In-Vitro activities of tetracyclines, macrolides, fluoroquinolones and clindamycin against *Mycoplasma hominis* and *Ureaplasma* ssp. isolated in Germany over 20 years

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Abstract

Antimicrobial resistance in genital mycoplasmas is increasing and shows global variation. We determined the susceptibilities of 469 mycoplasmas, comprising 290 *Mycoplasma hominis* and 179 ureaplasma isolates collected during 1983 and 1989–2004, to eleven antibacterials by agar dilution. Additionally, we analyzed the results of routine E-testing during 2005–2008. Doxycycline was the most active tetracycline with (MIC<sub>90</sub> of 1 and 8 mg/L for ureaplasmas and *M. hominis*, respectively. Significantly more *M. hominis* isolates (approximately 10–13%) than ureaplasmas (approximately 1–3%) were resistant to tetracyclines. Ofloxacin was effective against both species (>95% susceptibility). Ciprofloxacin was moderately active against *M. hominis* and less active against ureaplasmas (70.3% and 35.2% susceptibility, respectively). Clarithromycin and josamycin were the most potent macrolides (MIC<sub>90</sub> of 0.5 mg/L) against ureaplasmas. Erythromycin had the lowest activity (MIC<sub>90</sub> of 8 mg/L) against ureaplasmas like clindamycin which was the most potent agent against *M. hominis*. Cross-resistance was found between tetracyclines (53–93%), macrolides and erythromycin (70–100%), and between erythromycin and ciprofloxacin (43–55%). *M. hominis* became more resistant to tetracyclines and fluoroquinolones between 1989 and 2004, although there was little change during 2005–2008. Ureaplasmas became more resistant to ciprofloxacin during 1997 – 2004 and showed high resistance rates to erythromycin during 1989 – 2008. Doxycycline is still the drug of first-choice for the treatment of ureaplasmal infections and may be used for co-infection with *M. hominis*.

Keywords: Antimicrobials, cross-resistance, MICs, resistance development

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Introduction

*Mycoplasma hominis* and ureaplasmas frequently colonize the genital tract of humans. They are associated with genitourinary tract infections (urethritis, cervicitis, cystitis, bacterial vaginosis) and appear to have an aetiological role in postpartum infections of mothers and newborns [1–5].

*Mycoplasma* and *Ureaplasma* ssp. are susceptible to agents that interfere with protein synthesis, such as tetracycline, macrolides, aminoglycosides and chloramphenicol, and to the fluoroquinolones that act by inhibiting topoisomerases [4,6–12]. Mycoplasma and ureaplasma infections are usually treated with tetracycline, except in pregnant women, neonates or children in whom erythromycin is recommended [4,10,11]. However, there are increasing reports of resistance to these agents as a result of the presence of the tetM resistance determinant or chromosomal mutation [10,11,13–17]. Clindamycin, fluoroquinolones or other macrolides can be used to treat infections that do not respond to tetracycline or erythromycin [9,10]. However, there are few large studies on the antimicrobial susceptibilities of these species.

The purpose of the present study was: (i) to investigate the susceptibilities of a large number of clinical isolates of *M. hominis* and ureaplasmas to various antimicrobial agents, including tetracyclines, macrolides, clindamycin and fluoroquinolones, and (ii) to compare changes in the antimicrobial susceptibility patterns of these pathogens over several years, from 1983 and 1989–2008. The study aimed to: (i) clarify whether laboratory susceptibility testing is still worthwhile; (ii) clarify whether the recommended treatment regimes...
need to be modified; and (iii) determine the most appropriate agents for empirical therapy.

Materials and Methods

Bacterial strains
The 469 investigated strains of *M. hominis* (*n* = 290) and ureaplasmas (*n* = 179) were nonduplicate isolates from the urogenital tracts of adults collected prospectively during 1983 (when only *M. hominis* strains were available) and 1989–2004 (when both species were available), in Schleswig-Holstein, north Germany. After isolation on selective growth media and identification by conventional biochemical methods, followed by the growth inhibition test [18] using specific antisera as previously described [2], isolates were stored at −70°C in modified Hayflick’s liquid medium (B) (for mycoplasmas) and modified Shepard’s Broth (S) (for ureaplasmas) [2]. Isolates were tested with the same batch of drugs at the same time and under the same conditions to maintain comparability. Results from routine testing were also obtained from isolates collected during 2005–2008 (*M. hominis*, *n* = 74; ureaplasmas, *n* = 226).

Antimicrobial agents
The antimicrobial agents used were doxycycline (DOX) and azithromycin (AZM) (both from Pfizer, Karlsruhe, Germany), minocycline (MIN) (Wyeth-Pharma, Münster, Germany), tetracycline (TET) (Grüenthal, Aachen, Germany), clarithromycin (CLR) (Abbott, Wiesbaden, Germany), erythromycin (ERY) (Durachemie, Münster, Germany), josamycin (JOS) (Mack, Illertissen, Germany), roxithromycin (ROX) and ofloxacin (OFX) (Hoechst Marion Roussel, Frankfurt, Germany), clindamycin (CLI) (Pharmacia & Upjohn, Erlangen, Germany) and ciprofloxacin (CIP) (Bayer, Leverkusen, Germany). Each antimicrobial agent was prepared according to the manufacturer’s instructions at a stock concentration of 1600 mg/L and stored at −70°C before use; 0.9% NaCl was used as a diluent for all of the drugs.

Antimicrobial susceptibility testing
Antimicrobial susceptibility was determined by the standard agar dilution method by using modified A7-agar medium [19] as previously described [8]. Agar plates containing two-fold serial dilutions of antibiotics (range 0.001–16 mg/L) and antibiotic-free control plates were inoculated with a bacterial suspension (approximately $10^5–10^6$ CFU/mL) in S or B broth [2] with a multipoint inoculator (Titertek; Flow Laboratories, Meckenheim, Germany) [8]. The inoculated plates were incubated anaerobically at 37°C and read after 2 days under a stereo-microscope (Wild M3; Wild Heerbrugg, Switzerland) at ×16 and ×40 magnifications. The reference type strain of *M. hominis* PG 21 was included in each test. The MIC was defined as the lowest antibiotic concentration that completely inhibited the development of visible growth on the agar plates and was determined in duplicate for each isolate. Because no specific breakpoints have been established for mycoplasmas and ureaplasmas, the tentative breakpoints used for susceptible (S), intermediate (I) and resistant (R) strains were the MIC interpretive standards (mg/L) recommended by the CLSI (formerly NCCLS) for Enterobacteriaceae (for CIP, OFX, DOX, MIN and TET) and for *Staphylococcus* spp. (for AZM, CLR, ERY, JOS, ROX and CLI). These breakpoints were (S/I/R): for CLI, ≤0.5/1–2/≥4; for CIP, ≤1/2/≥4; for AZM, CLR, JOS, ROX and OFX, ≤2/4/≥8; for ERY, ≤0.5/1–4/≥8; and for DOX, MIN and TET, ≤4/8/≥16 [20].

Antimicrobial susceptibilities of isolates from 2005 to 2008 (*n* = 300) for DOX, CIP, ERY and CLR were determined routinely by the E-test method, according to the manufacturer’s instructions (AB Biodisk, Solna, Sweden). To estimate the absolute level of MICs, the values were rounded up to the next highest two-fold dilution when necessary.

Statistical analysis
The Mann–Whitney *U*-test was used for statistical analysis of MIC values. The chi-square test was used to compare the rates of susceptible and resistant strains to different antibiotics. *p* <0.05 was considered statistically significant.

Results

Antimicrobial effectiveness
MICs of the various antibacterials are shown in Table 1. DOX was the most active tetracycline against ureaplasmas and *M. hominis* (MIC$_{90}$ of 1 and 8 mg/L, respectively). TET and MIN showed two-fold higher MICs and, similar to DOX, were eight-fold more active against ureaplasmas than mycoplasmas. Significantly more *M. hominis* (approximately 10–13%) than ureoplasmas (approximately 1–3%) were resistant to tetracyclines. OFX was effective against both species (>95% susceptibility), and was significantly more active than CIP (70.3% of *M. hominis* and 35.2% of ureaplasmas were susceptible) (*p* <0.0001).

JOS was the only macrolide active against *M. hominis*. CLR and JOS were the most potent macrolides (MIC$_{90}$ of 0.5 mg/L) against ureaplasmas; CLR was significantly more active than JOS and DOX (*p* <0.0001) and JOS was more active than DOX (*p* 0.004). ERY had the lowest activity (MIC$_{90}$ of...
8 mg/L) against ureaplasmas like clindamycin which was the most potent drug against *M. hominis*.

**Cross-resistance**
A total of 17.9% (52/290) *M. hominis* strains were resistant to at least one tetracycline, with 36.5% (19/52) of the isolates showing multiple resistance; the level of cross-resistance varied in the range 55.3–93.1%.

Tetracyclines showed significantly lower cross-resistance with OFX (0–2.6%) than with CIP (10.5–18.2%) (Fig. 1a); approximately half the strains (44.8–55.3%) were inhibited by £1 mg/L CIP. No cross-resistance was detected between

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**TABLE 1. In-vitro activity of tetracyclines, macrolides, clindamycin and fluoroquinolones against 469 human isolates of genital *Mycoplasma* and *Ureaplasma* spp.**

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC50 (mg/L)</th>
<th>MIC90 (mg/L)</th>
<th>GM</th>
<th>% resistant strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxycycline</td>
<td>0.125</td>
<td>8</td>
<td>0.28</td>
<td>10.0</td>
</tr>
<tr>
<td>Minocycline</td>
<td>0.5</td>
<td>16</td>
<td>0.83</td>
<td>13.1</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.5</td>
<td>16</td>
<td>0.87</td>
<td>11.4</td>
</tr>
<tr>
<td>Josamycin</td>
<td>2</td>
<td>4</td>
<td>1.27</td>
<td>3.4</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>99.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>98.3</td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>98.3</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.25</td>
<td>0.5</td>
<td>0.23</td>
<td>0.0</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>1</td>
<td>2</td>
<td>0.80</td>
<td>1.7</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1</td>
<td>2</td>
<td>1.03</td>
<td>7.9</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>98.6</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>95.9</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.25</td>
<td>0.5</td>
<td>0.23</td>
<td>0.0</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>1</td>
<td>2</td>
<td>0.80</td>
<td>1.7</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1</td>
<td>2</td>
<td>1.03</td>
<td>7.9</td>
</tr>
</tbody>
</table>

GM, geometric mean of MIC (mg/L); %, percentage of strains resistant by CLSI (2007) criteria and as mentioned in the Materials and methods.

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**FIG. 1. Cross-resistance rates for *Mycoplasma hominis* (a) and *Ureaplasma* spp. (b) isolates to selected antibacterials such as doxycycline (DOX), tetracycline (TET), ciprofloxacin (CIP), ofloxacin (OFX), erythromycin (ERY) and azithromycin (AZM).** Additional drug abbreviations are provided in Tables 2 and 3. *Resistant isolates to at least one tetracycline. *Including OFX-resistant (n = 9) and -intermediate (n = 5) *M. hominis* isolates. Antibacterial with no detectable intermediate susceptibility (MIN, minocycline; CLR, clarithromycin; azithromycin) not included in the figure.
tetracyclines and CLI, which showed the greatest potency, inhibiting all these strains at ≤1 mg/L (MIC$_{90}$ 0.5 mg/L) (Fig. 1a).

CIP-resistant isolates were resistant to tetracyclines in 17.4–26.1% of strains and resistant to JOS in 30.4%. Isolates inhibited by ≥4 mg/L OFX were susceptible to tetracyclines (71–100%), CLI (79%) and JOS (71%). CIP had poor activity (approximately 43% susceptibility).

Overall, 23.5% (42/179) of ureaplasma isolates were resistant to at least one macrolide. The cross-resistance between ERY and tetracyclines varied in the range 2.7–13.5% (Fig. 1b). ERY-resistant isolates were susceptible to tetracyclines (81–89%), JOS (89.2%) and OFX (86.5%) but not to CIP (19% susceptibility). A significantly higher level of cross-resistance was detected between all the macrolides and ERY (70–100%). Tetracyclines, macrolides (except ERY) and OFX were more or less effective against CIP-resistant strains, inhibiting 86.2–96.6%, 69–93.1% and 79.3% of the strains, respectively; JOS, DOX and MIN had the highest efficiency (MIC$_{90}$ of 1–2 mg/L) and the lowest rate of cross-resistance. ERY was ineffective (55.2% cross-resistance; MIC$_{50/90}$ of 8/16 mg/L) (Fig. 1b).

**Susceptibility profiles over the test period**

A significant increase in tetracycline MIC$_{90}$ was seen among M. hominis isolates collected during 1989–2004 compared to those collected in 1983 (Table 2). During the three periods 1983 (47 isolates), 1989–1996 (126 isolates) and 1997–2004 (117 isolates), the changes in resistance were: resistance to DOX rose from 0% to 9.5% (p = 0.064) to 14.5% (p = 0.013); resistance to TET from 2.1% to 11.9% (p = 0.092) to 14.5% (p = 0.043); resistance to MIN from 0% to 15.9% (p = 0.008) to 15.4% (p = 0.01); resistance to CIP from 0% to 4% to 15.4% (p = 0.005); and resistance to OFX rose from 0% to 0.8% to 3.4%. A two-fold increase in MIC$_{90}$ for CLI and CIP and reduced susceptibility towards JOS with a resistance of 8.5% was observed in 1997–2004. The rate of resistance to DOX in M. hominis isolates during 2005–2008, determined by the E-test was 13.5% (MIC$_{50/90}$ of 0.125/16 mg/L) and to CIP was 13.3% (MIC$_{50/90}$ of 1/4 mg/L) (data not shown).

Ureaplasmas showed reduced susceptibility to all the classes of antibiotics during the studied period, leading to an increase in MIC geometric means (Table 3). The MIC$_{90}$ increased two-fold for some antibacterials during 1997–2004. Significantly diminished susceptibility was seen with DOX (p = 0.002), TET (p = 0.003), macrolides [CLR and ROX (p <0.001), JOS (p = 0.002), and AZM (p = 0.007)]. CIP (p <0.0001) and OFX (p <0.001); the difference was not significant for ERY (p = 0.151). However, it should be noted that a high percentage of isolates (44.8–51.1%) were inhibited at high concentrations of ≥4 mg/L ERY through the studied period. A significant increase in resistance (p = 0.003) was observed only for CIP (from 5.7 to 26.1%), whereas resistance to ERY remained approximately the same (19.5% and 21.7%). Resistance to DOX and CLR remained low (approximately 1.2%; MIC$_{50/90}$ of 0.25/10 mg/L and 3.2%; MIC$_{50/90}$ of 0.125/0.5 mg/L, respectively) during 2005–2008;

**TABLE 2. Susceptibility profiles of Mycoplasma hominis (n = 290) isolates collected during 1983 (n = 47), 1989–1996 (n = 126) and 1997–2004 (n = 117)**

<table>
<thead>
<tr>
<th>Drug*</th>
<th>Year</th>
<th>Cumulative % of isolates inhibited by MIC (mg/L)*</th>
<th>GM of MICs (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≤0.008</td>
<td>0.015</td>
</tr>
<tr>
<td>DOX</td>
<td>1983</td>
<td>– – –</td>
<td>8.5</td>
</tr>
<tr>
<td>TET</td>
<td>1983</td>
<td>– – –</td>
<td>0.8</td>
</tr>
<tr>
<td>MIN</td>
<td>1983</td>
<td>– – –</td>
<td>0.9</td>
</tr>
<tr>
<td>JOS</td>
<td>1983</td>
<td>– – –</td>
<td>0.9</td>
</tr>
<tr>
<td>CLI</td>
<td>1983</td>
<td>– – –</td>
<td>0.9</td>
</tr>
<tr>
<td>OFX</td>
<td>1983</td>
<td>– – –</td>
<td>0.8</td>
</tr>
<tr>
<td>CIP</td>
<td>1983</td>
<td>– – –</td>
<td>0.7</td>
</tr>
</tbody>
</table>

GM, geometric mean; DOX, doxycycline; TET, tetracycline; MIN, minocycline; JOS, josamycin; CLI, clindamycin; OFX, ofloxacin; CIP, ciprofloxacin; –, not detected.

MIC$_{50/90}$ and MIC$_{90}$ (bold) values of antibacterials against Mycoplasma (M.) hominis are indicated.

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ERY-resistance was 19% (MIC90 of 1.08 mg/L) during 2005–2008, which was comparable with results from earlier years (data not shown).

Discussion

In the present study, different antibacterials showed different levels of activity against these mycoplasmas and ureaplasmas. The majority of both M. hominis and ureaplasmas were most sensitive to OFX (approximately 95–97%), followed by tetracyclines (approximately 82–88% and 96–97%, respectively) and JOS (approximately 75 and 98%). Most were resistant to DOX, as well as OFX for treatment of genital mixed infections with M. hominis and ureaplasmas. The newer quinolones, levofloxacin (LVX) and moxifloxacin (MXF), which have good in vitro activity against both M. hominis (MIC90 of 0.5–1.0 and 0.03–0.06 mg/L, respectively) and ureaplasmas (MIC90 of 0.5–1.0 and 0.25–0.5 mg/L), superior to that of OFX [4, 7, 12, 24, Krausse R, Schubert S, unpublished data], could also be considered. CIP could be used for empirical treatment of mono-infection with M. hominis; CLI and JOS are alternatives because CLI had the greatest potency against M. hominis and no activity against ureaplasmas [11, 25], and JOS was the only effective macrolide against M. hominis, as noted in previous studies [7, 8, 26]. M. hominis is intrinsically resistant to ERY and other 14- and 15-membered
macrolides, whereas Ureaplasma spp. is naturally resistant to lincosamides such as CLI [4,10,15–17].

The most potent antibacterials against ureaplasmas were CLR, JOS and DOX, as previously reported [4,8,26]. CIP proved to be inactive, as reported previously [1,9], whereas OFX exhibited good activity. AZM was effective as well, with significantly better activity than OFX and TET, as in previous studies [7,9]. Recent studies reported equal activity for DOX and JOS, but a poor activity for CLR, AZM and ERY [1].

Other studies, investigating a large number (n = 100) of clinical isolates from different geographical regions, reported two-fold higher MICs for AZM compared to our studies [25]. Waites et al. [4] noted a four-fold lower MIC₉₀ for ERY and AZM and very low values for CLR. ERY is the most commonly used macrolide for treating ureaplasma infections, especially in pregnant women or neonates. However, the high MIC of this antibiotic against this commensal pathogen suggests that ERY is no longer the first choice for treatment of such infections, at least in our region. Thus, susceptibility testing is recommended for this drug before use. CLR and JOS or OFX could be used as alternatives, but not CIP (approximately 20% susceptibility). This strategy may not be transferable to other countries, such as Greece or Turkey, because of the poor activity of CLR and OFX [1,21]. A high percentage of our isolates (≥91%) inhibited by ≥4 mg/L ERY, were susceptible to these drugs.

The frequency of ERY-resistant ureaplasmas varies significantly between studies [1,4,21,27]. The activity of macrolides, especially ERY, is markedly affected by acidic pH [4,7], and this might partially explain the controversial results. The medium used in the present study had a higher pH (6.5) than that usually used (6.0) for ureaplasmas, and was unlikely to have affected the ERY MICs [7].

Differences in susceptibilities have been reported between the two Ureaplasma spp., Ureaplasma parvum (the most frequently isolated from clinical specimens) and Ureaplasma urealyticum and within the 14 distinct serovars [9,10,27,28]. However, the data are limited and conflicting. Unfortunately, comparison cannot be made in the present study because our isolates were not serotyped. In our region, ureaplasmas resistant to tetracyclines are rare, as recently reported (approximately 3%) [14]; thus, tetracyclines, especially DOX, continue to be the first choice for treating infection with these bacteria. DOX-resistant isolates were often accompanied by resistance to macrolides and CIP, but not to OFX. Therefore, OFX may be a therapeutic option in the management of ureaplasmal infections when tetracyclines cannot be used or macrolide resistance is a local problem.

Significant cross-resistance was detected between all the macrolides and ERY (up to 100%). CLR-resistant ureaplasmas were mainly resistant to all other macrolides and quinolones, so these drugs cannot be considered as an alternative; in these cases, DOX, which exhibited the lowest rate of cross-resistance, could be used. CIP showed limited cross-resistance with OFX; thus, OFX could also be used as a second alternative to tetracyclines. JOS could be used for CIP-resistant strains, but not vice versa; OFX-resistant ureaplasmas were all resistant to CIP, as previously reported [6].

Similar to previous studies [23], we found a high frequency of cross-resistance between tetracyclines for M. hominis; in these cases, OFX, CLI, or JOS may be considered. TET-resistant strains have been reported to have similar susceptibility to quinolones as the TET-susceptible strains [7,24,29]; this is in disagreement with our results, which showed that TET-resistant strains showed a significantly diminished susceptibility to quinolones.

Differences in antibacterial sensitivity were observed over the study period with more susceptible strains isolated in the earlier years. Resistance to CIP increased over the study period for both species. M. hominis has become more resistant to tetracyclines and fluoroquinolones over the years 1989–2004, with no changes in 2005–2008. A continuous rise in resistance, in M. hominis, to tetracyclines was reported previously [13,14]. There was a significant increase in MICs of all agents against ureaplasmas; however, there was no increase of resistance (except for CIP). DOX- and CLR-resistance remained low and unchanged during 2005–2008, as previously reported [14]. Generally, in our region, ureaplasmas have become significantly more resistant to CIP over the last few years and have shown very high ERY-resistance.

The susceptibility of ureaplasmas and M. hominis to antibacterials in different regions is difficult to compare because of different test methods, different breakpoints and a lack of standardization [4,7,30]. As far as we know, this is the first study to extensively analyse the resistance and cross-resistance to different classes of antibacterials, against a large number of clinical isolates of M. hominis and ureaplasmas over a long time period in Germany.

In conclusion, DOX is still the drug of first-choice for ureaplasmal infections and may be used for co-infection with M. hominis. CLI is very active against M. hominis and may be used as an alternative to tetracyclines. CLR and JOS exhibited an excellent efficacy against ureaplasmas; JOS is also active against mycoplasmas and may be used as an alternative to tetracyclines and ERY and in mixed infections, especially in pregnant women and neonates. OFX and the newer fluoroquinolones, LVX and MXF, are effective against both species and may be used in cases of tetracycline- and ERY-resistance. However, local in-vitro susceptibility testing is recommended, especially for ERY and CIP.
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Transparency Declaration

None to declare. The authors have no conflicts of interest to declare.

References


