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Effect of method, medium, pH and inoculum on the in-vitro antibacterial activities of fleroxacin and norfloxacin

P. Hohl* and A. M. Felber

F. Hoffmann-La Roche, Department of Pharmaceutical Research, CH-4002 Basel, Switzerland

The comparative in-vitro activities of fleroxacin and norfloxacin were determined by standard agar dilution and broth micro-dilution methods in four different culture media, varying the pH of the medium (pH 5 to pH 8) and the inoculum size. Four inoculum densities equivalent to approximately 10^3, 10^4, 10^5 and 10^6 colony forming units per well or spot were prepared for each of five reference test strains (*Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853).

The antibacterial activity of fleroxacin was similar overall to that of norfloxacin and rather uniform. The influence of the test conditions was moderate. However, the activity of both quinolones was uniformly lower at pH 5 in urine and against a high inoculum of 10^6 cfu/spot or well of *S. aureus*.

Introduction

Fleroxacin (Ro 23-6240, AM-833), a 6-fluoroquinolone currently undergoing clinical investigation, has been shown to possess high activity against a broad range of bacteria (Chin, Brittain & Neu, 1986; Manek, Andrews & Wise, 1986; Aldrige, Schiro & Sanders, 1987). Until now information on the influence of method, medium, pH, and inoculum on the outcome of antibacterial susceptibility testing of fleroxacin has been scarce. Susceptibility test data of this type serve to characterize the compound and its potential therapeutic role early in the clinical development, and the purpose of the present comparative study was to provide this information. In order to meet this objective the present study had to mimic *in vitro* the range of variations that can be expected to occur in the patient taking fleroxacin and determine the influence of changes in test conditions (such as the use of different media or inocula) on both the bacteriostatic and the bactericidal activity of fleroxacin. Norfloxacin was included as reference compound.

Methods

Commercially obtainable lyophilized standard laboratory control strains *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *S. aureus* ATCC 29213 (American Type Culture Collection, Rockville, Md 20852, USA) were grown as suggested by the

* Corresponding author: Peter Hohl, M.D., F. Hoffmann-La Roche & Co. Ltd., Department of Pharmaceutical Research, PF/ID, 70.44, Grenzacherstr. 124, CH-4002 Basel, Switzerland.

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supplier, and used throughout this study to determine the activity of fleroxacin (Lot No. 0499 720, potency 998 mg/g, from Kyorin) and norfloxacin (Merck, Sharp & Dohme, from an outside source). Standard dilution susceptibility test laboratory procedures (Jones *et al.*, 1985; Washington, 1985) as recommended by the NCCLS (1985) were employed.

MICs of the compounds were determined in four liquid media (Mueller-Hinton broth (BBL, 11443 Batch D5DMJH), Iso-Sensitest broth (Oxoid, CM 473 Batch 004 32115), pooled antibacterial-free filter-sterilized human urine from healthy volunteers, and reconstituted pooled human serum (TC Human Serum, Difco 5578, batch 71 57 35)) and on two solid media (Mueller-Hinton Agar (Difco 0252, lot 72 04 65) and Iso-Sensitest Agar (Oxoid, CM 471, lot 278-33523)).

Antibacterials were dissolved in methanol and 1 *N* NaOH or DMSO, respectively, immediately before use and diluted with sterile water, broth or serum by a serial twofold dilution scheme. Care was taken to avoid all unnecessary exposure of fleroxacin solutions to daylight. Final concentrations of antibacterials in broth and agar ranged from 128 to 0·008 mg/l. Drug-free control plates were included throughout the study.

The pH of each medium was measured with a pH meter (PHM 83 Autocal 'Radiometer') and a surface glass electrode (Tecan) and whenever necessary adjusted with 1 *N* HCl or 1 *N* NaOH except for human serum. A pH of 8·9 in serum when left standing in room air in microtitre plates was lowered to pH 7·4 by incubation in room air plus 10% added CO_2 but could not be lowered further (tests at pH 5, 6). In all experiments incubation was carried out at 35°C for 18 h.

The influence of a variable inoculum on MIC/MBC results was studied by dispensing 10 μl containing 10^3–10^6 cfu (photospectrometric adjustment of the inoculum from an overnight plate verified by colony counts) into a microtitre well (0·1 ml; 96 well plate) or 1–2 μl on to an agar plate (Denley multipoint inoculator).

The bactericidal activity of the two 6-fluoro-quinolones was assessed by subculturing 1–2 μl from all clear wells, as judged by the naked eye, of the microtitre plate on to antibacterial-free Mueller-Hinton agar plates. The MBC was taken as the antibacterial concentration that reduced the initial inoculum by $\geqslant$ 99·9%.

Results

The results of the comparative antibacterial susceptibility determinations of fleroxacin and norfloxacin are displayed in Tables I to V. The overall pattern of bacteriostatic and bactericidal activities of fleroxacin and norfloxacin against the different test organisms were similar and uniform, but with exceptions.

MIC values in agar under the same conditions in terms of pH and inoculum were generally identical to those in broth, and results on Mueller-Hinton agar were comparable with those on Iso-Sensitest agar. The activity of fleroxacin in serum at pH 7·4 was very similar to that in broth and thus entirely in keeping with the low degree of plasma protein binding (23%) reported by Weidekamm, Stöckel & Dell (1987). In urine at pH 7 the activity of both quinolones against a standard inoculum of 2×10^5 cfu/ml was two to fourfold lower than in broth whereas in urine at pH 5 the activity was as much as 32-fold lower against the *P. aeruginosa*, 16-fold lower against the staphylococci, and as much as 64-fold lower against the *E. coli* tested in broth at pH 7.

Table I. Influence of test conditions on the activity of fleroxacin and norfloxacin against *E. coli* ATCC 25922

Media	Inoculum (cfu/spot or well)	Q^+	Minimum inhibitory/bactericidal concentration^{++} (MIC/MBC) (mg/l) pH 5	pH 6	pH 7	pH 8
Mueller-Hinton agar	2×10^3	F	1	0·25	0·06	0·12
	3×10^3	N	2	0·5	0·03	0·015
Mueller-Hinton broth	2×10^3	F	0·5/1	0·06/0·12	0·06/0·06	0·06/0·12
	3×10^3	N	0·5/1	0·12/ND	0·015/0·03	0·015/0·015
Iso-Sensitest agar	3×10^3	F	NG	0·25	0·06	0·12
	3×10^3	N	NG	0·25	0·03	0·015
Iso-Sensitest broth	3×10^3	F	0·25/0·5	0·12/0·12	0·06/0·06	0·06/0·06
	3×10^3	N	1/2	0·12/0·25	0·03/0·03	0·015/0·015
Urine	2×10^3	F	⩾4/⩾4	1/1	0·25/0·25	0·25/0·25
	3×10^3	N	⩾4/⩾4	2/2	0·12/0·12	0·03/0·03
Human serum*	3×10^3	F	ND	ND	0·06/0·06	0·25/0·5
	3×10^3	N	ND	ND	0·03/0·03	0·015/0·03
Mueller-Hinton agar	2×10^4	F	1	0·25	0·06	0·12
	3×10^4	N	2	0·5	0·03	0·015
Mueller-Hinton broth	2×10^4	F	0·5/1	0·12/0·5	0·06/0·12	0·06/0·12
	3×10^4	N	0·5/1	0·12/ND	0·03/0·03	0·015/0·015
Iso-Sensitest agar	3×10^4	F	NG	0·25	0·06	0·12
	3×10^4	N	NG	0·25	0·06	0·03
Iso-Sensitest broth	3×10^4	F	0·5/0·5	0·12/0·12	0·06/0·06	0·06/0·06
	3×10^4	N	1/2	0·12/0·25	0·03/0·03	0·015/0·03
Urine	2×10^4	F	⩾4/⩾4	1/2	0·25/0·5	0·25/0·25
	3×10^4	N	⩾4/⩾4	2/2	0·12/0·12	0·03/0·03
Human serum*	3×10^4	F	ND	ND	0·06/0·06	0·25/0·5
	3×10^4	N	ND	ND	0·03/0·06	0·015/0·03
Mueller-Hinton agar	2×10^5	F	1	0·25	0·12	0·12
	3×10^5	N	4	0·5	0·06	0·03
Mueller-Hinton broth	2×10^5	F	0·5/1	0·12/0·12	0·12/0·12	0·06/0·12
	3×10^5	N	1/1	0·25/ND	0·03/0·03	0·015/0·03
Iso-Sensitest agar	3×10^5	F	0·03	0·25	0·06	0·12
	3×10^5	N	2	0·5	0·12	0·03
Iso-Sensitest broth	3×10^5	F	0·5/0·5	0·12/0·12	0·06/0·06	0·06/0·06
	3×10^5	N	1/2	0·25/0·25	0·06/0·06	0·03/0·03
Urine	2×10^5	F	⩾4/⩾4	1/1	0·25/0·5	0·25/0·25
	3×10^5	N	⩾4/⩾4	2/2	0·12/0·12	0·03/0·03
Human serum*	3×10^5	F	ND	ND	0·06/0·06	0·25/0·5
	3×10^5	N	ND	ND	0·03/0·06	0·03/0·03
Mueller-Hinton agar	2×10^6	F	16	4	1	4
	3×10^6	N	32	8	0·5	0·5
Mueller-Hinton broth	2×10^6	F	0·5/1	0·12/0·12	0·06/0·06	0·06/0·12
	3×10^6	N	1/1	0·25/ND	0·03/0·03	0·03/0·03
Iso-Sensitest agar	3×10^6	F	1	0·12	1	2
	3×10^6	N	4	4	0·5	0·25
Iso-Sensitest broth	3×10^6	F	0·5/1	0·12/0·25	0·06/0·06	0·06/0·06
	3×10^6	N	1/4	0·5/0·5	0·12/0·12	0·03/0·03
Urine	2×10^6	F	⩾4/⩾4	1/1	0·25/0·5	0·25/0·25
	3×10^6	N	⩾4/⩾4	2/2	0·12/0·12	0·03/0·03
Human serum*	3×10^6	F	ND	ND	0·06/0·12	0·5/1
	3×10^6	N	ND	ND	0·03/0·06	0·03/0·03

ND, Not done (see Methods); NG, no growth; Q^+, quinolone; $^{++}$, bactericidal test not done from agar dilution plates (see Methods); *, measured pH in serum + 10% CO_2 was 7·4 (8·9 in room air). F, fleroxacin; N, norfloxacin.

Table II. Influence of test conditions on the activity of fleroxacin and norfloxacin against *E. coli* ATCC 35218

Media	Inoculum (cfu/spot or well)	Q+	Minimum inhibitory/bactericidal concentration++ (MIC/MBC) (mg/l) pH 5	pH 6	pH 7	pH 8
Mueller-Hinton agar	2×10^3	F	1	0·25	0·06	0·12
	2×10^3	N	2	0·5	0·03	0·015
Mueller-Hinton broth	2×10^3	F	0·5/0·5	0·12/0·12	0·12/0·12	0·12/0·25
	2×10^3	F	1/1	0·12/ND	0·03/0·03	0·015/0·015
Iso-Sensitest agar	2×10^3	F	NG	0·25	0·06	0·12
	2×10^3	N	NG	0·25	0·03	0·03
Iso-Sensitest broth	2×10^3	F	0·5/0·5	0·12/0·12	0·06/0·06	0·06/0·12
	2×10^3	N	1/2	0·25/0·25	0·06/0·06	0·03/0·03
Urine	2×10^3	F	>4/>4	1/1	0·25/0·5	0·25/0·25
	2×10^3	N	≥4/≥4	1/1	0·12/0·12	0·03/0·03
Human serum*	2×10^3	F	ND	ND	0·06/0·12	0·25/0·5
	2×10^3	N	ND	ND	0·03/0·06	0·015/0·03
Mueller-Hinton agar	2×10^4	F	2	0·25	0·12	0·12
	2×10^4	N	4	0·5	0·03	0·015
Mueller-Hinton broth	2×10^4	F	0·5/0·5	0·12/0·12	0·12/0·12	0·25/0·25
	2×10^4	N	1/1	0·12/ND	0·03/0·03	0·015/0·015
Iso-Sensitest agar	2×10^4	F	NG	0·25	0·06	0·12
	2×10^4	N	NG	0·25	0·06	0·03
Iso-Sensitest broth	2×10^4	F	0·5/0·5	0·12/0·25	0·06/0·06	0·06/0·12
	2×10^4	N	1/2	0·25/0·25	0·06/0·06	0·03/0·03
Urine	2×10^4	F	≥4/≥4	1/2	0·25/0·5	0·25/0·25
	2×10^4	N	≥4/≥4	1/2	0·12/0·12	0·03/0·03
Human serum*	2×10^4	F	ND	ND	0·06/0·12	0·25/1
	2×10^4	N	ND	ND	0·03/0·06	0·03/0·03
Mueller-Hinton agar	2×10^5	F	4	0·25	0·12	0·25
	2×10^5	N	4	0·5	0·03	0·015
Mueller-Hinton broth	2×10^5	F	0·5/1	0·12/0·25	0·12/0·12	0·12/0·25
	2×10^5	N	2/2	0·25/ND	0·03/0·03	0·03/0·03
Iso-Sensitest agar	2×10^5	F	1	0·25	0·12	0·25
	2×10^5	N	2	0·5	0·12	0·06
Iso-Sensitest broth	2×10^5	F	0·5/0·5	0·12/0·25	0·06/0·06	0·06/0·12
	2×10^5	N	1/2	0·25/0·25	0·06/0·6	0·03/0·03
Urine	2×10^5	F	≥4/≥4	2/2	0·5/0·5	0·25/1
	2×10^5	N	≥4/≥4	2/2	0·12/0·12	0·03/0·03
Human serum*	2×10^5	F	ND	ND	0·06/0·12	0·5/4
	2×10^5	N	ND	ND	0·03/0·06	0·03/0·03
Mueller-Hinton agar	2×10^6	F	32	4	4	4
	2×10^6	N	32	16	4	0·5
Mueller-Hinton broth	2×10^6	F	1/>4	0·25/0·5	0·12/0·5	0·12/1
	2×10^6	N	2/>4	0·25/ND	0·03/0·25	0·03/0·12
Iso-Sensitest agar	2×10^6	F	2	4	2	4
	2×10^6	N	16	16	1	1
Iso-Sensitest broth	2×10^6	F	1/>4	0·25/1	0·12/0·25	0·12/0·25
	2×10^6	N	2/>4	0·5/2	0·12/0·12	0·03/0·12
Urine	2×10^6	F	≥4/≥4	2/4	0·5/4	0·25/2
	2×10^6	N	≥4/≥4	2/2	0·12/0·5	0·03/0·5
Human serum*	2×10^6	F	ND	ND	0·12/0·25	2/>4
	2×10^6	N	ND	ND	0·03/0·12	0·06/>4

See notes to Table I.

Table III. Influence of test conditions on the activity of fleroxacin and norfloxacin against *P. aeruginosa* ATCC 27853

Media	Inoculum (cfu/spot or well)	Q+	Minimum inhibitory/bactericidal concentration++ (MIC/MBC) (mg/l) pH 5	pH 6	pH 7	pH 8
Mueller-Hinton agar	8×10^2	F	16	4	2	4
	1×10^3	N	16	8	1	0·5
Mueller-Hinton broth	8×10^2	F	1/4	1/2	1/2	2/4
	1×10^3	N	0·5/0·5	0·25/0·25	≤0·25/≤0·25	≤0·25/≤0·25
Iso-Sensitest agar	1×10^3	F	NG	4	1	4
	1×10^3	N	NG	4	0·5	0·5
Iso-Sensitest broth	1×10^3	F	NG/NG	1/2	1/2	2/2
	1×10^3	N	NG/16	1/2	0·25/0·5	≤0·25/≤0·25
Urine	8×10^2	F	32/64	8/16	4/8	4/8
	1×10^3	N	32/64	8/8	1/2	≤0·25/0·5
Human serum*	1×10^3	F	ND	ND	0·5/2	16/32
	1×10^3	N	ND	ND	0·5/2	0·25/1
Mueller-Hinton agar	8×10^3	F	16	4	2	4
	1×10^4	N	32	8	1	0·5
Mueller-Hinton broth	8×10^3	F	1/4	1/2	1/2	2/8
	1×10^4	N	0·5/0·5	0·25/0·5	≤0·25/≤0·25	≤0·25/≤0·25
Iso-Sensitest agar	1×10^4	F	NG	4	2	4
	1×10^4	N	NG	4	0·5	0·5
Iso-Sensitest broth	1×10^4	F	NG/ 32	1/2	1/2	2/2
	1×10^4	N	NG/128	1/2	0·25/0·5	≤0·25/0·25
Urine	8×10^3	F	32/64	8/16	8/8	4/8
	1×10^4	N	32/64	8/16	1/2	0·5/0·5
Human serum*	1×10^4	F	ND	ND	1/2	16/64
	1×10^4	N	ND	ND	0·5/2	0·5/1
Mueller-Hinton agar	8×10^4	F	16	8	2	8
	1×10^5	N	32	8	1	0·5
Mueller-Hinton broth	8×10^4	F	2/4	1/2	2/2	2/4
	1×10^5	N	0·5/0·5	0·5/1	≤0·25/0·5	≤0·25/≤0·25
Iso-Sensitest agar	1×10^5	F	NG	4	2	4
	1×10^5	N	NG	4	1	0·5
Iso-Sensitest broth	1×10^5	F	NG/ 64	1/2	1/2	1/4
	1×10^5	N	NG/128	1/2	1/1	≤0·25/0·5
Urine	8×10^4	F	32/ 64	8/16	8/8	4/16
	1×10^5	N	32/128	8/32	1/2	0·5/2
Human serum*	1×10^5	F	ND	ND	2/4	32/64
	1×10^5	N	ND	ND	1/2	0·5/2
Mueller-Hinton agar	8×10^5	F	32	8	8	16
	1×10^6	N	64	16	2	1
Mueller-Hinton broth	8×10^5	F	4/4	1/4	4/4	4/4
	1×10^6	N	0·5/0·5	0·5/1	≤0·25/0·5	≤0·25/0·5
Iso-Sensitest agar	1×10^6	F	NG	8	4	8
	1×10^6	N	NG	8	2	1
Iso-Sensitest broth	1×10^6	F	0·5/64	2/8	2/2	4/4
	1×10^6	N	1/>128	4/4	1/2	<0·25/1
Urine	8×10^5	F	32/64	16/64	16/16	8/16
	1×10^6	N	64/>128	8/32	2/32	0·5/1
Human serum*	1×10^6	F	ND	ND	2/8	64/128
	1×10^6	N	ND	ND	1/4	1/2

See notes to Table I.

Table IV. Influence of test conditions on the activity of fleroxacin and norfloxacin against *S. aureus* ATCC 25923

Media	Inoculum (cfu/spot or well)	Q+	Minimum inhibitory/bactericidal concentration++ (MIC/MBC) (mg/l) pH 5	pH 6	pH 7	pH 8
Mueller-Hinton agar	3×10^2	F	2	0·5	0·5	0·5
	2×10^2	N	4	1	0·5	0·5
Mueller-Hinton broth	3×10^3	F	0·5/1	0·5/0·5	0·5/0·5	0·5/0·5
	2×10^3	N	1/1	0·5/0·5	0·25/0·5	0·25/0·5
Iso-Sensitest agar	1×10^2	F	NG	0·5	0·5	0·5
	4×10^2	N	NG	1	0·5	0·5
Iso-Sensitest broth	1×10^3	F	0·5/1	0·5/0·5	0·5/0·5	0·5/0·5
	4×10^3	N	0·5/1	0·5/0·5	0·5/0·5	0·5/0·5
Urine	3×10^3	F	8/2	1/1	1/1	1/2
	2×10^3	N	8/2	0·5/1	2/2	0·5/1
Human serum*	1×10^3	F	ND	ND	0·5/1	2/4
	4×10^3	N	ND	ND	0·5/1	0·25/1
Mueller-Hinton agar	3×10^3	F	2	0·5	0·5	0·5
	2×10^3	N	4	1	0·5	0·5
Mueller-Hinton broth	3×10^4	F	0·5/2	0·5/0·5	1/1	0·5/1
	2×10^4	N	1/1	0·5/0·5	0·25/1	0·25/1
Iso-Sensitest agar	1×10^3	F	1	1	0·5	1
	4×10^3	N	2	2	1	1
Iso-Sensitest broth	1×10^4	F	1/1	0·5/1	0·5/1	0·5/0·5
	4×10^4	N	2/2	0·5/1	0·5/1	0·5/0·5
Urine	3×10^4	F	8/4	1/1	1/2	1/2
	2×10^4	N	16/2	1/1	2/4	0·5/2
Human serum*	1×10^4	F	ND	ND	0·5/1	2/4
	4×10^4	N	ND	ND	0·5/1	0·5/2
Mueller-Hinton agar	3×10^4	F	4	1	0·5	1
	2×10^4	N	8	1	0·5	1
Mueller-Hinton broth	3×10^5	F	4/4	0·5/2	0·5/1	0·5/1
	2×10^5	N	4/4	0·5/1	0·25/1	0·25/1
Iso-Sensitest agar	1×10^4	F	2	1	1	1
	4×10^4	N	4	2	2	1
Iso-Sensitest broth	1×10^5	F	2/2	1/1	0·5/2	0·5/2
	4×10^5	N	2/2	1/1	0·5/1	0·5/1
Urine	3×10^5	F	32/4	4/2	2/2	2/2
	2×10^5	N	32/4	2/4	2/4	1/2
Human serum*	1×10^5	F	ND	ND	1/1	2/4
	4×10^5	N	ND	ND	0·5/4	0·5/2
Mueller-Hinton agar	3×10^5	F	>128	>128	>128	>128
	2×10^5	N	>64	>64	>64	>64
Mueller-Hinton broth	3×10^6	F	>32/>32	>32/>32	>32/>32	>32/>32
	2×10^6	N	32/32	32/32	32/32	32/32
Iso-Sensitest agar	1×10^5	F	8	>128	>128	>128
	4×10^5	N	>64	>64	>64	>64
Iso-Sensitest broth	1×10^6	F	>32/>32	>32/>32	>32/>16	>32/8
	4×10^6	N	>32/>32	>32/>32	>32/16	>32/8
Urine	3×10^6	F	>32/>32	>32/>32	>32/>32	>32/>32
	2×10^6	N	>32/>32	>32/>32	>32/>32	>32/>32
Human serum*	1×10^6	F	ND	ND	>32/>32	>32/>32
	4×10^6	N	ND	ND	>32/>32	>32/>32

See notes to Table I.

Table V. Influence of test conditions on the activity of fleroxacin and norfloxacin against *S. aureus* ATCC 29213

Media	Inoculum (cfu/spot or well)	Q+	Minimum inhibitory/bactericidal concentration++ (MIC/MBC) (mg/l) pH 5	pH 6	pH 7	pH 8
Mueller-Hinton agar	6×10^2	F	2	0·5	0·5	1
	7×10^2	N	4	1	0·5	0·5
Mueller-Hinton broth	6×10^3	F	2/2	0·5/0·5	0·5/0·5	0·5/0·5
	7×10^3	N	2/2	0·5/0·5	0·25/0·5	0·25/0·5
Iso-Sensitest agar	9×10^2	F	2	1	0·5	1
	8×10^2	N	8	1	1	1
Iso-Sensitest broth	9×10^3	F	2/2	0·5/0·5	0·5/0·5	0·5/1
	8×10^3	N	4/4	1/2	1/1	0·5/0·5
Urine	6×10^3	F	4/2	1/2	1/2	1/1
	7×10^3	N	16/2	<0·06/2	2/2	1/1
Human serum*	9×10^3	F	ND	ND	0·5/1	2/4
	8×10^3	N	ND	ND	0·5/0·5	0·5/0·5
Mueller-Hinton agar	6×10^3	F	2	0·5	0·5	1
	7×10^3	N	8	1	0·5	0·5
Mueller-Hinton broth	6×10^4	F	2/4	0·5/1	0·5/1	0·5/1
	7×10^4	N	2/4	0·5/1	0·25/0·5	0·25/0·5
Iso-Sensitest agar	9×10^3	F	4	1	1	1
	8×10^3	N	8	2	1	1
Iso-Sensitest broth	9×10^4	F	2/2	1/1	0·5/1	0·5/1
	8×10^4	N	4/8	1/2	1/1	0·5/0·5
Urine	6×10^4	F	8/4	2/2	2/2	1/2
	7×10^4	N	16/4	2/2	2/2	1/2
Human serum*	9×10^4	F	ND	ND	0·5/1	4/4
	8×10^4	N	ND	ND	0·5/0·5	0·5/0·5
Mueller-Hinton agar	6×10^4	F	4	1	1	2
	7×10^4	N	8	2	1	1
Mueller-Hinton broth	6×10^5	F	4/8	1/1	0·5/1	1/1
	7×10^5	N	8/16	1/2	0·25/1	0·25/1
Iso-Sensitest agar	9×10^4	F	4	2	1	2
	8×10^4	N	16	4	2	2
Iso-Sensitest broth	9×10^5	F	4/4	2/1	1/4	0·5/2
	8×10^5	N	8/16	2/2	2/1	1/1
Urine	6×10^5	F	16/4	4/4	2/2	1/2
	7×10^5	N	>32/16	4/8	2/4	2/2
Human serum*	9×10^5	F	ND	ND	1/2	4/4
	8×10^5	N	ND	ND	1/1	1/1
Mueller-Hinton agar	6×10^5	F	>128	>128	>128	>128
	7×10^5	N	16	>64	>64	>64
Mueller-Hinton broth	6×10^6	F	>32/>32	>32/>32	>32/>32	>32/>32
	7×10^6	N	>32/>32	>32/>32	>32/>32	>32/>32
Iso-Sensitest agar	9×10^5	F	8	>128	>128	>128
	8×10^5	N	>64	>64	>64	>64
Iso-Sensitest broth	9×10^6	F	>32/>32	>32/>32	>32/16	>32/16
	8×10^6	N	>32/>32	>32/>32	>32/8	>32/2
Urine	6×10^6	F	>32/>32	>32/>32	>32/32	>32/32
	7×10^6	N	>32/>32	>32/>32	>32/>32	>32/>32
Human serum*	9×10^6	F	ND	ND	>32/>32	>32/>32
	8×10^6	N	ND	ND	>32/>32	>32/>32

See notes to Table I.

The MIC results of fleroxacin and norfloxacin in liquid media against *P. aeruginosa* and *E. coli* remained constant even when the inoculum was increased from 10^3 to 10^6 cfu. However, in agar a corresponding 1000-fold increase in inoculum led to a 16-fold increase in MIC of both quinolones against the three Gram-negative enteric organisms, whereas against *S. aureus* the larger inoculum caused a 32-fold or even greater increase in MIC irrespective of the broth or agar used.

Fleroxacin was by and large two to fourfold less active at pH 5 than at pH 6 or pH 7. At pH 8 and in serum at pH 8·9 fleroxacin was also less active than at the near-physiological pH range of 7·0 (broth and agar) to 7·4 (serum). The decrease in activity seen at pH 5 was also seen with norfloxacin, but not at pH 8 (broth and agar) and pH 8·9 (serum), where norfloxacin proved to be two to fourfold more active than at pH 7.

Both compounds were bactericidal. Occasionally MBC/MIC ratios exceeded 1, presumably because of the technical difficulties inherent in MBC testing (Taylor *et al.*, 1983).

Discussion

The influence of method, medium, pH and inoculum on the antimicrobial activity of any new compound under investigation is crucial to determine the correct routine susceptibility test procedures and to maximise the correlation between susceptibility testing of the drug and the results of clinical therapy.

The antibacterial activity of fleroxacin under standard conditions paralleled that of norfloxacin except at pH 8 or greater where it was less by a factor of two or more, while that of norfloxacin was the same or even increased by a factor of two (e.g. against *P. aeruginosa*; Table III). This is in good agreement with Lacey, Lord & Howson (1984) who found the pH for optimum activity of norfloxacin to be pH 7·5–8·0.

The observation of a pH optimum for activity extended also to fleroxacin and norfloxacin activity in serum. Hence, in practical terms a serum bactericidal test incubated in room air will underestimate the activity of fleroxacin by a factor 4 or greater, but not that of norfloxacin, if no provision is made to correct for the alkalinity of serum during incubation such as by incubating the susceptibility test plate in a CO_2-incubator delivering approximately 10% CO_2.

Also noteworthy was that the activity of both quinolones was uniformly two to fourfold lower in urine than in broth or serum at pH 7, a difference that has been attributed to the higher Mg^{2+} content in urine than in broth or serum (Chin *et al.*, 1986). The loss of activity of both quinolones was even more pronounced in urine at pH 5 compared to broth at pH 6 or pH 7 and confirmed the earlier findings of Chin *et al.* (1986) working with other bacteria. The diminution in activity under standard conditions was less for fleroxacin than for norfloxacin and smaller against the more resistant strains (*P. aeruginosa, S. aureus*) than against the very susceptible strains (*E. coli*). Because in man both quinolones under study are primarily eliminated in urine at a pH near 7, the clinical relevance of these in-vitro data is likely to be minor. Nonetheless, quantitative monitoring of the resistance of urinary tract pathogens to either compound and correlation of the MIC result with clinical outcome may be justified at an early stage of clinical investigation in selected patients with a low urinary pH, a very large urine output and with pathogens in their urine barely susceptible under standard test conditions (MICs of 4 mg/l or greater).

The pH-dependency of the activity of the new quinolones shown in this study corroborates the findings of Gasser, Graversen & Madsen (1987) who attributed peak activity of fleroxacin at pH 7 to peak diffusion of the non-ionized fraction of the compound into bacteria. The clinical significance of the two to eightfold increase in resistance of *S. aureus* to fleroxacin and norfloxacin in urine remains to be investigated. That our observation could be of clinical importance can be hypothesized from unexplained clinical failures with ciprofloxacin in patients with uncomplicated urinary tract infections due to quinolone-susceptible *S. saprophyticus* (Garlando *et al.*, 1987) and from the fact that in this laboratory the susceptibility of *S. saprophyticus* has been very similar to that of *S. aureus* under standard conditions (data not shown).

Similarly, *S. aureus* and other staphylococci with a very high inoculum *in vivo*, at sites of infection other than the urinary tract might deserve close attention until the efficacy of the new compounds in the more difficult settings has been clearly established both in the normal and in the immunocompromised host.

Fleroxacin and norfloxacin both proved bactericidal under the varying conditions of the trial with the respective MBC/MIC ratio being 1 (mode), 2 and, rarely greater than 2, probably due to technical reasons (Taylor *et al.*, 1983). Several inversions of the ratio were observed primarily in tests with *S. aureus* at pH 5. Smith (1984) has shown that inversions of the MBC/MIC ratio of this type are due to ongoing filamentation in non-viable quinolone-exposed cells. Several of the MIC determinations at pH 5 but not at pH 6 against *S. aureus* therefore underestimated the true antibacterial potency of the new quinolones. Because no such inversions were noted near pH 7·4, at which susceptibility testing is normally carried out, this laboratory phenomenon is not prone to interfere adversely with susceptibility predictions for fleroxacin and norfloxacin under conditions of good laboratory practice.

Finally, note should be made that under standard laboratory conditions MIC results in liquid or solid media are very similar. Therefore the MIC breakpoints of fleroxacin (systemic infections: $S \leqslant 2$, $I = 4$, $R > 8$ mg/l; urinary infections: $S \leqslant 16$, $R > 16$ mg/l) as used for the tentative susceptibility interpretive criteria established by Fuchs *et al.* (1987) would appear applicable to agar as well as broth dilution susceptibility testing and to correlations with the 5 μg fleroxacin disc currently recommended for clinical trials.

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