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Investigation of mycoplasma and ureaplasma species using a molecular method in male patients suffering from urethritis symptoms: a cross-sectional study in the city of Antalya

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Abstract

Background This study aimed to determine whether *Mycoplasma (M) genitalium*, *M. hominis*, *Ureaplasma (U) urealyticum*, and *U. parvum* were present in male patients with symptoms of urethritis.

Methods First-void urine and genital discharge samples were collected from 94 men. The samples were examined for the presence of *M. genitalium*, *M. hominis*, *U. urealyticum*, and *U. parvum* using a multiplex polymerase chain reaction (PCR) method (BioGX Mycoplasma-Ureaplasma-OSR commercial multiplex PCR kit, BioGX, the Netherlands). The presence of *Trichomonas vaginalis* and *Neisseria (N) gonorrhoeae* was investigated using microscopy and culture methods. In the *M. genitalium*–positive samples, macrolide resistance was evaluated using the Macrolide-R/MG ELITe MGB Kit (ELITechGroup S.p.A., Italy) on the ELITe BeGenius system.

Results A total of 55 microorganisms were detected in 41.5% of the patients ($n=39$; *U. urealyticum* [$n=18$], *U. parvum* [$n=14$], *N. gonorrhoeae* [$n=9$], *M. hominis* [$n=8$], and *M. genitalium* [$n=6$]). The positivity rates of *M. genitalium* and *N. gonorrhoeae* were statistically higher in the patients with more than 3 partners in the last 12 months, and those of *U. urealyticum* and *N. gonorrhoeae* were higher in the patients with genital discharge ($p<0.05$). In addition, a significant relationship was found between *N. gonorrhoeae* positivity and genital itching and pain/discomfort during sexual intercourse ($p<0.05$). Macrolide resistance was detected in 2 (33.3%) of the 6 *M. genitalium*–positive samples.

Conclusion In this study, *U. urealyticum*, *N. gonorrhoeae*, and *M. genitalium* were detected in 19.1%, 9.6%, and 6.4% of the male patients who presented with symptoms of urethritis, respectively.

Keywords Urethritis, *Mycoplasma genitalium*, *Neisseria gonorrhoeae*, *Ureaplasma urealyticum*, Polymerase chain reaction

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Introduction

Sexually transmitted infections (STIs) spread predominantly through sexual contact, including vaginal, anal, and oral sex. More than 30 different bacteria, viruses, and parasites are transmitted through sexual contact [1]. The characteristic findings of urethritis include urethral discharge, dysuria, itching, and burning in the anterior urethra. However, not all men with urethritis present with symptoms [2, 3].

Infectious urethritis is typically caused by a sexually transmitted pathogen; thus, most cases occur in sexually active young men [4]. The most common cause of nongonococcal urethritis (NGU) is *Chlamydia (C) trachomatis*, followed by *Mycoplasma (M) genitalium* [3–5]. *Ureaplasma* (U) spp. and *M. hominis* may occur as commensals in the lower genital tract in many healthy sexually active adults [6]. The role of ureaplasmas in NGU has long been controversial [7]. *U. urealyticum* in high bacterial loads might cause a small proportion of male NGU cases, but in most men with *U. urealyticum* infection/colonization, NGU does not develop [6]. Evidence that *M. hominis* and *U. parvum* cause NGU is lacking [7]. Furthermore, almost half of all NGU cases have no specific etiology [4].

Multiplex polymerase chain reaction (PCR) is the fastest and sensitive diagnostic method for gonococcal urethritis and NGU. It allows the use of noninvasive samples, such as first-void urine samples and self-collected swabs [5]. The British Association for Sexual Health and HIV recommends the use of nucleic acid amplification tests as the only useful diagnostic method for *M. genitalium* infection in clinical samples [8].

Empirical treatment of NGU usually involves doxycycline or as an alternative azithromycin therapy. It is recommended that *M. genitalium* infection be considered in all patients with persistent urethritis despite initial empirical therapy, and if possible, a test to detect macrolide resistance should be performed. The prevalence of macrolide resistance in *M. genitalium* limits the usefulness of azithromycin-based regimens [9].

The main aim of this study was to determine the presence of *M. genitalium*, *M. hominis*, *U. urealyticum*, and *U. parvum* in male patients with symptoms of urethritis using molecular methods. In addition, this study investigated macrolide resistance in *M. genitalium*–positive samples. Other pathogenic agents of urethritis, such as *Neisseria (N) gonorrhoeae* and *Trichomonas (T) vaginalis*, were also examined in the samples.

Materials and methods

This study included 94 male patients who visited the urology outpatient clinics of 3 hospitals in the Antalya city center with symptoms of urethritis, such as genital discharge, itching, burning sensation during urination, pain, and redness of the penis. After obtaining informed consent from the patients, information on their demographics, socioeconomic status, sexual activity status, number of sexual partners, and symptoms was assessed. First-void urine and urethral swab samples (Copan Diagnostics, USA) were collected from the patients.

The presence of *M. genitalium*, *M. hominis*, *U. urealyticum*, *U. parvum*, and macrolide resistance in *M. genitalium* was investigated in first-void urine samples using PCR, and the presence of *T. vaginalis* was investigated using direct microscopy and culture methods. The presence of *N. gonorrhoeae* was investigated with Gram stain microscopy and culture methods using urethral swab samples.

Swab samples were inoculated onto modified Martin-Lewis agar (Becton Dickinson and Company, Franklin Lakes, NJ) for the detection of *N. gonorrhoeae* and then smeared for microscopic examination. The smear was Gram stained and examined under a light microscope. The modified Martin-Lewis agar plates were incubated at 35–37 °C in a 5–10% CO_2 environment for 48–72 h. Using Gram staining and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonik GmbH, Bremen, Germany), *N. gonorrhoeae* was identified from suspicious colonies grown in the medium. The cultured microorganisms were transferred to a designated area on a MALDI-TOF MS metal plate. The plate was then placed in the MALDI-TOF MS device, and mass spectrometry data were obtained using the Flex Analysis software.

The urine sample was centrifuged, and the sediment was examined under a light microscope with ×10 and ×40 objectives to detect *T. vaginalis* trophozoites. Culture for *T. vaginalis* was performed using a Diamond TYM medium prepared in the laboratory.

For multiplex PCR, the urine samples were stored at − 80ºC until further use. A BioGX Mycoplasma-Ureaplasma-OSR commercial multiplex PCR kit (BioGX, the Netherlands), a real-time multiplex PCR assay used on the BD MAX platform, was used for the qualitative detection of the presence of DNA from *M. genitalium* (MgPa operon gene), *M. hominis* (gap gene), *U. urealyticum* (UUR10_0680 gene), and *U. parvum* (UP063 gene). The samples were loaded into the device in accordance with the manufacturer's recommendations. At the end of the study, the results were evaluated in accordance with the manufacturer's recommendations. Macrolide resistance was examined using the Macrolide-R/MG ELITe MGB Kit (ELITechGroup S.p.A., Italy) on the ELITe BeGenius system, in accordance with the manufacturer's recommendations. Macrolide resistance–associated mutations in the *23 S rRNA* gene were determined in all samples that tested positive for *M. genitalium* using the Macrolide-R/MG ELITe MGB Kit, which allowed for the

simultaneous detection and identification of *M. genitalium* and the 7 mutations (A2058G, A2058C, A2058T, A2059G, A2059C, A2062G, and A2062T) responsible for macrolide resistance.

Statistical analyses

Statistical analyses were performed using SSPS version 23.0. The patients' characteristics and laboratory examination results were analyzed using descriptive statistics. Categorical data are presented as numbers (n) and percentages (%). Comparisons between the groups were performed using the chi-square test or Fisher exact test for categorical data and the Student *t*-test for numerical data. Statistical significance was set at $p < 0.05$.

Results

Of the patients included in the study, 71.0% were single; 53.8% reported having a new sexual partner in the last 3 months; 37.6% reported having a history of previous illness, such as gonorrhea, genital discharge, or warts; and 33.3% reported having had sex with a sex worker. The symptom reported was genital discharge in 53.8% of the patients, genital itching in 47.8%, pain or discomfort during sexual intercourse in 30.0%, and pain or discomfort during urination in 71.0%. The patients' demographic data, sexual behavior characteristics, and symptoms are presented in Table 1.

The Gram-stained microscopic examination revealed polymorphonuclear leukocytes and Gram-negative diplococci in 2 patients (2.1%). *N. gonorrhoeae* growth was observed on the modified Martin-Lewis agar plates in 9 patients (9.6%). The multiplex PCR tests detected 46 microorganisms in 37 patients (39.4%), a single bacterial species in 28 patients (29.8%), and multiple bacterial species in 9 patients (9.6%; *M. hominis* + *U. urealyticum* in 3 patients [3.2%], *M. hominis* + *U. parvum* in 2 patients [2.1%], and *U. parvum* and *U. urealyticum* in 4 patients [4.3%]; Table 2).

When the PCR and culture results were evaluated, 55 microorganisms were detected in 39 patients (41.5%). Seven of the 9 patients who tested positive for *N. gonorrhoeae* had at least one accompanying *Mycoplasma* or *Ureaplasma* species (*U. urealyticum* + *U. parvum* in 2 patients, *U. urealyticum* in 3 patients, and *M. genitalium* in 2 patients).

The mean age of the patients who were positive for any of the microorganism was lower than that of patients who were negative for any of the microorganisms (30.66 years

Table 1 Demographic data, sexual behavior characteristics, and symptoms of patients

Characteristics	*n*/*n**	%	Characteristics	*n*/*n**	%
Age (n)	91		**Age at first sexual intercourse (n)**	92	
18–25	28/91	30.8	<18	40 /92	43.5
26–34	28/91	30.8	18–25	46/92	50.0
35–44	20/91	22.0	26–34	5/92	5.4
≥44	15/91	16.4	35–44	1/92	1.1
Education Level (n)	88		**Frequency of sexual intercourse (n)**	90	
Primary School	18/88	20.5	Daily	3/90	3.3
High School	28/88	31.8	3–4 times a week	23/90	25.6
University	42/88	47.7	Once a week	31/90	34.4
			Less than once a week	33/90	36.7
Marital Status (n)	93		**Sexual intercourse in the last 6 months**	84/93	90.3
Married	27/93	29.0	**New sexual partner in the last 3 months**	50/93	53.8
Single	66/93	71.0	**Number of sexual partners to date (n)**	88	
Monthly income level (n)	82		1–3	28/88	31.8
Less than minimum wage	1/82	1.2	4–6	13/88	14.8
Minimum wage	34/82	41.5	7–9	3/88	3.4
Above minimum wage	47/82	57.3	≥10	44/88	50.0
History of HIV or other STIs	12/94	12.8	**Sexual intercourse with a sex worker (+)**	31/93	33.3
Symptoms			**History of gonorrhea or genital discharge, warts**	35/93	37.6
Genital discharge	50/93	53.8	**Sexual intercourse with someone having similar symptoms**	12/92	13.3
Genital itching	44/93	47.8	**Use of condom during sexual intercourse (+)**	38/92	41.3
Testicular pain	37/93	39.8	**Number of sexual partners in the last 12 months (n)**	90	
Testicular swelling	12/93	13.0	0	2/90	2.2
Pain/discomfort during intercourse	27/93	29.0	1	31/90	34.4
Pain/discomfort during urination	66/93	71.0	2–3	30/90	33.3
Redness on penis or testicles	23/93	24.7	4–5	7/90	7.8
Pain/discomfort in the pelvic area	18/93	19.4	≥6	20/90	22.2

* The patients did not answer all the questions in the survey. Analyses were calculated based on the data received from the survey questions

Table 2 Methods applied to patient samples and their results

Methods	Number of Patients (*n*: 94)
Microscopy, (*n*, %)	
Direct Microscopic Examination (Urine)	-
Gram-Stained Microscopic Examination (Genital Swab)	2 (2.1%)
Culture, (n, %)	
Trichomonas vaginalis	-
Neisseria gonorrhoeae	9 (9.6%)
Polymerase Chain Reaction (PCR), (n, %)	
Mycoplasma hominis	3 (3.2%)
Mycoplasma genitalium	6 (6.4%)
Ureaplasma urealyticum	11 (11.7%)
Ureaplasma parvum	8 (8.5%)
Multible agents, (n, %)	
Mycoplasma hominis + *Ureaplasma urealyticum*	3 (3.2%)
Mycoplasma hominis + *Ureaplasma parvum*	2 (2.1%)
Ureaplasma parvum + *Ureaplasma urealyticum*	4 (4.3%)

[range, 17–72 years] vs. 34.7 years [range, 17–69 years]). The microorganisms were most prevalent (41.8%) in the patients aged 26–34 years. *N. gonorrhoeae* and *M. hominis* were most prevalent in the patients between 18 and 25 years of age, whereas *M. genitalium*, *U. urealyticum*, and *U. parvum* were most prevalent in those between 26 and 34 years of ages (Table 3).

A statistically significant relationship was found between having more than 3 partners in the last 12 months and *M. genitalium* and *N. gonorrhoeae* positivity ($p < 0.05$). The patients with genital discharge had higher *U. urealyticum* and *N. gonorrhoeae* positivity rates ($p < 0.05$). In addition, a significant relationship was found between *N. gonorrhoeae* positivity and genital itching and pain during sexual intercourse ($p < 0.05$). No statistically significant relationships were found between *M. hominis* and *U. parvum* colonization and any of the symptoms (Table 3). Macrolide resistance was detected in 2 (33.3%) of the 6 *M. genitalium*–positive samples.

Discussion

In our study, the real-time PCR method, which previous studies have recommended, was used to investigate the presence of *Mycoplasma* spp. and *Ureaplasma* spp. The sensitivity and specificity rates of nucleic acid amplification tests for *M. genitalium*, *M. hominis*, *U. urealyticum*, and *U. parvum* ranged from 98 to 100% and from 97 to 100%, respectively [5].

In studies that used the PCR method in male patients with urethritis, the prevalence rates of *M. genitalium* were 10.5%, 12%, and 23% [2, 10–12]. The overall prevalence rate of *M. genitalium* was 10.5% in a study conducted with men who had sex with men [13]. In a study in Southern Ghana that used 2 different kits, the total *M. genitalium* infection rates were 3.1% and 3.4% [14]. In our study, which included symptomatic men, the *M. genitalium* positivity rate was 6.4%. The different results between the studies may be due to various factors, such as the patient group selected, the method used, and the patients' risky sexual behaviors.

Routine testing for *M. hominis*, *U. urealyticum*, and *U. parvum* is not recommended for patients with or without symptoms of urethritis. A high load of *U. urealyticum* might be associated with urethritis in men [15]. In the literature, the prevalence rate of *U. urealyticum* ranges widely from approximately 5–28% [10, 16, 17]. In our study, the multiplex PCR method was used in male patients who presented with symptoms of urethritis, and *U. urealyticum* was found in 19.1% of the patients, which is consistent with the literature. The load of *M. hominis* showed notable effects on sperm motility, morphology, and leukocyte count [18]. *M. hominis* can cause infertility in men, even in those who are asymptomatic, but the infertility can be improved with antibiotic treatment [19]. In our study, similar to the literature, the *M. hominis* and *U. parvum* colonization rates were 8.5% and 14.9%, respectively.

Genital discharge is one of the most common symptoms of urethritis and can be caused by various pathogenic agents of STI. Manhart et al. [11] reported that in men with *M. genitalium* urogenital infection, the most common symptom was urethral discharge (20.4%). Rietmeijer et al. [20] found a significant relationship between urethral discharge and *N. gonorrhoeae* positivity. In our study, a statistically significant relationship was found between the presence of genital discharge and *N. gonorrhoeae* and *U. urealyticum* positivity ($p < 0.05$).

The main risk factors of STIs include age between 20 and 35 years, having multiple partners, and a history of STI [21, 22]. de Souza et al. [22] found that pathogenic agents of STI were most common in the 20 to 29 year age group, and Manhart et al. [11] found that *M. genitalium* was most common in the 15 to 24 year age group. Among the patients in our study who tested positive for any of the microorganisms, those in the 26 to 34 year age group had the highest prevalence rate. The differences between age groups may be due to various reasons, such as the time of initiation of sexual activity, sociocultural features, and the sexual behavior characteristics of individuals in different populations. Risky sexual behaviors, especially increasing the number of sexual partners, are known to increase the risk of STIs. Several studies have found a correlation between the number of sexual partners and the risk of *M. genitalium* infection [23–25]. In our study, a statistically significant relationship was found between having 3 or more partners in the previous year and *M. genitalium* and *N. gonorrhoeae* positivity ($p < 0.05$).

Molecular studies have shown that organisms in patients with failed azithromycin treatment have

Table 3 Microorganisms detected in samples and associated clinical factors

	Microorganism (-) (*n*, %)	Any Microorganism (+) (*n*, %)				
		M. genitalium (*n*:6)	*M. hominis* (*n*:8)	*U. urealyticum* (*n*:18)	*U. parvum* (*n*:14)	*N. gonorrhoeae* (*n*:9)
Age 18–25	15 (28.3)	2 (33.3)	3 (37.5)	6 (33.3)	3 (21.4)	4 (44.4)
26–34	12 (22.6)	4 (66.6)	2(25.0)	7 (38.9)	7 (50.0)	3 (33.3)
35–44	15 (28.3)	-	1 (12.5)	2 (11.1)	3 (21.4)	2(22.2)
>44	11 (20.8)	-	2 (25.0)	3 (16.7)	1 (7.1)	-
Age at first sexual intercourse ≤25 Years	51 (94.4)	6 (100)	8 (100)	17 (100)	11 (78.6)	8 (100)
>25 Years	3 (5.6)	-	-	-	3 (21.4)	
Frequency of sexual intercourse	3 (5.6)	-	-	-	-	-
Daily	16 (29.6)	2 (33.3)	2 (28.6)	3 (17.6)	-	3 (37.5)
3–4 times week	16 (29.6)	3 (50)	3 (42.8)	6 (35.3)	7 (58.3)	5 (62.5)
Once a week	19 (35.2)	1 (16.7)	2 (28.6)	8 (47.1)	5 (41.7)	-
Less than once a week						
Number of partners in the last 12 months ≤3	42 (79.2)	2 (33.3)	6 (75)	10 (58.8)	7 (53.9)	3 (37.5)
>3	11 (20.8)	**4 (66.7)***	2 (25)	7 (41.2)	6 (46.1)	**5 (62.5)***
Sexual intercourse in the last 6 months	47 (87.1)	6 (100)	8 (100)	16 (88.9)	14 (100)	8 (88.9)
A new sexual partner in the last 3 months	23 (42.6)	5 (83.3)	6 (75)	12 (66.7)	10 (71.4)	7 (77.9)
History of previously diagnosed HIV or other STD	7 (13)	1 (16.7)	-	3 (16.7)	1 (7.1)	1 (11.1)
A history of previous illnesses such as gonorrhea or genital discharge or warts	21 (38.9)	1 (16.7)	2 (25)	7 (38.9)	6 (42.9)	5 (55.6)
Sexual intercourse with someone who has the above complaints	7 (13.5)	-	2 (28.6)	2 (11.1)	1 (7.1)	1 (11.1)
Sexual intercourse with a sex worker	17 (31.5)	1 (16.7)	4 (50)	7 (38.9)	5 (35.7)	2 (22.2)
Use of condoms during sexual intercourse	22 (41.5)	2 (33.3)	2 (28.6)	9 (56.3)	5 (38.5)	1 (12.5)
Genital discharge	23 (42.6)	5 (83.3)	4 (50)	**13 (72.2)***	7 (50)	**9 (100)***
Genital itching	23 (42.6)	3 (50)	5 (71.4)	9 (53)	8 (57.1)	**8 (88.9)***
Pain and discomfort during sexual intercourse	12 (23.1)	2 (33.3)	2 (25)	8 (47.1)	6 (42.9)	**5 (62.5)***
Pain and discomfort during urinating	36 (66.7)	3 (50)	6 (75)	14 (77.8)	13 (92.9)	8 (88.9)
Pain and discomfort in your hip area	12 (22.2)	1 (16.7)	1 (12.5)	3 (16.7)	2 (14.2)	2 (22.2)
Testicular pain	23 (42.6)	2 (33.3)	3 (37.5)	8 (44.4)	4 (28.5)	4 (44.4)
Swelling in the testicles	6 (11.3)	2 (33.3)	1 (12.5)	2 (11.1)	1 (7.1)	2 (22.2)
Redness on penis/testicles	13 (24.1)	1 (16.7)	2 (25)	7 (38.9)	4 (28.5)	4 (44.4)

*$p < 0.05$

N. gonorrhoeae; Neisseria gonorrhoeae, M. genitalium; Mycoplasma genitalium, U. urealyticum; Ureaplasma urealyticum, M. hominis; Mycoplasma hominis, U. parvum; Ureaplasma parvum,

mutations in the *23 S rRNA* gene [7]. In one study, macrolide resistance-associated mutations were identified in 48.8% of samples, of which A2059C was the most common (18.2%) [26]. A study that evaluated the performance of 3 commercial molecular diagnostic tests in detecting *M. genitalium* and macrolide resistance reported that the Macrolide-R/MG ELITe MGB Kit (ELITechGroup) had significantly higher sensitivity than the other tests in detecting macrolide resistance [27]. In our study, macrolide resistance was investigated using the Macrolide-R/MG ELITe MGB Kit and was detected in 2 (33.3%) of the 6 *M. genitalium*–positive samples. This rate may be misleading because of the small number of *M. genitalium*–positive samples.

The present study has several notable strengths and limitations that should be acknowledged. The study included cases identified within the 3 largest public hospitals in Antalya, which is a notable strength. Another strength was the prospective study design. The most important limitation of our study is that *C. trachomatis* was not investigated. In some cases where no microorganisms were detected, the cause of urethritis may be *C. trachomatis*. The other limitations of the study are that *N. gonorrhoeae* and *T. vaginalis* were investigated using microscopy and culture methods, none of which were molecular methods.

Conclusion

In our study, which involved various methods to assess male patients with symptoms of urethritis, we detected *U. urealyticum* in 19.1% of the cases, *N. gonorrhoeae* in 9.6%, and *M. genitalium* in 6.4%. Moreover, macrolide resistance was observed in 33.3% of the *M. genitalium* isolates.

Abbreviations

STIs	Sexually transmitted infections
NGU	Nongonococcal urethritis
C. trachomatis	*Chlamydia trachomatis*
N. gonorrhoeae	*Neisseria gonorrhoeae*
M. genitalium	*Mycoplasma genitalium*
U. urealyticum	*Ureaplasma urealyticum*
M. hominis	*Mycoplasma hominis*
U. parvum	*Ureaplasma parvum*
PCR	Polymerase Chain Reaction
T. vaginalis	*Trichomonas vaginalis*

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None.

Author contributions

All authors contributed to the study. The first draft of the manuscript was written by T.K. and H.Y. All authors read and approved the final manuscript.

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Data availability

Availability of data and materials: Yes, The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Faculty of Medicine of Akdeniz on 10.11.2021, with (decision number KAEK-798).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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