Activity of moxifloxacin against the urogenital mycoplasmas *Ureaplasma* spp., *Mycoplasma hominis* and *Mycoplasma genitalium* and *Chlamydia trachomatis*

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**ABSTRACT**

The activity of moxifloxacin was compared with that of other antimicrobial agents against 54 strains of *Ureaplasma* spp., 54 strains of *Mycoplasma hominis*, 14 strains of *Mycoplasma genitalium*, and 44 strains of *Chlamydia trachomatis*. Moxifloxacin inhibited 90% of all isolates at a concentration ≤1 mg/L, being the most active compound against *C. trachomatis* and sharing the highest activity with garenoxacin and gemifloxacin against mycoplasmas. Moxifloxacin killed the 30 mycoplasma isolates tested at a concentration ≤1 mg/L, except those resistant to fluoroquinolone. Thus, moxifloxacin has attracted interest as a potential therapy for mycoplasmal or chlamydial urogenital infections.

**Keywords**: *Chlamydia trachomatis*, fluoroquinolones, moxifloxacin, urogenital mycoplasmas

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The urogenital mycoplasmas *Mycoplasma hominis*, *M. genitalium* and *Ureaplasma* spp., and *Chlamydia trachomatis*, are involved in genitourinary infections in men and women. The antibiotics potentially active against mycoplasmas and *C. trachomatis* are relatively low in number, and include tetracyclines, macrolides and related antibiotics, and fluoroquinolones. Among fluoroquinolones, the newer products, e.g. moxifloxacin, have the highest *in vitro* activity against these microorganisms. The activity of moxifloxacin against human mycoplasmas [1–4] and *C. trachomatis* [5–7] has already been studied. These studies concerned a relatively low number of isolates and evaluated its bactericidal activity against mycoplasmas for only three *Ureaplasma* spp. and two *M. hominis* strains [4]. Our purpose was to extend the study of the *in vitro* activity of moxifloxacin to a larger number of strains of urogenital mycoplasmas, including *M. genitalium* and *C. trachomatis*, in comparison with other fluoroquinolones—ofloxacin against *C. trachomatis*; ofloxacin, and levofloxacin, gatifloxacin, gemifloxacin and garenoxacin against mycoplasmas—and with other antimicrobials, including erythromycin, azithromycin and doxycycline. Moreover, the MBCs of moxifloxacin for nine strains of *Ureaplasma* spp., seven strains of *M. hominis* and 14 strains of *M. genitalium* were determined.

Fifty-four strains of *Ureaplasma* spp., one *Ureaplasma parvum* and one *U. urealyticum* reference strain, 30 doxycycline-susceptible clinical isolates collected prospectively between 2003 and 2005, and 22 doxycycline-resistant clinical isolates collected prospectively between 1996 and 2005 at Pellegrin Hospital, Bordeaux, France, were studied. For *M. hominis*, 54 strains were evaluated, two reference strains, 32 doxycycline-susceptible clinical isolates collected prospectively between
2004 and 2005, and 20 doxycycline-resistant clinical isolates collected prospectively between 2002 and 2005 in the same hospital. The 52 clinical isolates of Ureaplasma spp. included 30 isolates of U. parvum and 22 of U. urealyticum after species determination by real-time PCR [8]. Clinical isolates of Ureaplasma spp. and M. hominis included two and one genetically characterized fluoroquinolone-resistant isolates, respectively. The quinolone resistance-determining regions of the four topoisomerase genes were amplified and sequenced for the three fluoroquinolone-resistant isolates, as previously described [9]. The 14 strains of M. genitalium included the seven genetically very closely related ATCC reference strains (G37, M30, R32G, TW 10-5, TW 10-6, TW 48-5 UTMB-10G), five Danish clinical isolates (M2282, M2288, M2300, M2321, M2341) [10] and two French clinical isolates obtained, 6 weeks apart, from the same patient (M6090 and M6151). Forty-four strains of C. trachomatis, four reference strains belonging to serovars D, E, F and H and 40 urogenital isolates obtained between 1985 and 2004 at Pellegrin Hospital, Bordeaux, France (seven isolated before 1990, 12 between 1990 and 1994, 12 between 1995 and 2000, and nine between 2001 and 2005) were studied.

All antimicrobial powders tested were obtained from their respective manufacturers and dissolved according to recommendations. Susceptibility testing was performed as previously described [11] using an agar dilution method for mycoplasmal strains and a broth dilution method for ureaplasmal strains. MBCs of the different antimicrobials were determined, as previously reported [4,11], for nine strains of Ureaplasma spp. (two reference strains and seven clinical isolates, including two with acquired resistance to fluoroquinolones) and seven strains of M. hominis (two reference strains and five clinical isolates, including a fluoroquinolone-resistant one), and for the 14 strains of M. genitalium. Bactericidal activity was identified as the MBC being no more than two dilutions (four-fold) greater than the MIC [1,2]. Susceptibility testing of C. trachomatis was performed on McCoy cells in 24-well microtitre plates, with an inoculum of 10^3 inclusion-forming units/mL, as previously described [12,13].

Comparative in vitro activities of moxifloxacin and other antimicrobials against 54 strains of Ureaplasma spp. (31 U. parvum, 23 U. urealyticum), 54 strains of M. hominis, 14 strains of M. genitali-um and 44 strains of C. trachomatis are shown in Table 1. Doxycycline was the most potent molecule, with an MIC₉₀ of 0.25 mg/L against doxycycline-susceptible Ureaplasma spp. isolates, whereas moxifloxacin, gemifloxacin and garenoxacin were the most active against the doxycycline-resistant isolates. Concerning M. hominis isolates, moxifloxacin and gemifloxacin were the second most potent molecules, with an MIC₉₀ of 0.06 mg/L, following garenoxacin (MIC₉₀, 0.015 mg/L) (Table 1). For both Ureaplasma spp. and M. hominis, no significant MIC differences

<table>
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<tr>
<th>Antimicrobials (no. of strains tested)</th>
<th>MIC ranges, MIC₉₀ and MIC₅₀ (mg/L) of moxifloxacin and other antimicrobials against 54 strains of Ureaplasma spp., 54 strains of Mycoplasma hominis, 14 strains of M. genitalium and 44 strains of Chlamydia trachomatis</th>
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<tbody>
<tr>
<td>Moxifloxacin (54)</td>
<td>0.06–2 0.25 1</td>
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<tr>
<td>Ofloxacin (54)</td>
<td>0.5–8 1 2</td>
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<tr>
<td>Levofloxacin (54)</td>
<td>0.25–4 0.5 2</td>
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<td>Gatifloxacin (54)</td>
<td>0.25–2 0.5 1</td>
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<tr>
<td>Gemifloxacin (54)</td>
<td>0.12–1 0.25 1</td>
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<tr>
<td>Garenoxacin (54)</td>
<td>0.06–1 0.25 0.5</td>
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<tr>
<td>Doxycycline (54)</td>
<td>0.03–0.5 0.12 0.25</td>
</tr>
<tr>
<td>Erythromycin (54)</td>
<td>2–16 8 16</td>
</tr>
</tbody>
</table>

Eight of the 30 U. parvum and 14 of the 22 U. urealyticum clinical isolates were doxycycline-resistant.

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were found between doxycycline-susceptible and doxycycline-resistant isolates, except for doxycycline. However, more doxycycline-resistant isolates were obtained from *U. urealyticum* (14 of 22) than from *U. parvum* (eight of 30); this observation will have to be confirmed by studying larger collections of strains. As compared with previous studies testing *Ureaplasma* spp. and *M. hominis* isolates obtained before 2000 [1–4], in which different methodologies were used, our results using more recent isolates were not significantly different from those obtained for fluoroquinolones, with the MIC90 of moxifloxacin ranging from 0.25 to 1 mg/L for *Ureaplasma* spp. and from 0.03 to 0.12 mg/L for *M. hominis*, depending on the study.

Against *M. genitalium*, erythromycin was the most active molecule, with an MIC90 of 0.015 mg/L. After erythromycin, moxifloxacin and doxycycline had similar activity, with MIC90s of 0.12 mg/L, followed by the three other newer quinolones, garenoxacin, gemifloxacin and gatifloxacin, confirming our previous data obtained with reference strains [2,4]. The sole study including a larger number of *M. genitalium* strains, which were genetically different from the present one [10], showed similar results, with moxifloxacin having the highest activity (MICs, 0.03–0.5 mg/L) among fluoroquinolones. It should be noted that the seven very closely related ATCC strains had some discrepancies, especially concerning the MBC values of gatifloxacin (MBCs ranging from 0.25 to 4 mg/L) and doxycycline (MBCs ranging from 0.12 to 1 mg/L) (Table 2), highlighting the reproducibility limits of the broth dilution method used to determine MBCs, at least of these two antibiotics.

As previously described [5,7], moxifloxacin inhibited 90% of *C. trachomatis* isolates at a value of 0.06 mg/L. It was the most potent antibiotic,
followed by doxycycline and both macrolides. Interestingly, no significant difference in the susceptibility profile of strains was noticed over the past 20 years (data not shown).

MBCs of moxifloxacin and other antimicrobials against nine strains of *Ureaplasma* spp., seven strains of *M. hominis* and 14 strains of *M. genitalium* are presented in Table 2. Except for three isolates identified during this study, *Ureaplasma* spp. 3868 and 3898, belonging to the *U. urealyticum* species, and *M. hominis* 3525, which acquired resistance to fluoroquinolones, the MBCs of moxifloxacin against *Ureaplasma* spp. and *M. hominis* ranged from 0.12 to 0.5 mg/L. All the moxifloxacin MBCs were less than or equal to four times the MICs found during the same experiment (MBC/MIC ratio, R ≤ 4, Table 2), indicating bactericidal activity. Although moxifloxacin kept its activity against the three fluoroquinolone-resistant isolates harbouring *gyrA* and/or *parC* mutations with MICs ≤ 1 mg/L (Table 2), the fluoroquinolone was no longer bactericidal *in vitro*, with an MBC/MIC ratio of 8. It should be noted that, for these isolates, none of the studied fluoroquinolones seemed to be bactericidal in spite of relatively low corresponding MICs, ranging from 0.25 to 4 mg/L (Table 2).

For the 14 *M. genitalium* strains studied, the MBCs of moxifloxacin ranged from 0.06 to 0.25 mg/L, with an MBC < 0.25 mg/L and an MBC/MIC ratio ≤ 4 (Table 2), showing the highest bactericidal activity among fluoroquinolones. The lowest MBCs against *M. genitalium* isolates were obtained with erythromycin (MBC < 0.03 mg/L). Although doxycycline was bactericidal *in vitro* against ten of the 14 *M. genitalium* strains studied, the obvious discrepancy between its *in vitro* and *in vivo* activities against *M. genitalium* should be mentioned, as treatment failures after doxycycline therapy have been commonly reported in clinical studies of *M. genitalium* non-gonococcal urethritis [14].

In summary, this study confirms the high *in vitro* activity of moxifloxacin against recent isolates of urogenital mycoplasmas and against a significant number of *C. trachomatis* isolates. Furthermore, the new data on its bactericidal activity against urogenital mycoplasmas show that moxifloxacin is bactericidal *in vitro* against all the mycoplasma and ureaplasma isolates, except those resistant to fluoroquinolones. Thus, moxifloxacin has attracted interest as a potential therapy for mycoplasmal or chlamydial urogenital infections, all the more because it has recently resolved azithromycin-resistant *M. genitalium* non-gonococcal urethritis in men following treatment failure with azithromycin [14].

**ACKNOWLEDGEMENTS**

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**TRANSPARENCY DECLARATION**

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**REFERENCES**


RESEARCH NOTE

Occupational infection due to Brucella abortus S19 among workers involved in vaccine production in Argentina

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ABSTRACT

The pathological consequences of exposure to the vaccine strain Brucella abortus S19 were evaluated in 30 employees from vaccine-manufacturing plants. Active brucellosis was diagnosed in 21 subjects, of whom only five recalled an accidental exposure. Clinical manifestations were mild, and only one patient presented a complication. After antimicrobial therapy, initially symptomatic patients either experienced clinical remission or had mild persistent symptoms. This is the first study reporting infection by B. abortus S19 among workers from vaccine-manufacturing plants, which in many cases was acquired from unnoticed exposures. Measures to improve the safety of B. abortus S19 handling should be implemented.

Keywords Biosafety, Brucella abortus S19 vaccine, human brucellosis, laboratory workers, occupational exposure

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Brucellosis, a zoonotic disease caused by different species of Brucella, affects several regions, including South America. Humans usually become infected by contact with tissues or secretions from cattle (Brucella abortus), goats and sheep (B. melitensis), pigs (B. suis) and dogs (B. canis). Clinical manifestations of human brucellosis range from asymptomatic to acute disease, complicated chronic disease or, occasionally, death.

The attenuated strain B. abortus S19 has been the vaccine most widely used to prevent bovine brucellosis. In some regions, this vaccine has been replaced by the RB51 strain, a rough mutant that is less virulent for cattle and does not interfere with serological screening [1]. In spite of their reduced virulence, both vaccines can be pathogenic for humans, as has also been observed for B. melitensis Rev-1 [2]. Infection due to these vaccines is usually acquired from conjunctival splashes, skin cuts or, occasionally, infectious aerosols, and generally occurs in individuals involved in animal vaccination [3–6]. An outbreak of brucellosis among employees of a laboratory manufacturing the Rev-1 vaccine has also been reported [7]. In contrast, there are no reports of human brucellosis due to S19 or RB51 strains acquired within vaccine production plants.

The goal of this study was to analyze the clinical, epidemiological and diagnostic aspects of workers with potential exposure to B. abortus S19 in laboratories producing the S19 vaccine in Argentina.

Thirty employees from S19-manufacturing plants (age 19–62 years, mean ± SD 33.5 ± 13.19 years, 20 males) were studied. They were referred between February 1999 and June 2006 because they had suggestive symptoms and/or serology positive for brucellosis. The clinical records of these individuals were retrospectively analyzed. Indi-