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Comparative in Vitro Activity of Norfloxacin (MK-0366) and Ten Other Oral Antimicrobial Agents Against Urinary Bacterial Isolates

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The in vitro activity of a new oral antimicrobial agent, norfloxacin (MK-0366), was compared with those of nalidixic acid, nitrofurantoin, co-trimoxazole, trimethoprim, sulfamethoxazole, cinoxacin, tetracycline, ampicillin, carbenicillin, and cephalexin against 628 urinary bacterial isolates. Norfloxacin was the most active antimicrobial agent tested against the gram-negative bacilli. It was less active than a few of the other antimicrobial agents against enterococci and *Staphylococcus aureus*.

Norfloxacin (MK-0366 or AM-715) is an antibacterial organic acid structurally related to nalidixic acid. Unlike nalidixic acid, norfloxacin exhibits a very broad spectrum of antibacterial activity against both gram-positive and gram-negative bacteria, including *Pseudomonas aeruginosa* and *Serratia marcescens* (2). In experimental systemic and urinary bladder-kidney infections in mice, norfloxacin was found to be five times as active as pipemidic acid and nalidixic acid (1). This study was conducted to evaluate the in vitro activity of norfloxacin against 628 urinary tract bacterial pathogens. Since norfloxacin is an oral antimicrobial agent, we compared it with 10 other oral antimicrobial agents currently available for use in the treatment of urinary tract infections.

Laboratory reference standards were provided by the following pharmaceutical companies: Merck Institute for Therapeutic Research (norfloxacin), Bristol Laboratories (ampicillin), Eli Lilly & Co. (cephalexin and cinoxacin), Burroughs Wellcome Co. (co-trimoxazole and trimethoprim), Hoffmann-La Roche, Inc. (sulfamethoxazole), Roerig (carbenicillin), Sterling-Winthrop Research Institute (nalidixic acid), Norwich-Eaton Pharmaceuticals (nitrofurantoin), and Pfizer Inc. (tetracycline). The antimicrobial agents were supplied as dry powders and stored at −20°C.

A total of 628 strains of bacteria were tested. All of the organisms were isolated from urine specimens obtained from patients at the Hennepin County Medical Center, Minneapolis, Minn., in 1981. The organisms were identified and stocked in the Clinical Microbiology Laboratory by standard methodology. The organisms were distributed as follows: 200 *Escherichia coli*, 100 *Klebsiella* spp., 50 *Enterobacter* spp., 50 *Proteus mirabilis*, 25 indole-positive *Proteus* spp., 23 *Citrobacter* spp., 48 *P. aeruginosa*, 10 *S. marcescens*, 50 enterococci, 22 penicillin G-resistant *Staphylococcus aureus*, and 50 *Staphylococcus epidermidis*. All organisms were maintained in stock by ultrafreezing methods.

The minimum inhibitory concentrations (MICs) of the antimicrobial agents were determined by the agar dilution technique. Twofold dilutions of the antimicrobial agents, from 0.125 to 128 μg/ml, were distributed into Mueller-Hinton agar (Difco Laboratories). The frozen isolates were thawed, grown overnight on Mueller-Hinton agar, and then suspended in Mueller-Hinton broth until the turbidity matched that of a 0.5 McFarland standard. A sample (1 μl) of the bacterial suspension (10^5 colony-forming units) was inoculated onto the antimicrobial agent-containing plates with a Steers replicator (3). *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *P. aeruginosa* ATCC 27853 were included as control organisms. The plates were incubated for 18 to 24 h at 35°C. The MIC was the lowest concentration of antimicrobial agent that inhibited visible growth.

Table 1 compares the MICs of the antimicrobial agents against 628 urinary isolates. Norfloxacin was highly active against the gram-negative isolates. It inhibited 90% of *E. coli*, *Enterobacter* spp., *P. mirabilis*, *Proteus* spp., and *Citrobacter* spp. at concentrations of ≤0.05 μg/ml. It inhibited 90% of *S. marcescens* and *Klebsiella* spp. at concentrations of ≤2 μg/ml. It was

TABLE 1. Comparative MICs of various antimicrobial agents against urinary isolates

Organism[a]	Antimicrobial agent	MIC (μg/ml)		
		Range	For following % of strains:	
			50	90
E. coli (200)	Norfloxacin	0.125–2	0.125	0.25
	Nalidixic acid	1–128	4	8
	Nitrofurantoin	8–>128	128	≥128
	Co-trimoxazole	0.25–8	0.5	2
	Trimethoprim	0.125–32	0.5	1
	Sulfamethoxazole	4–>128	32	>128
	Cinoxacin	1–32	2	4
	Tetracycline	2–>128	4	128
	Ampicillin	1–>128	4	128
	Carbenicillin	1–>128	8	>128
	Cephalexin	2–128	8	16
Klebsiella spp. (100)	Norfloxacin	0.125–8	0.5	2
	Nalidixic acid	2–>128	8	32
	Nitrofurantoin	32–>128	>128	>128
	Co-trimoxazole	0.25–>128	1	4
	Trimethoprim	0.25–32	0.5	1
	Sulfamethoxazole	2–>128	64	>128
	Cinoxacin	2–128	8	8
	Tetracycline	1–>128	8	16
	Ampicillin	2–>128	32	>128
	Carbenicillin	2–>128	>128	>128
	Cephalexin	2–>128	8	16
Enterobacter spp. (50)	Norfloxacin	0.125–1	0.25	0.25
	Nalidixic acid	1–32	4	16
	Nitrofurantoin	16–>128	>128	>128
	Co-trimoxazole	0.25–>128	1	4
	Trimethoprim	0.25–>128	2	2
	Sulfamethoxazole	8–>128	32	>128
	Cinoxacin	2–32	4	16
	Tetracycline	2–>128	8	16
	Ampicillin	4–>128	>128	>128
	Carbenicillin	1–>128	8	>128
	Cephalexin	1–>128	>128	>128
P. mirabilis (50)	Norfloxacin	0.125–2	0.25	0.25
	Nalidixic acid	2–128	8	16
	Nitrofurantoin	128–>128	128	≥128
	Co-trimoxazole	0.50–>128	1	4
	Trimethoprim	1–>128	4	32
	Sulfamethoxazole	32–>128	>128	>128
	Cinoxacin	2–>128	8	64
	Tetracycline	4–>128	128	≥128
	Ampicillin	0.5–>128	2	8
	Carbenicillin	0.5–>128	1	2
	Cephalexin	8–64	16	32
Proteus spp. (25)	Norfloxacin	0.125–8	0.125	0.5
	Nalidixic acid	2–>128	4	16
	Nitrofurantoin	64–>128	128	≥128
	Co-trimoxazole	0.5–>128	2	4
	Trimethoprim	1–>128	4	32
	Sulfamethoxazole	16–>128	>128	>128
	Cinoxacin	2–>128	8	64
	Tetracycline	1–>128	32	>128
	Ampicillin	16–>128	128	≥128
	Carbenicillin	1–>128	4	32
	Cephalexin	16–>128	>128	>128

TABLE 1—*Continued*

Organism[a]	Antimicrobial agent	MIC (μg/ml)		
		Range	For following % of strains:	
			50	90
Citrobacter spp. (23)	Norfloxacin	0.125–1	0.125	0.5
	Nalidixic acid	1–>128	4	16
	Nitrofurantoin	32–>128	64	>128
	Co-trimoxazole	0.25–>128	0.5	1
	Trimethoprim	0.25–>128	0.5	1
	Sulfamethoxazole	16–>128	64	>128
	Cinoxacin	2–128	8	16
	Tetracycline	2–>128	4	8
	Ampicillin	8–>128	64	>128
	Carbenicillin	2–>128	8	>128
	Cephalexin	8–>128	128	≥128
P. aeruginosa (48)	Norfloxacin	0.5–16	2	4
	Nalidixic acid	32–>128	>128	>128
	Nitrofurantoin	>128	>128	>128
	Co-trimoxazole	32–>128	>128	>128
	Trimethoprim	32–>128	>128	>128
	Sulfamethoxazole	64–>128	>128	>128
	Cinoxacin	32–>128	>128	>128
	Tetracycline	16–>128	64	>128
	Ampicillin	64–>128	>128	>128
	Carbenicillin	1–>128	64	>128
	Cephalexin	>128	>128	>128
S. marcescens (10)	Norfloxacin	0.125–2	0.25	2
	Nalidixic acid	2–8	2	4
	Nitrofurantoin	16–>128	>128	>128
	Co-trimoxazole	0.25–4	2	2
	Trimethoprim	0.25–8	2	4
	Sulfamethoxazole	32–>128	>128	>128
	Cinoxacin	2–16	8	16
	Tetracycline	2–>128	128	≥128
	Ampicillin	0.5–>128	64	>128
	Carbenicillin	1–>128	8	>128
	Cephalexin	4–>128	>128	>128
Enterococci (50)	Norfloxacin	1–8	4	8
	Nalidixic acid	>128	>128	>128
	Nitrofurantoin	8–32	16	32
	Co-trimoxazole	0.125–4	0.25	0.5
	Trimethoprim	0.125–32	0.25	1
	Sulfamethoxazole	0.25–>128	>128	>128
	Cinoxacin	64–>128	>128	>128
	Tetracycline	0.5–>128	64	128
	Ampicillin	0.25–2	2	2
	Carbenicillin	1–64	32	64
	Cephalexin	2–>128	128	≥128
S. aureus (22)	Norfloxacin	0.5–8	1	4
	Nalidixic acid	16–64	64	64
	Nitrofurantoin	32–>128	32	64
	Co-trimoxazole	0.5–1	0.5	1
	Trimethoprim	0.25–1	0.5	1
	Sulfamethoxazole	16–>128	32	>128
	Cinoxacin	64–>128	128	≥128
	Tetracycline	0.5–>128	2	4
	Ampicillin	0.25–128	4	32
	Carbenicillin	2–32	8	16
	Cephalexin	4–16	8	16

TABLE 1—*Continued*

Organism[a]	Antimicrobial agent	MIC (μg/ml)		
		Range	For following % of strains:	
			50	90
S. epidermidis (50)	Norfloxacin	0.5–8	1	2
	Nalidixic acid	32–>128	64	128
	Nitrofurantoin	8–64	32	64
	Co-trimoxazole	0.125–>128	0.5	128
	Trimethoprim	0.125–>128	0.5	>128
	Sulfamethoxazole	0.5–>128	128	≥128
	Cinoxacin	64–>128	128	≥128
	Tetracycline	0.5–>128	2	>128
	Ampicillin	0.125–>128	1	32
	Carbenicillin	0.5–>128	4	128
	Cephalexin	1–128	4	64

[a] The number of isolates is given in parentheses.

the most active antimicrobial agent tested against all of the above-named organisms. Co-trimoxazole and trimethoprim were the next most active antimicrobial agents against the *Enterobacteriaceae*. Against *P. aeruginosa*, norfloxacin inhibited 90% of the isolates at a concentration of 4 μg/ml, whereas all of the other antimicrobial agents inhibited 90% of the isolates at concentrations of >128 μg/ml.

Against the gram-positive cocci, norfloxacin inhibited 90% of enterococci at 8 μg/ml, 90% of *S. aureus* at 4 μg/ml, and 90% of *S. epidermidis* at 2 μg/ml. It was the most active antimicrobial agent tested against *S. epidermidis*. However, ampicillin, co-trimoxazole, and trimethoprim were more active than norfloxacin against enterococci. Co-trimoxazole and trimethoprim were also more active than norfloxacin against *S. aureus*. Norfloxacin was not as active against gram-positive cocci as it was against gram-negative bacilli.

Recently, there have been four other comparisons of the in vitro activity of norfloxacin against bacteria commonly recovered from urinary tract infections (R. L. Sweet, M. Ohm-Smith, and W. K. Hadley, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 21st, Chicago, Ill., abstr. no. 563, 1981; J. R. Dipersio and T. L. Krafczyk, 21st ICAAC, abstr. no. 564; I. Wilkinson and L. O. Gentry, 21st ICAAC, abstr. no. 565; J. Tenney, J. Klaff, B. Clayman, and J. Warren, 21st ICAAC, abstr. no. 566). As with our study, all found norfloxacin to be consistently more active than the oral antimicrobial agents currently available for use in the treatment of urinary tract infections. After a single oral dose of 800 mg, peak serum levels of norfloxacin approach 2.5 μg/ml, with a half-life of more than 4 h. Urinary concentrations under these conditions exceed 350 μg/ml (H. H. Gadebusch, Merck Sharp & Dohme, personal communication). This level in the urine is many times greater than the MIC of norfloxacin for all of the urinary isolates tested in our study. A preliminary report from Japan has shown that norfloxacin is effective in the treatment of urological infections in humans (Y. Nishimura, H. Kishi, O. Tsukada, T. Tominaga, and T. Niijima, 20th ICAAC, New Orleans, La., abstr. no. 76, 1980).

In summary, our in vitro results demonstrate that norfloxacin is highly active against urinary tract bacterial pathogens. Clinical trials to verify its efficacy as an oral antimicrobial agent for urinary tract infections are indicated.

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