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Antibiotic Resistance Profiles of *Mycoplasma hominis* and *Ureaplasma urealyticum* Strains Isolated from Patients with Urethritis/ Vaginitis Symptoms

Üretrit/Vajinit Belirtileri Olan Hastalardan İzole Edilen *Mycoplasma hominis* ve *Ureaplasma urealyticum* Suşlarının Antibiyotik Direnç Profilleri

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Abstract

Aim: *Mycoplasma hominis* and *Ureaplasma urealyticum* species, which are the most frequently isolated microorganisms from the urogenital system, are thought to cause urogenital diseases (urethritis, cervicitis, cystitis, bacterial vaginosis). The prevalence of these microorganisms, which are often isolated from the genital tract of sexually active women, differs between studies. In addition, the antibiotic susceptibility of these microorganisms also shows regional variations. In this study, it was aimed to determine the frequency of genital *U. urealyticum* and *M. hominis*, distribution by gender, and antibiotic resistance profile in patients with pre-diagnosis of urethritis and vaginitis.

Material and Method: This study is a retrospective observational study. Data were obtained retrospectively from hospital records. In the study, genital samples studied in the microbiology laboratory of a private hospital in Antalya for a period of three years between January 2017 and December 2019 were evaluated. Samples were taken into tubes that containing transport medium with sterile swabs. The presence of *M. hominis* and *U. urealyticum* was investigated using the AF Genital System (Liofilchem, Italy) (http://www.liofilchem.net/login/pd/ifu/74156_IFU.pdf) kit. Doxycycline, ofloxacin, erythromycin, tetracycline, clarithromycin, and clindamycin susceptibilities of the agents were determined in the samples which growth observed. Sowing of the swab samples taken from the patients was done in accordance with the manufacturer's recommendations. The strips were incubated at 37°C for 24–48 hours. At the end of the incubation, the results were evaluated according to the color changes.

Results: A total of 245 patients, 147 (60%) men, with a mean age of 31±10.22 years, were included in the study. Of the 245 genital sample material cultures sent, only *M. hominis* was isolated in 55 (22.4%), only *U. urealyticum* was isolated in 27 (11.02%), and both bacteria were isolated in 87 (35.5%). Antibiotics with the highest susceptibility rates of the studied strains which, for *M. hominis* doxycycline (83.1%), clindamycin (69.7%), tetracycline (61.9%) while for *U. urealyticum* was doxycycline (79.8%), clindamycin (71%), and tetracycline (65.7%). In the evaluation of factors according to gender, the rate of cultural positivity was statistically higher in women (p <0.05).

Conclusion: This study emphasized the importance of culture results in the treatment of genital infections caused by *U. urealyticum* and some genital mycoplasmas.

Keywords: *Mycoplasma hominis, Ureaplasma urealyticum*, antibiotic resistance profiles, vaginitis, urethritis

Öz

Amaç: Ürogenital sistemden en sık izole edilen mikroorganizmalar olan Mycoplasma hominis ve Ureaplasma urealyticum türlerinin ürogenital hastalıklara (üretrit, servisit, sistit, bakteriyel vajinozis) neden olduğu düşünülmektedir. Genellikle cinsel olarak aktif kadınların genital yollarından izole edilen bu mikroorganizmaların prevalansı çalışmalar arasında farklılık göstermektedir. Ayrıca bu mikroorganizmaların antibiyotik duyarlılıkları da bölgesel değişiklikler gösterir. Bu çalışmada, üretrit ve vajinit ön tanlılı hastalarda genital U. urealyticum ve M. hominis sıklığının, cinsiyetlere göre dağılımınının ve antibiyotik direnç profilinin belirlenmesi amaçlandı.

Gereç ve Yöntem Bu çalışma retrospektif gözlemsel çalışmadır. Veriler hastane kayıtlarından geriye dönük olarak elde edildi. Çalışmada Ocak 2017- Aralık 2019 tarihleri arasında üç yıllık süre boyunca Antalya'da özel bir hastanenin mikrobiyoloji laboratuvarında çalışılan genital örnekler değerlendirildi. Örnekler steril eküvyon ile taşıma besiyeri içeren tüplere alındı. AF Genital System (Liofilchem, İtalya) (http://www.liofilchem.net/login/pd/ifu/74156_IFU. pdf) kiti kullanılarak *M. hominis ve U. urealyticum* varlığı araştırıldı. Üreme saptanan örneklerde etkenlerin doksisiklin, ofloksasin, eritromisin, tetrasiklin, klaritromisin, klindamisin duyarlılıkları belirlendi. Hastalardan alınan sürüntü örneklerinin ekimleri üretici firma önerileri doğrultusunda yapıldı. Stripler 37° C'de 24-48 saat inkübe edildi. İnkübasyon sonunda kuyucuklardaki renk değişimine göre sonuçlar değerlendirildi.

Bulgular: Çalışmaya 147 (%60) erkek, yaş ortalaması 31±10,22 yaş olan 245 hasta dahil edildi. Gönderilen 245 genital örnek materyali kültürünün 55'inde (%22,4) sadece *M. hominis*, 27'sinde (%11,02) sadece *U. urealyticum*, 87'sinde (%35,5) ise her iki bakteri birlikte izole edildi. İncelenen kökenlerin duyarlılık oranlarının en yüksek olduğu antibiyotikler *M. hominis* için doksisiklin (% 83,1), klindamisin (% 69,7), tetrasiklin (% 61,9) iken; *U. urealyticum* için doksisiklin (% 79,8), klindamisin (% 71), tetrasiklin (% 65,7) idi. Cinsiyetlere göre etkenlerin değerlendirilmesinde ise, kadınlarda kültür pozitiflik oranı istatistiksel olarak daha yüksekti (p <.05).

Sonuç: Bu çalışma *U. urealyticum* ve bazı genital mikoplazmaların neden olduğu genital infeksiyonların tedavisinde kültür sonuçlarının önemini vurgulamıstır.

Anahtar Kelimeler: *Mycoplasma hominis, Ureaplasma urealyticum,* antibiyotik direnç profilleri, vajinit, üretrit



INTRODUCTION

The most frequently isolated microorganisms from the urogenital system are *Mycoplasma hominis* and *Ureaplasma urealyticum*. *M. hominis* and *Ureaplasma species* can be isolated from the lower genital tract in healthy and sexually active adults, and these agents are thought to cause some urogenital diseases (urethritis, cervicitis, cystitis, and bacterial vaginosis). It has been shown that *M. hominis* and *Ureaplasma species* have roles in infertility and respiratory system diseases in newborns.^[1,2]

Although often isolated from the genital tract of sexually active women, the prevalence of and *M. hominis* is closely related to sociodemographic factors such as income level and sexual partner diversity. The antibiotic susceptibility of these microorganisms also shows regional variations due to the differences in the antibiotic policies of the societies and the antibiotic use histories of the individuals.^[1-3] Current data on the frequency of *U. urealyticum* and *M. hominis*-related infections and the changes in antibiotic susceptibility rates contribute to the effective planning of the treatment of these infections.^[3] In this study, it was aimed to determine the frequency, gender distribution, and antibiotic resistance profile of genital *U. urealyticum* and *M. hominis* in patients with pre-diagnosis of urethritis and vaginitis.

MATERIAL AND METHOD

In order to conduct the study, ethical approval from the local ethics committee (date: 5/8/2021, number:11/4) was obtained. This study is a retrospective observational study. The data was obtained retrospectively from hospital records. In the study, genital samples sent to our center Microbiology Laboratory for a period of three years between January 2017 and December 2019 were evaluated. Urogenital samples were taken into tubes containing transport medium with sterile swabs. The presence of M. hominis and U. urealyticum was investigated using the AF Genital System (Liofilchem, Italy) kit. Antibiotic susceptibility tests were performed in accordance with the manufacturer's guidelines[4] and the evaluation of antibiotic susceptibility tests was done by the principal investigator. The Mycoplasma/ Ureaplasma identity verification and antibiotic susceptibility testing kits applied the following microbiological principle: In wells 1-Uu 103, 2-Uu 104, and 3-Uu 105, a color change from yellow to red denotes a semi-quantitative count of *Ureaplasma* spp. The semi-quantitative M. hominis count is demonstrated by a yellow to red color shift in wells 4-Mh 104 and 5-Mh 105.[4] Doxycycline, ofloxacine, erythromycin, tetracycline, clarithromycin, and clindamycin susceptibilities of the agents were determined in the samples which growth observed. Sowing of the swab samples taken from the patients was done in accordance with the manufacturer's recommendations.[4] The strips were incubated at 37°C for 24–48 hours. The results were evaluated according to the color changes at the end of the incubation period. In addition, patient files were evaluated in terms of demographic data and symptoms.

Inclusion Criteria

Having symptoms related to sexually transmitted disease.

Exclusion Criteria

Duplicate samples from the same patient.

Not having any symptoms related to sexually transmitted disease

Statistical Analysis

The SPSS package program (version 21.0; IBM, Armonk, NY) was used for statistical analysis of the research data. Percentage (%), frequency (f), arithmetic mean (X), χ^2 and the Fisher Exact test were used to analyze the statistical relationship between variables. P value of <0.5 was considered statistically significant.

RESULTS

During the study period, *M. hominis* and *U. urealyticum* examinations were studied in 387 samples. A total of 245 patients, 147 (60%) men, with a mean age of 31±10.22 years, were included in the study. Only *M. hominis* was isolated in 55 (22.4%) and only *U. urealyticum* was isolated in 27 (11.02%), and both bacteria were isolated in 87 (35.5%) of the 245 genital sample material cultures. The antimicrobial susceptibility results of *M. hominis* and *U. urealyticum* strains are summarized in **Table 1**.

It was found that the antibiotics with the highest susceptibility rates of the strains examined were doxycycline (83.1%), clindamycin (69.7%), and tetracycline (61.9%) for *M. hominis*; and doxycycline (79.8%), clindamycin (71%), and tetracycline (65.7%) for *U. urealyticum* (**Table 1**).

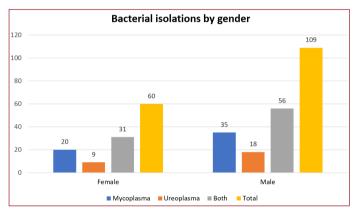
The lowest sensitivities were found to be erythromycin (54.4%) and ofloxacin (57.9%) for *U. urealyticum*, and erythromycin (43.6%) and clarithromycin (48.6%) for *M. hominis* (**Table 1**).

Antibiotics	Mycoplasma hominis (n=142)	Ureaplasma urealyticum (n=114)			
Doxycycline	118 (83.1%)	91(79.8%)			
Clindamycin	99 (69.7%)	81 (71%)			
Tetracycline	88 (61.9%)	75 (65.7%)			
Ciprofloxacin	87(61.2%)	71 (62.3%)			
Ofloxacin	87(61.2%)	66 (57.9%)			
Clarithromycin	69 (48.6%)	71 (62.3%)			
Erythromycin	62(43.6%)	62(54.4%)			
*Results with intermediate sensitivity were considered resistant.					

In the evaluation of bacterial growth isolation according to age groups, it was found that the highest rate of co-isolation of both *M. hominis* and *U. urealyticum*, only *U. urealyticum* and *M. hominis* was highest between the ages of 31-40 and the age range of 21-30 (**Table 2**).

Table 2. The distribution of <i>Mycoplasma hominis</i> and <i>Ureaplasma urealyticum</i> isolations by age groups.						
	Age between 18-20 years	Age between 21-30 years	Age between 31-40 years	Age between 41-50 years	Age >51 years	Total
Mycoplasma hominis	5	19	24	6	1	55
Ureaplasma urealyticum	0	11	14	2	0	27
Both Mycoplasma hominis and Ureaplasma urealyticum	6	34	29	16	2	87
Total	11	64	67	24	3	169

When factors were evaluated according to genders, bacterial growth detected in 109 cultures sent from 147 men and 60 cultures sent from 98 women. According to Fisher Exact test, p value was 0.0353 and culture positivity rate was statistically higher in females (p < .05) (**Table 3**, **Table 4** and **Graph 1**).



Graphic 1. The distribution of *Mycoplasma hominis* and *Ureaplasma urealyticum* isolations by gender.

Table 3. Gender positivity rates according to Fisher Exact test results					
	Positive	Negative	Total	P value	
Male	109	38	147	0.0353	
Female	60	38	98	0.0353	

Table 4. Distribution of <i>Mycoplasma hominis</i> and <i>Ureaplasma urealyticum</i> isolations by gender.					
	Mycoplasma hominis (n=55)	Ureaplasma urealyticum (n=27)	Mycoplasma hominis and Ureaplasma urealyticum (n=87)	Total	
Female	20	9	31	60	
Male	35	18	56	109	
Total	55	27	87	169	

DISCUSSION

Although many Mycoplasma species are considered members of the normal flora, some species cause serious genital diseases. There are a limited number of studies conducted in our country on *U. urealyticum* and *M. hominis*, which are among these agents. [1,3,5-12] In order to better understand and select the most effective medical treatments for these infections, the current investigation examined the prevalence and antimicrobial susceptibility of *U. urealyticum* and *M. hominis* isolated from urogenital samples from patients living in the Antalya province.

For the isolation of *U. urealyticum* and *M. hominis* in genital samples; methods such as the classical culture method, serological, molecular diagnostic methods, and readymade commercial test kits can be used. The gold standard in the diagnosis of these factors is culture. However, they are very difficult to produce in culture. Another method is the multiplex polymerase chain reaction (PCR) method. However, this method is also disadvantageous because it does not contain antimicrobial susceptibility information and has a high cost. In the detection of *U. urealyticum* and M. hominis from urogenital samples, systems including media and antimicrobial susceptibility tests can be used in the form of a special kit.[1,3,5,7] İçen et al.[10] defined similar species from vaginal samples by MALDI TOF-MS and multiplex PCR. However, these methods are costly and used for detailed examinations. In our study, we used a special ready kit because of the limited identification possibilities in a private hospital.

In similar studies conducted in our country, different samples with and without symptoms were selected. [1-3,5-8,10-16] In our study, samples which only sent from cases with symptoms (urethritis and vaginitis) were included in the study.

From these studies; Cetin et al.^[6] found the frequency of *M. hominis* to be 48.4%, the frequency of *U. urealyticum* to be 85.5%, and the positivity of both factors to be 13.2% in symptomatic patients, Ekşi et al.^[7] reported the reproduction frequency of *M. hominis* and/or *U. urealyticum* as 44.51% in women with cervicitis. In studies conducted in our country with different samples,^[3,4] *M. hominis* and *U. urealyticum* coinfection rates was reported as 2.9%,^[3] 4.5%,^[5] and 13.2%. ^[6]

In the current study, only patients with symptoms were included. *M. hominis* and/or *U. urealyticum* growth was detected in the cultures of 70.2% of the patients. In the cultures of genital sample material sent, only *M. hominis* was isolated in 22.4%, only *U. urealyticum* in 11.02%, and both bacteria were isolated in 35.5%. In a detailed analysis, it was found that co-growth was higher in our study than in similar studies^(3,5,6) performed in our country. This difference may be due to many different reasons that differs from the study method used to the characteristics of the patients.

In a recent study from our country, *M. hominis* strains were found to be resistant to all macrolide group antibiotics such as azithromycin, erythromycin, and clarithromycin.^[6] In the study of Ekşi et al.^[7] in *M. hominis* strains, 5% resistance

to roxithromycin and 28% of ofloxacin resistance have been reported. Meral et al.[12] reported that resistance to guinolones, which is an antibiotic that can be used in the treatment of *U. urealyticum* and *M. hominis*, is increasing. In a similar study conducted in women with vaginitis in 2011, Turan et al.[16] reported the ciprofloxacin resistance rates as 44.8% in M. hominis strains and 28.6% in U. urealyticum strains. Culture positivity rates in men and women with symptoms have not been compared in previous study. ^[17] In addition, we could not find a study based on age groups. Fluoroguinolones, in particular when M. hominis coinfection was present, exhibited the lowest action against U. urealyticum, according to Zhu et al.[18] Fluoroquinolones also shown a pattern of drug resistance against M. hominis that was comparable to *U. urealyticum*. Over the course of the test, antibiotic resistance did not change considerably. Notably, those who had both Mycoplasma infections concurrently showed a higher risk of multi-drug resistance. The above findings may help Chinese doctors adopt rational medicine usage and prevent the overuse of antibiotics in light of the epidemiological features of genital Mycoplasmas in male infertility patients.[18] In our study, the lowest sensitivities were found to be erythromycin (54.4%) and ofloxacin (57.9%) for *U. urealyticum*, and erythromycin (43.6%) and clarithromycin (48.6%) for M. hominis. Ciprofloxacin was found to be sensitive to around 60% of both bacteria (61.2% in M. hominis strains and 62.3% in *U. urealyticum* strains).

According to Zhu et al. study^[17] the frequency of U. urealyticum and M. hominis antibiotic resistance profiles was comparable to that of female-originated Mycoplasmas described by Wang et al.[19] in China, suggesting that these Mycoplasmas may be transmitted from males to women. Apart from the lowered activity of ciprofloxacin, which may be related to the drug's excessive use, no other significant variation in antibacterial activity was seen during the course of the trial. Therefore, variations in antibiotic usage regulations among different regions may potentially affect the features of antimicrobial susceptibility. In the current study, the culture positivity rate was statistically higher in women. In addition, when the growths were evaluated according to age groups, it was determined that both M. hominis, only *U. urealyticum*, and only *M. hominis* and *U.* urealyticum had the highest rate of co-growth between the ages of 31-40 and the age range of 21-30. This distribution by age may be related to the risky sexual behaviors of this age group.

In a bibliometric study conducted in our country, postgraduate thesis studies were evaluated. It has been determined that there are generally survey studies on sexually transmitted diseases in Turkey.^[20] For this reason, we think that studies examining the factors and resistance profiles in sexually transmitted diseases which similar to our study should be increased.

CONCLUSION

The low number of materials studied and the high positivity rate in a three-year period in a private hospital in Antalya suggest that there may be a need for a new approach to sexually transmitted diseases. In addition, this study emphasized the importance of culture results in the treatment of genital infections caused by *U. urealyticum* and some genital mycoplasmas. The antimicrobials with the highest sensitivity to both microorganisms were determined to be doxycycline, clindamycin, and tetracycline, respectively.

Culture positivity rates in men and women with symptoms have not been compared in previous studies. In the current study, the culture positivity rate was statistically higher in women. We think that this issue should be investigated in further studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Antalya Education and Research Hospital Ethics Committee (Date: 5.8.2021, Decision No: 11/4).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

Note: The 1-year (January 2017- December 2018) data of the study were presented as a poster presentation at the 34th ANKEM Congress held in Marmaris Grand Yazıcı Turban Hotel-Marmaris on May 1-5, 2019.

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