality was seen with any combination employed, although the triple combination of high-dose ampicillin (32 μg/ml), vancomycin, and gentamicin was inhibitory at 24 h. The combination of vancomycin (8 μg/ml) and ampicillin (either 8 or 32 μg/ml) without an aminoglycoside did not inhibit growth. Representative killing curves for TJ100 are shown in Fig. 1A.

For the highly vancomycin-resistant VanB strain OBO, there was also no inhibition by the ampicillin plus vancomycin combinations, although the triple combination achieved a reduction of >2 log CFU at 24 h (Table 2). For the moderately vancomycin-resistant VanB strain WC100, triple-combination therapy was also somewhat effective, resulting in a reduction of approximately 2 log CFU at 24 h, and the ampicillin-vancomycin combination was inhibitory (Table 2 and Fig. 1B). In this strain, the inhibitory activity of the two-drug combination is most likely attributable to the low vancomycin MIC. When WC100 was induced with 4 rather than 8 μg of vancomycin per ml, the addition of 32 μg of ampicillin per ml did not inhibit growth (Fig. 1B).

These results suggest that triple-combination therapy with ampicillin, vancomycin, and gentamicin is not reliably bactericidal for highly ampicillin-resistant, vancomycin-resistant, aminoglycoside-susceptible *E. faecium* strains and that ampicillin plus vancomycin combinations are not inhibitory for these strains. Differences between our results and those of other investigators do not appear to be due to differences in methods or media (2, 10, 12). In vitro killing in these strains appears to be a complex phenomenon that is affected by the level of vancomycin resistance, the level of ampicillin resistance, and the relative susceptibility to aminoglycosides. The aminoglycoside component of the regimen appears to be particularly important, as some killing could be achieved with triple-combination therapy even when there was no growth inhibition by the ampicillin-vancomycin combination. The finding that TJ100 and WC100 cells treated with ampicillin and vancomycin and then washed and resuspended in antibiotic-free media were significantly more susceptible to gentamicin than were untreated controls (data not shown) suggests that alterations in aminoglycoside uptake occur with exposure to even subinhibitory concentrations of cell wall-active agents.

The failure of triple-combination therapy was most clearly demonstrated in strain TJ100, a VanA clinical isolate from a

TABLE 2. Killing of enterococci by vancomycin, ampicillin, and gentamicin alone and in combination

Strain	Time (h)	Mean change in log CFU after treatment with[a]:								
		No drug	A(L)	A(H)	V	G	A(L) + V	A(H) + V	A(L) + V + G	A(H) + V + G
TJ100	0	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0
	4	+1.6	+1.3	+1.2	+1.1	−0.2	+0.7	+0.5	−0.6	−0.6
	24	+2.0	+1.6	+2.0	+1.9	+1.8	+1.5	+1.2	+0.4	−0.9
WC100	0	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0
	4	+1.8	+1.7	+0.0	+0.0	−0.4	−0.1	−0.1	−1.3	−1.1
	24	+2.0	+1.8	+1.3	+0.9	+1.7	+0.6	+0.3	−1.7	−2.3
OBO	0	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0	+0.0
	4	+0.7	+0.9	+1.0	+0.3	−0.2	+0.1	−0.2	−1.1	−1.2
	24	+2.0	+2.3	+1.9	+1.3	−0.3	+1.6	+1.1	−2.2	−2.5

[a] A(L) and A(H), ampicillin at 8 and 32 μg/ml, respectively; V, vancomycin at 8 μg/ml; G, Gentamicin at 4 μg/ml.

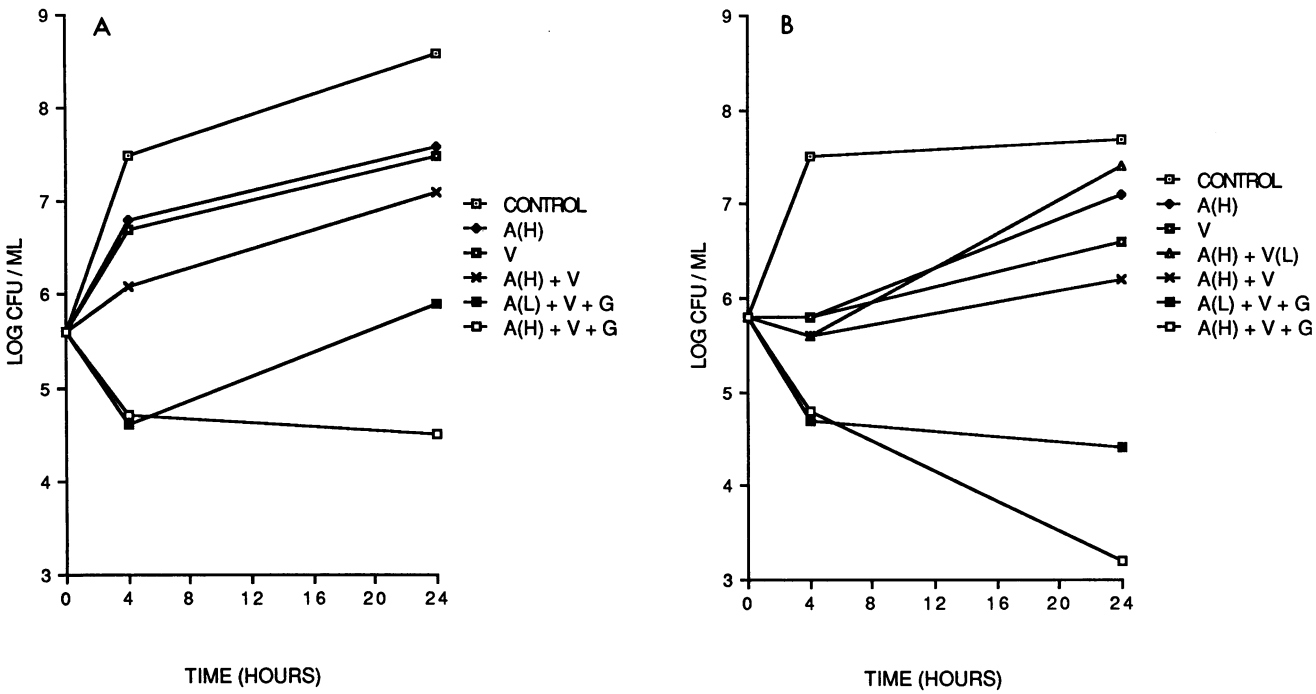

FIG. 1. Effects of combinations of ampicillin, vancomycin, and gentamicin against *E. faecium* TJ100 (A) and *E. faecium* WC100 (B). The control was antibiotic-free medium. Abbreviations: A(L) and A(H), ampicillin at 8 and 32 μg/ml, respectively; V(L) and V, vancomycin at 4 and 8 μg/ml, respectively; G, gentamicin (4 μg/ml).

multihospital outbreak of vancomycin-resistant enterococci. Similar results were obtained for several other highly ampicillin-resistant VanA isolates from the Philadelphia outbreak, as well as the phenotypically distinct VanA strain MCD1644 (data not shown). Interestingly, strain TJ153, a VanA *E. faecalis* strain containing the same vancomycin resistance plasmid as TJ100, did show marked enhancement of ampicillin lethality following vancomycin induction. This suggests that strain-to-strain differences in the activity of ampicillin-vancomycin regimens cannot be attributed to differences in vancomycin resistance genes but most likely reflect intrinsic differences in penicillin-binding proteins.

One proposed mechanism for the enhanced beta-lactam activity in induced VanA and VanB strains is that the ligase activity of *vanA* and *vanB* results in a pentapeptide intermediate other than acyl D-alanyl-D-alanine (1, 4) which may have diminished affinity for PBP 5, the penicillin-binding protein purported to be responsible for decreased beta-lactam activity in *E. faecium* (3, 14). However, the varied effects of vancomycin induction on ampicillin susceptibility suggest that there is significant heterogeneity among the penicillin-binding proteins of *E. faecium* with differing levels of beta-lactam resistance. This is consistent with recently published data on the penicillin-binding proteins of a variety of *E. faecium* strains (8).

This study demonstrates the need to pursue alternative treatment strategies for infections due to highly vancomycin-resistant enterococci. Although triple-combination therapy may be effective against selected strains, in vitro activity cannot be predicted in the absence of formal testing, and such regimens should not be used indiscriminately for all infections caused by vancomycin-resistant, aminoglycoside-susceptible *E. faecium*. In addition, beta-lactam- and vancomycin-resistant *E. faecium* isolates demonstrating high-level aminoglycoside resistance are being increasingly reported, and this factor will further limit the role of triple-combination regimens (9).

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