Prevalence and antibiotic susceptibility of *Mycoplasma hominis* and *Ureaplasma urealyticum* in pregnant women

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**Introduction**

Genital mycoplasmas represent a group of microorganisms that are commonly found in the genitourinary tract of preg-
M. hominis and U. urealyticum in pregnant women

The genital mycoplasmas that contribute most to these conditions are *Mycoplasma hominis* and *Ureaplasma urealyticum*. They are most commonly present in the genital tract of sexually active women.7–9 Colonization values worldwide for *M. hominis* range between 20% and 30% and for *U. urealyticum* range between 60% and 80%.10 The prevalence of these organisms is significantly associated with socioeconomic conditions, such as poverty, and increasing number of sexual partners.8,10 They pose a serious medical threat to the mother during gestation and to the fetus and neonate.8 The role of mycoplasmas in the aetopathogenesis of the urogenital system is still the subject of controversy.11 Controversy regarding the association of genital mycoplasmas with bacterial vaginosis also exists; some investigators claim that there is a relationship between the two,12 whereas others do not.13 Mycoplasmas lack a cell wall—the target of beta-lactam antibiotics and vancomycin. Tetracyclines, macrolides, and quinolones are the major antibiotics used in the treatment of urogenital infections caused by mycoplasmas.8 However, their therapeutic efficacy may be unpredictable due to increasing resistance.14 The extent of resistance varies geographically according to different antimicrobial therapy policies and the history of prior antimicrobial exposure in different populations.6,8

The aim of the present study was to detect the presence of *U. urealyticum* and *M. hominis* in symptomatic and control pregnant women, including an analysis by age group and gestation period. The association of these mycoplasmas with pregnancy complications and their antimicrobial susceptibility were also assessed.

**Subjects and methods**

One hundred pregnant women (50 symptomatic patients and 50 asymptomatic controls) were studied at outpatient visits to the gynecology and obstetrics clinics of Turgut Ozal Medical Center, Inonu University Hospital, Malatya, Turkey from July 2006 to May 2007. A questionnaire was completed for each woman, recording personal data, sexual history, symptoms, and other obstetric and gynecologic disorders. Babies born weighing less than 2500 g were classified as low birth weight infants according to the World Health Organization criteria. The mean ± standard deviation age of the patients was 28.6 ± 5.3 years (range 18–41 years) and of controls was 28.6 ± 5.6 years (range 18–39 years). Enrolled subjects were sexually active pregnant women with a gestation period of ≤36 weeks.

The patient group experienced symptoms of vaginitis, abnormal vaginal discharge, and/or itching or burning in the genital area. Controls were those attending routine checkups and had none of the above symptoms. It was stipulated that all women participating in the study should not have taken any antimicrobial agent prior to sampling that could affect the growth of mycoplasmas. Women who did not meet the inclusion criteria were excluded from the study, hence they were not consecutive cases. The approval of the Ethics Committee of Inonu University was obtained. All women gave informed consent prior to enrolling.

*M. hominis* and *U. urealyticum* are not routinely detected by conventional bacteriological methods. For this study a commercial kit, Mycoplasma IST-2 (BioMerieux, Marcy l’Etoile, France), was used according to the manufacturer’s instructions. The kit contains strips that give information on the presence or absence of *M. hominis* and *U. urealyticum* and also provide additional information on antibiotic susceptibility to doxycycline, josamycin, ofloxacin, erythromycin, tetracycline, ciprofloxacin, azithromycin, clarithromycin, and pristinamycin. In order to process specimens, two endocervical swabs were obtained from each individual. These were processed in the laboratory within 4 hours. One strip was placed directly into R1 tubes (transport medium) and subsequently delivered to the clinical laboratory for the identification of both *U. urealyticum* and *M. hominis* and to determine antimicrobial susceptibility. The other swab was cultured on blood, chocolate, and Sabouraud dextrose agars. A Gram stained smear was made. The presence of other organisms, such as *Gardnerella vaginalis*, was assessed according to Amsel’s criteria and conventional microbiological techniques.15,16

Swabs in the R1 transport medium were processed according to the manufacturer’s instructions. They were vortexed rapidly, and 3 ml of R1 was used to rehydrate the lyophilized growth medium R2 (provided in the Mycoplasma IST-2 kit). A Mycoplasma IST strip, consisting of 22 wells, was then inoculated with the rehydrated R2 growth medium (55 μl per well, overlaid with two drops of mineral oil). From the R2 positive tube, 0.1 ml was also inoculated onto A7 Mycoplasma agar plates (BioMerieux, Marcy l’Etoile, France) and incubated at 37°C in an atmosphere of 5% CO₂ for checking characteristic colony morphology. All media and the inoculated strip were incubated at 37°C in a CO₂ incubator and observed for color changes, and the results were interpreted after 24 and 48 h of incubation. Wells 1–5 provide information on the presence or absence of *M. hominis* and *U. urealyticum*, with an estimate of the density of each organism (≥10⁴ CFU), and wells 6–22 show the antimicrobial susceptibilities to doxycycline, josamycin, ofloxacin, erythromycin, tetracycline, ciprofloxacin, azithromycin, clarithromycin, and pristinamycin. The A7 plates were examined with a microscope twice daily for up to 5 days for characteristic colonies. Colonies presenting with a fried egg appearance suggest the presence of *M. hominis*, whereas colonies that are brown and tiny indicate the presence of *U. urealyticum*. *M. hominis* ATCC 15488 and *U. urealyticum* ATCC 27813 strains were used as controls.

Statistical analysis was performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were analyzed by Chi-square test. p-Values of <0.05 were considered statistically significant.

**Results**

Of the 100 subjects, 29 (29%) were positive for *M. hominis* and/or *U. urealyticum*. Of these, 27 women were in the patient group (54%) and two were in the control group (4%). *U. urealyticum* was isolated alone from 22/50 (44%) patients and 2/50 (4%) controls (p < 0.05). The frequency of
M. hominis detected alone in the patient group was 2/50 (4%); no M. hominis was detected in controls. Three women within the patient group (6%) were co-infected with both M. hominis and U. urealyticum. Thus 32 M. hominis and U. urealyticum were identified overall, 27 (84.4%) and five (15.6%) cum (4%); no M. hominis detected alone in the patient group was 2/50 M. hominis within the patient group (6%) were co-infected with both hominis and urealyticum.

The distribution of M. hominis and U. urealyticum according to age group is shown in Table 2. Of the symptomatic patients, six (12%) were aged 18—24 years, 12 (24%) were aged 25—29 years, six (12%) were aged 30—34 years, and three (6%) were over 35 years old. Significantly, approximately two thirds of U. urealyticum and M. hominis occurred in patients between the ages of 25 and 34 years (p < 0.05).

The distribution of M. hominis and U. urealyticum according to gestation period is shown in Table 3. Of the 32 mycoplasmas, 13 (48.1%) were seen in patients at between 33 and 36 weeks of gestation (p < 0.05). Twelve women in the patient group had a spontaneous abortion at a gestation period of less than 36 weeks (Table 4). Of these, eight (66.7%) cases had been colonized with M. hominis and/or U. urealyticum and only three cases were simultaneously infected with G. vaginalis. Other organisms detected in the patient group are presented in Table 5. These were as follows: G. vaginalis (seven strains, five in the patient group and two in controls), beta-hemolytic streptococcus (Streptococcus agalactiae; two strains, both in patients), Candida albicans (22 strains, 12 in patients and 10 in controls), and 13 strains of other Candida species. Some of the patients were co-infected with M. hominis and/or U. urealyticum (11 cases) as follows: three (27.3%) with strains of G. vaginalis, two (18.2%) with S. agalactiae, and six (54.5%) with C. albicans (Table 5).

Among symptomatic patients with positive cervical mycoplasmas, 11 (40.7%) were associated with low birth weight (Table 4).

Antimicrobial susceptibilities were determined using the Mycoplasma IST-2 kit and are shown in Tables 6 and 7. All strains were susceptible to doxycycline, tetracycline, and pristinamycin. Multiple resistance to two antibiotics was rarely seen, even when mixed genital mycoplasmas were present. Among M. hominis, the highest drug resistance rate was 100% to erythromycin, while the highest drug resistance rates in U. urealyticum were 92.6% to ciprofloxacin and 85.2% to ofloxacin. No resistance was observed to josamycin, although some strains had intermediate resistance.

### Discussion

In our study, 27 women (54%) in the patient group were positive for M. hominis, U. urealyticum, or both mycoplasmas. In controls, two women (4%) yielded only U. urealyticum (p < 0.05). None of the controls yielded M. hominis. Three cases (6%) were infected with both M. hominis and U. urealyticum. The highest prevalence of M. hominis and/or U. urealyticum was seen in pregnant women in the 18—34 years age group (Table 2). In comparing our findings with those of
many previous studies, which have reported between 10% and 50% of women to be colonized with *U. urealyticum* but colonization rates with *M. hominis* of less than 30%, we found a distinct disproportion in the incidence of these two mycoplasmas. *U. urealyticum* was more commonly detected than *M. hominis* in both the patients and controls. Our findings are fairly consistent with those of other studies conducted in Poland and Greece, but distinctly different to those of studies from the highlands of Papua New Guinea, Portugal, and Japan. The presence of genital mycoplasmas is associated with an increased risk of developing certain pathologic conditions of pregnancy, such as spontaneous abortion, preterm labor, and low birth weight. In the present study, 40.7% of pregnant women infected with *M. hominis* and/or *U. urealyticum* delivered a low birth weight infant. Moreover, eight out of 12 (66.7%) spontaneous abortion cases had been colonized with *M. hominis* and/or *U. urealyticum*. Although, it has been reported that *U. urealyticum* is associated with premature onset of labor, the exact role of mycoplasmas in such cases is not fully understood.

Simultaneous colonization with both *M. hominis* and *U. urealyticum* was not common (6% in symptomatic patients), but has been found to be as low as 2.92% in one population and as high as 60% in another. This discrepancy may be due to variations in socioeconomic conditions and living standards.

**Table 5** Co-infections of *Mycoplasma hominis* and/or *Ureaplasma urealyticum* with other pathogens

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Patients</th>
<th>Controls</th>
<th>Co-infection with mycoplasmas in patients, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gardnerella vaginalis</em> (n = 7)</td>
<td>5</td>
<td>2</td>
<td>3 (27.3%)</td>
</tr>
<tr>
<td><em>Candida albicans</em> (n = 22)</td>
<td>12</td>
<td>10</td>
<td>6 (54.5%)</td>
</tr>
<tr>
<td>Beta-hemolytic Streptococcus (n = 2)</td>
<td>2</td>
<td>0</td>
<td>2 (18.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>12</td>
<td>11 (100%)</td>
</tr>
</tbody>
</table>

**Table 6** Antimicrobial susceptibility of the total identified mycoplasmas determined using the Mycoplasma IST-2 kit

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxycycline</td>
<td>32 (100%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Josamycin</td>
<td>29 (90.6%)</td>
<td>3 (9.4%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>4 (12.5%)</td>
<td>2 (6.3%)</td>
<td>26 (81.3%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>16 (50%)</td>
<td>5 (15.6%)</td>
<td>11 (34.4%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>32 (100%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5 (15.6%)</td>
<td>0 (0)</td>
<td>27 (84.4%)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>17 (53.1%)</td>
<td>7 (21.9%)</td>
<td>8 (25%)</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>27 (84.4%)</td>
<td>1 (3.1%)</td>
<td>4 (12.5%)</td>
</tr>
<tr>
<td>Pristinamycin</td>
<td>32 (100%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Results are n (%).
M. hominis, they were found to be the most active agents against these pathogens in the current work, and could be used in empirical therapy. M. hominis is naturally resistant to erythromycin whereas U. urealyticum is moderately susceptible to macrolides but is resistant to quinolones. The significant difference related to susceptibility to macrolides and quinolones has been reported before. Resistance to macrolides such as ofloxacin and ciprofloxacin has been observed in our clinical isolates of U. urealyticum. Higher resistance of mycoplasmas to antimicrobials is due to mutations in antibiotic targets and may suggest their relation to higher pathogenicity.

Empirical therapy is important in the treatment of mycoplasmas since culture and identification methods are not routinely used for the detection of mycoplasmas in Turkish clinical laboratories. This is of paramount importance for successful therapy and clinicians recommend early administration of antibiotics for a good pregnancy outcome and to prevent the occurrence of complications. However, limitations of this study are the small number of samples and that we could not investigate other potential pathogens such as Ureaplasma parvum, since this was not available in the commercial kit used in the present study.

Conclusions

This study shows a low prevalence of mycoplasmas in pregnant women in our population, since this population is generally conservative and there is mostly one sex partner. The prevalence of genitourinary infections due to U. urealyticum was considerably higher as compared to M. hominis infection. The prevalence of U. urealyticum and M. hominis was significantly correlated with age and gestation period. M. hominis and U. urealyticum may be associated with spontaneous abortion and low birth weight. In inflammatory and pathological states of pregnancy, testing for the presence of mycoplasmas is necessary for a safe pregnancy outcome and should be included in the diagnostic protocol. Our results also indicate that doxycycline, tetracycline, and pristinamycin are the first choice drugs when empirical therapy is required.

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Conflict of interest: No conflict of interest to declare.

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