Detecting of Mycoplasma genitalium in male patients with urethritis symptoms in Turkey by polymerase chain reaction

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ABSTRACT

Objectives: The aim of this study was to investigate the incidence of *Mycoplasma genitalium* in the urine samples of 63 male patients who had urethritis symptoms. Along with *Neisseria gonorrhoeae (N. gonorrhoeae)* and *Chlamydia trachomatis (C. trachomatis)*. We also investigated *Mycoplasma hominis (M. hominis)* and *Ureaplasma urealyticum (U. urealyticum)*, both of which are known to cause urethritis.

Methods: Microorganisms were investigated in urine samples of the patients with polymerase chain reaction. The study was conducted between September 2003 - February 2004 at the Department of Microbiology and Clinical Microbiology Ankara University School of Medicine, Ankara, Turkey.

Results: A total of 63 urine samples were analyzed and 6 (9.52%) patients had *N. gonorrhoeae*, 4 (6.34%) had *C. trachomatis*, while 4 (6.34%) urines were positive in terms of *M. genitalium*. Nevertheless, 3 (4.76%) patients had *U. urealyticum* and 2 (3.17%) patients had *M. hominis.* One urine sample was positive in terms of both *N. gonorrhoeae* and *U. urealyticum*, and another urine sample was positive in terms of both *M. hominis* and *U. urealyticum*. The results were compared with the control group and found no statistically significant difference.

Conclusion: *Mycoplasma* species are found in normal flora of urogenital system and also as an agent of urogenital infection. In our study, we found low microorganism rates when compared with Europe and America. This difference may be due to the conservative sexual behavior in Turkey.

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Acute urethritis is one of the most frequent symptoms of sexually transmitted diseases and the etiology is still unknown for most of the cases. *Neisseria gonorrhoeae (N. gonorrhoeae)* is the etiological cause of gonococcal urethritis and other cases are defined as nongonococcal urethritis (NGU). In cases with NGU, *Chlamydia trachomatis (C. trachomatis)* first comes to mind.[1] However, starting from the 1990's, with the development of polymerase chain reaction (PCR), which is a highly sensitive and specific method, along with *C. trachomatis, Mycoplasma genitalium (M. genitalium)* was also shown to have a strong association with NGU.[1,2] Although, *M. genitalium* was first isolated in 1981 from the urine samples of 2 out of 13 male patients with NGU, detailed studies could not follow this finding depending on the difficulties in isolating this microorganism from cultures.[2-4] Today, following the *C. trachomatis*

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Table 1 - Primary sequences used for determining the pathogens (MWG Biotech AG, Germany).

Microorganism	Sequence 5'-3'	PCR product size (bp)
Mycoplasma genitalium	AGTTGATGAAACCTTAACCCCTTGG CCGTTGAGGGGTTTTCCATTTTTGC	281
Mycoplasma hominis	CAATGGCTAATGCCGGATACG GGTACCGTCAGTCTGCAAT	335
Chlamydia trachomatis	GATAGCGAGCACAAAGAGAGCTAA TTCACATCTGTTTGCAAAACACGG	282
Ureaplasma urealyticum	GTATTGGCAATCTTTATATGTTTTCG CAGCTGATGTAAGTGCAGCATTAAATTC	403
Neisseria gonorrhoeae	GCTACGCATACCCGCGTTGC CGAAGACCTTCGAGCAGA	282

PCR - polymerase chain reaction, bp -base pair.

screening and treatment programs performed by clinics specifically dealing with sexually transmitted diseases, the incidence of *C. trachomatis* in terms of NGU fell to 15% and along with *M. genitalium*, the incidence of other microorganisms like *Ureaplasma urealyticum (U. urealyticum), Trichomonas vaginalis* and *herpes simplex virus* are being investigated.[1,3] Depending on the association of *M. genitalium* and urethritis, we used PCR to detect *M. genitalium* in the urine samples of male patients with urethritis symptoms and compared our results with the control group consisting of asymptomatic patients. Along with *M. genitalium* we also investigated the incidence of *N. gonorrhoeae, C. trachomatis, U. urealyticum* and *Mycoplasma hominis (M. hominis)*.

Methods. ***Patients.*** Sixty-three sexually active heterosexual males who applied to the outpatient clinic of Department of Urology, Ankara University School of Medicine Ibn-i Sina Hospital, Ankara, Turkey, with complaints of urethral discharge and dysuria were included in the study. First urine sample in the morning was collected from all patients and was analyzed with PCR at the Molecular Diagnosis and Research Laboratory of the Department of Microbiology and Clinical Microbiology of the same University Hospital and the incidence of *N. gonorrhoeae , C. trachomatis, M. genitalium, M. hominis* and *U. urealyticum* were investigated. None of the patients were using antibiotic during sampling time. The control group consisted of 58 male patients who did not have any signs and symptoms in terms of urethritis and were inpatients at Department of Physical Medicine and Rehabilitation within the same hospital.

The DNA extraction. Phenol-chloroform extraction method with modifications was used.[5] Ten ml of first urine sample was centrifuged at 5000 rpm for 5 minutes. Pellet was dissolved in 500 µl of distilled water and 60-100 µl of this sample was used for DNA extraction.

Polymerase chain reaction. The final volume for PCR was 50 µl. The reaction consisted of 1 U of Taq DNA polymerase (Bioron GmbH), 50 pmol primer 1 µl of each (**Table 1**), 200 mmol of dNTP mix, and 2.5 mmol of MgCl2. Amplification was performed at Techgene Techne FTGENE 5D thermal cycler with the first denaturation being at 94^{o}C for 5 minutes and following this, 40 cycles were performed at 94 ^{o}C for 30 seconds, 58^{o}C for 30 seconds, 72^{o}C for 35 seconds and final extension was at 72^{o}C for 10 minutes.

Screening. Samples were electrophoresed with 2% agarose gel containing 0.5 µg ml^{-1} EtBr (ethidium bromide) and were exposed to 100 V electric current in 0.5X tris borate buffer (pH 8) and visualized with ultraviolet (UV) transilluminator. The "DNA MicroMarker-Hae III digest of pUC 18 plasmid (Ambresco)" was used as molecular marker.

Statistical Analysis. Categorical variables were evaluated by Fisher's exact test or chi-square test, where applicable.

Results. A total of 63 urine samples belonging to male patients with symptoms of urethritis were examined and 6 (9.52%) of them were positive for *N. gonorrhoeae*, 4 (6.34%) for *C. trachomatis*, 4 (6.34%) for *M. genitalium*, 3 (4.76%) for *U. urealyticum* and 2 (3.17%) for *M. hominis* (**Table 2**). A total of 58 cases were included in the control

Table 2 - Positive DNA rates of the patient and control groups

Microorganism	Patient Group n = 63		Control Group n = 58		Statistical Analysis
	Positive	(%)	Positive	(%)	*p*
Neisseria gonorrhoeae	6	(9.52)	2	(3.44)	.276
Chlamydia trachomatis	4	(6.34)	1	(1.72)	.367
Mycoplasma genitalium	4	(6.34)	1	(1.72)	.367
Ureaplasma urealyticum	3	(4.76)	0	-	.245
Mycoplasma hominis	2	(3.17)	0	-	.497
Total	**17***	**(27)**	**4**	**(6.9)**	**.004**

*One patient was positive in terms of both *Neisseria gonorrhoeae* and *Ureaplasma urealyticum*, and another patient was positive in terms of both *Mycoplasma hominis* and *Ureaplasma urealyticum*.

Table 3 - Findings at the NGU group.

Microorganism	Patient Group n = 57		Control Group n = 56	
	Positive	(%)	Positive	(%)
Chlamydia trachomatis	4	(7.01)	1	(1.78)
Mycoplasma genitalium	4	(7.01)	1	(1.78)
Ureaplasma urealyticum	3	(5.26)	0	-
Mycoplasma hominis	2	(3.50)	0	-
Total	**12***	**(21.1)**	**2**	**(3.6)**

*One case was positive in terms of both *Mycoplasma hominis* and *Ureaplasma urealyticum.*

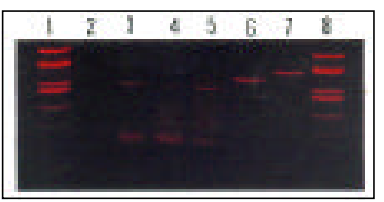

Figure 1 - Agarose gel electrophoresis of polymerase chain reaction products from 5 species selected from positive patients. Lane 1 and 8 molecular marker- 587, 458, 434, 298, 267-257, 171, 102, 80-, Lane 2: negative control, Lane 3: *Chlamydia trachomatis* (282bp), Lane 4: *Neisseria gonorrhoeae* (282bp), Lane 5: *Mycoplasma genitalium* (281bp), Lane 6: *Mycoplasma hominis* (335bp), Lane 7: *Ureaplasma urealyticum* (403).

group and 2 (3.44%) of them were positive for *N. gonorrhoeae*, 1 (1.72%) for *C. trachomatis* and 1 (1.72%) for *M. genitalium*, none of the patients in the control group were positive in terms of *U. urealyticum* or *M. hominis* (**Table 2**). Five microorganisms were analyzed and there was no statistically significant difference between patient and control groups (*N. gonorrhoeae*, p=0.276, *C. trachomatis*, p=0.367, *M. genitalium*, p=0.367, *U. urealyticum*, p=0.245, and *M. hominis*, p=0.497).

In the patient group, 17 (17/63; 27%) patients were positive in terms of at least one of the pathogens investigated, while in the control group, only 4 (4/58; 6.9%) cases were positive. This result did not show statistical significance (p=0.004). Cases with urine samples positive in terms of *N. gonorrhoeae* was left out of both groups and data were reanalyzed in terms of NGU, and the results are summarized at **Table 3**. Each microorganism showed no statistically significant difference between NGU and control groups, while a total analyses showed statistical significance at p=0.005 level. Although it did not show any statistical significance, the rate of *M. genitalium* and *C. trachomatis* being similar, in both the patient and in the NGU group seemed interesting. Polymerase chain reaction products representing positive reactions is shown in **Figure 1.**

Discussion. *Mycoplasma hominis, M. genitalium, Mycoplasma fermentans (M. fermentans)* and *U. urealyticum* belong to *Mycoplasma* and *Ureaplasma* species and they can be a part of the normal flora of the urogenital system, with the capacity to cause genital

infections.[6] Recently, the potential pathogenic role of *M. genitalium* in terms of diseases of the human urogenital tract has focused attention. A total of 16 *Mycoplasma* species are isolated from humans and 6 of them (*M. fermentans, Mycoplasma spermatophilum, U. urealyticum, M. genitalium, M. penetrans*, and *M. hominis*) are colonized at the urogenital tract, this tropism is explained by the presence of specific cytoadesins.[4,7] *Mycoplasma* infections are generally silent, they last short and have nonspecific symptoms, and as an element of normal flora at the same time, lead to difficulties in the diagnosis of these infections.[6] Cultures, direct diagnostic methods, and serologic tests are used for the classical diagnosis of *Mycoplasma* and *Ureaplasma* infections, but these methods not being fast enough and their sensitivity being low, lead to the usage and development of molecular diagnostic methods in recent years.[6,8] Along with other investigators, we also chose the PCR method because it allows us to study urine, which is an easily obtained sample, and in addition to its rapid results, PCR sensitivity is equal to or higher than *Mycoplasma* cultures. Mena et al [3] studied both urine samples and urethral swabs and showed that 18 patients were positive both at the urine and at the urethral swab, 3 patients were positive only at the urethral swab, and 2 patients were positive only at the urine sample; thus, the rate of *M. genitalium* infection on male patients with urethritis was 24% (23/97). These results demonstrate that at especially epidemiological studies, urine sample is appropriate in terms of detecting *M. genitalium*. There are other studies suggesting that urine samples are appropriate for detecting *C .trachomatis* depending on methods of DNA amplification.[9,10] Non-invasive way of sampling, do not require a clinician for sampling, does not require transport of samples from the clinic to the laboratory, thus, allowing us to work over the fresh sample at the laboratory and lead us to prepare the urine sample. Various studies have reported that *M. genitalium* is responsible from 11-35% of male NGU, which is not related with *C. trachomatis*.[3,11] Nevertheless, incidence of *M. genitalium* infection ranged from 0-9% among asymptomatic control patients.[3,11] In a study performed at Sweden, 17 out of 115 (14%) patients with urethritis symptoms were positive in terms of *M. genitalium* while only 1 case was positive at the control group consisting of 118 cases without urethritis.[12] In another study Bjornelius et al[13] examined 50 patients with NGU and the incidence of *M. genitalium* was 26% and on 50 cases as a control group, 10% of *M. genitalium* was observed. In a study from Italy by Busolo et al[8] incidence of *M. genitalium* was investigated by PCR at 56 patients with urethritis and 44 asymptomatic control cases and the rate was 10.7% at the patient group, while none of the cases in the control group were positive in terms of *M. genitalium*. Gambini et al[4] found that incidence of *M. genitalium* infection was 29.2% at symptomatic male patients while this rate was 4.3% at the asymptomatic control group. Thus, the authors stressed the pathogenic role of *M. genitalium* at male patients and stated that their study performed at Italy reflected the data of Mediterranean district. Turkey is another Mediterranean country and in our study this rate was 7.01% for symptomatic patients and 1.78% for asymptomatic patients of the NGU group, thus, our results are not in accordance with Gambini et al.[4] We found that the incidence of *M. genitalium* infection was 7.01% for patients with NGU symptoms and this number is lower than the rates reported in the literature. In a study performed in Jordan, incidence of *C. trachomatis* infection for symptomatic patients was reported as 4.6% and this was explained with the sexual behavior of this population being conservative when compared with Europe and America.[14] There may be some possible explanations regarding the differences of our observations from the observations reported by other authors. First of all, we chose a non-invasive sampling method and preferred urine sampling to urethral swabs. Although in the literature, the studies comparing urethral swabs and urine sampling,[9,10] reported that urine samples are appropriate, probability of detecting microorganisms can be higher when urethral swabs are studied.[3,8] Secondly, although most of the studies used gel electrophoresis for visualizing the PCR amplicone for *M. genitalium*, as we did, some studies reported that usage of southern blot techniques increases the sensitivity of detecting *M. genitalium* DNA.[1,3,4] The last and may be the most important explanation can be that, the difference between the results can depend on the different characteristics of study groups. The patient groups of the studies reported from West Europe, United Kingdom, and United States of America consist of both heterosexual and homosexual patients who were followed up by sexually transmitted diseases clinics, while our patient group only consisted of heterosexual patients attending to urology outpatient clinic with urinary tract complaints. When sexual behavior of Turkey being more conservative than the countries mentioned above is taken into consideration, we believe that our low infection rates are not surprising. Large scale studies with well defined patient groups and methods are needed to be planned to investigate the possible etiological roles of the *Mycoplasmas* and other NGU agents.

References

1. Totten PA, Schwartz MA, Sjöström KE, Kenny GE, Handsfield HH, Weiss JB, et al. Association of *Mycoplasma genitalium* with nongonococcal urethritis in heterosexual men. ***J Infect Dis*** 2001; 183: 269-276.
2. Jensen JS. *Mycoplasma genitalium*: the aetiological agent of urethritis and other sexually transmitted diseases. ***J Eur Acad Dermatol Venereol*** 2004; 18: 1-11.
3. Mena L, Wang X, Mroczkowski TF, Martin DH. *Mycoplasma genitalium* infections in asymptomatic men and men with urethritis attending a sexually transmitted diseases clinic in New Orleans. ***Clin Infect Dis*** 2002; 35: 1167-1173.
4. Gambini D, Decleva I, Lupica L, Ghislanzoni M, Cusini M, Alessi E. *Mycoplasma genitalium* in males with nongonococcal urethritis: Prevalence and clinical efficacy of eradication. ***Sex Transm Dis*** 2000; 27: 226-229.
5. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning A Laboratory Manual. 2nd ed. Book 2. New York (NY): Cold Spring Harbor Laboratory Press; 1989. p. E.3-E.4
6. Lippincott's Illustrated Reviews: Microbiology Strohl WA, Rouse H, Fisher BD, Harvey RA, Champe PC, editors. Mycoplasma. Chapter 19. Philadelphia (PA): Lippincott Williams and Wilkins; 2001. p. 229-234.
7. Deguchi T, Maeda SI. *Mycoplasma genitalium*: Another important pathogen of nongonococcal urethritis. ***J Urol*** 2002; 167: 1210-1217.
8. Busolo F, Camposampiero D, Bordignon G, Bertollo G. Detection of *Mycoplasma genitalium* and *Chlamydia trachomatis* DNAs in male patients with urethritis using the polymerase chain reaction. ***New Microbiol*** 1997; 20: 325-332.
9. Templeton K, Roberts J, Jeffries D, Forster G, Aitken C. The detection of *Chlamydia trachomatis* by DNA amplification methods in urine samples from men with urethritis. ***Int J STD AIDS*** 2001; 12: 793-796.
10. Sugunendran H, Birley HD, Mallinson H, Abbott M, Tong CY. Comparison of urine, first and second endourethral swabs for PCR based detection of genital *Chlamydia trachomatis* infection in male patients. ***Sex Transm Infect*** 2001; 77: 423-426.
11. Pepin J, Sobela F, Deslandes S, Alary M, Wegner K, Khonde N, et al. Etiology of urethral discharge in West Africa: the role of *Mycoplasma genitalium* and *Trichomonas vaginalis*. ***Bull World Health Organ*** 2001; 79: 118-126.
12. Johannisson G, Enstrom Y, Lowhagen GB, Nagy V, Ryberg K, Seeberg S, et al. Occurrence and treatment of *Mycoplasma genitalium* in patients visiting STD clinics in Sweden. ***Int J STD AIDS*** 2000; 11: 324-326.
13. Bjornelius E, Lidbrink P, Jensen JS. *Mycoplasma genitalium* in non-gonococcal urethritis – a study in Swedish male STD patients. I***nt J STD AIDS*** 2000; 11: 292-296.
14. Awwad ZM, Al-Amarat AA, Shehabi AA. Prevalence of genital chlamydial infection in symptomatic and asymptomatic Jordanian patients. ***Int J Infect Dis*** 2003; 7: 206-209.