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Synergistic Activities of Moxifloxacin Combined with Piperacillin-Tazobactam or Cefepime against *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Acinetobacter baumannii* Clinical Isolates

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The bactericidal activity of moxifloxacin alone and in combination with cefepime or piperacillin-tazobactam against clinical isolates of *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Acinetobacter baumannii* was evaluated by using time-kill methods and antimicrobial concentrations of one-half and one times the MIC. Synergy was observed in 58 to 88% of the strains and resulted in bactericidal activity against 60 to 100% of the strains. Combinations including moxifloxacin demonstrated enhanced bactericidal activity compared with that of either agent tested alone.

Gram-negative pathogens such as the *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* are commonly associated with intra-abdominal sepsis, pneumonia, and bloodstream infections among critically ill patients (1, 4). Moxifloxacin appears to be appropriate for monotherapy for uncomplicated infections due to its broad antimicrobial spectrum (2). However, for more severe nosocomial infections, moxifloxacin combined with a broad-spectrum β-lactam agent may offer the benefits of possibly synergistic activity and reduced emergence of resistance among gram-negative bacteria while providing adequate anaerobic activity in a simplified regimen. Moxifloxacin's activity against *A. baumannii* may also make it an attractive agent for use in combination regimens to treat multiresistant strains or to prevent the emergence of resistance (5). However, the activity of moxifloxacin in combination with other agents has not previously been evaluated. The objective of this study was to determine the activity of moxifloxacin alone and in combination with cefepime or piperacillin-tazobactam against clinical isolates of *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *A. baumannii*.

Antibiotic MICs were determined by broth microdilution in cation-supplemented Mueller-Hinton broth (Difco, Sparks, Md.) according to NCCLS methods (7). Five clinical isolates of *E. cloacae*, seven clinical isolates of *K. pneumoniae*, and seven clinical isolates of *A. baumannii* were selected based on MIC results in order to provide a range of susceptibilities to the different antimicrobials tested. This approach was taken to simulate clinical scenarios in which empirical drug therapy would be used against pathogens with unknown antibiotic susceptibilities.

Bactericidal activity was determined in duplicate by using time-kill experiments according to the NCCLS M26-A method (8). Antimicrobial concentrations tested were one-half and one times the previously determined MIC for each bacterial strain. The final inoculum was confirmed at time zero, and viable colonies were counted after 4, 8, and 24 h of incubation at 35°C. All tests were performed in duplicate, but the experiment was repeated and quadruplicate data were used for analysis if discordant results were obtained. Bactericidal activity was defined as a $\geq$3-$\log_{10}$ decrease in CFU per milliliter, while bacteriostatic activity was defined as a $<$3-$\log_{10}$ decrease in CFU per milliliter at 8 and 24 h. The lower limit of detection for the time-kill assays was 1.3 $\log_{10}$ CFU/ml. Synergism and antagonism were defined as a $\geq$100-fold increase and a $\geq$100-fold decrease, respectively, in bacterial killing at 8 and 24 h with the combination of drugs compared with that achieved with the most active single agent alone. Synergistic and bactericidal activities were evaluated at both 8 and 24 h to account for any drug inactivation or bacterial regrowth occurring by 24 h. Additivity was defined as a $<$10-fold increase in killing at 8 and 24 h with the combination relative to that achieved with the most active single antimicrobial alone. Areas under the $\log_{10}$ CFU per milliliter-time curves from time zero to 24 h (AUC_{0-24}) were calculated by the linear trapezoidal summation method in order to examine differences between antibiotic combinations in a more quantitative manner. Lower calculated AUC_{0-24} values represent more rapid and/or more complete bacterial killing than higher AUC_{0-24} values. A one-way analysis of variance was used with the Tukey test for post hoc analysis to compare AUC_{0-24} values between groups. Statistical P values of $\leq$0.05 were considered significant.

The MICs of study drugs for all 19 clinical isolates of *E. cloacae*, *K. pneumoniae*, and *A. baumannii* are listed in Table 1. No clinical strains of *E. cloacae* with decreased susceptibility to either cefepime or moxifloxacin could be identified for testing in this study. Likewise, no strains of *K. pneumoniae* with reduced susceptibility to cefepime could be identified. Other strains, particularly *A. baumannii* strains, were of various combinations of susceptibilities to moxifloxacin and the β-lactams.

Activities of piperacillin-tazobactam or cefepime in combination with moxifloxacin are summarized in Table 2. Depending on the concentrations tested, the combination of moxi-

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TABLE 1. MICs for *E. cloacae*, *K. pneumoniae*, and *A. baumannii*

Strain	MIC(s) (μg/ml)[a]		
	Piperacillin-tazobactam[b]	Cefepime[c]	Moxifloxacin[d]
E. cloacae			
U134	2, 4 (S)	1 (S)	0.0625 (S)
B8720	4, 4 (S)	0.25 (S)	0.0625 (S)
U155	32, 4 (I)	0.25 (S)	0.0625 (S)
U110	64, 4 (I)	0.5 (S)	0.0625 (S)
U093	128, 4 (R)	8 (S)	2 (S)
K. pneumoniae			
B9007	2, 4 (S)	0.0625 (S)	1 (S)
B9196	4, 4 (S)	0.25 (S)	1 (S)
ICU1	4, 4 (S)	0.125 (S)	2 (S)
ICU8	8, 4 (S)	0.25 (S)	1 (S)
B9152	16, 4 (S)	0.5 (S)	16 (R)
B9095	256, 4 (R)	8 (S)	4 (I)
B9127	256, 4 (R)	8 (S)	4 (I)
A. baumannii			
B8620	4, 4 (S)	8 (S)	4 (I)
B6994	16, 4 (S)	2 (S)	0.0625 (S)
B8560	32, 4 (I)	16 (I)	0.25 (S)
N134	32, 4 (I)	32 (R)	8 (R)
B8455	64, 4 (I)	4 (S)	2 (S)
50-0532	256, 4 (R)	16 (I)	1 (S)
ICU4	256, 4 (R)	32 (R)	4 (I)

[a] Susceptibility interpretive criteria are based on NCCLS recommendations. S, susceptible; I, intermediate; R, resistant.

[b] For organisms that were susceptible, MICs were ≤16, 4 μg/ml; for organisms that were intermediate, MICs were 34, 4 to 64, 4 μg/ml; for organisms that were resistant, MICs were ≥128, 4 μg/ml. MICs of piperacillin are listed first.

[c] For organisms that were susceptible, MICs were ≤8 μg/ml; for organisms that were intermediate, MICs were 16 μg/ml; for organisms that were resistant, MICs were ≥32 μg/ml.

[d] For organisms that were susceptible, MICs were ≤2 μg/ml; for organisms that were intermediate, MICs were 4 μg/ml; for organisms that were resistant, MICs were ≥8 μg/ml.

floxacin plus cefepime yielded synergistic activity against 63% of *E. cloacae* strains, 88% of *K. pneumoniae* strains, and 63% of *A. baumannii* strains tested, while the combination of moxifloxacin plus piperacillin-tazobactam was synergistic against 63, 75, and 67% of *K. pneumoniae*, *E. cloacae*, and *A. baumannii* strains, respectively. However, synergistic combinations of moxifloxacin plus cefepime or piperacillin-tazobactam resulted in bactericidal activity against 80, 100, and 60% of *K. pneumoniae*, *E. cloacae*, and *A. baumannii* strains, respectively. The levels of achievement of synergistic and/or bactericidal synergistic activity were similar among *K. pneumoniae*, *E. cloacae*, or *A. baumannii* strains and were irrespective of whether strains had reduced susceptibility to one or both drugs in the combination. Antagonism was not observed with any antimicrobial combination.

Because the MIC of moxifloxacin for *K. pneumoniae* strain B9152 was found to be higher than clinically relevant concentrations of the drug, time-kill studies were repeated with moxifloxacin concentrations of one-fourth of the MIC combined with β-lactam concentrations of one and two times the MIC to more accurately reflect clinically achievable drug concentrations. Bactericidal synergistic effects were observed with moxifloxacin concentrations of one-fourth of the MIC with piperacillin-tazobactam at one and two times the MIC as well as with cefepime at two times the MIC (data not shown).

TABLE 2. Bactericidal activities of piperacillin/tazobactam plus moxifloxacin and cefepime plus moxifloxacin against *E. cloacae*, *K. pneumoniae*, and *A. baumanii* strains

Strain	Effect of[a]:							
	Piperacillin/tazobactam + moxifloxacin				Cefepime + moxifloxacin			
	0.5× MIC		1× MIC		0.5× MIC		1× MIC	
	8 h	24 h	8 h	24 h	8 h	24 h	8 h	24 h
E. cloacae								
U134	A	A	A	S	A	S	A	S
	BS	BS	BS	BC	BS	BC	BC	BC
B8720	S	S	A*	A*	A*	S	A*	A*
	BC	BC	BC	BC	BC	BC	BC	BC
U155	A	A	A*	S	S	A	A*	S
	BS	BS	BC	BC	BS	BS	BC	BC
U110	A	A	A*	A	S	S	A*	S
	BS	BS	BC	BS	BC	BS	BC	BS
U093	A	S	A	A	A	S	A	A
	BS	BS	BS	BS	BS	BS	BS	BS
K. pneumoniae								
B9007	A	A	A	A*	A	S	A	A*
	BS	BS	BC	BC	BC	BC	BC	BC
B9196	S	A	A*	S	A	S	A*	A
	BC	BS	BC	BC	BC	BC	BC	BC
ICU1	S	S	A*	A*	S	S	A*	A*
	BC	BC	BC	BC	BC	BC	BC	BC
ICU8	A	A	A*	S	S	S	A*	S
	BS	BS	BC	BC	BC	BC	BC	BC
B9152	A	A	S	A*	S	A	S	S
	BS	BS	BC	BC	BS	BS	BC	BC
B9095	A	S	A*	A*	S	S	A*	A*
	BC	BS	BC	BC	BC	BS	BC	BC
B9127	S	S	A	A*	S	A	A*	A
	BC	BS	BS	BC	BS	BS	BC	BS
A. baumannii								
B8620	A	A	S	S	S	A	S	S
	BS	BS	BC	BC	BS	BS	BC	BC
B6994	A	S	A	S	A	A	S	S
	BS	BS	BS	BC	BS	BS	BS	BC
B8560	S	A	A	S	S	A	A	S
	BC	BS	BC	BC	BS	BS	BC	BC
N134	S	S	A	S	S	A	S	S
	BC	BC	BC	BC	BS	BS	BC	BC
B8455	A	S	A*	A*	S	A	A*	A*
	BS	BC	BC	BC	BS	BS	BC	BC
50-0532	S	S	A	S	A	A	A	S
	BS	BC	BS	BC	BS	BS	BS	BC
ICU4	S	A	A	S	A	A	S	S
	BS	BS	BC	BC	BS	BS	BC	BC

[a] S, synergistic; A, additive; BC, bactericidal; BS, bacteriostatic. *, due to the bactericidal activity of the single agent, synergistic activity could not be detected.

For quantitative evaluation by areas under the time-kill curves, no statistically significant differences in AUC_{0-24} values were found for any strain when antibiotic combinations were tested at one times the MIC for the organism. When tested at one-half of the MIC, the combination of cefepime plus moxifloxacin resulted in significantly lower AUC_{0-24} values, indicative of more rapid and/or extensive bacterial killing, for 8 of 19 strains (1 *E. cloacae* strain, 4 *K. pneumoniae* strains, and 3 *A. baumannii* strains), while piperacillin-tazobactam plus moxifloxacin resulted in significantly lower AUC_{0-24} values for 3 strains (two *E. cloacae* strains and one *K. pneumoniae* strain).

However, this difference was not significantly different ($P = 0.15$), and the activities of these two combination regimens were therefore considered to be equivalent.

The results of this study demonstrate that combinations of moxifloxacin and either cefepime or piperacillin-tazobactam achieve in vitro synergy in approximately 60 to 70% of the tested *E. cloacae* and *A. baumannii* clinical isolates and approximately 75 to 90% of the tested *K. pneumoniae* isolates. This synergistic activity resulted in bactericidal effects against 60 to 100% of the tested strains. It is of particular interest that this bactericidal activity was consistently observed even when MICs of one or both antibiotics in a combination were very high for bacterial strains. Appropriate selection of antibiotics for empirical treatment of gram-negative infections is often problematic because of the high rates of multidrug resistance seen in many institutions and the potential for choosing an antibiotic regimen to which pathogens may not be fully susceptible (1, 6). The potential to achieve bactericidal activity against these less-susceptible pathogens is a possible advantage favoring the use of potentially synergistic combinations of antibiotics rather than monotherapy.

In this experiment, moxifloxacin demonstrated synergistic and bactericidal activities against clinical isolates when tested at concentrations of one-half and one times the previously determined MIC. However, clinically achievable plasma concentrations at customary dosages would be important considerations in the use of agents intended to provide synergistic effects. Moxifloxacin achieves mean peak plasma concentrations of approximately 6.1 μg/ml with a regimen of 400 mg administered intravenously every 24 h (3). Moxifloxacin could thus be expected to achieve concentrations of up to one times the MICs for organisms with MICs of ≤4 μg/ml and one-half of the MICs for strains with MICs of up to 8 μg/ml but would not be expected to achieve adequate concentrations against strains with MICs of >8 μg/ml. Since concentrations of at least one-half of the MIC are clinically achievable against all strains tested in this study except one (*K. pneumoniae* B9152), moxifloxacin would have been suitable for use against the clinical isolates used in this experiment with some expectation of achieving synergistic activity as part of a combination regimen. Combination regimens including moxifloxacin would also have been suitable for use against *K. pneumoniae* B9152, since concentrations of one-fourth of the MIC were achievable against this strain. Based on the results of these in vitro experiments as well as pharmacokinetic considerations, moxifloxacin would be appropriate for patients with infections suspected or documented to be caused by *K. pneumoniae*, *E. cloacae*, or *A. baumannii* for whom therapy with a fluoroquinolone-containing regimen is desired.

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