Table 1: In vitro activity of norfloxacin against five isolates of *Chlamydia trachomatis*.

Strain	MIC (μg/ml)	Percentage of reduction of inclusion formation compared to control at norfloxacin concentrations (μg/ml) of*				
		10	5	2	1	0.5
1004	⩽ 20	99	74	–	–	0
2107	⩽ 20	99	90	–	–	0
3108	⩽ 20	94	77	53	12	0
4210	⩽ 20	98	50	–	18	0
5211	⩽ 20	98	61	13	12	7

*The number of inclusion formations in controls without antibiotics served as 100 %. Results are the mean of two experiments.

References

1. **Ito, A., Hirai, K., Inoue, M., Koga, H., Suzue, S., Irikura, T., Mitsuhashi, S.:** In vitro antibacterial activity of AM-715, a new nalidixic acid analog. Antimicrobial Agents and Chemotherapy 1980, 2: 103–108.
2. **Khan, M. Y., Siddiqui, Y., Gruninger, R. P.:** Comparative in vitro activity of MK-0366 and other selected oral antimicrobial agents against *Neisseria gonorrhoeae*. Antimicrobial Agents and Chemotherapy 1981, 2: 265–266.
3. **Haase, D., Urias, B., Harding, G., Ronald, A.:** Comparative in vitro activity of norfloxacin against urinary tract pathogens. European Journal of Clinical Microbiology 1983, 2: 235–241.
4. **Oriel, J. D., Reeve, P., Wright, J. T., Owen, J.:** Chlamydial infection of the male urethra. British Journal of Veneral Diseases 1976, 52: 46.
5. **Treharne, J. D., Day, J., Yeo, C. K., Jones, B. R., Squires, S.:** Susceptibility of chlamydiae of chemotherapeutic agents. In: Hobson, D., Holmes, K. K. (ed.): Nongonococcal urethritis and related infections. American Society for Microbiology, Washington, D.C., 1977, p. 214–222.

In Vitro Activity of Norfloxacin in Urine Compared to that of Cinnoxacin, Nalidixic Acid and Pipemidic Acid

Sir,

In a previous study (1) we found that the activity of some antibiotics is antagonised by human urine. In the present paper we report on the activity in urine of norfloxacin compared to cinnoxacin, nalidixic acid and pipemidic acid, antimicrobial agents generally used for the treatment of urinary tract infections.

The activity of the four antimicrobial agents was tested in DST agar and urine agar using 302 fresh isolates of the most common urinary tract pathogens. MICs were determined using the agar dilution technique (2). The MIC was determined simultaneously in DST agar (Oxoid) and in pooled human urine of pH 5.4–5.8, solidified by adding 1.2 % Bacto agar (Difco). The method for preparing the urine agar is described in detail elsewhere (1). The inoculum was adjusted to approximately 10^5 CFU per spot of a multipoint inoculator.

In order to rule out the possibility that a decrease in the activity of norfloxacin might be due to insolubility of norflaxacin in urine agar, the MIC of norfloxacin was also determined for seven selected strains in Mueller Hinton broth and unsupplemented fresh urine of pH 5.9.

Table 1 shows the activity of the four antimicrobial agents in DST agar and urine agar. Norfloxacin was the most active compound in DST agar, inhibiting all of the 302 strains tested except 15 *Pseudomonas aeruginosa* and three *Klebsiella pneumonia* strains with a concentration of ⩽ 1 mg/l.

All of the compounds investigated showed a loss of activity in urine agar. This was especially marked in the case of norfloxacin, the activity of which was only marginally better than that of nalidixic acid against *Escherichia coli* and *Klebsiella pneumoniae*. Whereas 90 % of *Enterobacter cloacae* strains were inhibited by 0.25 mg/l norfloxacin in DST agar, a concentration

Table 1: Comparative activity of norfloxacin, nalidixic acid, pipemidic acid and cinnoxacin against four bacterial species in urine agar and DST agar.

Organism (n)	Antimicrobial agent	Agar medium	No. of strains inhibited by an MIC (mg/l) of ⩽0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	⩾128
Escherichia coli (n = 97)	norfloxacin	urine					1	45	17	12	17	5		
		DST	65	17	11	2	1	1						
	nalidixic acid	urine						3	30	39	20		2	3
		DST					19	48	15	8	2		1	4
	pipemidic acid	urine								10	44	25	13	5
		DST				5	41	41	3	1	3	1		2
	cinnoxacin	urine						1	20	13	39	21	1	2
		DST					13	53	23	2	1			5
Enterobacter cloacae (n = 68)	norfloxacin	urine		1	2	9	14	11	22	5	4			
		DST	41	12	12	2	1							
	nalidixic acid	urine							3	19	41	5		
		DST					2	28	31	4	1	2		
	pipemidic acid	urine							1	13	27	23	4	
		DST				1	18	40	5	4				
	cinnoxacin	urine						2	5	20	29	8	4	
		DST						8	36	17	5	1	1	
Klebsiella pneumoniae (n = 87)	norfloxacin	urine				1	11	15	15	33	10	1	1	
		DST	26	33	20	2	3	1	2					
	nalidixic acid	urine							6	35	28	15		3
		DST						25	47	8	1	3	2	1
	pipemidic acid	urine							1	9	25	25	10	17
		DST					15	41	24	3	3	1		
	cinnoxacin	urine							1	24	40	19	2	1
		DST						3	43	35	1	1	3	1
Pseudomonas aeruginosa (n = 50)	norfloxacin	urine								2	15	9	21	3
		DST		7	12	6	10	7	5	1	1		1	
	nalidixic acid	urine										12	9	29
		DST										4	16	30
	pipemidic acid	urine										2	7	41
		DST							6	16	12	2	5	9
	cinnoxacin	urine										2	4	44
		DST										2	4	44

Table 2: Activity of norfloxacin in Mueller Hinton broth and unsupplemented urine.

Organism	Strain no.	MIC in broth (mg/l)	MIC in urine (mg/l)
Escherichia coli	6363	0.12	4
	6695	0.12	4
Klebsiella pneumoniae	9473	0.25	8
	550	1.0	16
Enterobacter cloacae	1844	1.0	32
	20	1.0	16
Serratia marcescens	10475	4.0	128

of 4 mg/l was needed in urine agar to obtain this degree of inhibition.
This phenomenon was also observed in urine (Table 2), the activity of norfloxacin in being considerably less in urine than in Mueller Hinton broth.
Some of the antibiotics used in routine practice are antagonised by urine, especially aminoglycosides; β-lactams are generally more active in urine than in broth or agar except against *Proteus* species (1). We have no explanation for this phenomenon so far. With regard to the clinical relevance of our results it has to be considered that after an oral dose of 400 mg, norfloxacin urine concentrations exceed 300 mg/l (3). Thus it would be possible in urine to inhibit all the strains we tested here.

P. M. Shah*
M. Ottrad
W. Stille

Zentrum der Inneren Medizin, Universitäts-Klinikum, Theodor-Stern-Kai 7, 6000 Frankfurt am Main, FRG.

References

1. **Shah, P. M., Schrinner, K. P., Helm, E. B., Stille, W.:** Untersuchungen zur antibakteriellen Aktivität von Penicillin und Cephalosporinen in Urin. Arzneimittelforschung 1976, 26: 1636–1638.
2. **Anhalt, J. P., Washington, J. A.:** Antimicrobial susceptibility tests of aerobic and facultatively anaerobic bacteria. In: Washington, J. A. (ed.): Laboratory procedures in clinical microbiology. Springer, New York, 1981, p. 279–308.
3. **Boppana, V. K., Swanson, B. N.:** Determination of norfloxacin a new nalidixic acid analog, in human serum and urine by high-performance liquid chromatography. Antimicrobial Agents and Chemotherapy 1982, 21: 808–810.

Effect of Norfloxacin on *Staphylococcus aureus* and *Pseudomonas aeruginosa* in Broth, Serum and in Combination with Human Polymorphonuclear Leukocytes

Sir,
Antibiotics act directly by killing or inhibiting the growth of microbial cells and indirectly by enhancing or stimulating host defenses. There appear to be two basic mechanisms by which antibiotics influence host defenses: alteration of microbial surface structures or toxin production and/or enhancement or suppression of host cell function (1, 2). Most investigators have studied the activity of beta-lactam antibiotics, macrolid antibiotics, tetracyclines, chloramphenicol and aminoglycosides on chemotaxis, phagocytosis or intracellular killing of phagocytic cells (3). Drugs almost exclusively used for treatment of urinary tract infections such as nalidixic acid and nitrofurantoin have only recently been investigated with respect to their effect on phagocytic and serum bactericidal activity of defibrinated human blood (4). We therefore compared the effect of norfloxacin, a new oral antibacterial organic acid structurally related to nalidixic acid, on *Pseudomonas aeruginosa* and *Staphylococcus aureus* in broth, serum and in combination with human polymorphonuclear leukocytes. Both strains, which were originally isolated from patients with nosocomial infections, were resistant to lysis by human serum. Culture conditions, preparation of leukocytes and serum and statistical methods have been described previously (5). The experimental protocol is summarized in Table 1.
The results of our experiments are outlined in Figures 1 and 2, and the statistical analysis is summarized in Table 2. Bactericidal activity of the four-fold MIC of norfloxacin on *Pseudomonas aeruginosa* and of the MIC on *Staphylococcus aureus* was better in broth (A) than in serum (B). The opposite was true for the effect of subinhibitory concentrations (1/4 MIC) on *Pseudomonas aeruginosa*, killing of which was significantly enhanced in serum in comparison to broth.