

# Investigation of the Frequency And Antibiotic Susceptibility of Mycoplasma/Ureaplasma in Urine Samples With Leukocyturia by Different Commercial Methods

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## Abstract

The aim of this study was to determine the prevalence and antibiotic susceptibility rates of *M. hominis* and *U. urealyticum* in urine samples with leukocyturia, but routine bacterial culture negative by commercial media. Urine samples that had leukocyturia (study group) and no leukocyturia (control group) were cultured by using two different culture medias. One-hundred-thirty-eight case samples and 70 control cases were processed. The Mycoplasma-IST kit revealed 68 samples with *U. urealyticum* and three with *M. hominis* (total 71 (51.4%)), whereas the Mycofast Evolution-2 kit revealed 63 and 3, respectively (total 66 (47.8%)). In the control group, *U. urealyticum* were isolated in 11 samples by the first kit and in 10 samples by the latter. Tetracycline (94%) and doxycycline (94%) were the most effective antibiotics against *U. urealyticum* based on the analysis of the first kit while doxycycline (95%) was the preferred antibiotic based on findings of the second kit. There was no difference between the two commercial media in the isolation of these microorganisms.

## INTRODUCTION

Genital mycoplasmas (*Ureaplasma urealyticum* and *Mycoplasma hominis*) are frequently isolated in the genitourinary tract, particularly in sexually active women. It is generally difficult to determine whether these agents cause colonization or infection. The incidence of infection is affected by the menstrual cycle and pregnancy, and the use of vaginal contraceptives. The prevalence of these organisms is significantly associated with socio-economic conditions, i.e. poverty, and large numbers of sexual partners (<sub>1, 2</sub>).

*M. hominis* and *U. urealyticum* differ from other bacteria in that they lack a rigid cell wall. They have limited biosynthetic abilities. Therefore, their cultivation requires an enriched medium containing precursor for nucleic acid, protein, and lipid biosynthesis, and the routine cultivation of mycoplasma and ureaplasmas remains limited to some laboratories. Because of difficulties in their cultivation, some commercial media have been developed. These media, which have assumed an important role in laboratory practices, are used in the isolation and identification of these agents and in testing their antibiotic susceptibilities. Phenol red, which changes color in alkaline pH, is generally used as an indicator in the media (<sub>3</sub>).

The aim of this study was to determine the prevalence and antibiotic susceptibility rates of *M. hominis* and *U. urealyticum* in urine samples with leukocyturia, but routine bacterial culture negative by commercial media (Mycoplasma-IST, Mycofast Evolution 2).

## METHODS

**Patient population:** Urine samples from outpatients at the Microbiology Department of our hospital for routine bacteriological cultivation were studied. A questionnaire to record the patient's identity, the presence of clinical manifestations and chronic illnesses, and pregnancy status was prepared and recorded data entered into a computer system.

**Collection of urine specimens:** Urine samples were collected from the midstream flow by the clean-catch technique.

**Processing of specimens:** Firstly, all urine samples were inoculated in 5% sheep blood agar and Eosin Methylene Blue (EMB) agar for routine bacteriological culture. The specimens were also investigated for leukocyturia by flowcytometry (Sysmex/UF-100/Toa Medical Electronics/Kobe 650-91/Japan) and microscopy. A small amount of each urine sample was stored at +4°C for the

cultivation of mycoplasmas/ureaplasmas. Flowcytometric analysis was carried out according to the manufacturer's instructions. Urine sediments were examined under a microscope (400x magnification) after the samples had been centrifuged for 5 minutes at 3000 rpm. The samples were evaluated as leukocyturia positive if they had >10 leukocytes per field microscopically or >120/ml by flowcytometric analysis. Then 5% sheep blood agar and EMB agar were incubated at 37°C for 18-24 hours. Urine samples stored at +4°C that had leukocyturia with negative urine culture (the study group) were selected. In addition, some of the specimens with leukocyturia and urine culture negative were also taken as control. These samples were cultured for mycoplasmas/ureaplasmas by Mycoplasma IST (bioMérieux/France) and Mycofast Evolution 2 (International Microbio/France) media according to the manufacturers' instructions. They were incubated at 37°C for 24-48 hours and the growing and antibiotic sensitivities of mycoplasmas/ureaplasmas were evaluated. After incubation, a subculture was made from M. hominis/U. urealyticum positive kits to 5% sheep blood agar to control contamination.

**Exclusion criteria:** Totally 23 samples were excluded from the study for various reasons: collecting samples inappropriately, receiving antibiotic therapy and hospitalization in the previous 15 days.

**Statistical analysis:** Statistical significance between the performances of Mycoplasma IST and Mycofast Evolution 2 were tested by McNemar's test. Differences between the study and the control group for each kit, as well as antibiotic sensitivities were evaluated by Pearson's chi-square test.

## RESULTS

Urine samples from 590 patients aged 1-58 years old were cultivated for routine bacteriological culture. Bacteria grew in 29.1% (172) of them. One hundred thirty-eight (from 22 male and 116 female patients) samples that had leukocyturia with negative urine culture were processed. Addition, 70 samples leukocyturia and urine culture negative samples were selected as control. M. hominis (two male, one female) were determined in three specimens in the study group by each kit, and none determined in the control group. The results of detected ureaplasmas are presented in

**Figure 1**

Table 1: Distribution in urine samples of detected by Mycoplasma IST and Mycofast Evolution 2.

	Study group (n:138)		Control group (n:70)	
Mycoplasma IST	68 (49.2%)	(2 M, 66 F)	11 (15.7%)	(11 F)
Mycofast Evolution 2	63 (45.6%)	(2 M, 61 F)	10 (14.3%)	(10 F)

\*M: male, F: female

Escherichia coli was detected in two, Enterococcus spp. in two, Candida spp. in one, and Klebsiella pneumoniae in one of the U. urealyticum positive samples after their subcultures from Mycoplasma IST to 5% sheep blood agar. On the other hand, one E. coli and one Enterococcus sp. were determined after subculturing from Mycofast Evolution 2.

Three of the isolated U. urealyticum strains were different patients' samples identified from each kit. The Mycoplasma IST kit revealed 68 samples with U. urealyticum and three with M. hominis, (total 71 (51.4%)). Of those patients, 43 (60.1%) were pregnant, 23 had urinary tract infection-related manifestations, and five had no distinguishing features. Diabetes mellitus was determined in 16 (of these three who were pregnant), hypertension in six, and hypothyroidism in one of the mycoplasma or ureaplasma positive cases. Three (4.2%) strains were isolated from the urine samples of 12 patients under 15 years-old, 44 (61.9%) were from 76 patients 16-30 years-old, 18 (25.3%) were from 33 patients 31-45 years-old, and 6 (8.4%) were from 17 patients over 45 years-old.

**Figure 2**

Table 2: The antibiotic susceptibility rates of strains isolated by Mycoplasma IST (n: 62)\*

	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Ofloxacin	22 (36%)	38 (61%)	2 (3%)
Erythromycin	36 (58%)	14 (23%)	12 (19%)
Tetracycline	58 (94%)	-	4 (6%)
Doxycycline	58 (94%)	2 (3%)	2 (3%)
Josamycin	55 (89%)	5 (8%)	2 (3%)
Pristinamycin	56 (90%)	2 (3%)	4 (7%)

\*Six samples that had bacteria grown in their subculture were excluded

**Figure 3**

Table 3: The antibiotic susceptibility rates of strains isolated by Mycofast Evolution 2 (n: 61)

	Sensitive n (%)	Resistant n (%)
Ofloxacin	56 (92%)	5 (8%)
Roxithromycin	53 (87%)	8 (13%)
Doxycycline	58 (95%)	3 (5%)

Three *M. hominis* strains were susceptible to all the antibiotics, but the sensitivity of *M. hominis* to macrolides was ignored because it is naturally resistant to all 14- and 15-membered macrolides. The antibiotic susceptibility rates of *U. urealyticum* strains are presented in Tables 2 and 3.

There was no statistical significance between Mycoplasma IST and Mycofast Evolution 2 ( $p=0.57$ ) in terms of isolating *M. hominis*/*U. urealyticum* in both the study group and the control group. On the other hand, statistical differences between the study group and the control group were significant by each kit ( $p=0.0001$  for both of them).

Statistical analysis of the antibiotic sensitivity of the two kits could be performed for only the ofloxacin and doxycycline. For ofloxacin, no statistical significance was observed between the two kits when we considered intermediate sensitive samples in Mycoplasma IST to be resistant ( $p=0.21$ ). If we considered them to be non-resistant, the difference was significant ( $p<0.001$ ). For doxycycline, no statistical significance was detected for either situation ( $p=0.49$  and  $p=0.5$ ), respectively.

## DISCUSSION

The presence of *M. hominis* and *U. urealyticum* in a large proportion of healthy women complicates the assessment of the pathogenic role of these organisms. To culture this group of nutritionally fastidious bacteria in the laboratory requires the use of complex growth media. For these reasons, routine culture for *M. hominis* and *U. urealyticum* is performed by relatively few laboratories, and antibiotic sensitivity testing of genital mycoplasmas is not carried out in routine laboratories. Therefore, various commercial media, which are more practical and faster for the isolation and evaluation of antibiotic susceptibility testing of these agents, were developed (<sup>4, 5</sup>). Of the commercial kits, the periods of isolation and antibiotic sensitivity of Mycoplasma IST and Mycofast Evolution 2 are approximately equal. Both of them are highly concordant with conventional cultures of mycoplasmas with regard to the isolation of *M. hominis*/*U. urealyticum* (<sup>1, 2, 4, 5</sup>). We used Mycoplasma IST and Mycofast Evolution 2 in our study, and we found no significant difference between the two methods ( $p=0.57$ ). However, the rate of isolation of Mycoplasma IST was partly higher, but the ratio of contamination was also like that. There was no significant difference in terms of cost, but Mycoplasma IST can determine the sensitivities of six antibiotics and Mycofast Evolution 2 just three.

It was pointed out that *M. hominis* is naturally resistant to macrolides, ureaplasmas are moderately susceptible to macrolides, and tetracycline resistance has been observed in both *M. hominis* and *U. urealyticum* (<sup>6</sup>). In our study, the three *M. hominis* strains were susceptible to all the antibiotics tested, except erythromycin.

For sensitivity to doxycycline, no significant difference was detected between the two kits ( $p=0.49$  and  $p=0.5$ ), respectively. However, for ofloxacin, no significant difference was observed between the two kits when we considered intermediate sensitive samples in Mycoplasma IST to be resistant ( $p=0.21$ ), whereas if we considered them to not be resistant, the difference was significant ( $p<0.001$ ).

Li et al. (<sup>7</sup>) reported that macrolides were more effective for *U. urealyticum* infection, and quinolones were more effective for *M. hominis* infection.

Dominigues et al. (<sup>2</sup>) determined that 90.7 and 24.1% of *M. hominis*/*U. urealyticum* isolates were resistant to erythromycin and tetracycline, respectively. They were not observed to be resistance to ofloxacin, although 50% of the strains had intermediate resistance. Bebear et al. (<sup>8</sup>) stated that the percentages of intermediate or resistant *U. urealyticum* strains were 3%, 72% (most intermediate), 0.9%, 0.3% and 34% (most intermediate) to doxycycline, erythromycin, josamycin, pristinamycin and ofloxacin, respectively. For *M. hominis*, these proportions were 2.7%, 100%, 2.7%, 0% and 0%, respectively. Clegg et al. (<sup>4</sup>) determined that the intermediate or resistance rates of the genital mycoplasma isolates to ofloxacin, erythromycin, tetracycline, doxycycline, josamycin, and pristinamycin were 71.9%, 98.1%, 14.6%, 10.1%, 19.1% and 6.7%, respectively. In a study performed in Turkey by Kilic et al. (<sup>1</sup>), resistance rates for *M. hominis* and *U. urealyticum* to ofloxacin, erythromycin, tetracycline, doxycycline, josamycin and pristinamycin were 16.7%, 12.5%, 4.2%, 4.2%, 0% and 8.8%, respectively. Resistance rates in our study were concordant with those given by Kilic et al., except for ofloxacin.

Differences among the sensitivity rates in separate studies may result from factors such as the populations studied, the kits used, and the study period. In particular, differences in the sensitivity to erythromycin may differ according to whether the evaluation of *M. hominis* and *U. urealyticum* is combined or separated. Indeed, in the study carried out by Bebear et al. (<sup>8</sup>), *M. hominis* and *U. urealyticum* were

evaluated separately, and *M. hominis* resistance was 100% to erythromycin, while it was 72% (most intermediate) to *U. urealyticum*.

As the cultivation of *U. urealyticum* and *M. hominis* requires special media, and their isolation is difficult and time-consuming, some problems arise. Therefore, clinicians treating *Ureaplasma* and *Chlamydia trachomatis* are advised to administer an antibiotic that belongs to the tetracycline group (1). Some studies have reported that *U. urealyticum* is significantly associated with non-gonococcal urethritis (NGU) (6). However, there is about 10% resistance to tetracyclines for *Ureaplasmas*, and this rate increases continuously. For this reason, macrolides (especially in children) or quinolones become alternative drugs (1). However, if there is a *M. hominis* infection, owing to the natural resistance of *M. hominis* to erythromycin, this antibiotic can not be an alternative (6).

*U. urealyticum* and *M. hominis* present serious medical complications for mothers during gestation and as well as for the fetuses and neonates (2, 10). Chua et al. (11) found maternal cervical colonization rates for *U. urealyticum* and *M. hominis* of 58% and 16%, respectively, while the overall transmission rates were 88% for *U. urealyticum* and 42% for *M. hominis*. We thought that they might constitute a risk for neonates, because most of the strains in our study were isolated during pregnancy and from sexually active young women.

Gonzalez et al. (12) stated that bacterial urinary tract infections were more frequent among the 20 to 40 age group, among women and diabetic patients. Most of the cases in our study (60%) were pregnant women. 87% of *U. Urealyticum* / *M. hominis* positive cases were aged 16-45 years. Of those cases, 32% had signs and symptoms of urinary tract infections and 23% were diabetic.

Urine analysis is very important for the diagnosis of urinary tract infections. We detected *U. urealyticum* or *M. hominis* in 71 out of 138 leukocyturic cases and in 11 out of 70 non-leukocyturic cases by *Mycoplasma* IST kit. These numbers were 66 and 10, respectively, by *Mycofast Evolution 2* kit. The statistical differences between two groups were significant ( $p=0.0001$ ).

## CONCLUSION

*U. urealyticum* and *M. hominis* are frequently isolated from the urogenital tract and should be investigated, especially in

patients with leukocyturia and routine negative bacterial negative. Furthermore, both of the commercial media may be used to isolate these microorganisms and they ought to be more widely used in laboratories.

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