ORIGINAL RESEARCH
General Urology

Doi: 10.4274/jus.1328
Journal of Urological Surgery, 2017;1:18-22

# Results of Multiplex Polymerase Chain Reaction Assay to Identify Urethritis Pathogens

## Üretrit Patojenlerinin Saptanmasında Multipleks Polimeraz Zincir Reaksiyonu Yöntemi

**Mehmet Sarıer[1], İbrahim Duman[1], Şafak Göktaş[2], Meltem Demir[3], Erdal Kukul[4]**

*[1]Kemerburgaz University Faculty of Medicine, Department of Urology, İstanbul, Turkiye*
*[2]Gelişim Medical Laboratories, Clinic of Clinical Microbiology and Infectious Diseases, İstanbul, Turkiye*
*[3]Kemerburgaz University Faculty of Medicine, Department of Biochemistry, İstanbul, Turkiye*
*[4]Medical Park Hospital, Clinic of Urology, Antalya, Turkiye*

**What's known on the subject? and What does the study add?**

We assume that using a multiplex polymerase chain reaction test, enabling results in a rapid and reliable way and performing the procedure with only one swap sample from the patient, provides the clinician with a big advantage as to identifying the pathogens within the ethiology of urethritis, which is a common sexually transmitted disease in males.

## Abstract

**Objective:** The purpose of this study was to evaluate the results of multiplex polymerase chain reaction (PCR) test applied to identify the pathogens in male patients who attended our urology clinic with a pre-diagnosis of urethritis related with sexual intercourse.

**Materials and Methods:** In this study, we included a total of 91 male patients, who sought medical advice in our clinic between August 2015 and October 2016 due to complaints of urethral discharge, dysuria and urethral itching, having a visible urethral discharge during the physical examination or a positive leukocyte esterase test (Combur-Test®-Roche) in the first urine sample. In the urethral swab samples of these patients, urethritis pathogens were searched with a multiplex PCR test. The multiplex PCR kit, which is able to identify nine pathogens and produced by PathoFinder® (Holland), was used in the process. The pathogens that could be detected by the kit were *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, Gardnerella vaginalis, Trichomonas vaginalis, Treponema pallidum,* and *Candida albicans.*

**Results:** The average age of the subjects was 35.1 (19-57) years. Sixty one out of 91 patients (67%) were found to have a pathogen in the urethral swab sample. In 45 patients (49.4%), only one pathogen, in 12 (13.1%) - two different pathogens and in 4 (4.3%) patients, 3 different pathogens were detected. The pathogens found were as follows: *Ureaplasma urealyticum* in 22 patients (27.1%), *Gardnerella vaginalis* in 15 (18.6%), *Neisseria gonorrhoeae* in 13 (16.1%), *Mycoplasma genitalium* (10 patients; 12.3%), *Mycoplasma hominis* (8 patients; 9.9%), *Chlamydia trachomatis* (8 patients; 9.9%), *Trichomonas vaginalis* (3 patients; 3.8%), and *Candida albicans* (2 patients; 2.4%). None of the patients were identified with *Treponema pallidum.* None of the pathogens were identified in 30 patients (32.9%) whose samples were examined by PCR method.

**Conclusion:** Sexually transmitted pathogens that are quite difficult to identify and that cause urethritis are possibly defined with only one swab sample in a short time using multiplex PCR method providing new possibilities and scopes for the diagnosis.

**Keywords:** Polymerase chain reaction, urethritis, mycoplasma, ureaplasma, *Gardnerella vaginalis*, sexual transmitted disease

## Öz

**Amaç:** Bu çalışmanın amacı; cinsel ilişkiye bağlı üretrit ön tanısı ile kliniğimize başvuran erkek hastalarda, patojenin tespiti için uygulanan multipleks polimeraz zincir reaksiyon (PCR) testinin sonuçlarının değerlendirilmesidir.

**Gereç ve Yöntem:** Kliniğimize Ağustos 2015-Ekim 2016 tarihleri arasında cinsel ilişki sonrası üretral akıntı, disüri, üretral kaşıntı şikayetleri ile başvuran, fizik muayenesinde görünür üretral akıntısı olan veya ilk idrar örneğinde lökosit esteraz testi (Combur-Test®-Roche) pozitif olan 91 erkek

**Correspondence:** Mehmet Sarıer MD, Kemerburgaz University Faculty of Medicine, Department of Urology, İstanbul, Turkiye
**Phone:** +90 533 332 49 60 **E-mail:** drsarier@gmail.com
**Received:** 04.01.2017 **Accepted:** 10.02.2017

**Presented in: 3rd Urological Surgery Congress, 2-6 November 2016, Antalya as a poster.**

**Cite this article as:** Sarıer M, Duman İ, Göktaş Ş, Demir M, Kukul E. Results of Multiplex Polymerase Chain Reaction Assay to Identify Urethritis Pathogens. J Urol Surg 2017;1:18-22.

*©Copyright 2017 by the Association of Urological Surgery / Journal of Urological Surgery published by Galenos Publishing House.*

hasta çalışmaya dahil edildi. Bu hastaların üretral swap örneğinde multipleks PCR testi ile üretrit patojenleri araştırıldı. Bu amaçla PathoFinder® (Hollanda) firmasının 9 patojeni tespit eden multipleks PCR kiti kullanıldı. Kitin saptadığı etkenler; *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, Gardnerella vaginalis, Trichomonas vaginalis, Treponema pallidum* ve *Candida albicans*'tı.

**Bulgular:** İnceleme yapılan 91 hastanın ortalama yaşı 35,1 idi (19-57). Doksan bir hastanın 61'inde (%67) üretral sürüntü örneğinde patojen saptandı. Bunlardan 45 (%49,4) hastada tek patojen, 12'sinde (%13,1) 2 farklı patojen ve 4'ünde (%4,3) ise 3 farklı patojen saptandı. Hastalarda saptanan patojenler sırasıyla, 22'si (%27,1) *Ureaplasma urealyticum*, 15'i (%18,6) *Gardnerella vaginalis*, 13'ü (%16,1) *Neisseria gonorrhoeae*, 10'u (%12,3) *Mycoplasma genitalium*, 8'i (%9,9) *Mycoplasma hominis*, 8'i (%9,9) *Chlamydia trachomatis*, 2'si (%2,4) *Candida albicans* ve 3'ü (%3,8) de *Trichomonas vaginalis*'ti. Hastaların hiçbirinde *Treponema pallidum* tespit edilmedi. Örnek alınan hastaların 30'unda (%32,9) PCR yöntemi ile hiçbir patojen saptanmadı.

**Sonuç:** Multipleks PCR yöntemi ile, saptanması oldukça zor olan cinsel yolla geçen ve üretrit nedeni patojenlerin tek bir üretral sürüntü örneğiyle, kısa sürede saptanır hale gelmesi, tanıya yeni olanaklar ve fırsatlar sağladığı görülmektedir.

**Anahtar Kelimeler:** Polimeraz zincir reaksiyonu, üretrit, mikoplazma, üreaplasma, *Gardnerella vaginalis*, cinsel yolla bulaşan hastalıklar

## Introduction

Sexually transmitted diseases (STDs) are significant cause of morbidity in sexually active individuals and still remain to be a major medical, social and economic burden all over the world. Urethritis, characterized by dysuria and urethral discharge, is the most common clinical picture and, globally annual incidence is estimated to be ~150 million cases (1). As a part of STDs, urethritis is widely classified as non-gonococcal urethritis (NGU) and gonococcal (2). STDs are highly varied and often involve more than one pathogen (3). The growing burden of STDs and ever-increasing costs have created a need for rapid and reliable laboratory techniques in order to detect the cause pathogens. There are continuing researches investigating methods that may detect more than one pathogen simultaneously in a single clinical patient. Polymerase chain reaction (PCR) assay has been found to be highly sensitive method detecting these STD pathogens (4). A multiplex assay has an additional advantage in screening since it involves simultaneous detection of multiple pathogens (5). The purpose of this study was to evaluate the results of multiplex PCR (MPCR) test performed to identify the pathogens in patients having complaints such as urethral discharge and dysuria after sexual intercourse.

## Materials and Methods

We included 91 male patients who were admitted to our clinic between August 2015 and October 2016 due to complaints of urethral discharge, dysuria, and urethral itching, having a visible urethral discharge during the physical examination and those with a positive leukocyte esterase test (Combur-Test®-Roche) in the first urine sample studied on urethritis pathogens using MPCR. The study was approved by Medical Park Hospital Ethics Committee (approval number: 189). Written informed consent was obtained from all patients before MPCR test and the study was performed in accordance with the principles of the Helsinki Declaration. To identify the pathogens in the swab samples, a MPCR kit which is able to identify nine pathogens and produced by PathoFinder® (Holland) was used. The pathogens that could be detected by the kit were *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, Gardnerella vaginalis, Trichomonas vaginalis, Treponema pallidum* and *Candida albicans*. The first nucleic acids (DNA and RNA) were extracted in accordance with the kit protocol. Afterwards, amplification, detection and data analysis processes were carried out using Rotor-Gene Real Time PCR System® (Qiagen, Germany) in compliance with the manufacturer's instructions. The results were examined retrospectively.

## Results

The average age of the patients was 35.1 (19-57) years. A total of 81 pathogens were identified in the urethral swab samples of 61 out of 91 patients (67%). Only one pathogen in 45 samples (49.4%), two different pathogens in 12 (13.1%), and 3 different pathogens in 4 (4.3%) samples were detected. The pathogens found were respectively as follows: *Ureaplasma urealyticum* in 22 patients (27.1%), *Gardnerella vaginalis* in 15 (18.6%), *Neisseria gonorrhoeae* in 13 (16.1%), *Mycoplasma genitalium* in 10 patients (12.3%), *Mycoplasma hominis* in 8 (9.9%), *Chlamydia trachomatis* in 8 (9.9%), *Trichomonas vaginalis* in 3 patients (3.8%) and *Candida albicans* in 2 (2.4%) (Figure 1). None of the patients were identified with *Treponema pallidum*. No pathogen was detected in 30 patients (32.9%) whose samples were examined using the PCR method.

## Discussion

Urethritis or inflammation of the urethra is a multifactorial condition which is sexually acquired in the majority cases. It is characterized by discharge, dysuria and/or urethral discomfort but may also be asymptomatic. The diagnosis of urethritis is confirmed by demonstrating an excess of polymorphonuclear leukocytes (PMNLs) in the anterior urethra. This is generally assessed by using a urethral swab. Urethritis is defined as

gonococcal when *Neisseria gonorrhoeae* is detected and as NGU when it is not detected. There exist many uncertainties in the event of NGU. Especially in cases having a low-grade inflammation, there are significant intra- and inter-observer mistakes in applying and reading the urethral slides and also in counting the PMNL (6). In many men with urethritis, a known pathogen is not detected (7). The high sensitivity levels of nucleic acid amplification tests like PCR enable the use of less invasive sampling, use of initial flow urine samples or swabs taken by one's own, which are not suitable for less sensitive tests like culture and antigen tests (8). MPCR, uses the advantage of the amplification of various target series under one single reaction condition set of more than one primer sets (9). Samples taken from patients having STDs (including symptomatic and asymptomatic cases) may frequently contain more than one pathogens (10). Therefore, in order to detect low levels of multiple (nine different) pathogens in one analysis, tests like MPCR having sufficient sensitivity and high specificity, have a significant potential in diagnosing STDs rapidly and reliably. MPCR also does not exhibit cross reaction with other relevant target species or many other common urogenital organisms.

Today, for patients diagnosed with urethritis, starting an antibiotic treatment is recommended before the results of culture tests (2). The results of culture tests take 3-7 days. During this period, it is within the bounds of possibility that the patient could contaminate others through new sexual intercourses. However, only the PCR method allows obtaining a result in less than 24 hours. This gives rise to the thought that it allows starting the correct treatment without delay. Identifying the pathogen in the early period will also make the sexual partner available for a correct treatment. Thus, the control of the infection will be easier. Here comes the cost as a significant issue. The cost of a PCR kit to the hospital is 280 TL (77$). Though this appears to be a high amount for a single test, being able to identify 9 different pathogens in one single sample shows that the test could be considered in terms of cost and profit. Apart from this, in resistive cases in which the patient has not recovered after empirical antibiotic treatment, or in urethritis cases where the pathogen cannot be identified through conventional methods, MPCR analysis could be considered.

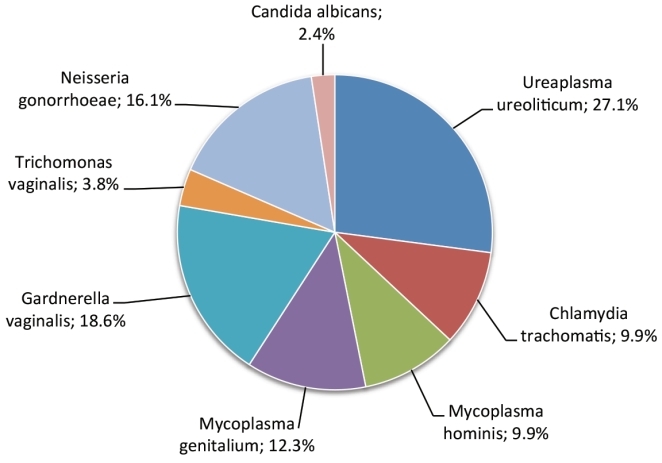


**Figure 1.** The disturbution of urethritis pathogens

*Neisseria* are gram-negative cocci that require nutritional supplement to be cultured in laboratory environment and their reproduction in culture is not easy. It is estimated that gonococcal urethritis accounts for approximately 5-20% of cases (11). 10% of males infected by gonorrhoeae are asymptomatic (12). *Chlamydia trachomatis* is generally defined as a required intracellular pathogen in the literature and it is responsible for 30-40% of NGU (13). PCR analysis has a high sensitivity and specificity in the diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

Studies of the role of ureaplasma in the pathogenesis of urethritis have revealed inconsistent results. Ureaplasms can be isolated from culture in 30-40% of asymptomatic males (14). In the literature, the prevalence of *Ureaplasma urealyticum* has been reported to be between 5% and 26% (2). *Ureaplasma* and *Mycoplasma species*, due to having no cell walls, are required intracellular parasites (15). Among the 9 pathogens detected by MPCR assay within this study, the most frequently identified ones are Ureaplasma and *Mycoplasma hominis* and *genitalium* (with a rate of 46.9 % within all urethritis pathogens) which indicates that intracellular parasitery infections occur much more frequently in the ethiopathogenesis of urethritis. It is rather difficult to isolate and identify these pathogens using conventional methods (16). MPCR assay is remarkably efficient in terms of making these pathogens detectable.

There is a lack of evidence for sexual transmission of genitourinary *Candida* infection. Transmission of *Candida* infection between partners in heterosexual intercourse is seen and genital *Candida albicans* isolates were alleged to transmit sexually in the studies where genotypes were used (17). However in the literature there is a limited number of publications about incidence of *Candida* infection in urethritis ethiology. In a study carried out by Obisesan et al., (18) the incidence of candida species in males was found to be 4.9% (18). In our study, however, the incidence of candida species was 2.4%.

There is increasing evidence that bacteria associated with bacterial vaginosis may cause NGU (19). However, there is no enough data on the association between *Gardnerella vaginalis*, and NGU in males. In a study including 80 heterosexual males and 79 controls carried out by Iser et al. (20), based on the microscobic criteria, the prevalence of *Gardnerella vaginalis* was found to be 14% (20) among heterosexual men with non-gonococcal urethral symptoms. In our study, the prevalence of *Gardnerella*

*vaginalis* was found to be 18.6%. *Gardnerella vaginalis* can be seen as commensal organisms in the genitourinary system. Since MPCR kits have been used in this study, a quantitative assessment could not be done for *Gardnerella vaginalis* as a microbial load. This could pose a handicap as to false positive result for such bacteria as *Gardnerella vaginalis* that can be commensal. With the method of quantitative PCR, we assume that the position of *Gardnerella vaginalis* in urethritis will be much more clearly understood.

In the literature, it has been reported that the pathogen associated with urethritis could not be identified in 20-30% of males (21). In a recent study by Wetmore et al. (7), the rate of patients in whom the pathogen could not be identified and who were considered as having idiopathic urethritis was 45.8% among all patients with urethritis. Whereas in our study, the rate of patients, so called idiopatic urethritis group in whom no pathogen could be detected, was found as 32.9%. The condition of idiopathic urethritis may not have an infectious etiology, or different from the ones infected by traditional STD pathogens, but this condition might result from unidentified infection agents in circulation in subgroups of population.

As explained before, identification of most pathogens causing STDs is not easy using routine microbiological diagnose methods. However, PCR assay is useful for identification of microorganisms that are difficult to cultivate and those grow slowly (22). In MPCR, more than one target sequence can be amplified by including more than one pair of primers in the reaction. MPCR has the potential of producing considerable savings in time and effort in the laboratory without compromising test utility. Furthermore, when the amount of the clinical sample is limited, multiplexing allows more targets to be analysed by using a single aliquot of sample material (23). When compared to uniplex PCR test, MPCR test has a general sensitivity of 96% and a specificity of 100% (24).

## Conclusion

Identifying pathogens causing sexually transmitted infections and urethritis in males and, are quite difficult to detect with one urethral swab sample in a short time using MPCR method shows that this method can provide new possibilities for the diagnosis.

### Ethics

Ethics Committee Approval: The study was approved by Medical Park Hospital Local Ethics Committee (Approval number: 189), Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Mehmet Sarıer, Concept: Mehmet Sarıer, Design: Erdal Kukul, Data Collection or Processing: Şafak Göktaş, Analysis or Interpretation: Meltem Demir, Literature Search: İbrahim Duman, Writing: Mehmet Sarıer.

Conflict of Interest: No conflict of interest was declared by the authors.

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

## References

1. Lee SJ, Cho YH, Ha US, Kim SW, Yoon MS, Bae K. Sexual behavior survey and screening for chlamydia and gonorrhea in university students in South Korea. Int J Urol 2005;12:187-193.
2. Horner PJ, Blee K, Falk L, van der Meijden W, Moi H. 2016 European guideline on the management of non-gonococcal urethritis. Int J STD AIDS 2016;27:928-937.
3. Khan A, Fortenberry JD, Juliar BE, Tu W, Orr DP, Batteiger BE. The prevalence of chlamydia, gonorrhea, and trichomonas in sexual partnerships: implications for partner notification and treatment. Sex Transm Dis 2005;32:260-264.
4. Chen MY, Donovan B. Changes in testing methods for genital Chlamydia trachomatis in New South Wales, Australia, 1999 to 2002. Sex Health 2005;2:251-253.
5. Kong F, Gilbert GL. Multiplex PCR-based reverse line blot hybridization assay (mPCR/RLB)--a practical epidemiological and diagnostic tool. Nat Protoc 2006;1:2668-8260.
6. Smith R, Copas AJ, Prince M, George B, Walker AS, Sadiq ST. Poor sensitivity and consistency of microscopy in the diagnosis of low grade non-gonococcal urethritis. Sex Transm Infect 2003;79:487-490.
7. Wetmore CM, Manhart LE, Lowens MS, Golden MR, Whittington WL, Xet-Mull AM, Astete SG, McFarland NL, McDougal SJ, Totten PA. Demographic, Behavioral, and Clinical Characteristics of Men With Nongonococcal Urethritis Differ by Etiology: A Case- Comparison Study. Sex Transm Dis 2011;38:180-186.
8. Mckechnie ML, Hillman R, Couldwell D, Kong F, Freedman E, Wang H, Gilbert GL. Simultaneous identification of 14 genital microorganisms in urine by use of a multiplex PCR-based reverse line blot assay. J Clin Microbiol 2009;47:1871-1877.
9. Markoulatos P, Siafakas N, Moncany M. Multiplex polymerase chain reaction: a practical approach. J Clin Lab Anal 2002;16:47-51.
10. Stellrecht KA, Woron AM, Mishrik NG, Venezia RA. Comparison of multiplex PCR assay with culture for detection of genital mycoplasmas. J Clin Microbiol 2004;42:1528-1533.
11. Bachmann LH, Manhart LE, Martin DH, Seña AC, Dimitrakoff J, Jensen JS, Gaydos CA. Advances in the Understanding and Treatment of Male Urethritis. Clin Infect Dis 2015;61(Suppl 8):S763-769.
12. Kim SJ, Lee DS, Lee SJ. The Prevalence and Clinical Significance of Urethritis and Cervicitis in Asymptomatic People by Use of Multiplex Polymerase Chain Reaction. Korean J Urol 2011;52:703-708.
13. Keane FE, Thomas BJ, Gilroy CB, Renton A, Taylor-Robinson D. The association of Chlamydia trachomatis and Mycoplasma genitalium with non-gonococcal urethritis: observations on heterosexual men and their female partners. Int J STD AIDS 2000;11:435-439.

14. Takahashi S, Takeyama K, Miyamoto S, Ichihara K, Maeda T, Kunishima Y, Matsukawa M, Tsukamoto T. Detection of Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum, and Ureaplasma parvum DNAs in urine from asymptomatic healthy young Japanese men. J Infect Chemother 2006;12:269-271.

15. Gdoura R, Kchaou W, Chaari C, Znazen A, Keskes L, Rebai T, Hammami A. Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis and Mycoplasma genitalium infections and semen quality of infertile men. BMC Infect Dis 2007;7:129.

16. Nassar F, Abu-Elamreen F, Shubair M, Sharif F. Detection of Chlamydia trachomatis and Mycoplasma hominis, genitalium and Ureaplasma urealyticum by Polymerase Chain Reaction in patients with sterile pyuria. Adv Med Sci 2008;53:80-86.

17. Li J, Fan SR, Liu XP, Li DM, Nie ZH, Li F, Lin H, Huang WM, Zong LL, Jin JG, Lei H, Bai FY. Biased Genotype Distributions of Candida albicans Strains Associated with Vulvovaginal Candidosis and Candidal Balanoposthitis in China. Clin Infect Dis 2008;47:1119-1125.

18. Obisesan OJ, Olowe OA, Taiwo SS. Phenotypic Detection of Genitourinary Candidiasis among Sexually Transmitted Disease Clinic Attendees in Ladoke Akintola University Teaching Hospital, Osogbo, Nigeria. J Environ Public Health 2015;2015:401340.

19. Manhart LE, Khosropour CM, Liu C, Gillespie CW, Depner K, Fiedler T, Marrazzo JM, Fredricks DN. Bacterial Vaginosis-Associated Bacteria in Men. Sex Transm Dis 2013;40:944-949.

20. Iser P, Read TH, Tabrizi S, Bradshaw C, Lee D, Horvarth L, Garland S, Denham I, Fairley CK. Symptoms of non-gonococcal urethritis in heterosexual men: a case control study. Sex Transm Infect 2005;81:163-165.

21. Bowie WR, Alexander ER, Stimson JB, Floyd JF, Holmes KK. Therapy for nongonococcal urethritis: double-blind randomized comparison of two doses and two durations of minocycline. Ann Intern Med 1981;95:306-311.

22. Daxboeck F, Zitta S, Stadler M, Iro E, Krause R. Mycoplasma hominis and Ureaplasma urealyticum in patients with sterile pyuria. J Infect 2005;51:54-58.

23. Henegariu O, Heerema NA, Dlouhy SR, Vance GH, Vogt PH. Multiplex PCR: critical parameters and step-by-step protocol. Biotechniques 1997;23:504-511.

24. Horii T, Ohtsuka H, Osaki M, Ohkuni H. Use of a dual priming oligonucleotide system to detect multiple sexually transmitted pathogens in clinical specimens. Lett Appl Microbiol 2009;49:46-52.