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## Comparative in-vitro activity of levofloxacin, other fluoroquinolones, doxycycline and erythromycin against *Ureaplasma urealyticum* and *Mycoplasma hominis*

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The susceptibility of 56 *Ureaplasma urealyticum* and 57 *Mycoplasma hominis* strains to levofloxacin, ofloxacin, ciprofloxacin, fleroxacin, doxycycline and erythromycin was determined by an agar dilution method. The reference strain used was *M. hominis* PG 21. Agar plates containing serial dilutions of antibiotics (range 0.03–16 mg/L), and control plates (without antibiotics) were inoculated with bacteria suspended in modified Shepard's broth using a multipoint inoculator. Levofloxacin showed greater activity against all *U. urealyticum* and *M. hominis* strains compared with all other antibiotics tested. The MIC<sub>90</sub> values for *U. urealyticum* were as follows: levofloxacin, 1 mg/L; ofloxacin, 2 mg/L; ciprofloxacin, 4 mg/L; fleroxacin, 4 mg/L; doxycycline, 1 mg/L; erythromycin, 8 mg/L. The MIC<sub>90</sub>s for *M. hominis* were: levofloxacin, 1 mg/L; ofloxacin, 2 mg/L; ciprofloxacin, 4 mg/L; fleroxacin, 4 mg/L; doxycycline, 4 mg/L; erythromycin,  $\geq$ 16 mg/L. In conclusion, the results of this study suggest that levofloxacin may be useful in the treatment of mycoplasma genital infections.

### Introduction

The mycoplasmas *Mycoplasma hominis* and *Ureaplasma urealyticum* are frequent isolates from patients with urethritis, cervicitis and cystitis, and also appear to have an aetiological role in post-abortion and post-partum fever, pyelonephritis and pelvic inflammatory disease.<sup>1–7</sup> Tetracyclines are the standard treatment of non-gonococcal urethritis (NGU) in men<sup>8</sup> and women.<sup>9</sup> However, worldwide, clinical isolates of both *M. hominis* and *U. urealyticum* increasingly show high-level resistance to tetracyclines due to the presence of the transposable resistance determinant, tet(M).<sup>10–12</sup>

Fluoroquinolones with excellent pharmacokinetic profiles and activity against both Gram-negative and -positive organisms have been developed.<sup>13</sup> One of these newer fluoroquinolones is levofloxacin, the L-isomer of ofloxacin. Previous studies have indicated that ofloxacin has good activity against mycoplasmas.<sup>14–18</sup>

The purpose of our study was to determine the susceptibilities of clinical isolates of *U. urealyticum* and *M. hominis* to levofloxacin compared with three other fluoroquinolones (ofloxacin, ciprofloxacin and fleroxacin), doxycycline and erythromycin. Antimicrobial susceptibility was deter-

mined using the agar dilution method, chosen because this technique produces conservative end-points which can be repeated consistently.<sup>19</sup>

### Materials and methods

#### Bacteria

In total, 56 *U. urealyticum* and 57 *M. hominis* isolates were tested. The *U. urealyticum* isolates were obtained from patients with urogenital infections, while *M. hominis* isolates were cultured from cervical, urethral and placental samples. A reference *M. hominis* strain, PG 21 (kindly provided by Dr E. Freundt, University of Aarhus, Denmark), was also included in the study. The isolates were stored at –70°C. *U. urealyticum* and *M. hominis* strains were grown in home-produced A7-Shepard's broth, modified as described by Black.<sup>20</sup>

#### Antimicrobial agents

The following antimicrobials were included in the study: levofloxacin (Hoechst Marion Roussel, Bad Soden, Ger-

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many), ofloxacin (Hoechst Marion Roussel), ciprofloxacin (Bayer, Leverkusen, Germany), fleroxacin (Hoffmann-La Roche, Basel, Switzerland), doxycycline (Pfizer, Karlsruhe, Germany) and erythromycin (Durachemie, Münster, Germany). After producing a stock solution in the appropriate medium, two-fold serial dilutions were made in distilled water.

#### Agar dilution susceptibility testing

The antimicrobial susceptibility pattern of each strain was determined by the agar plate dilution method using self-prepared Shepard's medium (A7), according to the method of Shepard & Lunceford.<sup>21</sup> Agar plates contained two-fold serial dilutions of antibiotic (in the range of 0.03–16 mg/L) and were inoculated with  $10^5$ – $10^6$  cfu/mL by a multipoint inoculator as described previously.<sup>20</sup> The reference strain, *M. hominis* PG 21, was included in each test. Cultures were incubated anaerobically at 37°C and read after 2 and 4 days under a stereomicroscope at  $\times 16$  and  $\times 40$  magnification. The MIC was defined as the lowest antibiotic concentration that completely inhibited the development of visible growth on the agar plates.

## Results and discussion

The absolute numbers and cumulative percentages of strains inhibited are presented in Tables I and II. The range, and MIC<sub>50</sub> and MIC<sub>90</sub> values of levofloxacin, ofloxacin, ciprofloxacin, fleroxacin, doxycycline and erythromycin are shown in Table III. For ofloxacin, ciprofloxacin and fleroxacin, the susceptibility patterns of *U. urealyticum* and *M. hominis* were similar and comparable to those of Gram-positive organisms such as streptococci and staphylococci.<sup>22–24</sup>

The fluoroquinolones investigated in this study, when ranked by their relative activities (MIC<sub>50</sub>, MIC<sub>90</sub>) against *U. urealyticum* and *M. hominis*, were in the following order: levofloxacin > ofloxacin > ciprofloxacin/fleroxacin. Our results are similar to those previously reported for ofloxacin and ciprofloxacin. Thus, for *M. hominis*, the MIC<sub>90</sub> values of ofloxacin and ciprofloxacin ranged from 0.5–1 mg/L<sup>25–32</sup> and 0.5–2 mg/L, respectively.<sup>20,21,29,30,33,34</sup> For *U. urealyticum*, the MIC<sub>90</sub> values of ofloxacin and ciprofloxacin ranged from 1–4 mg/L<sup>25–29,31,32</sup> and 4–8 mg/L, respectively.<sup>20,27–29,33,35</sup>

An even higher degree of susceptibility has been observed with other new fluoroquinolones such as sparfloxacin

**Table I.** Number and cumulative percentage of *U. urealyticum* strains ( $n = 56$ ) inhibited at each concentration (mg/L)

Antibiotic	Number (cumulative percentage)									
	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	≥16
Levofloxacin		3 (5)	12 (27)	11 (46)	21 (84)	9 (100)				
Ofloxacin	1 (2)	1 (4)	3 (9)	5 (18)	8 (32)	30 (86)	8 (100)			
Ciprofloxacin	1 (2)	0 (2)	2 (5)	1 (7)	3 (13)	14 (38)	29 (89)	6 (100)		
Fleroxacin			3 (5)	3 (11)	4 (18)	3 (23)	30 (77)	13 (100)		
Doxycycline	1 (2)	4 (9)	13 (32)	14 (57)	19 (91)	3 (96)	0 (96)	0 (96)	2 (100)	
Erythromycin	7 (12)	1 (14)	5 (23)	5 (32)	4 (39)	8 (54)	7 (66)	12 (88)	4 (95)	3 (100)

**Table II.** Number and cumulative percentage of *M. hominis* strains ( $n = 57$ ) inhibited at each concentration (mg/L)

Antibiotic	Number (cumulative percentage)									
	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	≥16
Levofloxacin				23 (40)	28 (89)	6 (100)				
Ofloxacin					6 (11)	34 (70)	14 (95)	3 (100)		
Ciprofloxacin					5 (9)	25 (53)	18 (84)	9 (100)		
Fleroxacin			1 (2)	0 (2)	0 (2)	4 (9)	28 (58)	24 (100)		
Doxycycline	1 (2)	1 (4)	16 (32)	22 (70)	4 (77)	4 (84)	0 (84)	3 (90)	0 (90)	6 (100)
Erythromycin										57 (100)

## Levofloxacin activity against mycoplasma

**Table III.** Ranges, MIC<sub>50</sub>s and MIC<sub>90</sub>s for *U. urealyticum* and *M. hominis*

Antibiotic	<i>U. urealyticum</i> MIC (mg/L)			<i>M. hominis</i> MIC (mg/L)		
	range	50%	90%	range	50%	90%
Levofloxacin	0.06–1	0.5	1	0.25–1	0.5	1
Ofloxacin	≤0.03–2	1	2	0.5–4	1	2
Ciprofloxacin	≤0.03–4	2	4	0.5–4	1	4
Fleroxacin	0.125–4	2	4	0.125–4	2	4
Doxycycline	≤0.03–8	0.25	1	≤0.03–≥16	0.25	4
Erythromycin	≤0.03–≥16	1	8	–	≥16	≥16

(MIC<sub>90</sub>: *M. hominis*, 0.03–0.063 mg/L; *U. urealyticum*, 0.25–1 mg/L),<sup>25–28,33,36</sup> clinafloxacin (MIC<sub>90</sub>: *M. hominis*, 0.31–0.06 mg/L; *U. urealyticum*, 0.25–0.5 mg/L),<sup>33,34</sup> grepafloxacin (MIC<sub>90</sub>: *M. hominis*, 0.125 mg/L; *U. urealyticum*, 2 mg/L),<sup>25</sup> moxifloxacin (MIC<sub>90</sub>: *M. hominis*, 0.06 mg/L; *U. urealyticum* doxycycline-susceptible, 0.25 mg/L; *U. urealyticum* doxycycline-resistant, 0.5 mg/L)<sup>36</sup> and trovafloxacin (MIC<sub>90</sub>: *M. hominis*, 0.03 mg/L).<sup>30</sup>

For both *M. hominis* and *U. urealyticum*, the MIC range of doxycycline was widespread (≤0.03–≥16 mg/L and ≤0.03–8 mg/L, respectively), which is in agreement with the results of other studies.<sup>33,35,37</sup> This wide range in MIC values indicates the problem of resistance to the tetracyclines<sup>10–12</sup> and the need for effective new compounds.

In conclusion, our data suggest that levofloxacin, as with other new fluoroquinolones, may be useful for the treatment of mycoplasma genital infections because it is active against both *U. urealyticum* and *M. hominis* at levels well within attainable serum and tissue concentrations.<sup>38</sup>

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