ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1984, p. 319–326
0066-4804/84/030319-08$02.00/0

Vol. 25, No. 3

Ciprofloxacin, a Quinolone Carboxylic Acid Compound Active Against Aerobic and Anaerobic Bacteria

NAI-XUN CHIN AND HAROLD C. NEU*

Departments of Medicine and Pharmacology, College of Physicians & Surgeons, Columbia University, New York, New York 10032*

Received 15 August 1983/Accepted 14 December 1983

The in vitro activity of ciprofloxacin, a quinolone-carboxylic acid derivative, was compared with those of norfloxacin, cefotaxime, cephalexin, ceftazidime, moxalactam, amoxicillin, and methicillin and other agents, as appropriate. The MICs of ciprofloxacin for 90% of members of the family *Enterobacteriaceae* and for *Pseudomonas aeruginosa*, *Neisseria* spp., and *Bacteroides fragilis* were between 0.005 and 0.8 μg/ml, whereas streptococci and staphylococci were all inhibited by ≤6.3 μg/ml. Ciprofloxacin was 4- to 32-fold more active than norfloxacin and inhibited gentamicin-, ameikacin-, cefotaxime-, and moxalactam-resistant members of the family *Enterobacteriaceae* and *P. aeruginosa* and methicillin-resistant *Staphylococcus aureus*. The activity of ciprofloxacin was not affected by serum but decreased in the presence of acid urine. The frequency of resistance to ciprofloxacin was between 10^{-7} and 10^{-9}.

Nalidixic acid, a pyridone-carboxylic acid compound, was introduced into medical use in 1963. It was not used clinically to any great extent because of the rapid development of resistance to the compound (5). In the 1970s, pipemidic acid was synthesized in Japan (6). This compound, which provided reasonable concentrations in tissue, was used to treat infections other than urinary tract infections, as was nalidixic acid and other compounds of this class, such as oxolinic acid and cinoxacin.

The development of norfloxacin, a pyridone-carboxylic acid derivative, showed that it was possible to develop compounds of this class which, by virtue of their broad spectrum of activity and the low incidence of resistance to them, would be clinically useful (2, 4). Although the expanded-spectrum cephalosporins inhibit many clinically important bacteria, resistance has already been encountered among *Enterobacter* and *Pseudomonas* spp. (3). For this reason, we sought to study cirprofloxacin, a novel quinolone compound, and to compare its activity with those of other agents currently in use.

MATERIALS AND METHODS

Compounds. All compounds were obtained from their respective manufacturers. Ciprofloxacin was a gift of Bayer; norfloxacin was obtained from Merck Sharp & Dohme.

Fresh dilutions of each compound were prepared daily in sterile medium. Bacterial isolates were obtained from patients treated at The Columbia-Presbyterian Medical Center, New York. Both recent isolates and isolates that are multiply resistant to antibiotics had been saved over the past 5 years and were tested. Only one isolate from a patients was tested to avoid multiple copies of the same strain.

Susceptibility testing. Antimicrobial susceptibility tests were performed by an agar dilution method with Mueller-Hinton agar, except where otherwise specified. A final inoculum of 10^5 CFUs, prepared by dilution of a fresh overnight broth culture, was applied to agar with a replicating spot device. Broth dilutions were performed with 10^5 CFU in tubes each having a volume of 1 ml. Agar plates and tubes were incubated at 35°C for 18 h. The MIC was defined as the lowest concentration of antimicrobial agent that inhibited development of visible growth on agar or in the tubes. The MBC was determined by plating 0.1 ml of broth from clear tubes to blood agar plates and was defined as the lowest concentration that inhibited all growth. Anaerobic susceptibility tests were performed with Mueller-Hinton agar supplemented with hemin and vitamin K_1. Incubation took place in GasPack jars (BBL Microbiology Systems) at 35°C for 48 h. Susceptibility tests of streptococcal species were performed with Mueller-Hinton agar supplemented with 5% sheep blood, whereas susceptibility tests of *Haemophilus* and *Neisseria* spp. were performed in the presence of 10% CO_2 on chocolate agar.

MIC determinations. MIC determinations in urine were performed with urine obtained in the morning from male and female volunteers which was sterilized by passage through a 0.22-μm (pore size) membrane filter (Millipore Corp.). The inoculum used was 10^5 CFU. Incubation and determination of MICs and MBCs were done in a manner similar to those used for Mueller-Hinton broth.

Organisms were made resistant to ciprofloxacin and norfloxacin by daily passage in increasing concentration of drug.

Protein binding. Protein binding was determined by the dialysis technique with a membrane with a molecular weight exclusion of 50,000. The filtrate and the antibiotic remaining in the dialysis sack were assayed by a microbiological method against standards prepared in 0.05 M phosphate buffer (pH 7.4). Dialysis was performed with the same phosphate buffer.

Determination of mutation frequency. Spontaneous mutants resistant to ciprofloxacin were detected by plating 0.1 ml of an overnight growth of cultures onto agar plates containing ciprofloxacin at concentrations four and eight times the MIC.

RESULTS

Ciprofloxacin had an extremely broad range of antibacterial activity, inhibiting aerobic and anaerobic cocci and bacilli at low concentrations (Table 1). A total of 90% of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter cloacae* isolates were inhibited by ≤0.05 μg/ml. Ciprofloxacin was consistently four- to eightfold more active

* Corresponding author.

TABLE 1. Comparative activities of ciprofloxacin and other antibiotics

Organism (no.)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Acinetobacter anitratus (19)	Ciprofloxacin	0.005–6.3	0.4	1.6
	Norfloxacin	0.1–12.5	1.6	6.3
Aeromonas hydrophila (5)	Ciprofloxacin	≤0.002–0.02	≥0.002	0.02
Bacteroides fragilis (23)	Ciprofloxacin	≤0.01–0.8	0.8	0.8
	Norfloxacin	6.3–≥100	6.3	50
Other *Bacteroides* spp. (12)	Ciprofloxacin	0.1–0.8	0.8	0.8
	Norfloxacin	6.3–>100	6.3	25
Branhamella catarrhalis (10)	Ciprofloxacin	<0.01–0.05	0.01	0.05
	Ampicillin	>25	25	
Citrobacter diversus (25)	Ciprofloxacin	≤0.02–0.05	0.01	0.02
	Norfloxacin	0.02–0.2	0.1	0.2
	Cefotaxime	≤0.1	≤0.1	≤0.1
	Ceftazidime	≤0.1–0.2	0.1	0.1
	Cephalexin	3.1–≥100	3.1	50
	Amoxicillin	≥100	≥100	≥100
Citrobacter freundii (22)	Ciprofloxacin	≤0.002–0.2	0.01	0.1
	Norfloxacin	0.02–0.4	0.1	0.4
	Cefotaxime	0.1–12.5	0.2	3.1
	Ceftazidime	0.1–12.5	0.4	3.1
	Moxalactam	0.1–12.5	0.4	3.1
	Amoxicillin	≥100	≥100	≥100
Enterobacter aerogenes (17)	Ciprofloxacin	0.005–0.2	0.01	0.05
	Norfloxacin	0.1–0.8	0.2	0.4
	Cefotaxime	≤0.1–6.3	0.2	6.3
	Ceftazidime	≤0.1–3.1	0.4	3.1
	Moxalactam	0.05–50	0.1	6.3
	Amoxicillin	12.5–≥100	≥100	≥100
	Trimethoprim	0.8–25	3.1	12.5
Enterobacter cloacae (28)	Ciprofloxacin	0.005–0.4	0.01	0.05
	Norfloxacin	0.02–1.6	0.2	0.4
	Cefotaxime	≤0.1–12.5	0.2	12.5
	Ceftazidime	≤0.1–12.5	0.4	12.5
	Moxalactam	≤0.1–12.5	0.1	6.3
Escherichia coli (40)	Ciprofloxacin	≤0.01–0.2	≤0.01	0.02
	Norfloxacin	0.05–0.8	0.1	0.2
	Cefotaxime	≤0.1–0.8	≤0.1	0.1
	Ceftazidime	≤0.1–0.4	0.1	0.2
	Cephalexin	6.3–≥100	6.3	25
	Moxalactam	≤0.1–0.4	≤0.1	0.2
	Amoxicillin	1.6–≥100	≥100	≥100
	Trimethoprim	0.2–3.1	0.2	1.6
Haemophilus influenzae (10)	Ciprofloxacin	≤0.01	≤0.01	≤0.01
	Cefotaxime	≤0.1	≤0.1	≤0.1
	Cephalexin	≤0.1–≥100	12.5	50
	Amoxicillin	≤0.1–25	0.4	25
Klebsiella oxytoca (25)	Ciprofloxacin	0.01–0.05	0.01	0.05
	Norfloxacin	0.05–0.4	0.1	0.4
	Cefotaxime	≤0.1–1.6	<0.1	0.2
	Cephalexin	12.5–100	12.5	100
	Amoxicillin	50–≥100	100	≥100
Klebsiella pneumoniae (29)	Ciprofloxacin	0.005–0.1	0.02	0.05
	Norfloxacin	0.1–0.8	0.2	0.4
	Cefotaxime	≤0.1–1.6	0.1	0.2
	Ceftazidime	0.1–1.6	0.2	0.2
	Cephalexin	0.8–100	3.1	25
	Moxalactam	≤0.1–1.6	0.1	0.2
	Trimethoprim	0.8–≥100	1.6	3.1

TABLE 1—*Continued*

Organism (no.)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Listeria monocytogenes (31)	Ciprofloxacin	0.1–1.6	0.2	0.4
	Amoxicillin	≤0.1–0.2	≤0.1	0.2
Morganella morganii (25)	Ciprofloxacin	≤0.002–0.1	0.01	0.02
	Norfloxacin	0.01–0.4	0.05	0.02
	Cephalexin	>100	≥100	≥100
	Amoxicillin	≥100	≥100	≥100
Neisseria meningitidis (4)	Ciprofloxacin	≤0.01	≤0.01	≤0.01
Proteus mirabilis (17)	Ciprofloxacin	≤0.01–0.1	0.05	0.05
	Amoxicillin	0.8–≥100	0.8	1.6
Proteus vulgaris (13)	Ciprofloxacin	0.01–0.05	0.02	0.02
	Norfloxacin	0.05–3.1	0.05	0.1
	Ceftazidime	0.1–6.3	0.1	0.4
	Cephalexin	12.5–≥100	100	≥100
	Amoxicillin	12.5–≥100	100	≥100
	Trimethoprim	1.6–12.5	3.1	12.5
Providencia rettgeri (16)	Ciprofloxacin	0.01–0.2	0.02	0.2
	Norfloxacin	0.05–1.6	0.2	0.4
	Cephalexin	25–≥100	100	≥100
	Amoxicillin	12.5–≥100	100	≥100
	Trimethoprim	0.04–100	3.1	6.3
Providencia stuartii (22)	Ciprofloxacin	0.005–0.8	0.1	0.4
	Norfloxacin	0.02–1.6	0.05	1.6
	Cefotaxime	≤0.1–0.8	0.1	0.2
	Cephalexin	>100	100	≥100
	Moxalactam	<0.1–0.2	<0.1	0.2
	Trimethoprim	0.8–100	1.6	6.3
Pseudomonas aeruginosa (70)	Ciprofloxacin	0.02–1.6	0.2	0.8
	Norfloxacin	0.2–6.3	1.6	3.1
	Ceftazidime	0.2–100	1.6	12.5
	Carbenicillin	>100	<100	>100
	Piperacillin	0.8–>100	12.5	>100
	Gentamicin	0.4–≥100	3.1	25
Pseudomonas cepacia (12)	Ciprofloxacin	0.1–6.3	0.8	6.3
Pseudomonas maltophilia (11)	Ciprofloxacin	0.05–3.1	0.8	3.1
Salmonella spp. (43)	Ciprofloxacin	≤0.002–0.05	0.01	0.02
	Norfloxacin	≤0.01–0.2	0.05	0.1
	Cefotaxime	<0.1	<0.1	<0.1
	Cephalexin	6.2–25	6.3	12.5
	Amoxicillin	0.8–25	1.6	≥100
	Trimethoprim	0.1–>25	0.2	1.6
Salmonella typhi (5)	Ciprofloxacin	0.01–0.02	0.01	0.02
Serratia marcescens (27)	Ciprofloxacin	0.05–0.4	0.1	0.4
	Norfloxacin	0.1–1.6	0.4	0.8
	Cefotaxime	0.1–25	1.6	6.3
	Ceftazidime	0.1–12.5	0.8	3.1
	Moxalactam	0.1–25	0.8	3.1
	Trimethoprim	0.8–≤100	6.3	≤100
Shigella spp. (26)	Ciprofloxacin	≤0.002–0.05	0.005	0.02
	Norfloxacin	0.01–0.1	0.02	0.1
	Cefotaxime	≤0.1	<0.1	0.1
	Cephalexin	3.1–12.5	6.3	12.5
	Ampicillin	0.8–25	1.6	≥100
	Trimethoprim	0.2–>25	0.2	0.8

TABLE 1—*Continued*

Organism (no.)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus (45)	Ciprofloxacin	0.1–0.8	0.4	0.8
	Norfloxacin	0.05–3.1	0.8	3.1
	Cephalexin	0.8–12.5	3.1	12.5
	Cefaclor	0.8–25	1.6	12.5
	Methicillin	3.1–12.5	6.3	12.5
Methicillin-resistant *Staphylococcus aureus* (35)	Ciprofloxacin	0.1–0.8	0.4	0.8
	Methicillin	25–>100	>100	>100
Staphylococcus epidermidis (19)	Ciprofloxacin	0.1–0.8	0.2	0.4
	Norfloxacin	0.2–6.3	0.8	3.1
	Cephalexin	0.1–50	1.6	25
	Methicillin	0.1–≥100	0.8	>100
Streptococcus pyogenes (15)	Ciprofloxacin	0.2–1.6	0.8	1.6
	Norfloxacin	0.4–25	1.6	6.3
	Cephalexin	0.2–0.8	0.2	0.8
Streptococcus agalactiae (8)	Ciprofloxacin	0.1–0.8	0.8	0.8
	Cephalexin	1.6–3.1	1.6	3.1
	Amoxicillin	≤0.01–0.05	≤0.01	0.02
Streptococcus spp. group C (12)	Ciprofloxacin	0.05–0.4	0.05	0.4
Streptococcus faecalis group D (26)	Ciprofloxacin	0.8–25	1.6	6.3
	Norfloxacin	1.6–50	6.3	25
	Cephalexin	100–>100	100	>100
	Amoxicillin	0.2–0.4	0.2	0.4
Streptococcus bovis group D (7)	Ciprofloxacin	0.8–3.1	1.6	3.1
	Amoxicillin	≤0.1–0.4	≤0.1	0.4
Streptococcus anginosus group G (3)	Ciprofloxacin	0.2–0.4	0.4	0.4
Streptococcus pneumoniae (8)	Ciprofloxacin	0.2–3.1	0.8	3.1
	Amoxicillin	≤0.01–1.6	0.2	0.4
Viridans group streptococci (8)	Ciprofloxacin	1.6	1.6	1.6
Yersinia enterocolitica (16)	Ciprofloxacin	≤0.01	0.01	0.01
	Amoxicillin	25–≥100	100	≥100

[a] 50% and 90%, MIC required to inhibit 50 and 90% of isolates, respectively.

TABLE 2. Activity of ciprofloxacin against selected bacteria resistant to new β-lactams and aminoglycosides

Organism	MIC (μg/ml) of[a]:			
	Ciprofloxacin	Amikacin	Cefotaxime	Moxalactam
Acinetobacter anitratus	0.4	>16	>128	>128
Bacteroides thetaiotaomicron	0.8	>128	>128	>128
Citrobacter freundii 1	0.1	>16	>128	>128
Citrobacter freundii 2	0.05	>16	>128	>128
Enterobacter aerogenes	0.05	>16	>128	64
Enterobacter cloacae 1	0.05	>16	>128	>128
Enterobacter cloacae 2	0.05	>16	128	64
Klebsiella pneumoniae	0.05	>16	4	4
Proteus vulgaris	0.02	4	>128	32
Pseudomonas aeruginosa 1	0.8	>16	>128	>128
Pseudomonas aeruginosa 2	0.8	>16	>128	>128
Pseudomonas cepacia	0.8	>16	>128	>128
Pseudomonas maltophilia	0.8	>16	>128	>128
Serratia marcescens 1	0.4	>16	>128	64
Serratia marcescens 2	0.4	>16	>128	32
Staphylococcus aureus	0.8	>16	>128	>128

[a] The MICs of piperacillin and cefoperazone were >128 μg/ml for all organisms.

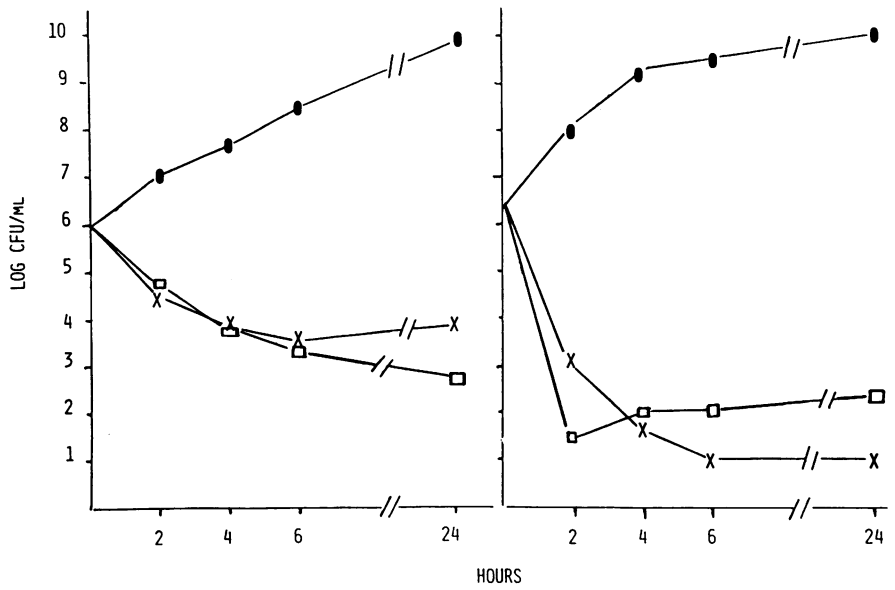

FIG. 1. Exposure of *Staphylococcus aureus* to ciprofloxacin (X) and norfloxacin (□). Each agent was added at time 0 at a concentration that was four times the MIC. Ciprofloxacin was present at 1.6 (A) and 6.4 (B) μg/ml, and norfloxacin was present at 6.4 (A) and 25 (B) μg/ml. ●, Control without antimicrobial agent.

than norfloxacin and inhibited the growth of *Citrobacter freundii* and *Enterobacter cloacae*, which were resistant to cefotaxime and ceftazidime. Ciprofloxacin was more active than the other agents tested against *Haemophilus influenzae*, inhibiting 90% of both β-lactamase-negative and β-lactamase-positive isolates at concentrations of ≤0.01 μg/ml. Ciprofloxacin also inhibited trimethoprim-resistant *K. pneumoniae* and *Klebsiella oxytoca*. *Morganella morganii* and *Proteus vulgaris* isolates resistant to cefotaxime were inhibited by ciprofloxacin (Tables 1 and 2), which was several-fold more active than ceftazidime againt *Proteus* and *Providencia* species (Table 1). *Serratia marcescens* isolates resistant to moxalactam, cefotaxime, and trimethoprim were inhibited by 0.4 μg of ciprofloxacin per ml. Ciprofloxacin was fourfold more active than norfloxacin against *Pseudomonas aeruginosa* isolates, inhibited gentamicin-, moxalactam-, and ceftazidime-resistant organisms, and was 32-fold more active than aztreonam or ceftazidime. *Pseudomonas cepacia* and *Pseudomonas maltophilia* isolates which were resistant to cefotaxime, moxalactam, aztreonam, and norfloxacin were inhibited by concentrations of 6.3 and 3.1 μg/ml, respectively (Table 2).

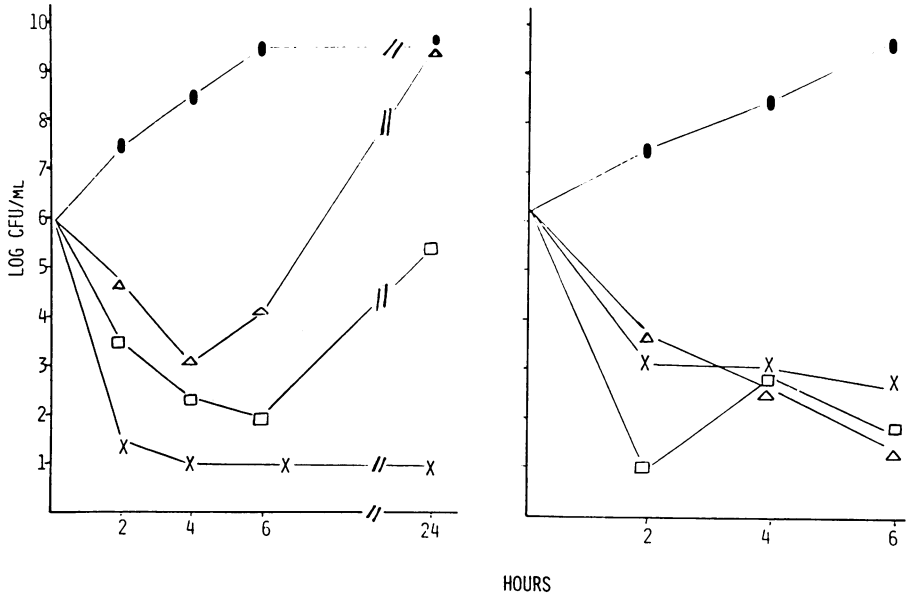

FIG. 2. (A) Exposure of *K. pneumoniae* to ciprofloxacin at 0.05 μg/ml (X), norfloxacin at 0.2 μg/ml (□), and nalidixic acid at 12.5 μg/ml (△) at time 0. Each agent was present at a concentration that was four times the MIC. ●, Control. (B) Exposure of *Pseudomonas aeruginosa* to ciprofloxacin at 0.2 μg/ml (X), norfloxacin at 3.2 μg/ml (□), and gentamicin at 6.4 μg/ml (△) at time 0. Each agent was present at a final concentration that was four times the MIC. ●, Control.

TABLE 3. Activity of ciprofloxacin in urine at different pHs

Organism	MIC in Mueller-Hinton broth	Ciprofloxacin concn (μg/ml) in urine at pH of:					
		5.5		6.5		7.5	
		MIC	MBC	MIC	MIC	MIC	MBC
Escherichia coli 5441	≤0.01	3.1	3.1	1.6	12.5	1.6	3.1
Enterobacter cloacae 4646	0.01	6.3	25.0	1.6	3.1	0.4	3.1
Klebsiella pneumoniae 5740	0.05	6.3	6.3	1.6	6.3	0.8	3.1
Morganella morganii 5738	0.01	0.4	1.6	≤0.005	1.6	0.2	3.1
Serratia marcescens 5738	0.1	1.6	3.1	0.02	1.6	0.2	3.1
Pseudomonas aeruginosa 5267	0.4	6.3	25.0	1.6	6.3	1.6	3.1

Enteric diarrheal species such as *Salmonella* spp., including *Salmonella typhi* and *Shigella sonnei* and *Shigella flexneri*, were inhibited by <0.05 μg of ciprofloxacin per ml. These included ampicillin-, chloramphenicol- (not shown), and trimethoprim-resistant isolates. *Yersinia enterocolitica* isolates were inhibited by <0.01 μg of ciprofloxacin per ml. *Acinetobacter calcoaceticus* subsp. *anitratus* and *lwoffii* isolates were inhibited by ciprofloxacin concentrations that were fourfold lower than those of norfloxacin, and all ceftazidime-resistant isolates were inhibited by <1.6 μg/ml. Ciprofloxacin MICs for other organisms (data not shown) were as follows: *Alcaligenes denitrificans*, 0.8 μg/ml; *Brucella melitensis*, 0.05 μg/ml; 0.05 g of *Neisseria lactamica*, <0.1 μg/ml; *Pseudomonas fluorescens*, 0.8 μg/ml; and *Pseudomonas stutzeri*, 0.8 μg/ml.

Ciprofloxacin showed excellent activity (MIC for 90% of isolates, 0.8 μg/ml) against *Staphylococcus aureus*, including methicillin-resistant isolates, and against methicillin-resistant *Staphylococcus epidermidis*. Ciprofloxacin was several-fold more active than norfloxacin against various hemolytic and nonhemolytic streptococci and against *Streptococcus pneumoniae*. Ciprofloxacin was less active than β-lactams against streptococcal species but was as active as amoxicillin against *Listeria monocytogenes*.

Ciprofloxacin was the most active agent tested against *Bacteriodes* spp., including *B. fragilis*, *B. thetaiotaomicron*, *B. vulgatus*, *B. melaniongenicus*, *B. disiens*, *B. oralis*, *B. distasonis*, and *B. bivius*.

Comparative killing curves of ciprofloxacin and norfloxacin against *Staphylococcus aureus* and *Streptococcus faecalis* (Fig. 1) were determined with concentrations that were four times the MICs. Both agents produced a 3- to 4-log decrease in CFU in 6 h, and regrowth did not occur in 24 h. The concentrations of ciprofloxacin and norfloxacin used against *Streptococcus faecalis* and *Klebsiella* and *Pseudomonas* spp. can be achieved in serum (unpublished data). Regrowth of *K. pneumoniae* did not occur at 24 h when ciprofloxacin was used but did occur when both nalidixic acid and norfloxacin were used (Fig. 2). Ciprofloxacin at a concentration of 0.2 μg/ml, as compared with norfloxacin and gentamicin at respective concentrations of 3.2 and 6.4 μg/ml, caused a four-log decrease in CFU of *Pseudomonas aeruginosa*.

Ciprofloxacin MBCs were identical or only fourfold higher than MICs for 75% of the isolates. An eightfold increase in MBC would have to be considered a relative increase, since the increase was from 0.005 to 0.1 μg/ml. MBCs were not raised beyond a level of 1.5 μg/ml, a concentration which can be achieved in serum with a dose of 500 mg orally (unpublished data), for any of the members of the family *Enterobacteriaceae*.

An inoculum of 10^7 CFU produced an eightfold increase in

TABLE 4. Frequency of resistance to ciprofloxacin

Organism (MIC [μg/ml])	Fold above MIC	Resistance frequency at 48 h
Enterobacter cloacae (0.025)	4	$1 \times <10^{-9}$
	8	$1 \times <10^{-9}$
Escherichia coli (0.025)	4	2.92×10^{-7}
	8	3.33×10^{-8}
Klebsiella pneumoniae (0.025)	4	1.06×10^{-7}
	8	3.33×10^{-8}
Providencia stuartii (0.1)	4	1.78×10^{-7}
	8	1.48×10^{-7}
Pseudomonas aeruginosa (0.8)	4	$1 \times <10^{-9}$
	8	$1 \times <10^{-9}$
Serratia marcescens (0.2)	4	$1 \times <10^{-9}$
	8	$1 \times <10^{-9}$
Staphylococcus aureus (0.4)	4	1.82×10^{-7}
	8	1.67×10^{-8}
Streptococcus faecalis (0.8)	4	$1 \times <10^{-9}$
	8	$1 \times <10^{-9}$

TABLE 5. Activity of ciprofloxacin against strains made resistant to quinolone compounds

Organism	MIC (μg/ml) in Mueller-Hinton agar of:		
	Nalidixic acid	Norfloxacin	Ciprofloxacin
Enterobacter aerogenes 6541	50	3.1	0.1
Parent	≥100	50	3.1
Mutant	≥100	12.5	1.6
Enterobacter aerogenes 5429	12.5	0.2	1.6
Parent	≥100	50	6.3
Mutant	≥100	50	6.3
Providencia stuartii 5544	≥100	3.1	1.6
Parent	≥100	≥100	25
Mutant	≥100	25	12.5
Providencia stuartii 5543	≥100	3.1	1.6
Parent	≥100	≥100	25
Mutant	≥100	25	12.5
Klebsiella pneumoniae UO-9	25	0.8	0.2
Parent	≥100	12.5	3.1
Mutant	≥100	25	3.1
Morganella morganii 6050	3.1	0.2	0.1
Parent	≥100	50	12.5
Mutant	≥100	100	6.3
Staphylococcus aureus 3540	≥100	6.3	0.8
Parent	≥100	25	3.1
Mutant	≥100	100	25

TABLE 6. Activity of ciprofloxacin against *E. coli* mutants resistant to nalidixic acid

E. coli strain[a]	DNA gyrase marker	MCI/MBC (μg/ml) of:		
		Nalidixic acid	Cinoxacin	Ciprofloxacin
KL166	A 13	100	50	<0.01
		<100	>100	<0.01
KL163	A 12	6.3	3.1	<0.01
		25	25	<0.01
KL164	B 14	25	50	<0.01
		25	>100	<0.01
KLNI748	B 41	6.3	3.1	<0.01
		12.5	25	<0.01
N4177	B 203	6.3	3.1	<0.01
	B 221	50	50	<0.01

[a] Isolates were obtained from B. Bachmann, Yale University, New Haven, Conn.

MICs as compared with MICs obtained with 10^5 CFU. Conversely, MICs obtained with an inoculum of 10^3 CFU were twofold lower than those obtained with an inoculum of 10^5 CFU. Although the MICs were eightfold greater at 10^7 CFU, they did not exceed 3.1 μg/ml.

The activity of ciprofloxacin in 50% normal human serum was identical to that in Mueller-Hinton broth. For example, MICs and MBCs for *Staphylococcus aureus* were 0.2 and 0.8 μg/ml, respectively, in broth and 0.2 and 0.8 μg/ml, respectively, in serum plus broth. Similar results were obtained with *Streptococcus faecalis*, *Serratia marcescens*, *Escherichia coli*, and *Pseudomonas aeruginosa*. In contrast, the activity of ciprofloxacin was lower in the presence of urine, particularly acidic urine (Table 3). For example, an *Escherichia coli* strain with an MIC of <0.01 μg/ml in Mueller-Hinton broth at pH 7.4 had MICs of 1.6 μg/ml in urine at pH 7.5 and 3.1 μg/ml in urine at pH 5.5. Of 30 isolates tested at pH 5.5, however, the MBCs were >6.3 μg/ml for only 10% of *Pseudomonas aeruginosa* and *Enterobacter cloacae* isolates.

The type of media used to determine MICs, Mueller-Hinton, brain-heart infusion, tryptic soy digest, Columbia, or nutrient medium, did not alter MICs or MBCs for members of the family *Enterobacteriaceae* or for *Pseudomonas aeruginosa* or *Staphylococcus aureus*. MICs and MBCs were 8- to 16-fold greater at pH 5.5 as compared with pH 7.5, regardless of whether Mueller-Hinton or nutrient broth was used.

Ciprofloxacin resistance. The frequency of ciprofloxacin resistance of various bacteria was determined for two isolates of eight species (Table 4). The highest frequency of resistance, 10^{-7} was found for *Escherichia coli*, *K. pneumoniae*, and *Providencia stuartii*, but the other organisms, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus faecalis*, had a ciprofloxacin resistance frequency of $<10^{-9}$. The activity of ciprofloxacin against organisms made resistant to nalidixic acid and norfloxacin is shown in Table 5. Ciprofloxacin inhibited a number of norfloxacin-resistant isolates and all of the parent nalidixic acid-resistant strains. The activity of ciprofloxacin was also tested against *Escherichia coli* mutants (Table 6) which contain altered DNA gyrase and are resistant to nalidixic acid owing to changes at the DNA gyrase A or B locus. Ciprofloxacin inhibited all of the isolates at concentrations <0.01 μg/ml, indicating that *Escherichia coli* mutants with altered DNA gyrase are not resistant to ciprofloxacin.

Protein binding of ciprofloxacin was determined to be 28% at 3 μg/ml, a level attainable in serum with a dose of 1 g orally (unpublished data).

DISCUSSION

Ciprofloxacin is markedly different from the older quinolone derivatives, such as nalidixic acid, cinoxacin, or oxalinic acid. It encompasses a much larger spectrum of antibacterial activity which includes streptococcal, staphyloccocal, and *Pseudomonas* species. Ciprofloxacin differs from norfloxacin in that it inhibits *Bacteroides* spp. and certain organisms resistant to both nalidixic acid and norfloxacin. The compound has excellent activity against methicillin-resistant *Staphylococcus aureus* and, furthermore, inhibits many bacteria, such as *Enterobacter aerogenes*, *Enterobacter cloacae*, and *C. freundii*, that are resistant to the new β-lactamase-stable cephalosporins, e.g., cefotaxime and moxalactam. Ciprofloxacin also inhibits moxalactam-resistant *B. thetaiotaomicron*. Results similar to these have been published by Wise et al. (9) and Bauernfeind and Petetmullar (1).

Although the activity of ciprofloxacin is markedly reduced in acid urine at pH 5.5, it inhibits bacteria at concentrations of <25 μg/ml, which are readily attainable in serum (unpublished data), and does not show a discrepancy between MICs and MBCs.

Quinolone compounds are known to affect DNA gyrase and DNA nicking-closing enzymes (7). Whether ciprofolxacin attaches more avidly to one or both enzymes has not been established. Results of studies by our group with other new quinolones (N.-X. Chin, and H. C. Neu, submitted for publication) suggest that increased permeability of these compounds is not the explanation. Rather, we would postulate that the cylopropyl substituent has altered DNA gyrase activity, and we have studies under way to clarify this point. Interestingly, ciprofloxacin seems to be less readily affected by resistance than is norfloxacin (8).

In view of the appearance of *Enterobacteriaceae* strains that are resistant to the oxa cephems and aminothiazolyl cephalosporins, drugs such as ciprofloxacin may be extremely important since the potential for resistance is low, as shown by studies such as this one. Furthermore, the quinolones will not select plasmid-carrying enzymes and may even reduce the appearance of such species. Because of their excellent activities against enteric diarrhea-producing species, compounds such as ciprofloxacin may be extremely helpful in controlling diarrheal disease.

LITERATURE CITED

1. **Bauernfeind, A., and C. Petetmullar.** 1983. In vitro activity of ciprofloxacin, norfloxacin, and nalidixic acid. Eur. J. Clin. Microbiol. **2**:111–115.
2. **Ito, A., K. Hirai, M. Inoue, H. Koga, S. Suze, T. Irkura, and S. Mitsuhashi.** 1980. In vitro antibacterial activity of AM-715, a new nalidixic acid analog. Antimicrob. Agents Chemother. **17**:103–108.
3. **Neu, H. C.** 1983. Structure-activity relations of new β-lactam compounds and in vitro activity against common bacteria. Rev. Infect. Dis. **5**(Suppl):319–337.
4. **Neu, H. C., and P. Labthavikul.** 1982. In vitro activity of norfloxacin, a quinolone carboxylic acid, compared with that of β-lactams, aminoglycosides, and trimethoprim. Antimicrob. Agents Chemother. **22**:23–37.
5. **Ronald, A. R., M. Turck, and R. G. Petersdorf.** 1956. A critical evaluation of nalidixic acid in urinary tract infections. N. Engl. J. Med. **297**:1081–1089.
6. **Shimizu, M., S. Nakamura, Y. Takasi, and N. Kurobe.** 1975. Pipemidic acid: absorption, distribution, and excretion. Antimi-

crob. Agents Chemother. **7**:441–446.
7. **Sugino, A., C. L. Prebles, K. N. Kremzer, and N. R. Cozzarelli.** 1977. Mechanism of action of nalidixic acid: purification of *E. coli* NalA gene production and its relationship to DNA gyrase and a novel nicking-closing enzyme. Proc. Natl. Acad. Sci. U.S.A. **74**:4767–4771.
8. **Tenney, J. J., R. W. Maack, and G. R. Chippendale.** 1983. Rapid selection of organisms with increasing resistance on subinhibitory concentrations of norfloxacin in agar. Antimicrob. Agents Chemother. **23**:188–189.
9. **Wise, R., J. M. Andrews, and L. J. Edwards.** 1983. In vitro activity of Bay 09867, a new quinolone derivative, compared with those of other antimicrobial agents. Antimicrob. Agents Chemother. **23**:559–564.