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In-vitro activities of ciprofloxacin, levofloxacin, lomefloxacin, ofloxacin, pefloxacin, sparfloxacin and trovafloxacin against Gram-positive and Gram-negative pathogens from respiratory tract infections

J. A. A. Hoogkamp-Korstanje

Department of Medical Microbiology, University Hospital St Radboud, University of Nijmegen, Geert Grooteplein 24, Nijmegen, The Netherlands

Trovafloxacin, sparfloxacin, ciprofloxacin and levofloxacin were equally active against *Moraxella catarrhalis*, *Haemophilus influenzae*, *Legionella pneumophila*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Serratia marcescens*. Ciprofloxacin was the most active compound against *Pseudomonas aeruginosa* (MIC_{90} = 1 mg/L), followed by trovafloxacin (MIC_{90} = 4 mg/L). Trovafloxacin was twice as active as sparfloxacin against *Streptococcus pyogenes* (MIC_{90} = 0.12 mg/L), *Streptococcus pneumoniae* (MIC_{90} = 0.12 mg/L) and *Staphylococcus aureus* (MIC_{90} = 0.06 mg/L) (except quinoione-resistant, methicillin-resistant *S. aureus*, for which the MIC_{90} was 8 mg/L). Trovafloxacin was the most active compound against *Enterococcus faecalis*: 80% of strains were susceptible to 0.25 mg/L. There was complete cross-resistance between all fluoroquinolones.

Introduction

A number of fluoroquinolones have recently been shown to be clinically effective for the treatment of community- and hospital-acquired respiratory tract infections.[1,2] Most experience has been obtained with ciprofloxacin, ofloxacin and pefloxacin. Their activity against Gram-positive bacteria is limited, however. In the last five years several new quinolones with activity against Gram-positive bacteria have been evaluated *in vitro*, but improved activity against Gram-positive bacteria has often appeared to be associated with decreased activity against Gram-negative bacteria.[3] Some drugs had high activity *in vitro* against Gram-positive and Gram-negative microorganisms[4] but showed serious side effects in humans, requiring their withdrawal. In the present study the in-vitro activities of sparfloxacin and trovafloxacin were compared with those of older fluoroquinolones against Gram-positive and Gram-negative respiratory pathogens from community-acquired and hospital-acquired pneumonia.

Materials and methods

A total of 498 clinical isolates from patients with respiratory tract infections, hospitalized in the University Hospital of Nijmegen, were studied.

MICs were determined in duplicate using a routine broth dilution method in microtitre plates.[4] Media used were Isosensitest broth (Oxoid), supplemented with 2% lysed horse blood and Isovitalex (2.5%, BBL) for *Haemophilus influenzae* and buffered starch yeast extract broth for *Legionella pneumophila*.

Antimicrobial stock solutions were prepared by dissolving powdered ciprofloxacin, levofloxacin, ofloxacin and trovafloxacin in water, and powdered lomefloxacin, pefloxacin and sparfloxacin in 0.1 M NaOH. Ciprofloxacin was provided by Bayer AG (Leverkusen, Germany), levofloxacin and ofloxacin by Hoechst Pharma (Amsterdam, The Netherlands), pefloxacin and sparfloxacin by Rhône–Poulenc Rorer (Amstelveen, The Netherlands), lomefloxacin by Searle Nederland (Maarssen, The Netherlands) and trovafloxacin by Pfizer (Capelle, The Netherlands).

The microtitre plates were filled with 100 μL of double-strength antibiotic test solution in each well. The inocula were prepared by taking four colonies of overnight cultures grown on appropriate media, which were added to 3 mL of sterile 0.85% NaCl to a McFarland turbidity standard of 0.5 (1.5×10^8 cfu/mL) and further diluted in 10 mL of double-concentrated test broth to a final organism concentration of 3×10^6–5×10^6 cfu/mL. Each well was inoculated with 100 μL of this suspension (final inocu-

Tel: +31-80-614356; Fax: +31-80-540216.

lum size 1.5×10^6–2.5×10^6 cfu/mL). The inoculum size and purity were controlled by plating 1 μL of the bacterial suspension on appropriate media. The plates were incubated at 37°C and growth was assessed after 24 and 48 h of incubation.

The MIC was defined as the lowest concentration preventing visible growth in the test medium. Control strains used were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212.

Results

The comparative activities of the strains are given in the Table.

Gram-negative organisms

Against Enterobacteriaceae, *H. influenzae*, *Moraxella catarrhalis* and *L. pneumophila*, ciprofloxacin, sparfloxacin and trovafloxacin were more active than levofloxacin, ofloxacin, lomefloxacin and pefloxacin. Ciprofloxacin, sparfloxacin and trovafloxacin were about twice as active as ofloxacin and lomefloxacin and at least eight times as active as pefloxacin. Levofloxacin was as active as ciprofloxacin against *Serratia marcescens*, *H. influenzae* and *M. catarrhalis*, but showed less activity against the other Gram-negative organisms tested. Ciprofloxacin was the most active compound against *P. aeruginosa*, being twice as active as trovafloxacin, levofloxacin and sparfloxacin and four times as active as ofloxacin and lomefloxacin. Taking 2 mg/L as the breakpoint for susceptibility, the activities of trovafloxacin, sparfloxacin, ciprofloxacin and levofloxacin towards *Klebsiella pneumoniae*, *Enterobacter cloacae*, *S. marcescens*, *H. influenzae*, *M. catarrhalis* and *L. pneumophila* were similar.

Gram-positive organisms

Trovafloxacin was twice as active as sparfloxacin against *Streptococcus pyogenes*, *S. pneumoniae*, *E. faecalis* and *S. aureus*. Seven of the MRSA strains and all the methicillin-susceptible strains were equally susceptible to trovafloxacin and sparfloxacin, with MIC_{90}s of 0.06–0.12 mg/L, but the three methicillin- and quinolone-resistant *S. aureus* (MQRSA) strains were inhibited only by 4 mg/L, 8 mg/L and 16 mg/L trovafloxacin and 8 mg/L, 16 mg/L and 32 mg/L sparfloxacin, respectively. These three strains were resistant to the other quinolones with MICs of 8–≥32 mg/L. Both penicillin-resistant and pencillin-susceptible *S. pneumoniae* strains were susceptible to trovafloxacin and sparfloxacin, but they were less susceptible to the other quinolones. Trovafloxacin was the most active quinolone against *E. faecalis*.

Discussion

Trovafloxacin and sparfloxacin showed high activity against Gram-positive bacteria without loss of Gram-negative spectrum. The most important feature of their antimicrobial spectrum was their activity against pneumococci irrespective of penicillin-susceptibility, with 90% of the strains susceptible to 0.12 mg/L of trovafloxacin and 0.25 mg/L of sparfloxacin. This has also been found by

Table. Antibacterial activities of seven fluoroquinolones against 498 respiratory pathogens (agents are shown in descending order of activity)

Bacterium (*n*)	Drug	MIC (mg/L) MIC_{90}	MIC (mg/L) range	% Susceptible to ≤2 mg/L
Haemophilus influenzae (50)	sparfloxacin	0.015	0.015–0.03	100
	ciprofloxacin	0.03	0.015–0.03	100
	trovafloxacin	0.03	0.015–0.03	100
	levofloxacin	0.03	0.015–0.06	100
	ofloxacin	0.06	0.015–0.06	100
	lomefloxacin	0.12	0.03–0.12	100
	pefloxacin	0.5	0.12–1	100
Moraxella catarrhalis (50)	sparfloxacin	0.03	0.015–0.03	100
	trovafloxacin	0.03	0.015–0.03	100
	ciprofloxacin	0.12	0.03–0.12	100
	levofloxacin	0.12	0.03–0.12	100
	ofloxacin	0.12	0.06–0.25	100
	lomefloxacin	0.25	0.12–0.5	100
	pefloxacin	1	0.12–1	100

Table. *Continued*

Bacterium (*n*)	Drug	MIC (mg/L) MIC_{90}	MIC (mg/L) range	% Susceptible to ⩽2 mg/L
Legionella pneumophila (50)	sparfloxacin	0.015	0.015–0.03	100
	trovafloxacin	0.015	0.015	100
	ciprofloxacin	0.03	0.015–0.06	100
	levofloxacin	0.03	0.015–0.03	100
	ofloxacin	0.03	0.03–0.06	100
	lomefloxacin	0.06	0.06–0.12	100
	pefloxacin	0.5	0.25–0.5	100
Streptococcus pneumoniae (10 penicillin resistant, 39 penicillin susceptible)	trovafloxacin	0.12	0.06–0.25	100
	sparfloxacin	0.25	0.12–0.5	100
	ciprofloxacin	2	0.5–2	100
	levofloxacin	1	0.5–2	100
	ofloxacin	2	1–4	96
	lomefloxacin	8	4–⩾32	0
	pefloxacin	⩾32	16–⩾32	0
Streptococcus pyogenes (20)	trovafloxacin	0.12	0.03–0.5	100
	sparfloxacin	0.25	0.12–0.5	100
	ciprofloxacin	0.5	0.12–2	100
	levofloxacin	1	0.25–2	100
	ofloxacin	1	0.5–4	95
	lomefloxacin	8	2–16	10
	pefloxacin	⩾32	8–⩾32	0
Enterococcus faecalis (47)	trovafloxacin	16	0.12–16	81
	sparfloxacin	⩾32	0.25–⩾32	81
	ciprofloxacin	⩾32	0.25–⩾32	79
	levofloxacin	⩾32	0.5–⩾32	81
	ofloxacin	⩾32	1–⩾32	55
	lomefloxacin	⩾32	2–⩾32	2
	pefloxacin	⩾32	2–⩾32	0
Staphylococcus aureus MSSA (20)	trovafloxacin	0.06	0.015–0.06	100
	sparfloxacin	0.12	0.03–0.5	100
	ciprofloxacin	1	0.25–1	100
	levofloxacin	0.5	0.12–1	100
	ofloxacin	1	0.25–4	95
	lomefloxacin	2	0.5–16	90
	pefloxacin	2	0.5–16	90
S. aureus MRSA (10)	trovafloxacin	8	0.015–16	70
	sparfloxacin	16	0.03–⩾32	70
	levofloxacin	16	0.25–⩾32	70
	ofloxacin	⩾32	0.25–⩾32	70
	ciprofloxacin	⩾32	0.25–⩾32	70
	lomefloxacin	⩾32	0.5–⩾32	60
	pefloxacin	⩾32	1–⩾32	50
Klebsiella pneumoniae (50)	sparfloxacin	1	0.015–⩾32	90
	ciprofloxacin	2	0.015–⩾32	90
	trovafloxacin	2	0.015–⩾32	92
	levofloxacin	2	0.015–⩾32	90
	ofloxacin	4	0.03–⩾32	86
	lomefloxacin	8	0.06–⩾32	82
	pefloxacin	4	0.12–⩾32	88

Table. *Continued*

Bacterium (*n*)	Drug	MIC (mg/L) MIC$_{90}$	MIC (mg/L) range	% Susceptible to ⩽2 mg/L
Serratia marcescens (51)	ciprofloxacin	4	0.015–8	86
	sparfloxacin	4	0.015–16	82
	trovafloxacin	4	0.03–16	82
	levofloxacin	4	0.03–8	84
	ofloxacin	8	0.06–16	80
	lomefloxacin	8	0.06–⩾32	73
	pefloxacin	⩾32	0.12–⩾32	39
Enterobacter cloacae (50)	ciprofloxacin	1	0.015–⩾32	98
	sparfloxacin	1	0.015–⩾32	98
	trovafloxacin	1	0.015–16	90
	levofloxacin	2	0.03–⩾32	90
	ofloxacin	4	0.03–⩾32	88
	lomefloxacin	8	0.06–⩾32	78
	pefloxacin	8	0.25–⩾32	76
Pseudomonas aeruginosa (51)	ciprofloxacin	1	0.03–⩾32	90
	trovafloxacin	4	0.12–⩾32	86
	levofloxacin	8	0.12–⩾32	78
	ofloxacin	16	0.12–⩾32	78
	sparfloxacin	8	0.12–⩾32	78
	lomefloxacin	16	0.5–⩾32	64
	pefloxacin	⩾32	1–⩾32	16
Control strains				
E. coli ATCC 25922	ciprofloxacin	0.015–0.03		
	sparfloxacin	0.015		
	trovafloxacin	0.015		
	levofloxacin	0.03		
	ofloxacin	0.03–0.06		
	lomefloxacin	0.06–0.25		
	pefloxacin	0.25		
P. aeruginosa ATCC 28753	ciprofloxacin	0.12–0.25		
	trovafloxacin	0.25–0.5		
	sparfloxacin	0.5–1		
	levofloxacin	0.5–1		
	ofloxacin	1		
	lomefloxacin	1–2		
	pefloxacin	8		
S. aureus ATCC29213	trovafloxacin	0.015 -0.03		
	sparfloxacin	0.06		
	ciprofloxacin	0.25 -0.5		
	levofloxacin	0.25 -0.5		
	ofloxacin	0.5		
	pefloxacin	1		
	lomefloxacin	1		
E. faecalis ATCC 29212	trovafloxacin	0.12–0.25		
	sparfloxacin	0.25–0.5		
	ciprofloxacin	0.5–1		
	levofloxacin	1		
	ofloxacin	2–4		
	pefloxacin	4		
	lomefloxacin	4		

others.[5,6] Serum concentrations of trovafloxacin and sparfloxacin in humans of ⩾2 mg/L after oral dosing of 300 mg[7] should therefore be expected to exceed the MIC for all pneumococci. These quinolones may therefore be welcome drugs in countries where a substantial percentage of pneumococci have become resistant to penicillin. Trovafloxacin is in clinical trials and in Europe the use of sparfloxacin is currently limited to the treatment of community-acquired pneumonia following the incidence of phototoxicity after its launch in France.

Like others[8] we found MRSA strains less susceptible and MQRSA strains not susceptible to all quinolones as a result of complete cross-resistance between the older and newer quinolones.

Enterococcal infections have become increasingly important. Most enterococci tested were isolated from our intensive care units where ciprofloxacin is often used. We observed a significant rise in MIC towards enterococci since its introduction. In 1986 100% of enterococci were susceptible to ⩽1 mg/L, compared with 50% in 1996, with 29% moderately susceptible and 21% not susceptible (MIC > 2 mg/L). Similar reports have come from others.[9] The strains susceptible to ciprofloxacin were also susceptible to trovafloxacin and sparfloxacin with MICs two or four times lower; the strains insusceptible to ciprofloxacin were also insusceptible to the newer drugs, indicating cross-resistance.

A number of Gram-negative organisms may be responsible for hospital-acquired pneumonia. Among them *K. pneumoniae*, *Enterobacter* sp., *Serratia marcescens* and *P. aeruginosa* predominate. Resistance of these species to quinolones has been reported.[10] Although we used ciprofloxacin with restriction for treatment of hospital-acquired pneumonia, we have also observed a substantial rise in MIC (eight- to 30-fold with 10% resistance) towards these problem organisms during the last ten years. Trovafloxacin and sparfloxacin were no more active than ciprofloxacin towards these strains.

In conclusion, trovafloxacin and sparfloxacin were more active against Gram-positive respiratory pathogens than were the older fluoroquinolones; their activities against Gram-negative organisms, except *P. aeruginosa*, were similar to that of ciprofloxacin. The latter drug may remain the drug of choice for treatment of *P. aeruginosa* infections.

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