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In Vitro Activity of CI-934, a New Quinolone, Compared with That of Other Quinolones and Other Antimicrobial Agents

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The in vitro activity of CI-934, a new 4-quinolone, was determined against gram-positive and gram-negative bacteria. The MICs for 90% of the isolates tested were 0.25 μg/ml for *Streptococcus pneumoniae*, 0.5 μg/ml for *Streptococcus faecalis*, 0.25 μg/ml for staphylococci, including methicillin-resistant strains, and ≤1.0 μg/ml for *Escherichia coli*, *Salmonella* and *Shigella* spp., *Klebsiella* spp., *Proteus* spp., and *Citrobacter* spp. CI-934 had activity superior to that of other quinolones against streptococci by four- to eightfold. Against members of the family *Enterobacteriaceae*, ciprofloxacin was 2- to 18-fold more active; ofloxacin and norfloxacin were twofold more active or similar to CI-934. CI-934 inhibited ampicillin-cephalothin-resistant urinary isolates of *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* and cefoxatime-resistant *Acinetobacter* spp., *Citrobacter freundii*, *Enterobacter cloacae*, *Proteus vulgaris*, and *Morganella morganii*. The medium, inoculum size, and oxygen concentration, as well as the addition of serum, had no major effect on the activity of CI-934. Magnesium at a concentration of 9 mM increased MICs and MBCs four- to eightfold, and testing at pH 6 increased MICs as much as 32- to 64-fold for some organisms in comparison with MICs at pH 7. The frequency of spontaneous mutation to resistance was comparable to that for other new quinolones, but resistant isolates could be selected by repeated subculture.

In the past several years a number of extremely active quinolone antimicrobial agents have been synthesized (8, 18–20). These agents have been shown in many situations to inhibit the majority of the members of the family *Enterobacteriaceae*, including organisms resistant to beta-lactams and aminoglycosides (8, 9, 11). Some of the agents also have had excellent activity against *Pseudomonas aeruginosa* and other nonfermenting gram-negative species (8). However, these agents have tended to have much lower activity against gram-positive species such as the hemolytic streptococci and, in particular, *Streptococcus pneumoniae* (2, 3, 6, 8, 13). CI-934 is a new quinolone which has been preliminarily reported to have activity against many gram-positive organisms in addition to possessing activity against gram-negative bacteria (5). The purpose of this study was to investigate the in vitro activity of CI-934 and to compare its activity with that of other agents, with particular reference to gram-positive microorganisms.

MATERIALS AND METHODS

Antimicrobial agents. CI-934 was obtained from Warner-Lambert Research Laboratories, Ann Arbor, Mich.; ciprofloxacin was obtained from Miles Pharmaceuticals, West Haven, Conn.; ofloxacin was obtained from Ortho Diagnostics, Inc., Raritan, N.J.; norfloxacin and imipenem were obtained from Merck Sharp & Dohme, Rahway, N.J.; and cefotaxime was obtained from Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J. Other antimicrobial agents were purchased from their respective manufacturers. Fresh dilutions of all compounds were made daily.

Bacterial strains. Bacterial isolates were obtained from patients recently hospitalized at the Columbia-Presbyterian Medical Center, New York, N.Y. Some multiply resistant isolates which had been collected over the past 5 years were also used. However, only one isolate from a patient was included to avoid multiple copies of the same strain.

Antimicrobial susceptibility tests. Antimicrobial activity was measured by an agar dilution method with Mueller-Hinton agar, unless specified otherwise. All agents were run simultaneously. An inoculum of 10^5 CFU prepared by dilution of a fresh overnight broth culture was applied to agar with a replicating spot device. Broth dilutions were performed with approximately 5×10^5 CFU in 1-ml tubes. Agar plates and tubes were incubated at 35°C for 18 h. The MIC was defined as the lowest concentration of the antimicrobial agent that inhibited the development of visible growth on agar or in tubes. The MBC was determined by transferring 0.01 ml from a clear tube to agar plates. The MBC was the concentration that inhibited all growth (10). Susceptibility tests of *Haemophilus* and *Neisseria* species and *Branhamella catarrhalis* were performed in the presence of 5% CO_2 on chocolate agar. Susceptibility tests of streptococcal species were performed with Mueller-Hinton agar supplemented with 5% sheep blood. The susceptibility of anaerobic bacteria was determined with GasPak jars (BBL Microbiology Systems, Cockeysville, Md.), with incubation of cultures at 35°C for 48 h on brucella agar supplemented with hemin and vitamin K.

Synergy studies. Synergy studies were performed in agar. Serial twofold dilutions of the agents were used with the following concentrations (micrograms per milliliter): penicillin G, 128; gentamicin 16; chloramphenicol, 32; rifampin, 16; ticarcillin, 256; piperacillin, 256; and CI-934, 8. Synergy was defined as a fourfold reduction in the MICs of both agents. Antagonism was defined as a fourfold increase in the MIC of at least one agent.

Selection of resistant organisms. The development of resistance to CI-934 was determined by repeated subculture in the presence of the compound. An inoculum of approximately 5×10^5 CFU was exposed to CI-934 at twofold increasing concentrations. From the highest concentration

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TABLE 1. Activity of CI-934 compared with that of other agents against gram-positive organisms

Organism (no. of isolates tested)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus (25)	CI-934	0.06–1	0.12	0.25
	Ciprofloxacin	0.12–1	0.12	0.25
	Ofloxacin	0.25–0.5	0.25	0.5
	Norfloxacin	0.12–2	0.5	1
	Cefotaxime	0.5–4	2	4
	Imipenem	0.03–0.25	0.06	0.12
Staphylococcus aureus (methicillin-resistant) (25)	CI-934	0.03–0.25	0.06	0.12
	Ciprofloxacin	0.12–1	0.5	1
	Ofloxacin	0.5–4	2	4
	Norfloxacin	2.0–4	4	4
	Cefotaxime	8–>128	>128	>128
	Imipenem	0.03–8	0.12	8
Staphylococcus epidermidis (17)	CI-934	≤0.03–0.5	0.12	0.25
	Ciprofloxacin	0.06–0.5	0.06	0.25
	Ofloxacin	0.12–0.5	0.25	0.5
	Norfloxacin	0.25–4	0.5	2
	Cefotaxime	0.25–>128	2	>128
	Imipenem	0.03–0.25	0.03	0.12
Streptococcus pyogenes (30)	CI-934	≤0.015–2	0.12	0.5
	Ciprofloxacin	1–4	1	4
	Ofloxacin	1–4	2	4
	Norfloxacin	4–8	4	8
	Cefotaxime	≤0.03–0.25	0.03	0.25
	Imipenem	≤0.03–2	0.06	0.12
Streptococcus agalactiae (17)	CI-934	0.03–4	0.25	0.5
	Ciprofloxacin	1–4	1	2
	Ofloxacin	2–4	2	2
	Norfloxacin	8–16	16	16
	Cefotaxime	0.03–0.5	0.03	0.12
	Imipenem	0.06–0.5	0.06	0.12
Streptococcus groups C, F, and G (35)	CI-934	<0.015–4	0.12	0.25
	Ciprofloxacin	0.06–2	1	2
	Ofloxacin	0.5–2	1	2
	Norfloxacin	1–16	8	16
	Cefotaxime	≤0.015–0.5	≤0.015	0.25
	Imipenem	≤0.015–0.06	0.015	0.06
Streptococcus bovis (17)	CI-934	≤0.015–1	0.12	1
	Ciprofloxacin	1–2	2	2
	Ofloxacin	2–8	2	4
	Norfloxacin	4–16	4	16
	Cefotaxime	≤0.03–>8	0.12	0.5
	Imipenem	≤0.03–1	1	1
Streptococcus faecalis (32)	CI-934	0.06–1	0.12	0.5
	Ciprofloxacin	0.5–1	1	1
	Ofloxacin	2–4	2	4
	Norfloxacin	4–8	4	8
	Cefotaxime	32–>128	>128	>128
	Imipenem	0.5–1	1	1
Streptococcus pneumoniae (15)	CI-934	0.06–0.25	0.12	0.25
	Ciprofloxacin	1–2	1	1
	Ofloxacin	2–4	2	4
	Norfloxacin	4–16	8	16
	Cefotaxime	≤0.03–0.06	≤0.03	0.06
	Imipenem	≤0.03	≤0.03	≤0.03
Viridans group streptococci (14)	CI-934	0.12–1	0.5	1
	Ciprofloxacin	0.5–4	2	4
	Ofloxacin	0.25–2	2	2
	Norfloxacin	1–16	8	16
	Cefotaxime	0.015–8	0.015	0.25
	Imipenem	≤0.03–1	0.03	0.03
Listeria monocytogenes (15)	CI-934	0.25–1	0.5	1
	Ciprofloxacin	0.25–1	0.5	1
	Ofloxacin	0.5–2	0.5	1
	Norfloxacin	2–8	2	4
	Cefotaxime	8–>128	16	64
	Imipenem	0.03–2	0.06	0.5
Corynebacterium JK (11)	CI-934	0.06–0.12	0.06	0.06
	Ciprofloxacin	0.12–1	0.25	1
	Ofloxacin	0.25–2	0.25	1
	Norfloxacin	0.5–4	1	4
	Cefotaxime	>128	>128	>128
	Imipenem	>32	>32	>32
Clostridium spp.[a] (25)	CI-934	0.25–>16	4	8
	Ciprofloxacin	0.25–16	4	8
	Ofloxacin	0.25–>16	4	8
	Norfloxacin	0.5–64	2	64
	Cefotaxime	0.5–64	8	64
	Imipenem	≤0.06	0.06	8
Peptostreptococci (14)	CI-934	0.06–8	1	1
	Ciprofloxacin	0.06–4	0.5	0.5
	Ofloxacin	0.25–4	0.5	2
	Norfloxacin	0.5–8	2	4
	Cefotaxime	0.5–>64	0.5	4
	Imipenem	≤0.06	≤0.06	≤0.06

[a] Included five *C. difficile* isolates.

showing visible growth, approximately 5×10^5 CFU was reexposed to CI-934 at the same and higher concentrations. This was repeated daily for 12 days. Organisms were subsequently plated on antibiotic-free medium for three transfers and retested for susceptibility. The original isolate and the resistant isolate were tested against the other quinolones.

The occurrence of spontaneous single-step resistance to CI-934 was determined by plating 10^8 to 10^9 CFU, obtained by centrifugation of an overnight culture, onto Mueller-Hinton agar which contained eight times the MIC of CI-934 for the particular organism being tested. Two isolates of each species were tested. CFUs were determined after 48 h of incubation at 35°C.

RESULTS

CI-934 showed excellent in vitro activity against the majority of gram-positive and gram-negative aerobic microorganisms. Ninety percent of the gram-positive species tested were inhibited by ≤1 μg of CI-934 per ml (Table 1). Similarly 90% of the members of the *Enterobacteriaceae* were inhibited by 2 μg/ml with the exception of *Providencia rettgeri*, *Pseudomonas aeruginosa*, and other *Pseudomonas* species, which had MICs for 50% of the isolates tested (MIC_{50}s) of ≤8 μg/ml and MIC_{90}s of 16 μg/ml (Table 2). Anaerobic species such as *Bacteroides fragilis* and *Clostridium* species, which included *C. perfringens*, *C. difficile*, and *C. septicum*, had MICs of 8 to 16 μg/ml as did the peptostreptococci (14 isolates).

CI-934 was consistently more active than ofloxacin or

TABLE 2. Activity of CI-934 compared with that of other agents against gram-negative species

Organism (no. of isolates tested)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Escherichia coli[a] (25)	CI-934	<0.015–1	0.06	0.12
	Ciprofloxacin	<0.015–1	0.06	0.12
	Ofloxacin	0.03–0.06	0.06	0.06
	Norfloxacin	0.03–1	0.06	0.06
	Cefotaxime	<0.015–8	0.03	0.12
	Imipenem	0.12–0.5	0.25	0.5
Klebsiella pneumoniae[a] (25)	CI-934	0.06–2	0.25	0.5
	Ciprofloxacin	0.015–1	0.015	0.25
	Ofloxacin	0.12–0.5	0.12	0.25
	Norfloxacin	0.06–1	0.12	0.5
	Cefotaxime	0.03–8	0.03	0.12
	Imipenem	0.015–1	0.12	0.5
Klebsiella oxytoca[a,b] (20)	CI-934	0.06–1	0.25	0.05
	Ciprofloxacin	0.015–0.06	0.015	0.06
	Ofloxacin	0.03–0.12	0.06	0.06
	Norfloxacin	0.03–0.25	0.12	0.25
	Cefotaxime	≤0.015–0.06	0.03	0.06
	Imipenem	0.12–0.25	0.12	0.12
Enterobacter cloacae[a,b] (20)	CI-934	0.12–1	0.25	0.5
	Ciprofloxacin	<0.015–0.5	0.015	0.03
	Ofloxacin	0.06–2	0.06	0.5
	Norfloxacin	0.03–4	0.06	0.5
	Cefotaxime	0.06–>16	2	>16
	Imipenem	0.12–16	2	16
Enterobacter aerogenes[a,b] (25)	CI-934	0.12–1	0.25	1
	Ciprofloxacin	0.03–0.12	0.03	0.12
	Ofloxacin	0.06–0.5	0.06	0.25
	Norfloxacin	0.06–0.5	0.06	0.5
	Cefotaxime	0.03–>16	0.12	16
	Imipenem	0.25–8	2	4
Serratia marcescens[a,b] (25)	CI-934	0.5–8	1	2
	Ciprofloxacin	≤0.015–1	0.06	0.25
	Ofloxacin	0.15–4	0.25	1
	Norfloxacin	0.06–8	0.12	0.5
	Cefotaxime	0.12–64	0.5	16
	Imipenem	0.12–4	0.12	1
Citrobacter freundii[a,b] (20)	CI-934	0.06–2	0.25	0.5
	Ciprofloxacin	0.12–4	0.12	1
	Ofloxacin	0.12–0.5	0.12	0.5
	Norfloxacin	0.06–0.5	0.06	0.5
	Cefotaxime	0.12–64	0.25	16
	Imipenem	0.12–02	0.5	2
Citrobacter diversus[a] (20)	CI-934	0.06–0.5	0.12	0.25
	Ciprofloxacin	≤0.015–0.015	≤0.015	0.015
	Ofloxacin	0.03–0.5	0.03	0.06
	Norfloxacin	≤0.015–0.12	0.15	0.12
	Cefotaxime	0.03–1	0.12	0.5
	Imipenem	0.12–2	0.12	0.25
Proteus mirabilis (25)	CI-934	0.03–1	0.5	1
	Ciprofloxacin	≤0.015–0.12	≤0.015	0.015
	Ofloxacin	0.03–2	0.06	0.25
	Norfloxacin	0.03–0.12	0.03	0.06
	Cefotaxime	≤0.015–0.06	≤0.015	0.03
	Imipenem	0.03–4	0.5	2
Proteus vulgaris[a,b] (20)	CI-934	0.03–4	0.25	1
	Ciprofloxacin	≤0.015–1	0.03	0.5
	Ofloxacin	0.03–0.5	0.03	0.25
	Norfloxacin	≤0.015–4	0.03	2
	Cefotaxime	0.03–32	0.06	2
	Imipenem	0.12–2	0.5	8
Providencia rettgeri[a,b] (15)	CI-934	0.5–8	2	8
	Ciprofloxacin	<0.03–0.25	0.03	0.25
	Ofloxacin	0.06–4	0.25	2
	Norfloxacin	0.12–2	0.5	2
	Cefotaxime	0.06–2	0.5	2
	Imipenem	1–4	1	2
Providencia stuartii[a,b] (20)	CI-934	0.12–8	1	2
	Ciprofloxacin	≤0.015–1	0.12	1
	Ofloxacin	0.12–8	0.5	4
	Norfloxacin	0.03–2	0.5	0.5
	Cefotaxime	≤0.015–4	0.25	2
	Imipenem	0.5–2	1	2
Morganella morganii[a,b] (20)	CI-934	0.12–1	0.12	0.5
	Ciprofloxacin	≤0.015–0.06	0.015	0.03
	Ofloxacin	≤0.015–0.25	0.06	0.12
	Norfloxacin	≤0.015–0.5	0.06	1
	Imipenem	0.06–4	1	1
Salmonella spp. (25)	CI-934	0.03–0.25	0.25	0.25
	Ciprofloxacin	≤0.015–0.25	<0.015	0.015
	Ofloxacin	0.03–1	0.06	0.12
	Norfloxacin	≤0.015–1	<0.015	0.06
	Cefotaxime	≤0.015–1	0.06	0.25
	Imipenem	0.06–0.25	0.06	0.25
Shigella spp. (20)	CI-934	0.06–2	0.12	0.5
	Ciprofloxacin	≤0.015–0.25	≤0.015	≤0.015
	Ofloxacin	0.03–1	0.03	0.03
	Norfloxacin	≤0.015–1	≤0.015	0.03
	Cefotaxime	≤0.15–12	≤0.015	0.06
	Imipenem	≤0.06–0.5	≤0.06	0.25
Yersinia enterocolitica (16)	CI-934	≤0.03–0.5	0.12	0.5
	Ciprofloxacin	≤0.015	≤0.015	≤0.015
	Ofloxacin	0.06	0.06	0.06
	Norfloxacin	≤0.03–0.06	<0.03	0.06
	Cefotaxime	≤0.06–2	≤0.06	≤0.06
	Imipenem	0.03–0.25	0.12	0.25
Aeromonas hydrophila[a,b] (13)	CI-934	0.015–0.12	0.06	0.12
	Ciprofloxacin	≤0.015	≤0.015	≤0.015
	Ofloxacin	0.015–0.06	0.015	0.015
	Norfloxacin	0.015–0.03	0.015	0.03
	Cefotaxime	0.06–16	<0.06	16
	Imipenem	0.12–4	0.5	2
Pseudomonas aeruginosa[a,b] (30)	CI-934	4–64	8	16
	Ciprofloxacin	0.06–1	0.06	1
	Ofloxacin	0.5–8	1	2
	Norfloxacin	0.25–4	0.25	2
	Cefotaxime	8–>128	64	>128
	Imipenem	1–32	4	8
Pseudomonas spp.[a,b,c] (11)	CI-934	1–16	8	16
	Ciprofloxacin	0.015–4	0.5	4
	Ofloxacin	0.12–2	1	4
	Norfloxacin	0.12–16	4	16
	Cefotaxime	1–>128	16	>128
	Imipenem	0.25–>128	2	>128

Continued

TABLE 2—*Continued*

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TABLE 2—*Continued*

Organism (No. of isolates tested)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
"*Acinetobacter anitratum*"[a,b] (21)	CI-934	0.25–4	0.5	1
	Ciprofloxacin	0.12–2	0.12	0.25
	Ofloxacin	0.12–2	0.25	0.5
	Norfloxacin	0.12–64	4	16
	Cefotaxime	8–>64	16	64
	Imipenem	0.12–0.25	0.12	0.25
Haemophilus influenzae[d] (17)	CI-934	0.03	0.03	0.03
	Ciprofloxacin	≤0.03	≤0.03	≤0.03
	Ofloxacin	≤0.03	≤0.03	≤0.03
	Norfloxacin	≤0.03	≤0.03	≤0.03
	Cefotaxime	≤0.03	≤0.03	≤0.03
	Imipenem	0.012–2	0.25	0.5
Branhamella catarrhalis (12)	CI-934	0.03–0.12	0.12	0.12
	Ciprofloxacin	0.015–0.5	0.03	0.5
	Ofloxacin	0.25–4	0.25	1
	Norfloxacin	0.5–4	1	4
	Cefotaxime	<0.03–0.5	0.12	0.25
	Imipenem	<0.03–0.12	<0.03	0.06
Neisseria gonorrhoeae[d] (15)	CI-934	≤0.015–0.12	<0.015	0.03
	Ciprofloxacin	≤0.015	≤0.015	≤0.015
	Ofloxacin	≤0.15	≤0.015	0.03
	Norfloxacin	≤0.015	≤0.015	0.03
	Cefotaxime	≤0.015	≤0.015	≤0.015
	Imipenem	≤0.015–0.25	≤0.015	0.12
Bacteroides fragilis[a,b] (29)	CI-934	4–16	8	16
	Ciprofloxacin	4–64	4	16
	Ofloxacin	4–64	4	8
	Norfloxacin	8–64	4	64
	Cefoxitin	4–16	4	16
	Imipenem	<0.06–0.25	0.06	0.12
Bacteroides spp.[a,b] (15)	CI-934	2–64	16	16
	Ciprofloxacin	2–64	4	8
	Ofloxacin	2–64	4	8
	Norfloxacin	2–64	32	64
	Cefoxitin	2–64	4	16
	Imipenem	0.06–0.5	0.25	0.5

[a] MIC_{50} >16 μg/ml for ampicillin.
[b] MIC_{50} >16 μg/ml for cephalothin.
[c] *P. maltophila* (six isolates) and *P. cepacia* (five isolates).
[d] Included 10 beta-lactamase-positive isolates.

norfloxacin against the gram-positive species and more active than ciprofloxacin against some isolates. CI-934 inhibited staphylococci, including methicillin-resistant strains, at concentrations ≤0.25 μg/ml. Staphylococci resistant to imipenem and to cephalothin also were inhibited. CI-934 was 8- to 32-fold more active against the hemolytic streptococcal groups A, B, C, F, and G than the other quinolone agents. It was less active than imipenem or cefotaxime against a number of the streptococci. CI-934 was also more active than the other quinolones against *Streptococcus pneumoniae*, *Streptococcus faecalis*, and *Streptococcus bovis*, with MIC_{50}s of 0.12 μg/ml in contrast to MICs of 1 to 8 μg/ml for the other agents. *Listeria monocytogenes* and the *Corynebacterium* JK species resistant to cephalothin, cefotaxime, and imipenem were inhibited by relatively low concentrations of CI-934.

Against the members of the *Enterobacteriaceae*, CI-934 had activity comparable to that of norfloxacin (Table 2). In general it was less active than ofloxacin and ciprofloxacin against most enteric microorganisms. Similar to the other quinolones, CI-934 inhibited *Enterobacter cloacae* and *Citrobacter freundii*, both of which were resistant to cefotaxime. The activity of CI-934 against *Haemophilus influenzae* and *Neisseria gonorrhoeae*, including beta-lactamase-producing isolates, was comparable to that of the other quinolones. The species against which CI-934 was consistently less active than the other quinolones or other agents tested were *Pseudomonas aeruginosa*, other *Pseudomonas* species, and *Providencia rettgeri*.

Effect of growth conditions. The effect of the type of agar growth medium used was determined for 10 strains each of *Staphylococcus aureus*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Escherichia coli*, *Citrobacter freundii*, and *Serratia marcescens*. The MICs were within a twofold range for Mueller-Hinton, brain heart infusion, nutrient, and brucella agars. The MICs for the aforementioned organisms determined under anaerobic conditions were either identical or within a twofold range of those determined aerobically. At pH 8 compared with pH 7, the MICs were fourfold lower for 15 of the 60 isolates. In contrast, at pH 6, as compared with pH 7, the MICs of the aforementioned organisms were four- to eightfold for all isolates and as much as 32- to 64-fold greater for half of the isolates (Table 3).

The effect of inoculum size on MICs was determined for the 60 aforementioned isolates with an agar medium. At 10^3 and 10^5 CFU there were no major differences in MICs. At 10^7 CFU compared with 10^5 CFU, 13% (eight) of the isolates had a fourfold increase in the MIC, but none of the isolates had an MIC increase greater than fourfold.

The activity of CI-934 was determined in urine (pH 5.5), sterilized by filtration through a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.) and compared to the activity in Mueller-Hinton broth (pH 7.4). There was an eightfold increase in MICs for single isolates of *S. aureus*, *E. coli*, *Proteus mirabilis*, *K. pneumoniae*, *Citrobacter freundii*, and *S. marcescens*. Increasing the concentration of Mg^{2+} from the 0.3 mM in unsupplemented Mueller-Hinton broth to 9 mM, the concentration of Mg^{2+} in urine, caused the CI-934 MICs to increase 4- to 16-fold for all of the aforementioned organisms. However, at 1.5 mM Mg^{2+}, a concentration found in normal serum, the CI-934 MICs were identical to those in Mg^{2+}-supplemented Mueller-Hinton broth. When the pH of Mueller-Hinton broth was lowered to 5.5 and the Mg^{2+} concentration was increased to 9 mM, the MICs for the aforementioned organisms were similar to those in urine.

In contrast, when the activity of CI-934 was determined in 50% heat-inactivated human serum, there was no increase in the MICs for single isolates of *S. aureus*, *Streptococcus faecalis*, *E. coli*, *K. pneumoniae*, and *Enterobacter aero-*

TABLE 3. Effect of pH on MICs of CI-934

Organism[a]	Mode MIC (μg/ml) at pH:		
	6	7	8
Staphylococcus aureus	2	0.12	0.06
Escherichia coli	16	0.25	0.06
Klebsiella pneumoniae	16	1	0.12
Enterobacter aerogenes	16	0.5	0.12
Citrobacter freundii	16	0.5	0.06
Serratia marcescens	16	2	0.25

[a] 10 isolates of each species.

genes, or for *Streptococcus pneumoniae* and *H. influenzae* determined with Schaedler broth (BBL).

Bactericidal activity. The MBCs for CI-934 determined in Mueller-Hinton broth against gram-positive and gram-negative species were identical to or twofold greater than the MICs for *S. aureus*, *Streptococcus faecalis*, *E. coli*, *K. pneumoniae*, and *Citrobacter freundii* (ten isolates of each were tested).

Development of resistance. Organisms were repeatedly subcultured in broth containing CI-934. There were 8- to 64-fold increases in the MICs for *S. aureus*, *Enterobacter cloacae* (data not shown), *Citrobacter freundii*, *Enterobacter aerogenes*, and *K. pneumoniae* (Table 4). There were comparable increases in the MICs of ofloxacin and ciprofloxacin. The increases in the MICs were stable, within a twofold dilution, after subculture three times on antibiotic-free medium. The increases in the MICs of other quinolones are also shown in Table 4.

The frequency of resistance to eight times the MIC of CI-934 was $<10^{-9}$ for *E. coli*, *K. pneumoniae*, *Citrobacter freundii*, *S. marcescens*, *Proteus vulgaris*, *S. aureus*, and *Streptococcus faecalis* (two isolates of each, with an inoculum of 1×10^9 to 5×10^9 CFU).

Synergy studies. The effects of combinations of CI-934 with gentamicin, rifampin, chloramphenicol, and penicillin G were studied with *S. aureus*, *Staphylococcus epidermidis*, *S. marcescens*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Providencia stuartii* (10 isolates of each species). The combinations of CI-934 with the other antimicrobial agents did not show antagonism or synergy. No synergy was found for the combination of CI-934 and either ticarcillin or piperacillin against *Pseudomonas aeruginosa* (12 isolates).

TABLE 4. Comparative activity of CI-934 and other quinolones against organisms exposed repeatedly to subinhibitory concentrations of CI-934[a]

Organism	Isolate	MIC (μg/ml) of:			
		CI-934	Ciprofloxacin	Ofloxacin	Norfloxacin
Escherichia coli	Parent	0.06	0.015	0.06	0.06
	Mutant	1	0.5	1	1
Klebsiella pneumoniae	Parent	0.12	0.015	0.06	0.12
	Mutant	8	2	8	8
Enterobacter aerogenes	Parent	0.25	0.06	0.12	0.12
	Mutant	8	1	4	8
Citrobacter freundii	Parent	0.25	0.06	0.25	0.25
	Mutant	4	1	4	4
Proteus mirabilis	Parent	0.12	0.06	0.12	0.25
	Mutant	4	2	8	8
Pseudomonas aeruginosa	Parent	8	0.25	4	4
	Mutant	64	4	16	32
Staphylococcus aureus	Parent	0.25	0.25	0.5	0.5
	Mutant	4	2	4	4

[a] Organisms were selected as described in Materials and Methods by repeated subculture in increasing concentrations of CI-934 over 12 days.

DISCUSSION

There has been increased interest in the quinolones as antibacterial agents in the past few years (8, 19, 20). CI-934 is a novel 7-substituted quinolone antibacterial agent. This study demonstrated that its in vitro activity was comparable to that of agents such as norfloxacin and ofloxacin against the majority of the members of the *Enterobacteriaceae*. These results are similar to those of Cohen et al. (5), who found CI-934 MICs of 0.2 μg/ml for staphylococci and 0.4 μg/ml for *Streptococcus pneumoniae* and other streptococci, with the exception of enterococcal species. They also found relatively poor activity for CI-934 against *Pseudomonas aeruginosa* and other pseudomonads. In general in this study CI-934 was less active than ciprofloxacin against members of the *Enterobacteriaceae*. Like the other quinolones, CI-934 inhibited microorganisms such as the *Enterobacter* and *Citrobacter* species which are resistant to expanded-spectrum cephalosporins. CI-934 had appreciably less activity against *Pseudomonas aeruginosa* than did the other quinolones with MICs in the range of 8 to 16 μg/ml (1, 6, 8, 9, 11, 17). Interestingly, the MIC_{90} against "*Acinetobacter anitratum*" (*Acinetobacter calcoaceticus*) was 1 μg/ml; CI-934 was superior to markedly norfloxacin but less active than ofloxacin or ciprofloxacin.

The major improvement in activity shown by CI-934 over previous agents of this type was against hemolytic streptococci and *Streptococcus pneumoniae*. Fifty percent of the streptococci and methicillin-resistant staphylococci were inhibited by 0.12 μg/ml. The activity of quinolones against gram-positive species has been of considerable concern because the MICs of quinolones against streptococci, including *Streptococcus pneumoniae*, often are ≥2 μg/ml. This would be of particular benefit in certain respiratory tract infections in which *Streptococcus pneumoniae*, as well as *H. influenzae* or *Branhamella catarrhalis*, may be involved and in soft tissue infections where streptococci, staphylococci, and members of the *Enterobacteriaceae* may be simultaneously present.

We did not test the activity of CI-934 against penicillin-resistant *Streptococcus pneumoniae*. Gombert and Aulicino (7) reported that ciprofloxacin inhibited these species at 0.5 to 1 μg/ml. We noted, in contrast to Cohen et al. (5), that CI-934 had MICs of 8 to 16 μg/ml against *Clostridium difficile* strains, similar to the other quinolones.

CI-934 had in vitro properties similar to those of other quinolone antimicrobial agents in that there was a minor effect of inoculum size on inhibitory activity (3, 4, 11). Like that of the other quinolones, the activity of CI-934 was reduced in the presence of magnesium and at an acid pH, conditions which both exist in urine, and its activity in urine was reduced as has been shown for other agents of this type (11, 14). In contrast, there was no effect of serum on the inhibitory activity of CI-934. Cohen et al. (5) noted that serum and bile had a minimal effect on the MICs of CI-934, and MICs were similar under aerobic and anaerobic conditions. Quinolones have been effective in eradicating most urinary pathogens because of the relatively high (>100 μg/ml) urinary concentrations (20). Thus, the magnesium and pH effects do not seem to interfere with their activity in such infections.

In this study, as in other studies, organisms which had major increases in MICs could be selected by repeated stepwise exposure to the quinolone. As others have shown, increases in MICs comparable to those for other quinolones are found for these organisms (1, 12, 16, 17). The frequency of resistance to CI-934, similar to that reported for other quinolone agents, was low (13, 15). The precise meaning of this stepwise increase in MICs will have to be determined from future clinical investigations. Mouse protection studies

reported by J. C. Sesnie, M. A. Shapiro, C. L. Heifetz, and T. F. Mich (Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 82, 1984) have shown that CI-934 is highly effective in protecting mice against streptococcal infections. If the pharmacokinetic profile of CI-934 is reasonable, its anti-gram-positive and anti-gram-negative activity may make it a useful agent to treat respiratory tract and cutaneous infections. Further pharmacological and clinical studies are needed to determine the potential use of CI-934.

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