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Activity of Clinafloxacin against Multidrug-Resistant *Enterococcus faecium*

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Enterococci resistant to ampicillin, vancomycin, and/or aminoglycosides are a growing clinical problem. We studied the in vitro activity of the new fluoroquinolone clinafloxacin (PD 127,391) against 15 clinical isolates of multidrug-resistant *Enterococcus faecium*. In kill-kinetic studies, clinafloxacin (1 μg/ml) was bactericidal against 7 of 12 susceptible isolates, although substantial regrowth occurred in 4 isolates at 48 h. The addition of ampicillin (20 μg/ml) resulted in bactericidal activity in all 12 isolates, and no regrowth was seen. For three isolates resistant to clinafloxacin, effective killing was not observed at these concentrations of antibiotics. Clinafloxacin with ampicillin shows promising activity against many of these multiply resistant enterococci.

Nosocomial outbreaks of increasingly resistant enterococci have been recently documented (4, 5, 7). Prolonged treatment with a cell wall agent is recommended for infections with enterococci possessing high-level aminoglycoside resistance (8). However, effective therapeutic regimens have not been established for enterococci with concomitant resistance to vancomycin, ampicillin, and aminoglycosides. The combination of ampicillin and ciprofloxacin has been previously shown to be bactericidal against many of these isolates in vitro; however, this activity was dependent on concentrations which were near the peak levels achieved clinically (6). Newer quinolones have been reported to have greater activity against gram-positive bacteria (9, 13) and deserve further investigation. We evaluated the activities of two of these agents against strains of multidrug-resistant *Enterococcus faecium*.

Fifteen isolates of *E. faecium* were collected from two hospitals in Brooklyn, N.Y., and from the Centers for Disease Control, Atlanta, Ga., as previously described (6), and were identified to the species level according to established methods (2). Macrodilution MIC testing was performed in cation-supplemented Mueller-Hinton broth (50 mg of Ca^{2+} and 25 mg of Mg^{2+} per liter) with an inoculum of approximately 5×10^5 CFU/ml in a final volume of 1 ml. The MIC was read as the lowest concentration that prevented turbidity after 18 h of incubation at 37°C; selected isolates were incubated for an additional 24 h. The following antibiotics (from the indicated manufacturers) were tested: ampicillin (Bristol-Myers Squibb, Princeton, N.J.), vancomycin (Eli Lilly & Co., Indianapolis, Ind.), ciprofloxacin (Miles Inc., West Haven, Conn.), gentamicin (Schering-Plough Corp., Bloomfield, N.J.), and sparfloxacin and clinafloxacin (Parke-Davis, Ann Arbor, Mich.). Isolates for which the MIC of clinafloxacin was ≤1 μg/ml were considered susceptible to this agent.

Time-kill studies were performed with log-phase cultures at approximately 10^6 CFU/ml in supplemented Mueller-Hinton broth. Because the MICs of sparfloxacin were generally 1 dilution higher than those of clinafloxacin, only the latter quinolone was tested in the time-kill studies. The antibiotics tested were ampicillin at 20 and 40 μg/ml, clinafloxacin at 0.25 and 1 μg/ml, and the combination of both antibiotics. These concentrations of clinafloxacin are well within the levels achievable in humans (1). For three isolates with higher-level quinolone resistance (ciprofloxacin, MIC > 16 μg/ml; clinafloxacin, MIC > 2 μg/ml), a higher concentration of clinafloxacin (3 μg/ml) was also tested. Cultures was incubated in glass tubes at 37°C. The cultures were vortexed at 20 h of incubation and prior to colony count determinations. Aliquots were obtained at 0, 4, 24, and 48 h of incubation, diluted in normal saline, and streaked onto tryptic soy agar with 5% sheep blood. In separate studies, antibiotic carryover was tested for using similar antibiotic concentrations, and no carryover effect was detected. The lowest number of detectable organisms was 33 CFU/ml. Killing rates for the ampicillin-plus-clinafloxacin combination were confirmed with repeated studies. Bactericidal activity was defined as a decrease of $\log_{10}$ CFU per milliliter of ≥3 at 24 h. Results are expressed as the change in $\log_{10}$ CFU per milliliter (mean ± standard deviation).

A microbiological assay using well diffusion with *Escherichia coli* ATCC 25922 was used to measure the levels of bioactive clinafloxacin in the broth during the time-kill studies. Samples were collected at 0, 24, and 48 h in selected experiments.

The MICs of the indicated drugs for the 15 isolates, expressed as the MIC for 50% of the isolates, MIC for 90% of the isolates, and range, were as follows: ampicillin, 64, 256, and 64 to 256 μg/ml; vancomycin, 512, 1,024, and 64 to 1,024 μg/ml; ciprofloxacin, 4, >32, and 2 to >32 μg/ml; sparfloxacin, 2, >64, and 0.5 to >64 μg/ml; and clinafloxacin, 1, 8, and 0.5 to 16 μg/ml. For selected isolates, the clinafloxacin MICs were unchanged after extended incubation. For all isolates, the MICs of gentamicin were >2,000 μg/ml.

For analysis of the time-kill experiments, the isolates were divided into clinafloxacin-susceptible (n = 12) and clinafloxacin-resistant (n = 3) groups. For the susceptible isolates, clinafloxacin (1 μg/ml) resulted in a change in $\log_{10}$ CFU per milliliter of -2.9 ± 0.40 at 24 h and was bactericidal for 7 of 12 isolates (Table 1). Of eight isolates which were kept under incubation for 48 h, four demonstrated regrowth of at least 1 $\log_{10}$ CFU/ml. The MICs for these four strains did not change following the 48 h of incubation, and there was no growth of these organisms when plated directly onto tryptic soy agar containing 2 times the MIC of clinafloxacin. The concentration (mean ± standard deviation) of bioactive clinafloxacin also remained stable over the course of the extended incubation

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TABLE 1. Results of time-kill experiments involving 12 isolates of *E. faecium* susceptible to clinafloxacin

Treatment[a]	Change in $\log_{10}$ CFU/ml (mean ± SD) at indicated time	
	4 h	24 h
GRCNT	+2.3 ± 0.3	+2.7 ± 0.3
AMP20	+1.1 ± 0.4	+1.4 ± 0.9
AMP40	+1.1 ± 0.5	+0.4 ± 1.1
CLIN.25	+1.5 ± 0.3	+1.9 ± 0.2
CLIN1	−1.5 ± 0.4	−2.9 ± 0.4
AMP20 + CLIN.25	+0.5 ± 0.3	+1.9 ± 0.2
AMP20 + CLIN1	−2.0 ± 0.6	−3.8 ± 0.6
AMP40 + CLIN1	−2.0 ± 0.6	−4.0 ± 0.6

[a] GRCNT, growth control; AMP20 and AMP40, ampicillin at 20 and 40 μg/ml, respectively; CLIN.25 and CLIN1, clinafloxacin at 0.25 and 1 μg/ml, respectively.

(1.1 ± 0.05 μg/ml at 0 h, 1.1 ± 0.04 μg/ml at 24 h, and 1.2 ± 0.07 μg/ml at 48 h). Therefore, the regrowth of selected isolates at 48 h was not due to loss of bioactive clinafloxacin but may have been related to small increments in resistance not detected by our methodology. The addition of ampicillin (20 μg/ml) resulted in bactericidal activity for all 12 isolates with no regrowth at 48 h (Table 1). The combination of ampicillin with a subinhibitory concentration of clinafloxacin was ineffective (Table 1).

For the clinafloxacin-resistant isolates, clinafloxacin at 1 μg/ml was ineffective when used alone (changes in $\log_{10}$ CFU per milliliter of +1.3 ± 0.4 and +2.0 ± 0.4 at 4 and 24 h, respectively). The addition of ampicillin (20 μg/ml) resulted in a change of +1.3 ± 0.8 $\log_{10}$ CFU/ml at 24 h and was inhibitory in only one of three of these isolates. At 3 μg/ml, clinafloxacin resulted in changes of +0.9 ± 0.6 and +0.3 ± 2.3 $\log_{10}$ CFU/ml at 4 and 24 h, respectively. Ampicillin (at 40 μg/ml) resulted in a change of +1.6 ± 0.2 $\log_{10}$ CFU/ml at 24 h. The combination of clinafloxacin (3 μg/ml) and ampicillin (40 μg/ml) resulted in a change of −2.2 ± 2.3 $\log_{10}$ CFU/ml at 24 h and was bactericidal for one of three of these resistant isolates.

The emergence of multidrug-resistant enterococci as nosocomial pathogens limits therapeutic options. The in vitro activity of fluoroquinolones against enterococci has been largely disappointing (3, 11). The results of quinolone monotherapy for experimental enterococcal infections have also been poor (3). Combining a quinolone with other antibiotics has led to enhanced activity against selected strains. Fernandez-Guerrero et al. found enhanced activity against two sensitive isolates of *E. faecalis* when ciprofloxacin was combined with gentamicin (3). However, this observation was highly inoculum dependent, and the degree of killing was inferior to that of penicillin with gentamicin (3). Similarly, treatment of experimental endocarditis due to these strains with ciprofloxacin and gentamicin was inferior to therapy with penicillin and gentamicin (3). Livornese et al. recently described in vitro bactericidal activity with the combination of ciprofloxacin plus gentamicin, rifampin, or both against vancomycin-resistant *E. faecium* lacking high-level resistance to gentamicin (7). These combinations did display efficacy in an animal model of endocarditis with the same bacterial strain (16). Unal et al. reported synergy with vancomycin and ciprofloxacin against vancomycin- and ciprofloxacin-resistant *E. faecium*; at high concentrations, ciprofloxacin prevented the induction of vancomycin resistance (14). However, the concentrations used in these experiments were not clinically relevant (14). Finally, the addition of ampicillin with ciprofloxacin has resulted in enhanced activity in vitro against some isolates of multidrug-resistant *E. faecium* (6). However, this effect was highly dependent upon the concentrations of the antibiotics, which were near the peak levels achievable clinically. The effectiveness of ampicillin plus ciprofloxacin in treating experimental endocarditis did not approach that traditionally seen with penicillin plus an aminoglycoside against sensitive enterococci (10).

Newer quinolone antibiotics which possess greater activity against gram-positive bacteria are being developed. The MICs of clinafloxacin for many enterococci range from 0.12 to 4 μg/ml (9, 13). The peak level in serum in humans following a 200-mg dose is 2.5 μg/ml, and the half-life is 6.1 h (1). There is little information regarding the therapeutic efficacy of the newer quinolones for enterococcal infections. In one report, sparfloxacin and clinafloxacin, with and without gentamicin, each had only a modest effect in an animal model of enterococcal endocarditis with strains lacking high-level gentamicin resistance (15). The development of resistance, as already seen with ciprofloxacin (12), may also limit the potential effectiveness of these antibiotics. Further studies will be needed to determine if these new quinolones, with or without beta-lactam antibiotics, will be useful in the treatment of serious resistant enterococcal infections.

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