In-vitro activity of newer quinolones against aerobic bacteria

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Nalidixic and five newer 4-quinolones, ciprofloxacin, enoxacin, norfloxacin, ofloxacin and pefloxacin were tested against 576 recent clinical aerobic bacterial isolates. The 4-quinolones were regularly active ($MIC_{90} < 4$ mg/l) against the following bacteria: *Staphylococcus aureus, S. epidermidis, S. saprophyticus,* different Enterobacteriaceae, *Haemophilus influenzae, Campylobacter jejuni, Pseudomonas aeruginosa, Agrobacter* spp., *Aeromonas* spp., *Plesiomonas* spp., *Neisseria meningitidis.* Other bacteria were usually intermediately susceptible or resistant: different streptococci, *Listeria monocytogenes, Nocardia asteroides, P. maltophilia, Achromobacter xylosoxydans* and *Alcaligenes denitrificans.* Ciprofloxacin was the most potent compound, followed by ofloxacin and pefloxacin, norfloxacin and enoxacin being less active. All the 4-quinolones were much more active than nalidixic acid. The MBC/MIC ratios of the 4-quinolones were between 1 and 2 with a majority of strains, and between 2 and 3 with *Streptococcus agalactiae, Str. faecalis* and *L. monocytogenes.* A two- to eight-fold increase of MIC was observed by increasing the inoculum 10,000-fold with most of the strains tested. Susceptible bacterial population of *Klebsiella pneumoniae, Enterobacter cloacae, Serratia marcescens* and *P. aeruginosa* contained more clones resistant to nalidixic acid (10^4 to 10^8 at four times the MIC) than to 4-quinolones (10^5 to 10^9 at four times the MIC). Supplementing the media with $MgSO_4$ produced smaller inhibition zone diameters with a disc diffusion method than those obtained with non-supplemented agar, with all quinolone or strains. Less regular effect, or no effect was obtained after supplementation with $ZnSO_4$ or $Ca(NO_3)_2$.

Introduction

In contrast to nalidixic acid and early derivatives such as cinoxacin, rosoxacin or flumequine, the activity of newer 4-quinolone compounds does not limit them to the oral therapy of urinary tract infections caused by Enterobacteriaceae. Recently developed 4-quinolone are characterized by a wider spectrum including *Pseudomonas* spp., *Legionella* spp. (Greenwood & Laverick, 1983), Gram-positive bacteria and obligate intracellular organisms such as *Mycobacterium* spp. (Gay, DeYoung & Roberts, 1984) or *Chlamydia* spp. (Heesen & Muytiens, 1984; von Roosbroeck, Privinciael & Caekenberghe, 1984) and *Mycoplasma* spp. (Ridgway *et al.,* 1984). In contrast, the activity against anaerobic bacteria is limited. The extremely low MICs of 4-quinolones against aerobic bacteria and their pharmacokinetic properties suggests their use in numerous clinical situations. In the present study we compare the *in-vitro* activity of ciprofloxacin, enoxacin, norfloxacin, ofloxacin and pefloxacin against routine clinical isolates of aerobic Gram-positive and Gram-negative organisms.

Material and methods

Antimicrobial agents. Standard powder of the following drugs were obtained from their manufacturers: Nalidixic acid (Winthrop, Switzerland), ciprofloxacin (Bayer AG, Germany), enoxacin (Roger Bellon, France), norfloxacin (Merck Sharp and Dohme, Switzerland), ofloxacin (Roussel-Hoechst, France) and pefloxacin (Rhone Poulenc, France). Antibiotic solutions were prepared in water or in broth and used immediately.

Bacterial strains. Five hundred and seventy-six clinical strains collected from patients hospitalized in the University Hospital of Geneva or, occasionally, in other Swiss hospitals, and seven ATCC control organisms were used. They were kept frozen in skim milk at −70°C. Before study, organisms were thawed, streaked onto sheep blood agar and incubated overnight at 35°C.

Antimicrobial susceptibility tests. Antimicrobial activity was measured by the microdilution method in Mueller–Hinton broth (NCCLS, 1983). Disposable material was used exclusively in order to avoid cross-contamination with quinolones. The final inoculum of 10^5–10^6 cfu/ml was prepared from a trypticase soy broth inoculated 4 h before and controlled by counting the bacteria in a calibrated volume. The inoculum for *Haemophilus influenzae* was prepared and tested in Brain Heart Infusion broth supplemented with 5% nicotinamide diphosphate and 10% haemin. The minimal inhibitory concentration (MIC) was read after 18 h of incubation at 35°C in air or at 42°C in 10% CO_2 atmosphere for *Campylobacter jejuni*. The minimal bactericidal concentration (MBC) was determined by subculturing 0·01 ml on Mueller–Hinton agar and defined as 99·9% reduction in the initial inoculum. *Nocardia asteroides* was tested by agar dilution on Mueller–Hinton agar with 10^4 cfu/spot with 35°C incubation for three days.

Effect of calcium and magnesium. Defined medium (Iso-sensitest agar, Oxoid) containing 0·3 mmol Ca^{2+}, 1·2 mmol Mg^{2+} and 0·014 mmol Zn^{2+} respectively was supplemented in order to obtain a final concentration of 0·8 or 2·8 mmol/l $Ca(NO3)_2$, 2·8 or 5·0 mmol/l $MgSO_4$, and 0·03 or 0·07 mmol/l $ZnSO_4$.

Inoculum effect. This effect was measured on Mueller–Hinton agar with 10^8 or 10^4 cfu per inoculum.

Results

MICs of quinolones

Against Gram-positive genera, nalidixic acid had very poor activity as shown in Table I. In contrast 96 out of 97 staphylococci were susceptible to 4-quinolones; the exception was one methicillin resistant *Staphylococcus aureus* which was resistant to the quinolones tested. Activity of quinolones was similar against *S. epidermidis* and three groups of *S. aureus* classified according to their susceptibility to penicillin G and methicillin. *S. saprophyticus* was found to be one- or two-fold less susceptible than *S. epidermidis*. Streptococci were far less susceptible than staphylococci, most of the strains being in the intermediate susceptibility range. MICs of quinolones were similar against *Streptococcus pneumoniae*, *Str. agalactiae* and *Str. faecalis*. The majority of *Listeria monocytogenes* and *N. asteroides* were resistant to all quinolones tested except for a few sensitive strains. Quinolones were more active against the Enterobacteriaceae (Table II) than against Gram-positive bacteria. Most strains being inhibited by

Table I. Activity of quinolones against Gram positive bacteria

Organisms (number)	Antibiotic	MIC$_{50}$ (mg/l)	MIC$_{90}$ (mg/l)	Range (mg/l)
Penicillin-sensitive	nalidixic acid	64	>128	32->128
S. aureus (20)	ciprofloxacin	0·25	0·5	0·125–0·5
	enoxacin	1	4	0·5–4
	norfloxacin	1	2	0·5–2
	ofloxacin	0·25	1	0·125–1
	pefloxacin	0·5	1	0·25–4
Penicillin-resistant	nalidixic acid	64	128	32–128
Oxacillin-sensitive	ciprofloxacin	0·25	0·5	0·125–1
S. aureus (19)	enoxacin	1	2	0·5–4
	norfloxacin	2	2	0·5–4
	ofloxacin	0·25	0·5	0·125–0·5
	pefloxacin	0·5	0·5	0·25–1
Oxacillin-resistant	nalidixic acid	64	128	32->128
S. aureus (24)	ciprofloxacin	0·25	0·5	0·125–4
	enoxacin	1	2	0·5–32
	norfloxacin	1	2	0·5–>32
	ofloxacin	0·25	0·5	0·125–2
	pefloxacin	0·5	1	0·25–8
S. epidermidis (22)	nalidixic acid	64	128	64->128
	ciprofloxacin	0·125	0·25	0·125–0·25
	enoxacin	1	1	0·5–2
	norfloxacin	0·5	2	0·5–2
	ofloxacin	0·125	0·25	0·125–1
	pefloxacin	0·5	1	0·5–2
S. saprophyticus (12)	nalidixic acid	>128	>128	128->128
	ciprofloxacin	0·25	0·5	0·125–0·5
	enoxacin	2	4	1–4
	norfloxacin	2	4	0·5–4
	ofloxacin	0·5	1	0·25–1
	pefloxacin	2	4	1–4
Str. pneumoniae (31)	nalidixic acid	128	>128	32->128
	ciprofloxacin	0·5	1	0·06–1
	enoxacin	8	16	4–16
	norfloxacin	4	8	2–32
	ofloxacin	0·5	1	0·015–1
	pefloxacin	4	8	4–8
Str. agalactiae (19)	nalidixic acid	>128	>128	>128
	ciprofloxacin	0·5	0·5	0·25–1
	enoxacin	32	32	8–32
	norfloxacin	4	16	4–16
	ofloxacin	1	1	0·25–2
	pefloxacin	8	16	4–32
Str. faecalis (22)	nalidixic acid	>128	>128	>128
	ciprofloxacin	0·5	0·5	0·25–0·5
	enoxacin	8	8	4–8
	norfloxacin	4	4	2–4
	ofloxacin	1	2	1–2
	pefloxacin	4	4	2–4

Table I—*contd*.

Organisms (number)	Antibiotic	MIC_{50} (mg/l)	MIC_{90} (mg/l)	Range (mg/l)
L. monocytogenes (26)	nalidixic acid	>128	>128	>128
	ciprofloxacin	0·5	1	0·25–1
	enoxacin	8	16	4–16
	norfloxacin	4	8	2–16
	ofloxacin	1	2	0·5–2
	pefloxacin	8	8	2–8
N. asteroides (19)	nalidixic acid	>128	>128	64–>128
	ciprofloxacin	4	8	0·25–32
	norfloxacin	32	64	2–64
	ofloxacin	4	16	0·5–64
	pefloxacin	32	64	2–64

Table II. Activity of quinolones against Enterobacteriaceae

Organisms (number)	Antibiotic	MIC_{50} (mg/l)	MIC_{90} (mg/l)	Range (mg/l)
E. coli (30)	nalidixic acid	4	4	2–>128
	pipemidic acid	2	2	0·25–>128
	ciprofloxacin	0·015	0·03	0·004–1
	enoxacin	0·125	1	0·125–8
	norfloxacin	0·06	0·5	0·06–4
	ofloxacin	0·06	0·06	0·015–2
	pefloxacin	0·5	0·125	0·06–8
Salmonella spp. (22)	nalidixic acid	4	8	2–8
	pipemidic acid	2	4	0·25–4
	ciprofloxacin	0·008	0·015	0·004–0·015
	enoxacin	0·25	0·25	0·125–0.25
	norfloxacin	0·06	0·125	0·06–0·125
	ofloxacin	0·03	0·06	0·015–0·25
	pefloxacin	0·125	0·25	0·06–0.25
Shigella spp. (26)	nalidixic acid	2	4	2–4
	pipemidic acid	0·25	0·25	0·125–0·25
	ciprofloxacin	0·008	0·03	0·004–0·06
	enoxacin	0·125	0·125	0·06–0·25
	norfloxacin	0·06	0·06	0·06–0.125
	ofloxacin	0·06	0·125	0·03–1
	pefloxacin	0·125	0·125	0·06–0·125
Y. enterocolitica (7)	nalidixic acid	2	4	1–4
	pipemidic acid	0·25	2	0·25–4
	ciprofloxacin	0·015	0·03	0·008–0.03
	enoxacin	125	0·25	0·125–0·25
	norfloxacin	0·125	0·125	0·06–0·125
	ofloxacin	0·06	0·125	0·03–0·125
	pefloxacin	0·125	0·25	0·125–0·25

Table II—*contd.*

Organisms (number)	Antibiotic	MIC_{50} (mg/l)	MIC_{90} (mg/l)	Range (mg/l)
Klebsiella spp. (29)	nalidixic acid	4	8	1–16
	pipemidic acid	0·5	4	0·25–16
	ciprofloxacin	0·015	0·06	0·008–0·25
	enoxacin	0·25	0·5	0·06–1
	norfloxacin	0·125	1	0·03–8
	ofloxacin	0·125	0·25	0·015–0·5
	pefloxacin	0·25	0·5	0·03–1
Enterobacter spp. (27)	nalidixic acid	8	32	4–32
	pipemidic acid	2	4	0·25–16
	ciprofloxacin	0·015	0·03	0·004–0·06
	enoxacin	0·25	0·5	0·125–1
	norfloxacin	0·125	0·5	0·06–1
	ofloxacin	0·125	0·5	0·03–0·5
	pefloxacin	0·25	1	0·125–1
Ser. marcescens (23)	nalidixic acid	4	16	2–32
	pipemidic acid	0·5	16	0·25–32
	ciprofloxacin	0·125	1	0·015–>2
	enoxacin	0·5	2	0·125–4
	norfloxacin	0·25	1	0·125–2
	ofloxacin	0·5	>2	0·125–>2
	pefloxacin	0·5	1	0·125–4
Citrobacter freundii (13)	nalidixic acid	8	32	4–64
	pipemidic acid	0·5	0·5	0·25–1
	ciprofloxacin	0·008	0·015	0·004–0·5
	enoxacin	0·25	0·5	0·125–2
	norfloxacin	0·25	1	0·125–1
	ofloxacin	0·06	0·25	0·03–1
	pefloxacin	0·125	1	0·06–2
Proteus spp. (30)	nalidixic acid	8	16	2–32
	pipemidic acid	0·5	4	0·25–4
	ciprofloxacin	0·015	0·03	0·008–0·25
	enoxacin	0·5	0·5	0·125–1
	norfloxacin	0·125	0·25	0·03–0·5
	ofloxacin	0·125	0·25	0·03–0·25
	pefloxacin	0·25	0·25	0·125–0·5
Morganella morganii (16)	nalidixic acid	4	8	2–16
	pipemidic acid	0·25	0·5	0·25–2
	ciprofloxacin	0·015	0·03	0·004–0·03
	enoxacin	0·25	0·5	0·125–0·5
	norfloxacin	0·06	0·125	0·03–0·125
	ofloxacin	0·125	0·25	0·06–0·25
	pefloxacin	0·25	0·5	0·06–0·5
Providencia rettgeri (6)	nalidixic acid	4	8	2–>128
	pipemidic acid	0·5	0·5	0·5–32
	ciprofloxacin	0·03	0·25	0·008–2
	enoxacin	0·25	0·5	0·125–16
	norfloxacin	0·125	0·125	0·06–16
	ofloxacin	0·25	1	0·015–>2
	pefloxacin	0·25	0·25	0·125–16

Table III. Activity of quinolones against other aerobic and microaerophilic Gram-negative bacteria

Organisms (number)	Antibiotic	MIC$_{50}$ (mg/l)	MIC$_{90}$ (mg/l)	Range (mg/l)
H. influenzae (18)	nalidixic acid	1	1	0·5–2
	pipemidic acid	2	4	2–128
	ciprofloxacin	0·008	0·008	0·004–0·015
	enoxacin	0·125	0·125	0·06–0·5
	norfloxacin	0·06	0·06	0·03–1
	ofloxacin	0·015	0·03	0·015–0·5
	pefloxacin	0·03	0·03	<0·015–1
C. jejuni (24)	nalidixic acid	8	16	4–128
	pipemidic acid	16	64	8–128
	ciprofloxacin	0·25	1	0·25–2
	enoxacin	1	8	0·5–8
	norfloxacin	1	8	0·5–8
	ofloxacin	0·5	2	0·25–2
	pefloxacin	0·5	2	0·25–4
P. aeruginosa (19)	nalidixic acid	128	128	64–128
	pipemidic acid	32	64	16–64
	ciprofloxacin	0·125	0·25	0·06–0·25
	enoxacin	1	2	0·5–2
	norfloxacin	1	2	0·5–2
	ofloxacin	2	2	0·06–>2
	pefloxacin	2	4	1–4
P. maltophilia (7)	nalidixic acid	8	16	8–16
	pipemidic acid	>64	>64	>64
	ciprofloxacin	4	4	2–8
	enoxacin	8	8	4–16
	norfloxacin	16	32	16–>32
	ofloxacin	>2	>2	>2
	pefloxacin	2	4	2–4
Other *Pseudomonas* spp. (not *aeruginosa*) (8)	nalidixic acid	4	64	0·5–64
	pipemidic acid	4	8	2–64
	ciprofloxacin	0·125	2	0·03–2
	enoxacin	0·5	2	0·06–4
	norfloxacin	0·5	2	0·06–4
	ofloxacin	0·25	1	0·06–2
	pefloxacin	1	2	0·06–4
Achromo. xylosoxydans and *Alcalig. denitrificans* (6)	nalidixic acid	16	32	16–32
	pipemidic acid	128	>128	>128
	ciprofloxacin	2	>2	1–>2
	enoxacin	16	>32	16–>32
	norfloxacin	>32	>32	>32
	ofloxacin	4	32	4–32
	pefloxacin	8	16	4–16
Acinetobacter spp. (15)	nalidixic acid	4	8	0·5–8
	pipemidic acid	16	128	1–128
	ciprofloxacin	0·06	0·06	0·004–0·125
	enoxacin	0·5	4	0·004–4
	norfloxacin	2	16	0·03–32
	ofloxacin	0·25	1	0·015–1
	pefloxacin	0·25	1	0·03–4

Table III—*contd.*

Organisms (number)	Antibiotic	MIC$_{50}$ (mg/l)	MIC$_{90}$ (mg/l)	Range (mg/l)
Agrobacter spp. (20)	nalidixic acid	16	16	8–32
	pipemidic acid	4	4	2–32
	ciprofloxacin	0·06	0·06	0·03–0·06
	enoxacin	0·25	0·5	0·25–0·5
	norfloxacin	0·5	1	0·125–1
	ofloxacin	0·25	0·5	0·25–1
	pefloxacin	0·25	0·25	0·125–0·5
Aeromonas and	nalidixic acid	1	2	0·5–2
Plesiomonas spp. (19)	pipemidic acid	0·125	0·25	0·125–0·5
	ciprofloxacin	0·004	0·008	<0·001–0·015
	enoxacin	0·06	0·125	0·03–0·125
	norfloxacin	0·03	0·06	<0·015–0·06
	ofloxacin	0·015	0·015	0·008–0·03
	pefloxacin	<0·015	0·06	<0·015–0·06
N. meningitidis (18)	nalidixic acid	1	2	0·5–2
	ciprofloxacin	0·002	0·004	0·002–0·004
	enoxacin	0·03	0·06	<0·015–0·06
	norfloxacin	0·03	0·03	<0·015–0·03
	ofloxacin	0·008	0·015	0·004–0·015
	pefloxacin	0·03	0·03	<0·015–0·06

<1 mg/l. Some genera were rather more susceptible, such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp. and *Yersinia enterocolitica*, while others were distinctly more resistant, such as *Enterobacter* spp., *Serratia marcescens* and *Providencia rettgeri*. Although not shown in Table II, ampicillin, carbenicillin, cephalothin, cefotaxime, gentamicin and amikacin were tested simultaneously with the quinolones. Many of the strains of enterobacteria used were resistant to penicillins, cephalothin and gentamicin. No relationship between resistance to these agents and to 4-quinolones could be found. However, several strains showed a high level of resistance to nalidixic acid not seen with the 4-quinolones. As well as the Enterobacteriaceae, many other aerobic Gram-negative bacteria were susceptible to quinolones (Table III). These agents were extremely active against *Aeromonas* spp., *Plesiomonas* spp., *Neisseria meningitidis* and *H. influenzae*. Among the pseudomonas, *P. maltophilia* was more resistant than *P. aeruginosa*. The quinolones showed activity against *C. jejuni* and *Agrobacter* spp. comparable to that against the majority of enterobacteria, while *Achromoxylosoxydans* and *Alcaligenes denitrificans* were generally resistant. *Acinetobacter* spp. were susceptible to wide range concentrations of quinolones.

Comparing the activity of the different quinolones tested on a weight basis, ciprofloxacin was the most potent compound, followed by ofloxacin and pefloxacin. Norfloxacin and enoxacin were less active than pefloxacin. Differences between these compounds were more pronounced with Gram-negative bacteria than with Gram-positive genera. All the 4-quinolones were more active than nalidixic acid.

MBCs of quinolones. The MBC/MIC ratios of pefloxacin, norfloxacin and enoxacin were between 1 and 2 for the majority of strains, and between 2 and 3 with *Str.*

Table IV. Mean MDC/MIC ratios for five quinolones

Organisms	Number tested	Nalidixic acid	Cipro-floxacin	Enoxacin	Nor-floxacin	Pefloxacin
Penicillin-sensitive S. aureus	14	1·7	1·5	1·64	1·64	1·4
Penicillin-resistant Methicillin-sensitive S. aureus	11	2·3	2·4	1·45	2·0	1·9
Penicillin-resistant Methicillin-resistant S. aureus	16	1·7	2·7	1·6	2·2	1·7
S. epidermidis	17	1·7	2·9	1·7	1·9	1·4
S. saprophyticus	11	inactive	2·1	1·6	1·7	1·2
Str. agalactiae	14	inactive	2·0	N.D.	2·6	2·5
Str. faecalis	16	inactive	1·6	3·5	2·1	2·9
Str. pneumoniae	11	inactive	1·3	1·3	1·3	1·2
L. monocytogenes	10	inactive	2·1	2·4	2·2	1·9
E. coli	12	2·1	1·0	1·3	1·6	1·8
Shigella spp.	24	1·8	N.D.	1·1	1·3	1·1
Salmonella spp.	29	2·2	1·0	1·6	1·2	1·5
Enterobacter spp.	13	1·5	1·0	1·3	1·1	1·1
Klebsiella spp.	15	3·0	1·1	1·3	1·3	1·7
Serratia spp.	18	3·2	N.D.	1·8	2·1	2·1
Proteus spp.	20	2·0	1·4	1·6	1·5	1·8
P. aeruginosa	10	1·7	4·5	1·6	1·8	1·9
Pseudomonas spp. (not aeruginosa)	15	2·7	2·5	1·8	2·1	1·9

* N.D. = not determined.

agalactiae, Str. faecalis and *L. monocytogenes* (Table IV). In comparison with these three agents, bactericidal activity of ciprofloxacin was somewhat different, with higher MBC/MIC ratios against most staphylococci and Pseudomonas, but lower ratios against *Str. agalactiae, Str. faecalis*, and enterobacteria.

Inoculum effect and mutational frequencies. Determinations of MICs with two inocula of 10^4 and 10^8 cfu showed an inoculum effect particularly obvious with *Enterobacter cloacae* and *Ser. marcescens* (Table V). The inoculum effect was most

Table V. Fold increase of MICs with an inoculum of 10^8 cfu per spot as compared to an inoculum of 10^4 cfu per spot

Strains	Number tested	nalidixic acid	Fold increase of MICs range with:			
			cipro-floxacin	enoxacin	nor-floxacin	pefloxacin
E. coli	4	1–4	2–4	1–2	1–2	2
K. pneumoniae	4	4–8	2–8	1–4	2–8	2–8
E. cloacae	4	8–32	1–16	1–32	2–8	4–16
Ser. marcescens	4	8–16	4–16	8–32	8	4–8
P. aeruginosa	4	4–16	4–8	2–8	4–16	2–8
S. aureus	4	2–4	2–4	1–4	2–4	2–4
Str. faecalis	4	resistant	2–8	8–16	2–4	2–8

Table VI. Log values of the number of colonies growing on Mueller-Hinton Agar containing quinolone concentrations four or eight times the MICs (inoculum: 10^9 cfu)*

Strains (number)	nalidixic acid		cipro-floxacin		enoxacin		nor-floxacin		ofloxacin		pefloxacin	
	4× MIC	8× MIC	4× MIC	8× MIC	4× MIC	8× MIC	4× MIC	8× MIC	4× MIC	8× MIC	4× MIC	8× MIC
E. coli (4)	0	0	0	0	0	0	0	0	0	0	0	0
K. pneumoniae (4)	1–2	0	0–1	0	0–2	0	0–2	0–1	0–2	0–1	0–2	0
E. cloacae (4)	1–2	1–2	0–2	0–2	0–2	0–2	0–2	0–1	0–3	0–2	0–2	0–2
Ser. marcescens (4)	2–4	1–3	0–4	0–2	2–4	0–4	2–4	0–1	0–4	0–2	1–3	0–1
P. aeruginosa (4)	1–2	0–2	1–3	0	1–2	0–1	1–2	0–2	0–2	0–1	0–2	0–1
S. aureus (4)	3–5	0	0–2	0	0	0	0–1	0–1	0	0	0–2	0–1

* No colonies grew at 16 times the MIC with either compound.

marked with nalidixic acid while the differences found between the 4-quinolones were not significant. Data of Table V correlated well with determination of mutation frequencies shown in Table VI. *E. coli* populations contained less than 10^9 mutants resistant to the quinolones tested. Within the other strains studied, *Ser. marcescens* and *E. cloacae* had the highest frequency of mutants, and we found more clones resistant to nalidixic acid (10^4–10^8 at a concentration of four times the MICs) than to 4-quinolones (10^5–10^9 at four times the MICs). All the 4-quinolones tested selected resistant mutants at similar frequencies. No colonies grew at 16 times the MICs with either compounds.

Influence of cation supplementation. Addition of 0·03 or 0·06 mmol of $ZnSO_4$ did not alter the size of the inhibition zone diameters produced by ciprofloxacin, ofloxacin, pefloxacin and gentamicin when testing six strains in a disc-diffusion method (Figure 1). Supplementation with $Ca(NO_3)_2$ reduced the inhibition zone diameters of ofloxacin, pefloxacin and gentamicin but not those of ciprofloxacin. Supplementation with $MgSO_4$ produced significantly smaller inhibition zone diameters than those obtained with non-supplemented agar in all cases, whatever the antibiotic or the strain considered.
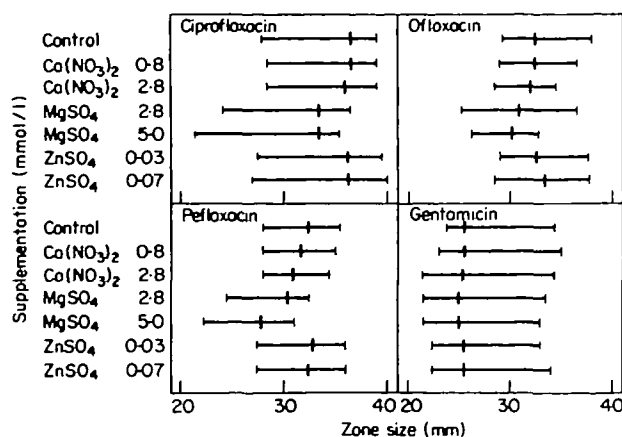

Figure 1. Influence of cation supplementation on the inhibition zone size obtained by disc diffusion with four antibiotics.

Discussion

Newer 4-quinolones are characterized by a broader spectrum of activity when compared to nalidixic acid. The data of this study confirm the similarity of the antibacterial spectrum for the 4-quinolone compounds tested (Wise, Andrews & Edwards, 1983; Barry *et al.*, 1984; Bauernfeind & Petermüller, 1984; van Caekenberghe & Pattyn, 1984; Chin & Neu, 1984; Hoogkamp, 1984). 4-quinolones are active against all Enterobacteriaceae, including *Enterobacter* spp. or *Ser. marcescens* which are often resistant also to newer cephalosporins. In particular organisms causing diarrhoea including *Campylobacter* spp. are very sensitive and may therefore be useful in this clinical situation. Against *P. aeruginosa* the activity is modest and not related to concomittant resistance to carbenicillin or gentamicin. Other non-fermenters such as *P. maltophilia*, *Achromobacter* spp. or *Alcaligenes* spp. are more than ten times less sensitive and often resistant to quinolones. The spectrum of 4-quinolones also includes Gram-positive organisms including staphylococci and to a lesser degree streptococci and *Listeria* spp. The MICs of *Nocardia* spp. are close to the achievable peak serum levels of quinolones and therefore cannot be recommended for clinical trials. As found by other authors (Bauernfeind & Petermüller, 1984; van Caekenberghe & Pattyn, 1984) the activity on a weight basis is highest for ciprofloxacin, followed by pefloxacin and ofloxacin, norfloxacin, enoxacin and the least active nalidixic acid. However, this observation will have to be matched with pharmacokinetics and clinical results, because other examples have taught, that *in-vitro* activity is not a sufficient criterion for the evaluation of new antimicrobial agents.

The bactericidal activity of quinolones is in general within 1–2-fold the MIC. Only *Str. agalactiae*, *Str. faecalis*, and *L. monocytogenes* have MBC two to three times higher than the MIC. Therefore the use of quinolones particularly for enterococcal endocarditis has first to be confirmed in the animal model. Ciprofloxacin has higher MBC–MIC ratios against *P. aeruginosa* when compared to the other 4-quinolones.

Emergence of resistance during therapy has been noted with nalidixic acid (Slack, 1984), and newer 4-quinolones (Acar, Kitzis & Goldstein, 1985; Lauwers *et al.*, 1985). In this study we have evaluated the frequency of resistant mutations by measuring the increase of MIC by raising the inoculum 10,000 fold. The increase was two- to eight-fold for most of the organisms tested. However, *Enterobacter* spp. and *Ser. marcescens* both well known for emergence of resistance during therapy with cephalosporin (Sanders & Sanders, 1985), again have a higher increase of the MIC up to 32 times and this could well explain the clinical failures. The mechanism of resistance is not known, but could be explained by a defect in bacterial penetration as the observation is common to all 4-quinolones tested (Smith, 1984).

The influence of pH, cations and various media has been mentioned by various authors. We have measured the influence of increased calcium, magnesium or zinc concentration on the zone size of a disc diffusion test. At the tested concentrations only magnesium reduced the zone size significantly for all compounds. The mechanism of impaired activity in presence of magnesium is unknown. Possibly, magnesium might interfere at least at two levels, either on the outer membrane as is the case with aminoglycosides, or at the level of DNA-gyrase–DNA interaction. The clinical significance of the magnesium effect is unknown. However, increased magnesium concentrations in the urine, together with a low pH, could be responsible for impaired therapeutic response in difficult to treat urinary tract infections. In addition, in testing

susceptibility of 4-quinolones, concentration of the magnesium in the medium should be standardized.

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