

Prevalence and antibiotic susceptibility of *Mycoplasma hominis* and *Ureaplasma urealyticum* in genital samples collected over 6 years at a Serbian university hospital

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ABSTRACT

Background: *Mycoplasma hominis* and *Ureaplasma urealyticum* are implicated in a wide array of infectious diseases in adults and children. Since some species have innate or acquired resistance to certain types of antibiotics, antibiotic susceptibility testing of mycoplasma isolated from the urogenital tract assumes increasing importance. **Aims:** To evaluate the prevalence and antibiotic susceptibility of *M. hominis* and *U. urealyticum* in genital samples collected between 2007 and 2012. **Methods:** Three hundred and seventy three patients presenting with symptoms of sexually transmitted diseases, infertility or risky sexual behaviour, who had not taken antibiotics in the previous 6 weeks and had ≥ 10 WBC per high power field on genital smears were studied. Urethral samples were taken in men and endocervical samples in women. The mycoplasma IST-2 kit was used for organism identification and for testing susceptibility to doxycycline, josamycin, ofloxacin, erythromycin, tetracycline, ciprofloxacin, azithromycin, clarithromycin and pristinamycin. **Results:** *U. urealyticum* was isolated from 42 patients and *M. hominis* from 11 patients. From 9.8% of isolates, both organisms were grown. All *M. hominis* isolates were resistant to tetracycline, clarithromycin and erythromycin while *U. urealyticum* was highly resistant to clarithromycin (94.6%), tetracycline (86.5%), ciprofloxacin (83.8%) and erythromycin (83.8%). *M. hominis* was sensitive to doxycycline (83.3%) and ofloxacin (66.7%) while most *U. urealyticum* strains were sensitive to doxycycline (94.6%). **Limitations:** Inability of the commercial kit used in the study to detect other potentially pathogenic urogenital mycoplasmas (*Ureaplasma parvum*, *Mycoplasma genitalium*). **Conclusion:** There is significant resistance of *U. urealyticum* and *M. hominis* to tetracycline and macrolides. The most active tetracycline for genital mycoplasmas was found to be doxycycline, which continues to be the drug of first choice.

Key words: Antibiotic susceptibility, genital mycoplasmas, university hospital

INTRODUCTION

Mycoplasmas belong to the class of Mollicutes and they are the smallest free-living micro-organisms. The urogenital mycoplasmas, *Mycoplasma hominis* and *Ureaplasma urealyticum* are part of the normal commensal flora of the genital tract of sexually active healthy adults. Moreover, *M. hominis* and

U. urealyticum are implicated in a wide array of infectious diseases in adults and children. They are suspected to be the causative agents of non-gonococcal urethritis, pregnancy complications, prenatal infections, infertility, bacterial vaginosis and pelvic inflammatory disease.^[1,2] The incidence of infection

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is affected by the menstrual cycle, pregnancy and the use of vaginal contraceptives.^[3]

Urogenital mycoplasmas, unlike conventional bacteria, do not have a cell wall and are not susceptible to penicillins, cephalosporins, vancomycin and rifampicin. *Mycoplasma* and *Ureaplasma* spp. are currently susceptible to agents that interfere with protein synthesis such as tetracyclines, macrolides, aminoglycosides and chloramphenicol and to fluoroquinolones that inhibit topoisomerases.^[4,5] Some mycoplasmal species are selectively and innately resistant to an antibiotic to which other species are sensitive. An example of this is *M. hominis*, all strains of which are resistant to erythromycin. *Mycoplasma* also can develop resistance to antibiotics to which they are usually considered sensitive.^[4] Due to this fact, testing urogenital mycoplasmas for antibiotic susceptibility is assuming greater importance.

The aims of this study were to investigate the prevalence and antibiotic susceptibilities of genital *U. urealyticum* and *M. hominis* between 2007 and 2012.

METHODS

The study was conducted on 373 patients attending the Clinic of Dermatovenereology in Belgrade from January 2007 to May 2012, whose urethral and cervical swabs were analyzed for the presence of *U. urealyticum* and *M. hominis*.

All investigated patients either had symptoms of genital infection or attended the clinic for microbiological investigation for infertility, risky sexual behavior or because their sexual partners suffered from sexually transmitted diseases. The patients participating in this study had not taken any antimicrobial agent for at least 6 weeks prior to investigation and had greater than or equal to 10 white blood cells (WBC) per high power field ($\times 1000$) in Gram-stained urethral and cervical smears.

Patients who did not meet the inclusion criteria were excluded from the study. The approval of the Ethics Committee of Clinical Center of Serbia was obtained.

Three urethral or cervical samples were taken from all patients by inserting a dacron swab 2-3 cm into the urethra or endocervix and rotating it in order to obtain a greater number of cells. The first sample was used

for Gram staining and also for plating on modified Thayer-Martin agar, chocolate agar and Sabouraud's dextrose agar. *Neisseria gonorrhoeae* was detected by the presence of intracellular Gram-negative diplococci on the Gram preparation and by growth in culture on modified Thayer-Martin agar and chocolate agar under microaerophilic conditions. *Candida* spp. was confirmed by the finding of Gram-positive yeast cells and pseudohyphae and by growth on Sabouraud's dextrose agar.

The direct immunofluorescence technique (Chlamydia Direct IF Identification, BioMerieux, Mercey L'etoile, France) was used for detection of *Chlamydia trachomatis* in the second endocervical and urethral samples. After air drying, the samples were fixed with methanol for 5 minutes. The fixed smears were incubated with 25 μ l of chlamydiae direct fluorescent-antibody reagent in a moist chamber for 30 minutes in the dark and rinsed gently in a bath of phosphate buffer saline for 1 minute. Subsequently, they were mounted with glycerol and examined using immunofluorescence microscopy.

The third sample was subjected to the Mycoplasma IST-2 commercial kit (Bio Merieux, Marcy L'Etoile, France) containing substrates with growth factors for mycoplasma to ensure optimal sensitivity and inhibitors of polymorphic flora to ensure optimal selectivity for *M. hominis* and *U. urealyticum* detection. The test also provided information regarding the density of each organism ($>10^4$ colony-forming units [CFU]) and its susceptibility to doxycycline, josamycin, ofloxacin, erythromycin, tetracycline, ciprofloxacin, azithromycin, clarithromycin and pristinamycin. The third genital sample was mixed with transport medium R1 and vortexed rapidly. Then, 3 ml of R1 was used to rehydrate the lyophilized growth medium, R2. Rehydrated R2 medium was inoculated onto a mycoplasma IST-2 kit, consisting of 22 wells (50 μ l per well, overlaid with mineral oil). The remainder of the broth and the inoculated strip were incubated at 37°C, observed for color changes from yellow to red and the results were interpreted after 24 and 48 h of incubation.

RESULTS

Of the 373 patients enrolled in the study, 241 (64.6%) were men and 132 (35.4%) were women. Their average age was 33.8 and 38.4 years, respectively. Of

the 373 specimens tested, 48 (12.9%) were positive for urogenital mycoplasmas. Of these, 37 (77.8%) were positive for *U. urealyticum*, 6 (22.9%) were positive for *M. hominis* and 5 (10.4%) were positive for both. According to gender, *U. urealyticum* was detected in 25 of 241 men and in 17 of 132 women, while *M. hominis* was isolated in 8 of 241 men and 3 of 132 women. The distribution of *U. urealyticum* and *M. hominis* according to gender is presented in Table 1. The vast majority of *U. urealyticum* and *M. hominis* infections occurred in patients between the ages of 16 and 39 [Table 2].

Other microorganisms found along with mycoplasma in genital samples were *C. trachomatis* (4 of 48 patients) and *Candida* spp. (also 4 of 48 patients).

Antimicrobial resistance of isolated mycoplasma is shown in Table 3. Among the 37 isolates of *U. urealyticum*, 34 (94.6%) were sensitive to doxycycline. Furthermore, josamycin and azithromycin were found to be highly and moderately potent against *U. urealyticum*, respectively (70.3% and 67.6%). *M. hominis* was sensitive to doxycycline (83.3%) and moderately sensitive to ofloxacin (66.7%). Among *M. hominis*, the drug resistance rate was 100% to erythromycin, tetracycline, clarithromycin while *U. urealyticum* was highly resistant to clarithromycin (94.6%), tetracycline (86.5%), ciprofloxacin (83.8%) and erythromycin (83.8%). Doxycycline and josamycin were the most potent antibiotics (80%) in mixed infections with both *U. urealyticum* and *M. hominis*.

DISCUSSION

Although *U. urealyticum* and *M. hominis* can be found as commensal bacteria in healthy adults, these bacteria are well-known causative agents of non-gonococcal genital infection. Therefore, the presence of 10 or more leukocytes in Gram-stained urethral or cervical smears and dense growth of mycoplasmas (>10⁴ CFU) on culture were important parameters that we used in our study to support the diagnosis of commensal mycoplasmal infection.^[6]

The prevalence of *U. urealyticum* and *M. hominis* in our group of patients was 11.3% and 2.9%, respectively. Among samples positive for mycoplasmas, *U. urealyticum* was isolated more frequently than *M. hominis*. Our results are consistent with those of

Table 1: Gender distribution of urogenital mycoplasmas

Number of patients	Total (n)	Male (%)	Female (%)
Total analyzed	373	241 (64.6)	132 (35.4)
Positive finding	48	29 (12.9)	19 (14.4)
<i>U. urealyticum</i>	37	21	16
<i>M. hominis</i>	6	4	2
Both bacteria	5	4	1

M. hominis: *Mycoplasma hominis*, *U. urealyticum*: *Ureaplasma urealyticum*

Table 2: Distribution of urogenital mycoplasmas in the different age groups

Age	<i>M. hominis</i> (%)	<i>U. urealyticum</i> (%)	<i>M. hominis</i> + <i>U. urealyticum</i> (%)	Total (%)
16-27	2 (33.3)	10 (27.0)	4 (80.0)	16 (33.3)
28-39	4 (66.7)	11 (29.7)	1 (20.0)	16 (33.3)
40-51		11 (29.7)		11 (22.9)
52-63		4 (10.8)		4 (8.3)
64-79		1 (2.7)		1 (2.1)
Total	6 (100)	37 (100)	5 (100)	48 (100)

M. hominis: *Mycoplasma hominis*, *U. urealyticum*: *Ureaplasma urealyticum*

Table 3: Antibiotic resistance of *M. hominis* and *U. urealyticum*

Antibiotic	Number of strains resistant (%)		
	<i>M. hominis</i> (n=6)	<i>U. urealyticum</i> (n=37)	<i>M. hominis</i> + <i>U. urealyticum</i> (n=5)
Doxycycline	1 (16.7)	2 (5.4)	1 (20)
Josamycin	4 (66.7)	11 (29.7)	1 (20)
Ofloxacin	2 (33.3)	18 (48.6)	4 (80)
Erythromycin	6 (100)	31 (83.8)	5 (100)
Tetracycline	6 (100)	32 (86.5)	5 (100)
Ciprofloxacin	3 (50)	31 (83.8)	5 (100)
Azithromycin	3 (50)	12 (32.4)	4 (80)
Clarithromycin	6 (100)	35 (94.6)	5 (100)
Pristinamycin	3 (50)	19 (51.4)	2 (40)

M. hominis: *Mycoplasma hominis*, *U. urealyticum*: *Ureaplasma urealyticum*

other studies conducted in Italy, Poland, and Greece but distinctly different from studies based in Papua New Guinea and Portugal.^[7-11] In the Portuguese study, mycoplasma were detected in vaginal swabs in 57.4% of women; more often *M. hominis* (31.5%) than *U. urealyticum* (27.8%). The Papua New Guinea study also showed a high prevalence of *U. urealyticum* and *M. hominis* (78% and 70%, respectively). It was found that the prevalence of *M. hominis* varied between 2% and 84% in patients with non-gonococcal genital infection in different studies.^[12] This vast difference in ratio has been attributed to different laboratory methods and variation in geographical and cultural features in studied patients.^[3,8] Mycoplasma IST-2 commercial kit is considered a simple, sensitive, and

specific test and there was no difference between the results of culture and those of the mycoplasma IST with regard to the isolation of Mycoplasma.^[10] The highest prevalence of *U. urealyticum* and *M. hominis* was seen in the 16–39 years age group [Table 1]. This finding is similar to the results of Turkish and Italian studies.^[7,13] Regarding antimicrobial susceptibility, *U. urealyticum* and *M. hominis* were most sensitive to doxycycline (94.6% and 83.3%, respectively). Although doxycycline is the most commonly used antibiotic in the treatment of non-gonococcal genitourinary infections, it continues to be the most effective agent for *U. urealyticum* and *M. hominis*. We would like to emphasize our finding of high level resistance of mycoplasmas to tetracycline and macrolides with tetracycline resistance rates of 100% and 86.5% in *M. hominis* and *U. urealyticum* respectively. Investigators from other areas have also observed that resistance of mycoplasmas to tetracyclines has increased.^[14] The rates of tetracycline resistance were 22.7% and 25% among ureaplasmas and *M. hominis*, respectively, in one Tunisian study.^[15] While the results of European studies suggest lower rates of tetracycline resistance, ureaplasma isolates collected from a broad geographical area of the USA between 2000 and 2004, showed a higher resistance rate of 45%.^[16,17] We were unable to find any previous reports that revealed a level of tetracycline resistance in mycoplasmas as high as in our study. The reason for this is likely to be the acquisition of a streptococcal *tet M* gene because of frequent and systemic use of tetracycline in recent decades.^[18] Contrary to other studies which suggest that erythromycin or clarithromycin are good alternatives for treating ureaplasma infections, our results revealed significant resistance to these drugs.^[11,19] Macrolides and lincosamides are widely used antibiotics for *U. urealyticum* infections, especially among children and those allergic to tetracyclines and quinolones. Unfortunately, recently, widespread macrolide resistance in *U. urealyticum* has been reported.^[11,16,19] One of the mechanisms of macrolide resistance in *U. urealyticum* is transition mutations in 23S ribosomal RNA.^[20] While *U. urealyticum* was resistant to quinolones, *M. hominis* was moderately sensitive to ofloxacin (66.7%).^[21] Therefore, ofloxacin may be the only alternative treatment for *M. hominis* infection. High level resistance of *U. urealyticum* to quinolones in our study can be explained by the widespread usage of these drugs in urinary tract infections. This finding is similar to that of De Francesco *et al.* and Kechagia *et al.*^[7,9] The new macrolide pristinamycin

was not effective against *U. urealyticum* and *M. hominis* despite the fact that it is not in use in our country. High resistance of *M. hominis* to pristinamycin was also observed in the Turkish study.^[19] Unlike our results, De Francesco *et al.* reported that pristinamycin had potent activity against both *U. urealyticum* and *M. hominis*.^[7] However, we found that josamycin was effective against *U. urealyticum* (70.3%) and could be an alternative treatment for *U. urealyticum* infections. Furthermore, in combined infections with both bacteria, doxycycline and josamycin were the most effective. It is believed that both mycoplasmas could develop resistance to these new generation antibiotics because of cross-border interaction with resistant agents.

The inability of the used commercial kit to detect other potentially pathogenic urogenital mycoplasmas (*Ureaplasma parvum*, *Mycoplasma genitalium*) is the main limitation of this study.

Our results indicate that doxycycline is still the drug of choice for *U. urealyticum* and *M. hominis* genital infections. Significant resistance of *U. urealyticum* and *M. hominis* to tetracycline and macrolides has been found. In spite of the fact that these antibiotics are believed to be standard treatment options for non-gonococcal genital infections, their effectiveness may have declined due to long-term usage of these drugs and rapid development of resistance. Antibiotic susceptibility testing of genital mycoplasmas should be undertaken to detect resistance and inform treatment decisions.

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Conflicts of interest

There are no conflicts of interest.

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