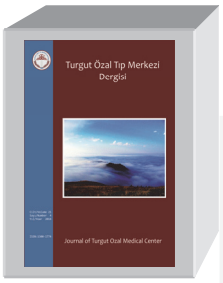

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Investigating the Presence of Mycoplasma Hominis-Ureaplasma Urealyticum and in Vitro Antimicrobial Susceptibilities in Patients With Sterile Pyuria

Mehmet Burak Selek, Bayhan Bektöre, Tuğba Kula Atik, Barış Yalçın, Orhan Baylan, Mustafa Özyurt

GATA Haydarpaşa Training Hospital, Department of Medical Microbiology, Istanbul, Turkey

Abstract

Aim: In this study, we aim to investigate the presence of *Mycoplasma hominis* and *Ureaplasma urealyticum* antimicrobial susceptibilities at mid-stream urine samples from outpatients who were admitted to Urology, Obstetrics and Gynecology, and Internal Medicine outpatient clinics with dysuria complaints with sterile pyuria negative in first culture results.

Material and Method: To test *M. Mycoplasma hominis* and *Ureaplasma urealyticum* isolation and antibiotic susceptibility we have used Mycoplasma System Plus (Liofilchem Diagnostics, Italy) kit, which is based on biochemical reaction interpretation.

Results: A total of 228 urine samples, from 139 (61%) women and 89 (39%) men with sterile pyuria, were evaluated in the laboratory. Patients were classified according to their ages, 79 (34.6%) of the patients were in 15-35 age group, 60 (26.3%) of the patients were in 36-55 age group, and 89 (39%) were above 56. Seasonal distribution of isolates were also assessed. While *Mycoplasma hominis* isolation rate in winter months was 13.8% (n:22), in spring it was %8.8 (n:5); *Ureaplasma urealyticum* isolation rate in winter was 17% (n:27) and %17.5 (n:10) in spring. No isolates could be found in the samples from the summer. The least susceptible antimicrobial to *Mycoplasma hominis* was ofloxacin while clarithromycin was found relatively less effective on *Ureaplasma urealyticum* among the tested antimicrobials.

Conclusion: In our study, we have considered the urogenital Mycoplasma and Ureaplasma species as pathogens in cases with steril pyuria through implementing a biochemical diagnostic kit in all the laboratories for the isolation, identification, and antimicrobial susceptibility tests.

Key Words: Mycoplasma Hominis; Ureaplasma Urealyticum; Sterile Pyuria; Urinary Infection.

Steril Piyürili Hastalarda Mycoplasma Hominis-Ureaplasma Urealyticum Varlığının ve Antibiyotiklere in Vitro Etkinliğinin Araştırılması

Özet

Amaç: Bu çalışmada, dizüri şikayeti ile hastanemiz üroloji, kadın hastalıkları ve doğum ve dahiliye polikliniklerine başvuran ve ilk idrar kültürlerinde üreme saptanmayan steril piyürili hastalardan istenen orta akım idrarında, *Mycoplasma hominis* ve *Ureaplasma urealyticum* varlığının ve antibiyotik duyarlılıklarının araştırılması amaçlandı.

Gereç ve Yöntem: *Mycoplasma hominis* ve *Ureaplasma urealyticum* bakterilerini izole etmek ve antibiyotik duyarlılığını belirlemek amacıyla biyokimyasal temelli test kiti olan Mycoplasma System Plus (Liofilchem Diagnostics, İtalya) kullanıldı.

Bulgular: Laboratuvara gelen 139'u (%61) kadın ve 89'u (%39) erkek toplam 228 steril piyürili hasta örneği değerlendirmeye alındı. Hastalar yaşlarına göre gruplara ayrıldı, 79'unun (%34.6) 15-35 yaş grubunda, 60'ının (%26.3) 36-55 yaş grubunda, 89'unun (%39) 56 yaş ve üzerinde olduğu tespit edildi. Üremelerin mevsimlere göre dağılımında; *Mycoplasma hominis* izolasyon oranı kış aylarında %13.8 (n:22), ilkbahar aylarında %8.8 (n:5) iken *Ureaplasma urealyticum* izolasyon oranı kış aylarında %17 (n:27), ilkbahar aylarında %17.5 (n:10) olarak bulundu. Yaz aylarında her iki etken de izole edilemedi. İzole edilen bakterilerden *Mycoplasma hominis*'e etkinliği en düşük olan antimikrobiyal ajan ofloksasin olarak tespit edilirken; *Ureaplasma urealyticum* için ise klaritromisinin etkinliği test edilen antimikrobiyaller içinde göreceli olarak düşük bulundu.

Sonuç: Çalışmamızda steril piyürisi olan ve rutin kültürlerde üremesi saptanmayan hastalarda etken olarak ürogenital mikoplazma-üreaplazma türlerinin akla getirilmesi gerektiği ve izolasyon, identifikasyon ve duyarlılık testlerine yönelik hemen hemen tüm laboratuvar seviyesinde biyokimyasal tanı kitleri kullanılabileceğini düşünüyoruz.

Anahtar Kelimeler: Mycoplasma Hominis; Ureaplasma Urealyticum; Steril Piyüri; Uriner Enfeksiyonu.

INTRODUCTION

Mycoplasma and Ureaplasma species, which belong to the family Mycoplasmataceae in the Mollicutes class, are the smallest known prokaryotes. The family Mycoplasmataceae contains two genera: *Mycoplasma* and *Ureaplasma* (1). To date, 15 species of mycoplasma have been isolated, but only four of them are important to humans: *Mycoplasma pneumoniae, Mycoplasma hominis (M.hominis), Ureaplasma urealyticum (U.urealyticum)* and *Ureaplasma parvum*. These bacteria have three-layer cell membranes with sterols, but they lack cell walls. Unlike other microorganisms, they cannot be stained using Gram or reproduced in culture medium because they lack cell walls. This also makes them intrinsically resistant to beta lactam antibiotics. These microorganisms are very common in nature, and they are

found in human oral and genital mucosa as commensals (1,2). Despite being associated with many diseases of the urogenital system, they are often isolated in the lower urogenital tract of healthy adults, as well. *U.urealyticum* can be found in 40-80% of women and *M.hominis* in 21-53% of women while it is lower among men (1,3). The rate of colonization is higher among young women and in societies with lower socioeconomic status (4,5). Genital mycoplasmas can be found in many places, particularly in the lower genital tract of sexually active individuals and the colonization rate is directly related to the number of sexual partners (1). It can also be isolated in the upper genital tract of patients with Salpingitis (5,6). As they are opportunistic pathogens, the incidence and severity of the disease is dependent on the immune status of the host (1).

Ureaplasma species have been associated with 10% of male non-gonococcal urethritis and diseases related to the upper genitourinary tracts of women (1). *U.urealyticum* is a causative agent of non-gonococcal urethritis, chorioamnionitis and low birth weight. Moreover, both *U.urealyticum* and *M.hominis* are associated with infertility and neonatal respiratory diseases (5,7).

Pyuria is defined as the presence of more than 5 leukocytes per high field of microscope with X400 magnifier lens. Sterile pyuria describes no bacterial growth in routine bacteriology cultures when leukocytes are present or growth less than 1000 colonies per milliliter (cfu/ml). Mycoplasma and Ureaplasma infections may cause various conditions such as urinary tuberculosis and interstitial cystitis (9).

This study aims at investigating the prevalence and antibiotic resistance of *M.hominisis* and *U.urealyticum* in the mid-stream urine of the patients with sterile pyuria. The study population comprised of patients with dysuria and admitted to the Departments of Urology, Gynecology, Obstetrics Polyclinic and Internal Medicine at GATA Haydarpaşa Training Hospital, Istanbul. No productivity was detected in the initial culture of the mid-steam urine samples collected from these patients.

MATERIAL AND METHODS

Our study was designed as a retrospective study that covered a two-year period. We used the midstream urine samples taken from 228 patients with sterile pyuria at medical microbiology laboratories between 2012 and 2013. No growth was detected in the 48-hour incubation period, and more than five 5 leukocytes were detected in urine microscopy of the initial samples collected from these patients. Mycoplasma System Plus (Liofilche Diagnostics, Italy), a biochemical test kit, was used to isolate *M.hominis* and *U.urealyticum* and to determine their antibiotic resistance. The same samples were also submitted to routine urine culture. The processing and evaluation of the samples were performed according to the kit procedure. Identification and antibiotic susceptibilities of the bacteria were interpreted according to color changes in the wells. The presence of *M. hominis* or *U. urealyticum* was demonstrated by color change in the wells on the kit used to identify each bacterium. If the color of identification wells does not change, it indicates that the agent has not grown. If there is no color change in the wells with antibiotics, it means that breeding bacterium is sensitive to the antibiotic in that well. There are also three quantitation wells on the kit to determine the number of colonies. Color changes in these wells (from yellow to bright pink) help calculate bacterial density: a) low growth (10^4 colony-forming units/milliliter, cfu/mL), b) moderate growth (10^4-10^5 cfu/ml), c) high growth (10^5 cfu/mL). In our study, the growth rates over 10^4 cfu / mL, were considered positive. Bacterial growth together with color changes in the wells containing antibiotics was interpreted as an indication of resistance to antibiotics. The wells with two different doses of antibiotics were used to analyze the antibiotic sensitivity of the isolated bacterium.

Statistical analysis of the data obtained from the study was performed using SPSS for Windows 16.0 software. In addition to descriptive statistical analyses (frequency, percentage), the Pearson chi-square test was performed to analyze the collected data. Statistical significance level was set at $p < 0.05$.

RESULTS

228 samples from the patients with sterile pyuria were included in the evaluation. Of 228 participants, 139 (61%) were women and 89 (39%) were men. 79 of the patients (34.6%) were in the 15-35 age group, 60 (26.3%) of them in the 36-55 age group and 89 (39%) of them were 56 years old or older.

U.urealyticum was isolated in 39 samples (17.1%) *and* *M.hominis* was isolated in 27 samples (11.8%). While *M.hominis* was isolated in 13.7% of the female patients (n:19), and 9% of male patients (n:8), *U.urealyticum* was isolated in 23.7% of female patients (n = 33) and in 6.7% of male patients (n = 6) (Table 1).

M.hominis growth by age group is as follows: a) 8.9% (n:7) in the 15-35 age group, b) 6.7% (n:4) in the 36-55 age group, c) 18% in the group of 56-year-olds and older (n:16) (Table 2). The distribution of growth according to the season is follows: a) the isolation rate of *M.hominis* (positive number of samples / number of samples) in winter was 13.8% of (n:22) and 8.8% in spring (n:5) b) the isolation rate of *U.urealyticum* in winter was 17% (n:27) and 17.5% in spring (n:10). Neither bacterium could be isolated in the summer. While *M.hominis* could not be isolated in the autumn, the growth rate for *U.urealytic* in the autumn was detected to be 33.3% (n:2). Of the samples where growth was detected, 27 (40.9%) were sent from the Department of Gynecology, 21 (31.8%) from the Urology Service, and 18 (27.3%) from the Internal Medicine Service.

Ofloxacin was found to be the antimicrobial agent with the lowest *M.hominis* efficiency. Clarithromycin was

found to be relatively the lowest antimicrobials tested for *U.urealyticum* efficiency. The susceptibility of the other antibiotics is shown in Table 3.

No yeast cells and *Trichomonas vaginalis,* which may cause contamination, were found in the direct examination of the wells containing the samples where growth was detected.

Table 1. The gender-wise distribution of isolated bacteria

Pathogen			Gender			*p**
			Female	Male	Total	
M.hominis	null	n (%)	120 (86,3)	81 (91,0)	201 (88,2)	0.286
	present	n (%)	19 (13,7)	8 (9,0)	27 (11,8)	
Total			139	89	228	
U.urealyticum	null	n (%)	106 (76,3)	83 (93,3)	189 (82,9)	**0.001****
	present	n (%)	33 (23,7)	6 (6,7)	39 (17,1)	
Total			139	89	228	

*Assessed by using the Pearson chi-square test.
**Gender-wiser *U.urealyticum* distribution is statistically meaningful (p< 0.05)

Table 2. Age-wise distribution of isaolated bacteria

Pathogen			Age Group				*p**
			15-35	36-55	56 and over	Total	
M.hominis	null	n (%)	72 (91,1)	56 (93,3)	73 (82,0)	201 (88,2)	0.066
	present	n (%)	7 (8,9)	4 (6,7)	16 (18,0)	27 (11,8)	
Total			79	60	89	228	
U.urealyticum	null	n (%)	70 (88,6)	47 (78,3)	72 (80,9)	189 (82,9)	0.229
	present	n (%)	9 (11,4)	13 (21,7)	17 (19,1)	39 (17,1)	
Total			79	60	89	228	

*Assessed by using the Pearson chi-square test.

Table 3. The antibotical susceptibility of the isolated *Mycoplasma hominis* and *Ureaplasma urealyticum* strains (%)

	Antibiotics																	
Pathogen(n)	Tetracycline 4 µg/mL	Tetracycline 8 µg/mL	Pefloxacine 8 µg/mL	Pefloxacine 16 µg/mL	Ofloxacin 1 µg/mL	Ofloxacin 4 µg/mL	Doxycycline 4 µg/mL	Doxycycline 8 µg/mL	Erythromycin 8 µg/mL	Erythromycin 16 µg/mL	Klaritromisin 8 µg/mL	Clarithromycin 16 µg/mL	Minocycline 4 µg/mL	Minocycline 8 µg/mL	Clindamycin 4 µg/mL	Clindamycin 8 µg/mL	Azithromycin 4 µg/mL	Azithromycin 8 µg/mL
***M.hominis* (27)**	42.8	50	57.1	64.2	7.1	14.2	50	50	50	64.2	35.7	50	57.1	78.5	64.2	64.2	42.8	42.8
***U.urealyticum* (39)**	92.3	92.3	92.3	92.3	88.5	88.5	96.2	96.2	80.8	80.8	76.9	76.9	96.2	96.2	84.6	84.6	80.8	80.8

DISCUSSION

Mycoplasmas are bacteria that exist as commensals in the mouth and genital tracts of humans. They may cause illnesses with the influence of various factors, particularly with those related to the host. *M.hominis* and *U.urealyticum* are known to be the most frequently isolated mycoplasma specimen (1.4).

When isolated from the genital tract, mycoplasmas, *M.hominis* in particular, may cause sexually transmitted urogenital infections, pyelonephritis, Bartholin's abscess on, cervicitis to, pelvic inflammatory disease, neonatal conjunctivitis, meningitis, puerperal infection and brain abscess. Ureaplasmas are microorganisms that can be found in the genital tract as colonizing or infectious agents. Colonization is directly related to sexual relation, and it is more common at reproductive age. It may cause nongonokoksik urethritis, üretroprostatit, epididymitis in men and cervicitis and pelvic inflammatory disease in women (4).

Despite the fact that both microorganisms can be isolated in special medium culture, diagnosis is difficult in routine laboratories because of their slow growth rate and the specific requirements and challenges in the preparation of medium culture. New rapid biochemical-based test methods were developed for quick and easy diagnosis (5).

As a result of our study, *M.hominis* was detected in 27 (11.8%) patients and *U.urealyticum* was detected in 39 (17.1%) patients. There are numerous studies conducted to determine the prevalence of these bacterial infections in our country and the world. In these studies, the isolation rate of *U.urealyticum* was found to range between 29.7% and 48.4% and *M.hominis* between 1.6% and 11%. *U.urealyticum* and *M.hominis* detection rates presented in some studies: 45.9% *U.urealyticum*, 1.6%, (a study conducted on 61 patients, Ardıç et al, 2014), 48.4% *U.urealyticum*, 4.4% *M.hominis* (a study conducted on 382 patients, Karabay et al, 2006), 39.2% *U.urealyticum*, 5.4% *M.hominis* (a study conducted on 130 patients, Ekşi et al, 2006), 29.7% *U.urealyticum*, 9.8% *M.hominis* (a study conducted on 461 patients, Afacan et al, 2007), 42.6% *U.urealyticum*, 4.1% *M.hominis* (a study conducted on 461 patients, Turan et al, 2011). *U.urealytic* was generally isolated at a higher rate than *M.hominis* in these studies (5.10-13).

Similarly, *U.urealyticum* was isolated at a higher rate than *M.hominis* in our study. While the isolation rate of *M.hominis* was found to be similar to the previous studies, the isolation rate of *U.urealyticum* was lower than previous studies. The isolation rate might have resulted from the fact that the participant patients were symptomatic and their treatment started early.

In our review of the literature, we did not find any reference to variations in the seasonal distribution of *U.urealytic* and *M.hominis.* Thus, we concluded that the seasonal variation in our study might have resulted from the uneven distribution of the number of samples. There were only six samples sent for laboratory examination in summer and autumn, which prevented a precise statistical evaluation.

Tests based on nucleic acid amplification are used in all areas of microbiology, including the studies on *U.urealyticum and M.hominis*. In the studies that compare the two methods, PZT (polymerase chain reaction) tests have been found to be more sensitive than culture (14). In our study, it was necessary to perform a PZT test to determine whether the low rate of *U.urealytic* was false or not.

While *M.hominis* was isolated by 13.7% of the female patients and 9% of the male patients, *U.urealyticum* was isolated by 23.7% of the female patients and 6.7% of the male patients. *U.urealytic* isolation by women was found to be significantly high. Ureaplasmas were found in the virginal flora of approximately 66% of healthy and sexually active women, and *M.hominis* was found with a lower proportion (10%) (15). The high rates found in the female patients in our study are similar to those in previous studies (3).

In our study, there was no statistically significant variation in the growth of both agents by the three age groups. It is known that the colonization of *M.hominis and U.urealyticum* is directly related to sexual activity and thus more common among young women at the age of reproductivity (4.5). In our study, the rate of *U.urealyticum* positivity was found to be 21.7% in the 36-55 age group, while the rate of *M.hominis* positivity was found to be highest among the age group of 56-year-olds and older (18.0%). The high colonization rates in the group of fifty-six-year-olds and older is due to the different groups in the study.

U.urealyticum was usually susceptible to antibiotics of macrolides group, while *M.hominis* are generally resistant to this group (13,16). Similarly, our study showed that *macrolides* resistance was found to be higher in *M.hominis* compared to *U.urealyticum.* Yet, clarithromycin was found to be the antimicrobial agent with lowest efficiency against *U.urealyticum.*

The increasing antibiotic resistance in urogenital mycoplasma-ureaplasma infections may cause serious clinical consequences. We think that this resistance might be due to the lack of an effective treatment plan as culture and in vitro susceptibility tests are not frequently performed and thus clinical diagnosis of the infections caused by these two agents is not supported by laboratory diagnosis.

The results obtained from our study indicate that mycoplasma-ureaplasma specimen might be present in patients with sterile pyuria with no growth detected in routine culture and that biochemical diagnostic kits can be used in isolation, identification and susceptibility testing at almost all laboratory levels. Both species are considered to be sensitive to tetracycline, but the prevalence of an increasing number of isolates with tetM gene, which causes high levels of tetracycline resistance, have been reported (10,13). In our study, the tetracycline had higher efficiency against *U.urealytic* than *M.hominis*.

In our study, ofloxacin was found to be the antimicrobial agent with the lowest efficiency against *M.hominis*. In the study conducted by Karabay et al, ofloxacin resistance was found to be 41.2% in the *M.hominis* strains isolated from patients with vaginitis. In the same study, *erythromycin* was found to be the antimicrobial agent with the lowest efficiency against *M.hominis* (88.2%) (11). Although the rate of resistance against in *M.hominis* isolates against erythromycin was found to be lower (10) than other studies, the resistance against macrolide antibiotics (clarithromycin, erythromycin and azithromycin) was higher than other antimicrobials (5,11,17).

CONCLUSION

We believe that rising resistence to antibiotics in urogentical microplasma-ureaplasma infections can lead to clinical issues. In our belief, the reasons behind this can be listed as the rare application of culture and in vitro susceptibility tests, discarding laboratory diagnosis during the diagnosis process as an outcome of the previous reason, and, eventually, failure in planning efficient treatments.

We suggest that practitioners should consider types of urogentical microplasmas-ureaplasmas as active substances in patients with sterile pyuria in whose cultures there was no growth. Therefore, we recommend the use of biochemical diagnosis kits for isolation, identification, and susceptibility tests in almost every laboratory stage of the diagnosis process.

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Correspondence/İletişim

Mehmet Burak SELEK
GATA Haydarpaşa Training Hospital, Department of Medical Microbiology, ISTANBUL, TURKEY
E-mail: mbselek@gata.edu.tr

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