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Novel Antibiotic Regimens against *Enterococcus faecium* Resistant to Ampicillin, Vancomycin, and Gentamicin

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Enterococci have emerged as significant nosocomial pathogens. Enterococci with resistance to commonly used antibiotics are appearing more frequently. We encountered at our institution several infections caused by *Enterococcus faecium* with high-level resistance to ampicillin, vancomycin, and gentamicin. The optimal antibiotic therapy for serious infections with unusually resistant enterococci has not been established. Using time-kill studies, we tested the effectiveness of various antibiotic combinations against 15 isolates of multidrug-resistant enterococci. No antibiotic was consistently effective when used alone. The combination of ampicillin plus ciprofloxacin was bactericidal for the 12 isolates for which the ciprofloxacin MIC was ≤8 μg/ml. The combination of ciprofloxacin plus novobiocin also demonstrated activity against these isolates. No combination was found to be bactericidal for the remaining three isolates, which were highly ciprofloxacin resistant. These antibiotic combinations may be important for the future treatment of serious infections caused by these resistant pathogens.

Nosocomial infections with enterococci have been reported with increasing frequency in the past several years (24). Antibiotics used against enterococci are typically bacteriostatic when used alone. Therefore, the mainstay of therapy for serious enterococcal infections includes a penicillin (or a glycopeptide antibiotic) in combination with an appropriate aminoglycoside. However, enterococci are becoming increasingly resistant to traditional antimicrobial agents. High-level aminoglycoside resistance has become commonplace (15, 20). Penicillin resistance may or may not be β-lactamase mediated (1, 6, 18, 22).

Vancomycin resistance was recently reported for enterococci (8, 13, 23, 28). Two distinct patterns of vancomycin resistance have been documented for most enterococci. Class A strains have the *vanA* gene, are able to transfer resistance by conjugation, and are also resistant to teicoplanin (13, 26). Class B strains generally do not transfer resistance and remain susceptible to teicoplanin (23, 28); however, transfer of resistance was recently observed for *Enterococcus faecalis* and *E. faecium* (21). Teicoplanin resistance has also been noted to develop in a clinical isolate of *E. faecium* (9) with *vanB*.

Over the course of 10 months, we identified six isolates of *E. faecium* with high-level resistance to ampicillin, vancomycin, and gentamicin. Five of these isolates originated from patients with bacteremia. There is no proven effective therapy for such multidrug-resistant enterococci. In this investigation, we determined the effect of various combinations of antibiotics on the killing of these and other *E. faecium* isolates resistant to ampicillin, gentamicin, and vancomycin.

MATERIALS AND METHODS

Patients. From August 1991 through May 1992, six clinical isolates of *E. faecium* resistant to ampicillin and vancomycin were collected at our hospital. The six isolates were widely separated in both time and location at our institution during the 10-month period. Five patients were located on the medical service (including one patient in the medical intensive care unit) and one patient was in the surgical intensive care unit at the time of the positive cultures. The length of hospitalization ranged from 9 to 41 days. Five patients were bacteremic, including two patients with urosepsis, and one patient had wound colonization. All patients had received beta-lactam antibiotics, primarily ampicillin and broad-spectrum cephalosporins, in the month prior to the occurrence of the positive cultures. Three patients had also received vancomycin and three had received gentamicin during the preceding month.

From February through June 1992, five additional isolates of *E. faecium* were obtained from patients at a neighboring community hospital in Brooklyn, N.Y. The patients were located on different wards in the hospital, and all five were bacteremic.

Organisms. The organisms were identified as enterococci by standard microbiological techniques and determined to be *E. faecium* by established methods (3). Four additional multidrug-resistant isolates were generous gifts from the Nosocomial Pathogens Laboratory Branch, Centers for Disease Control, Atlanta, Ga. Finally, *E. faecalis* ATCC 29212 was studied as a control organism.

Susceptibility testing. In vitro broth macrodilution susceptibility testing in tubes was performed with an inoculum of approximately 5×10^5 CFU/ml in a final volume of 1 ml of cation-supplemented Mueller-Hinton broth (50 mg of Ca^{2+} and 25 mg of Mg^{2+} per liter). The MIC was read as the lowest concentration of each antimicrobial agent that prevented turbidity after 18 h of incubation at 37°C. The following antibiotics were tested: vancomycin (Eli Lilly & Co., Indianapolis, Ind.), ampicillin (Bristol-Myers Squibb, Princeton, N.J.), novobiocin (The Upjohn Co., Kalamazoo, Mich.), ciprofloxacin (Miles Inc., West Haven, Conn.), gentamicin (Schering-Plough Corp., Bloomfield, N.J.), and rifampin and teicoplanin (Marion Merrell Dow, Cincinnati, Ohio). The presence of penicillinase was determined by nitrocefin disk testing (BBL Microbiology Systems, Cockeysville, Md.).

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TABLE 1. MICs for 15 isolates of *E. faecium*

Isolate	MIC (μg/ml) of:					
	Ampicillin	Vancomycin	Teicoplanin	Ciprofloxacin	Novobiocin	Rifampin
VA1	128	256	8	4	1	>8
VA2	64	256	8	4	1	>8
VA3	64	256	4	4	1	>8
VA4	64	512	4	4	2	>8
VA5	64	512	1	4	1	>8
VA6	256	512	64	8	16	>8
MMC1	128	1,024	>512	4	2	8
MMC2	64	1,024	128	2	1	>8
MMC3	64	512	64	≥32	2	>8
MMC4	256	1,024	128	32	2	8
MMC5	64	512	128	≥32	1	>8
CDC1	64	64	<4	4	2	>8
CDC2	64	256	<4	2	1	>8
CDC3	64	256	<4	4	2	>8
CDC4	256	1,024	1,024	4	2	>8

Killing rates. Time-kill studies were performed with log-phase cultures adjusted to approximately 10^6 CFU/ml in supplemented Mueller-Hinton broth. The following antibiotic concentrations were used for the time-kill experiments: vancomycin, 20 μg/ml; ampicillin, 20, 40, 80, and 160 μg/ml; novobiocin, 50 μg/ml; ciprofloxacin, 1 and 3 μg/ml; and teicoplanin, 20 μg/ml. These concentrations were chosen because they generally reflect intermediate to maximal clinically achievable concentrations in blood. The higher concentrations of ampicillin were also tested to demonstrate any dose-response relationship between ampicillin and killing rates. Clinically achievable levels of novobiocin in serum range from 20 to 100 μg/ml (12). Each antibiotic was tested alone in the time-kill experiments. The following two-drug combinations were also analyzed: ampicillin plus ciprofloxacin, ampicillin plus novobiocin, ampicillin plus vancomycin, ampicillin plus teicoplanin, vancomycin plus ciprofloxacin, teicoplanin plus ciprofloxacin, and novobiocin plus ciprofloxacin. In addition, the following three-drug combinations were studied: ampicillin plus vancomycin plus ciprofloxacin, ampicillin plus novobiocin plus ciprofloxacin, and ampicillin plus teicoplanin plus ciprofloxacin. Ten-milliliter cultures were incubated in glass tubes at 37°C. Cultures were vortexed at 20 h of incubation and just prior to colony count determinations. Aliquots of the cultures were obtained at 0, 4, and 24 h of incubation. Tenfold dilutions (minimums of 1:10 and 1:100 for each study) were made in normal saline for each quantitative culture, streaked onto tryptic soy agar with 5% sheep blood, and incubated at 37°C for 24 h. Antibiotic carryover effects were tested for and detected only in undiluted specimens from time-kill experiments involving novobiocin. Therefore, the lowest detectable number of organisms for studies involving novobiocin was 330 CFU/ml. For the remaining antibiotics, the lowest number of detectable organisms was 33 CFU/ml. Appropriate growth and sterility controls were used for each time-kill study. Killing rates for the ampicillin-ciprofloxacin combination were determined at least twice. Bactericidal killing was defined as a change in $\log_{10}$ CFU of >3 at 24 h. Results are expressed as the change in $\log_{10}$ CFU (mean ± standard deviation).

RESULTS

For all 15 isolates of *E. faecium*, the MIC of both ampicillin and vancomycin was ≥64 μg/ml (Table 1); none of these isolates were found to produce penicillinase. All isolates expressed high-level resistance to gentamicin (MIC, >2,000 μg/ml) as well as resistance to rifampin. Seven isolates were resistant to teicoplanin.

Time-kill studies revealed that no antibiotic was consistently effective when used alone (Table 2). Because of differences in killing rates, the 15 isolates were grouped on the basis of ciprofloxacin susceptibility for further analysis. Ciprofloxacin MICs were ≤8 μg/ml for group 1 isolates (n = 12), and ≥32 μg/ml for group 2 isolates (n = 3). All group 2 isolates were resistant to teicoplanin as well. For the group 1 isolates, only combinations involving higher concentrations of ampicillin and ciprofloxacin showed bactericidal activity. The combination of ampicillin (40 μg/ml) plus ciprofloxacin (3 μg/ml) displayed the highest bactericidal activity, with changes in $\log_{10}$ CFU of -1.90 ± 0.83 and -3.96 ± 0.50 at 4 and 24 h, respectively. Repeat time-kill experiments with the same 12 isolates resulted in changes in $\log_{10}$ CFU of -2.01 ± 0.95 and -4.10 ± 0.73, respectively, for the same combination. The decrease in CFU was much higher than that seen with either antibiotic alone at 24 h. Lower concentrations of ampicillin and ciprofloxacin were not effective (Table 2). When combined with ciprofloxacin, increasing levels of ampicillin did not result in increased killing for the group 1 isolates.

At 24 h, the combination of ciprofloxacin (3 μg/ml) plus novobiocin resulted in a change in $\log_{10}$ CFU of -2.54 ± 0.82. A 100-fold-larger decrease in CFU was demonstrable for 3 of the 12 isolates with this combination than with each antibiotic alone at 24 h. The killing rate was not appreciably different when the lower concentration of ciprofloxacin was combined with novobiocin (Table 2). The combination of ampicillin plus vancomycin was generally ineffective. Other ineffective combinations included vancomycin plus ciprofloxacin, teicoplanin plus ciprofloxacin, ampicillin plus teicoplanin, and ampicillin plus novobiocin (data not shown).

For the group 1 isolates, none of the three-drug combinations were more effective than ampicillin plus ciprofloxacin. Despite the susceptibility of some of the isolates to teicoplanin, combinations with teicoplanin were no more effective than those with vancomycin (data not shown). Similarly, the addition of novobiocin to the ampicillin-ciprofloxacin combination did not improve the killing rate.

In comparison, none of the combinations were bactericidal against the group 2 isolates. At 24 h, a high dose of

TABLE 2. Results of time-kill experiments

Treatment[a]	Change in $\log_{10}$ CFU/ml, mean ± SD, at the indicated time for isolates of group[b]:			
	1 (n = 12)		2 (n = 3)	
	4 h	24 h	4 h	24 h
GRCNT	+2.32 ± 0.26	+2.69 ± 0.31	+1.94 ± 0.30	+2.46 ± 0.12
AMP20	+1.05 ± 0.44	+1.39 ± 0.93	ND	ND
AMP40	+1.12 ± 0.49	+0.46 ± 1.12	+1.46 ± 0.29	+1.59 ± 0.17
AMP80	−0.10 ± 0.52	−1.07 ± 1.40	+0.61 ± 0.86	−0.95 ± 1.79
AMP160	−0.56 ± 0.48	−2.21 ± 1.05	+0.09 ± 0.76	−2.27 ± 1.35
CIP1	+0.57 ± 0.72	+1.93 ± 0.55	ND	ND
CIP3	−0.55 ± 1.27	+0.45 ± 1.51	+1.74 ± 0.40	+2.37 ± 0.24
VANC	+0.32 ± 0.91	+1.85 ± 0.77	+1.56 ± 0.63	+2.53 ± 0.13
TEIC	+0.20 ± 0.76	+0.31 ± 1.70	+0.78 ± 0.69	+1.80 ± 0.35
NOV	−0.09 ± 0.22	−1.37 ± 0.59	−0.10 ± 0.37	−2.09 ± 0.39
AMP20-CIP3	+1.05 ± 0.46	+1.34 ± 1.09	ND	ND
AMP40-CIP1	−0.47 ± 0.62	−1.85 ± 1.27	ND	ND
AMP40-CIP3	−1.90 ± 0.83	−3.96 ± 0.50	+0.66 ± 0.46	+0.75 ± 0.59
AMP80-CIP3	−1.33 ± 0.48	−3.68 ± 0.60	+0.07 ± 0.40	−1.73 ± 0.54
AMP160-CIP3	−1.46 ± 0.40	−3.83 ± 0.56	−0.18 ± 0.42	−2.63 ± 0.62
NOV-CIP1	−0.59 ± 0.30	−2.51 ± 0.65	ND	ND
NOV-CIP3	−0.95 ± 0.60	−2.54 ± 0.82	−0.25 ± 0.19	−2.34 ± 0.35
AMP40-VANC	−0.17 ± 0.67	−0.84 ± 1.75	+0.32 ± 0.90	+1.22 ± 0.49
AMP40-VANC-CIP3	−0.54 ± 0.17	−3.45 ± 0.62	+0.28 ± 0.49	+0.96 ± 0.39

[a] GRCNT, growth control; AMP20, AMP40, AMP80, or AMP160, ampicillin at 20, 40, 80, or 160 μg/ml, respectively; CIP1 or CIP3, ciprofloxacin at 1 or 3 μg/ml; VANC, vancomycin; TEIC, teicoplanin; NOV, novobiocin.
[b] For group 1, the ciprofloxacin MIC was ≤8 μg/ml; for group 2, the ciprofloxacin MIC was ≥32 μg/ml. ND, not done.

ampicillin (160 μg/ml) resulted in a change in $\log_{10}$ CFU of −2.27 ± 1.35; when ampicillin was combined with ciprofloxacin, there was a change in $\log_{10}$ CFU of −2.63 ± 0.62. The combination of ciprofloxacin and novobiocin produced a change in $\log_{10}$ CFU of −2.34 ± 0.35 at 24 h. No three-drug combination showed higher activity for the group 2 isolates.

Time-kill experiments with the same antibiotic combinations were performed for *E. faecalis* ATCC 29212. Ampicillin at 20 μg/ml resulted in changes in $\log_{10}$ CFU of −1.11 and −3.25 at 4 and 24 h, respectively. Ampicillin at 40 μg/ml caused changes in $\log_{10}$ CFU of −1.41 and −4.25, respectively. Ciprofloxacin at 1 μg/ml led to changes in $\log_{10}$ CFU of −3.25 and −1.19, respectively. Ciprofloxacin at 3 μg/ml resulted in changes in $\log_{10}$ CFU of −2.97 and −4.56, respectively. The combinations of ampicillin plus ciprofloxacin were indifferent. Novobiocin alone caused changes in $\log_{10}$ CFU of −0.14 and −1.38 at 4 and 24 h, respectively. The addition of novobiocin to ciprofloxacin did not enhance the killing of this strain.

DISCUSSION

Enterococci are now the second most common nosocomial pathogens in the United States (24). Increasing resistance patterns have made therapy problematic. Cases of infections with unusually resistant enterococci have occurred (8, 14, 19); these include the 10 cases of bacteremia in this report. To date, recommendations for the treatment of ampicillin-, vancomycin-, and aminoglycoside-resistant enterococci have not been established (10). Our study suggests that the combination of ampicillin plus ciprofloxacin may be an effective combination against many of these multidrug-resistant enterococci.

The overall activity of quinolone antibiotics against enterococci is disappointing. When used alone, ciprofloxacin displays poor killing of enterococci (4, 17). Similarly, the combination of ciprofloxacin plus beta-lactam antibiotics has generally been ineffective against enterococci. For example, Moody et al. (16) found indifference for the combination of ciprofloxacin plus azlocillin against 10 strains of *E. faecalis* and *E. avium*. Similarly, Hodin and Painter (11) found indifference for the combination of ampicillin plus ciprofloxacin against 25 strains of *E. faecalis*. Wise and Andrews (29) found indifference or "partial synergy" for 10 strains of *E. faecalis* with the combination of penicillin plus ciprofloxacin. All of these investigators used the checkerboard technique for determining antibiotic activity. Fernandez-Guerrero et al. (4) performed time-kill studies with lower concentrations of ciprofloxacin plus penicillin against two susceptible strains of *E. faecalis* and found the activity of the combination to be similar to that of penicillin alone. This observation was also reflected during in vivo experiments done by the same authors (4). In contrast, Grimm (7) demonstrated synergy of ampicillin plus ciprofloxacin against 10 strains of gentamicin-resistant enterococci not identified to the species level. The susceptibility of these strains to ampicillin was not stated. Livornese et al. (14) found synergy of ciprofloxacin plus rifampin plus gentamicin for one strain of *E. faecium*. This strain differed from ours by the lack of high-level gentamicin and rifampin resistance. In our study, the combination of ampicillin plus ciprofloxacin was bactericidal against all *E. faecium* strains without high-level ciprofloxacin resistance (MIC, ≤8 μg/ml). While only three strains with high-level ciprofloxacin resistance (MIC, ≥32 μg/ml) were studied, there was a trend toward slightly increased killing when ciprofloxacin was added to each ampicillin concentration. This result may indicate some interaction between ampicillin and ciprofloxacin even for these strains. For *E. faecalis* ATCC 29212 and for two other ampicillin-susceptible strains of *E. faecalis* (data not shown), we found that the combination of ampicillin plus ciprofloxacin was indifferent. Whether synergy of ampicillin plus ciprofloxacin is a property restricted only to *E. faecium* remains to be determined.

For our group 1 isolates, the addition of vancomycin or teicoplanin to the combination of ampicillin plus ciprofloxacin diminished killing at 4 h. This result was somewhat surprising, since the addition of vancomycin to ampicillin or penicillin generally has resulted in indifference and occasionally synergy in other studies (2, 5, 8). A possible explanation for this finding is that the addition of vancomycin may have slowed bacterial replication and removed the bacteria from the log phase of growth, thereby interfering with the activity of ampicillin plus ciprofloxacin. The precise molecular basis for the effect that we observed remains unknown.

Novobiocin is a DNA gyrase inhibitor that has been available for the treatment of gram-positive pathogens for 30 years (12). Because of toxicity and the development of beta-lactam antibiotics, novobiocin is rarely used at present. We demonstrated appreciable killing (-2.54 $\log_{10}$ CFU) of our group 1 isolates with the combination of ciprofloxacin plus novobiocin. Venuti et al. (27) reported similar findings when using a 10-fold-lower concentration of novobiocin, including synergy for some isolates when novobiocin was combined with a quinolone. While further studies will be needed to determine whether this finding is a consistent one, this combination may be potentially useful in patients allergic to penicillin.

Antibiotic activity in vitro does not always correlate with in vivo effectiveness. The effects of protein binding, distribution, tissue penetration, and the constantly changing antibiotic concentrations that occur in vivo are not accounted for during in vitro studies. In particular, the efficacy of novobiocin may be limited by its high degree of protein binding (12). Furthermore, the growing prevalence of ciprofloxacin resistance (25) and the possibility of the development of resistance during therapy may limit the usefulness of this drug. Nevertheless, there is no proven effective therapy for multidrug-resistant enterococci. This problem underscores the need to investigate new combinations and develop new agents for these multidrug-resistant strains.

In summary, we found that combinations of ciprofloxacin plus ampicillin or novobiocin have significant in vitro activity against many multidrug-resistant enterococci. The activity of these combinations is decreased by high-level ciprofloxacin resistance. For strains with high-level ciprofloxacin resistance, very high doses of ampicillin—with or without ciprofloxacin—may prove useful. Further in vivo studies examining the effectiveness of these antibiotic combinations will be necessary before firm treatment recommendations can be established.

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