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Antibacterial Activity of Norfloxacin

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Norfloxacin, a new quinoline derivative, was studied in vitro, and determinations of agar dilution minimal inhibitory concentrations (MICs) and broth dilution MICs and MBCs were made. Nalidixic acid and cinoxacin were used as comparative agents. Norfloxacin was found to be extremely active against all strains tested of *Escherichia coli, Klebsiella* spp., *Proteus mirabilis*, indole-positive *Proteus* spp. *Serratia* spp., *Citrobacter* spp., and *Enterobacter* spp., with MICs normally below 1 μg/ml. It also was found to be highly active against *Pseudomonas aeruginosa, Staphylococcus saprophyticus*, and enterococci, which are all resistant to nalidixic acid and cinoxacin. The MICs for norfloxacin obtained by broth dilution were slightly higher than those obtained by agar dilution, whereas the reverse was true for nalidixic acid and cinoxacin. The MBCs of norfloxacin were only slightly higher than the MICs, even at high inocula. The in vitro activity of norfloxacin was not dependent on the inoculum size, whereas both the MICs and the MBCs of nalidixic acid increased markedly for many of the strains tested when the inoculum was increased in broth dilution from 10^3 to 10^6 colony-forming units per ml. Norfloxacin seems to be a promising antibacterial agent for the treatment of urinary tract infections, especially those caused by *Pseudomonas* spp. and other species today requiring the use of injectible antibiotics.

Norfloxacin is a newly developed antibacterial agent that is chemically related to nalidixic acid and cinoxacin. Norfloxacin has been demonstrated in vitro to be extremely active against *Enterobacteriaceae*, with minimal inhibitory concentrations (MICs) for *Escherichia coli, Klebsiella* spp., and *Proteus* spp. normally below 1 μg/ml (2–4, 6–8). In addition, norfloxacin seems to include in its spectrum species which are resistant to nalidixic acid, e.g., *Pseudomonas* spp. and enterococci (2, 4, 8). The present investigation was undertaken to study the in vitro activity of norfloxacin against gram-positive and gram-negative aerobic bacteria and to evaluate the effect of the technique used for MIC determinations on the susceptibility of these species to norfloxacin, nalidixic acid, and cinoxacin.

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MATERIALS AND METHODS

Bacterial strains. All strains tested were clinical isolates typed by routine bacteriological techniques. Some strains had been stored frozen on deep agar before the study, but none of them had been recultivated more than twice.

Antibiotics. Norfloxacin (MK-0366) was obtained from Merck & Co. Inc., Rahway, N.J.; nalidixic acid was obtained from Sterling-Winthrop AB, Skärholmen, Sweden; and cinoxacin was obtained from Eli Lilly & Co., Stockholm, Sweden. All drugs were supplied as dry powder with known potency for investigational use.

Medium. In all experiments, Mueller-Hinton agar or broth (pH 7.4) made from the same batch of medium (Difco Laboratories, Detroit, Mich.) was used.

Determinations of MICs and MBCs. Overnight broth cultures were used in all MIC determinations. Agar dilution MICs were determined by using agar plates with an incorporated antibiotic in twofold dilutions. Overnight broth cultures of the strains to be tested were diluted to give an inoculum of 10^4 colony-forming units (CFU) per application when 0.001-ml samples were applied to the agar surface with a modified Steers replicator. MICs were determined after overnight incubation at 37°C and were defined as the lowest antibiotic concentrations completely inhibiting growth. Broth dilution MICs were determined with overnight broth cultures of the strains to be tested. The cultures were diluted to give inoculum sizes of 10^3 and 10^6 CFU/ml. Antibiotics were added in twofold dilutions, and each test tube contained 1 ml. The MIC was determined after overnight incubation at 37°C and was defined as the lowest antibiotic concentration inhibiting visual bacterial growth. Minimal bactericidal concentrations (MBCs) were assayed by transferring 0.1 ml from each tube without visible growth in the

TABLE 1. Agar dilution MICs of norfloxacin, nalidixic acid, and cinoxacin

Species (no. of strains)	Agent[a]	MIC (μg/ml)		
		Range	50%	90%
E. coli (40)	NOR	0.03–2	0.06	0.5
	NAL	1–>32	4	>32
	CIN	2–>32	4	>32
K. pneumoniae (20)	NOR	0.03–4	0.125	1
	NAL	2–>32	4	>32
	CIN	2–>32	4	>32
Enterobacter spp. (10)	NOR	0.06–0.5	0.125	0.5
	NAL	8–>32	8	>32
	CIN	4–>32	8	>32
Proteus mirabilis (20)	NOR	0.125–2	0.125	2
	NAL	4–>32	4	>32
	CIN	4–>32	8	>32
Proteus spp., indole + (20)	NOR	0.06–0.125	0.06	0.125
	NAL	4–>32	8	>32
	CIN	4–>32	8	>32
Citrobacter spp. (5)	NOR	0.06–0.5	0.125	0.5
	NAL	4–8	4	8
	CIN	4–8	4	8
Acinetobacter spp. (5)	NOR	2–8	4	8
	NAL	8–>32	>32	>32
	CIN	8–>32	>32	>32
Serratia spp. (11)	NOR	0.125–1	0.25	1
	NAL	2–>32	4	>32
	CIN	4–>32	8	>32
Pseudomonas aeruginosa (20)	NOR	0.5–8	1	5
	NAL	>32	>32	>32
	CIN	>32	>32	>32
Enterococci (20)	NOR	0.25–4	2	4
	NAL	>32	>32	>32
	CIN	>32	>32	>32
S. saprophyticus (20)	NOR	0.25–4	2	4
	NAL	>32	>32	>32
	CIN	>32	>32	>32

[a] NOR, Norfloxacin; NAL, nalidixic acid; CIN, cinoxacin.

broth dilution MIC series to agar plates and were defined as the lowest antibiotic concentrations at which no bacterial growth occurred after incubation overnight at 37°C.

RESULTS

The accumulated percentages of bacterial strains inhibited by norfloxacin with the agar dilution technique are shown in Table 1.

Agar dilution MICs. Of the strains of *Staphylococcus saprophyticus* tested, 90% were inhibited by 4 μg of norfloxacin per ml; only 1 of 20 strains had an MIC of 8 μ/ml. The drug was more active against enterococci; 2 μg/ml inhibited the growth of 90% of the strains.

When *E. coli* was tested, only 5 of 40 strains required norfloxacin concentrations above 0.125 μg/ml for inhibition of growth. The MICs were 2 μg/ml for two strains, 1 μg/ml for one strain, and 0.5 μg/ml for two strains. Similar results were obtained with *Klebsiella* and *Enterobacter* spp., *Proteus mirabilis*, indole-positive *Proteus* spp., *Citrobacter* spp. (only five strains tested), and *Serratia* spp., for which MICs above 0.5 μg/ml were found for only 9 of 85 strains tested. The five strains of *Acinetobacter* spp. were inhibited by 0.25 to 4 μg/ml, whereas the MICs of norfloxacin when 20 strains of *Pseudomonas aerugino-*

TABLE 2. Effects of inoculum size and technique used on MICs and MBCs of norfloxacin, nalidixic acid, and cinoxacin

Species (no. of strains)	Technique[a]	Inoculum[b]	MIC (μg/ml) range for[c]:			MBC (μg/ml) range for:		
			NOR	NAL	CIN	NOR	NAL	CIN
E. coli (5)	AD	10^4	0.03–0.125	2–8	2–8			
	BD	10^3	0.125–0.5	2–8	2–64	0.125–0.5	8–32	2–64
	BD	10^6	0.25–1	2–8	8–64	0.5–1	16–64	8–>64
K. pneumoniae (5)	AD	10^4	0.125–0.25	4–8	0.25–4			
	BD	10^3	0.125–0.5	4–8	4–16	0.25–1	8–16	8–32
	BD	10^6	0.5–4	8–64	4–32	1–4	128–128	16–32
Enterobacter spp. (5)	AD	10^4	0.125–0.25	4–8	4–16			
	BD	10^3	0.25–1	4–8	4–16	0.25–4	16–>128	16–32
	BD	10^6	0.25–1	64	8–64	0.5–2	>128	32–128
Proteus mirabilis (5)	AD	10^4	0.125–0.25	4–16	4–64			
	BD	10^3	0.06	4–8	2–4	0.125	8–>128	4–16
	BD	10^6	0.125–0.25	16–128	4–8	0.5–2	>128	8–32
Proteus spp., indole + (5)	AD	10^4	0.06–0.126	4–64	4–64			
	BD	10^3	0.03–1	8–64	2–128	0.03–1	32–128	2–128
	BD	10^6	0.125–1	64	2–>128	0.125–1	128–>128	8–128
Acinetobacter spp. (4)	AD	10^4	0.25–4	8–64	8–64			
	BD	10^3	0.5–4	4–8	32–64	2–8	64–128	64–>128
	BD	10^6	2–8	16	64–128	4–128	64–>128	64–>128
Citrobacter spp. (4)	AD	10^4	0.06–0.125	4–8	4–8			
	BD	10^3	0.03–0.125	2–16	4	0.03–0.125	16–128	4
	BD	10^6	0.03–1	32–64	8–16	0.125–1	<128	16–32
Serratia spp. (5)	AD	10^4	0.125–0.5	2–64	4–64			
	BD	10^3	0.25–1	2–64	2–64	0.25–2	8–>128	8–128
	BD	10^6	0.25–2	64–128	16–64	0.25–8	>128	32–128
Pseudomonas aeruginosa (5)	AD	10^4	0.5–1	NT[d]	NT			
	BD	10^3	0.5–2	NT	NT	4–16	NT	NT
	BD	10^6	2–8	NT	NT	16–32	NT	NT
Enterococci (4)	AD	10^4	2	NT	NT			
	BD	10^3	2	NT	NT	4	NT	NT
	BD	10^6	2–4	NT	NT	8–16	NT	NT
S. saprophyticus (4)	AD	10^4	1–2	NT	NT			
	BD	10^3	1	NT	NT	1–2	NT	NT
	BD	10^6	1–2	NT	NT	4	NT	NT

[a] AD, Agar dilution; BD, broth dilution.
[b] Inoculum sizes are given as CFU per application for agar dilution MICs and CFU per milliliter for broth dilution MICs and MBCs.
[c] NOR, Norfloxacin; NAL, nalidixic acid; CIN, cinoxacin.
[d] NT, Not tested.

sa were tested were 8 μg/ml for two strains and 0.5 to 4 μg/ml for the remaining strains.

When the agar dilution MICs of norfloxacin were compared with those of nalidixic acid and cinoxacin, it was obvious that the two latter agents were considerably less active (Table 1). Nalidixic acid and cinoxacin showed no obvious differences when compared with each other.

Broth dilution MICs and MBCs. Four to five strains of each species were selected for this part of the study, which, in addition to agar MIC determinations, included assays of broth dilution MICs and MBCs at two inoculum sizes, 10^3 and 10^6 CFU/ml. *Pseudomonas aeruginosa*, *S. saprophyticus*, and enterococci were not tested against nalidixic acid or cinoxacin, since all strains were resistant to these agents with MIC values of 64 μg/ml or higher.

When agar and broth dilution MICs obtained with the lower inoculum were compared, similar

or lower MICs were obtained with nalidixic acid for most of the strains, and only two strains had broth MICs of more than twice the agar MICs (Table 2). The broth MIC of norfloxacin was four times higher than the agar MIC for three strains and eight times higher for four strains. The broth MIC of cinoxacin was 32 times higher for four strains, 8 times higher for one strain, and 4 times higher for three strains.

A 1,000-fold increase of the inoculum in broth dilution MIC determinations resulted in 8- or 16-fold increases of the MIC for 17 of the strains with nalidixic acid. Such increases were seen for seven of the strains with norfloxacin and for only two strains with cinoxacin.

The MBCs were only slightly higher than the MICs when norfloxacin and cinoxacin were tested. With nalidixic acid, only four *E. coli* strains had MBCs below 64 μg/ml, and with a majority of the strains, the MBC fell outside the highest concentration tested (usually 128 μg/ml). Cinoxacin seemed slightly more bactericidal than nalidixic acid, with MBCs below 64 μg/ml at the high inoculum for all strains of *Proteus mirabilis, Klebsiella* spp., and *Citrobacter* spp. tested.

DISCUSSION

This study demonstrated that norfloxacin is extremely active against gram-negative aerobic pathogens and that it includes in its spectrum important gram-positive species causing urinary tract infections. Compared with nalidixic acid and cinoxacin, norfloxacin was more active against all strains tested with regard to both bacteriostatic and bactericidal activity. The small differences between the MICs and the MBCs of norfloxacin indicate that development of resistance against this drug might be less common than is the case with nalidixic acid. However, comparing our results with those of King et al. (6) and Neu and Labthavikul (7), we demonstrated higher MBCs than MICs for many of the strains tested, whereas only a few strains in the other studies had higher MBCs than MICs. A possible explanation for this discrepancy is that we used a volume of 0.1 ml for the MBC test, whereas the inocula in the previous reports were 0.001 and 0.01 ml, respectively. There is a possibility, although we did not investigate it, that in some cases, the higher MBCs could be due to selection of mutants that are less susceptible to norfloxacin. Norfloxacin also differs from nalidixic acid and cinoxacin in that it includes in its antibacterial spectrum *Pseudomonas aeruginosa* and gram-positive pathogens, species which are completely resistant to the two comparative agents (2, 4, 8).

When the in vitro activity of norfloxacin is related to available documentation of the pharmacokinetics of the drug in humans, it is obvious that urine concentrations well above the MICs and MBCs of all strains tested in this study will be maintained for at least 12 h after a single oral dose of 100 mg, after which about 15 μg of norfloxacin per ml in urine was reported in the 12 to 24-h collection period (1). However, the drug is incompletely absorbed from the gastrointestinal tract, and the serum concentrations are low (1). Therefore, higher doses would be required to guarantee therapeutic concentrations in tissues and tissue fluids in the treatment of a systemic infection, e.g., pyelonephritis. Although low peripheral concentrations are achieved, it has been demonstrated in experimental infections that norfloxacin at concentrations close to the MIC is bactericidal and effectively eliminates *E. coli, Klebsiella pneumoniae*, and other pathogens from the site of the infection (2, 5; 22nd ICAAC, abstr. no. 682).

Further clinical evaluation of norfloxacin seems worthwhile partly because of its high antibacterial activity, and especially since it offers one of the very few alternatives for oral treatment of infections caused by *Pseudomonas aeruginosa*, infections which today may require hospitalization only for the purpose of administration of injectible antibiotics, owing to lack of available oral agents.

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