Original Article

**Ureaplasma urealyticum**: Presence among Sexually Transmitted Diseases

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**SUMMARY:** The aim of this study was to detect the presence of *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Mycoplasma hominis, Trichomonas vaginalis,* and *Ureaplasma urealyticum* in genital specimens of symptomatic patients. This study also examined the role of *U. urealyticum* in infections of the lower genital tract. Cervical and urethral samples from 96 patients (46 males, 50 females) were tested using the Seeplex(®) STD6 ACE kit. Consent forms were received and a questionnaire was applied. All statistical analyses were performed using the SPSS statistical software program (version 17.0). Among the samples tested, at least 1 pathogen was detected in 49% of the samples; specifically, the rate of detection of *U. urealyticum, M. hominis, C. trachomatis, N. gonorrhoeae, M. genitalium,* and *T. vaginalis* was 29.1%, 10.4%, 8.3%, 7.3%, 6.3%, and 4.2%, respectively. *U. urealyticum* was detected as the sole pathogen in samples from 10% of female patients and 28.3% of male patients (*p* = 0.035). *U. urealyticum* was present in 54.5% (18/33) of samples in which a single pathogen was detected and 71.4% (10/14) of samples in which multiple pathogens were detected. Among men, significant differences in discharge, dysuria, and pruritus were not noted among those with negative results (84.6%, 69.2%, and 38.5%, respectively), among those positive for only *U. urealyticum* (100%, 66.7%, and 26.7%, respectively), and those positive for *N. gonorrhoeae, C. trachomatis, M. genitalium,* and *T. vaginalis* (100%, 93.3%, and 26.7%, respectively). Detection of *U. urealyticum,* either alone or together with other pathogens, in a symptomatic group of patients is an important finding, particularly in men.

**INTRODUCTION**

Sexually transmitted diseases (STDs) refer to the various clinical syndromes caused by pathogenic microorganisms that are transmitted through sexual intercourse. A syndromic approach to the management of STDs and improvement of STD treatment protocols is a useful tool, particularly in developing countries where laboratory facilities are limited. However, approaches using laboratory diagnosis and testing to manage STDs are predominant in developed countries. STD symptoms and syndromes (depending on the sex of the patient), in addition to urethral discharge in males and vaginal discharge in females, include testicular pain and swelling, genital ulcer, lower abdominal pain, inguinal bubo, and anorectal infections (1). Among the causative agents of STDs, *N. gonorrhoeae* and *C. trachomatis* are associated with urethritis and cervicitis, in particular. However, in a minority of cases, other agents can cause non-gonococcal urethritis (NGU), including herpes simplex virus, Epstein Barr virus, and adenosivirus. The diagnosis and treatment procedures for the aforementioned STDs are reserved for situations in which these infections are suspected (e.g., contact with trichomoniasis, urethral lesions, or severe dysuria and meatitis, which might suggest genital herpes) or when NGU is not responsive to recommended therapy (2).

*Ureaplasma* species are frequently found in the commensal flora of the lower genital tract, and their role among other sexually transmitted infections is still under discussion (1,3). The formerly known *U. urealyticum* biovars, biovar 1 and biovar 2, were identified as separate species, *U. parvum* and *U. urealyticum,* respectively by polymerase chain reaction (PCR) (4). *Ureaplasma* spp. has been the most extensively studied and controversial organism among pathogens associated with NGU; their role in NGU has not yet been demonstrated in a meta-analysis (5). According to Zhang et al., the association of *Ureaplasma* spp. with NGU depends on the species detected and that *U. urealyticum,* but not *U. parvum,* is an etiological agent in NGU (5). Furthermore, it has been reported that *U. urealyticum* can cause infections in the lower genital tract and is a pathogen of male urethritis (4,6). However, *U. urealyticum* has been reported as a probable cause of bacterial vaginosis, and there are limited data indicating bacterial vaginosis might cause cervicitis in females (5).

To prevent unnecessary treatment of *U. parvum* infections, most of which are colonized as commensals and mistakenly diagnosed as *U. urealyticum,* it is important to differentiate between the 2 pathogens in the diagnosis of STIs (3). Because cointfection with differ-
ent pathogens is possible with STIs, it is essential to use different clinical samples and tests for accurate diagnoses, which is both time consuming and costly. One technique involves culturing endocervical or urethral swab specimens on selective media, which provides an accurate diagnosis of bacterial STIs; however, it has many disadvantages. Nevertheless, it has been stated that nucleic acid amplification tests (NAATs) are more sensitive than previously available diagnostic tests (e.g., culture, antigen detection, and nucleic acid hybridization) by approximately 20%-30% (3). In addition, NAATs are very convenient for the noninvasive collection of samples. Multiplex real-time PCR was equal or superior to NAATs, and currently there are kits used for STI diagnosis that enable simultaneous detection of multiple pathogens in a single specimen and therefore are faster, reducing labor and reagent costs (3). The Seeplex® STD6 ACE Detection assay (Seegene, Seoul, Korea) is a multiplex PCR assay based on a semi-automated detection system that employs 6 pairs of dual priming oligonucleotide (DPO™) primers specifically targeted to unique genes of C. trachomatis, N. gonorrhoeae, M. genitalium, U. urealyticum, M. hominis, and T. vaginalis. The Seeplex® STD6 ACE Detection assay was reported to be a novel, cost-effective, fast diagnostic tool with high sensitivity and specificity for the simultaneous detection of STI pathogens (7–9). In the assay, 2 separate primer segments with distinct annealing properties are incorporated into a single primer and joined by a poly-linker, which blocks the extension of non-specifically primed templates and increases the specificity of the test (10).

The objective of this study was to detect the presence of U. urealyticum, C. trachomatis, N. gonorrhoeae, M. genitalium, M. hominis, and T. vaginalis in the urethral and cervical swab samples of patients with urethritis, cervicitis, or vaginitis symptoms using the Seeplex® STD6 ACE Detection assay. Additionally, the study aimed to demonstrate the association between U. urealyticum positivity and patient characteristics, and to draw attention to the role of the agent in lower genital tract infections.

MATERIALS AND METHODS

A total of 96 patients (46 males and 50 females) who were admitted to the urology and obstetric outpatient clinic at Ankara Training and Research Hospital between February and May 2013, with urethral and/or vaginal discharge, dysuria, pruritus, dyspareunia bleeding during sexual intercourse, or lower abdominal pain were included in the study.

The potential agents were investigated from cervical and urethral swab samples using a multiplex PCR assay, the Seeplex® STD6 ACE Detection assay. The female patients were also investigated for bacterial vaginosis and Candida infections. A consent form was signed by the patients and a questionnaire was applied during a face-to-face interview. Clinical samples were collected before the initiation of antibiotic treatment.

Cervical and urethral samples: (i) Sample collection: The cervical and urethral samples were taken by sterile-nylon-flocked swabs (Copan Flock Tecnologies, MicroRheologics, Brescia, Italy). The samples were placed in 2 mL of transportation medium (Universal Transport Medium™, Copan, Italy). Samples were stored at 2°C–8°C for up to 7 days if they were not processed immediately after collection. (ii) Pretreatment of clinical samples and DNA extraction: The caps from the specimen tubes were carefully removed to avoid contamination, and then 1 mL of swab specimen was centrifuged for 10 minutes at 5,000 × g. The supernatant was discarded, the pellet was resuspended in 1 mL of 1 × phosphate-buffered saline (PBS) by vortexing thoroughly for 15–30 sec, and 200 μL was used for the DNA extraction. Genomic DNA is extracted from pretreated specimens with GeneAll® Ribospin™ vRD extraction kit (GeneAll Biotechnology Co., LTD, Seoul, Korea), which uses glass filter membrane technology and spin column extraction, according to the kit protocol. Next, 1 mL of pretreated specimen was centrifuged 15 minutes at 15,000 × g, the supernatant was discarded, and the pellet was resuspended in 200 μL 1 × PBS by vortexing for 15–30 sec.

Multiplex PCR assay: DNA amplification was performed with Seeplex® STD6 ACE Detection kit (Seegene, Seoul, Korea) according to the manufacturer’s instructions. The kit contains 6 pairs of DPO™ primers specifically targeted to unique genes of C. trachomatis, N. gonorrhoeae, M. genitalium, U. urealyticum, M. hominis, and T. vaginalis. An internal control was present in the PCR mix for the detection of PCR inhibiting conditions (981 bp). For the negative control, sterile deionized water was used. To verify the integrity of the primers used in the PCR assay, positive DNA controls from the kit were assayed in the presence of all primer pairs. The amplification products were separated and detected with the Screen Tape System®; the amplification products were separated and detected by automated gel electrophoresis using the Tapestation detection system (Lab901, Bilson Glen, Loanhead, UK). The results were analyzed automatically with ScreenPlex® software.

Vaginal samples: Vaginal swab samples were taken by cotton swabs and transported to the laboratory in Stuart transport medium. The vaginal samples were evaluated for bacterial vaginosis and Candida infections by using the evidence from both a direct microscopic investigation and a culture. The vaginal cultures were evaluated for the presence of normal flora, yeasts, and Gardnerella vaginalis. In addition to the growth of G. vaginalis on the blood agar medium, the presence of “clue cells” in a vaginal smear was used as a supportive evidence for bacterial vaginosis. Positive germ tube test from white, creamy yeast colonies were considered Candida albicans and negative germ tube tests were considered Candida spp. (non-albicans).

Statistical analysis: Frequency, percentage, significance tests (i.e., chi-square, Fisher’s exact, and Student’s t-test), and odds ratio were performed with the SPSS statistical software program (Windows, version 17). A p-value of 0.05 was accepted as statistically significant.
RESULTS

The study consisted of 50 females and 46 males for a total of 96 patients. There was no statistically significant difference between the mean age of the females (32.5 ± 8.5 years, min = 18, max = 50) and the males (30.8 ± 9.5 years, min = 18, max = 50; p = 0.358). All patients were sexually active, and 70.8% of them were married. Lower income (39.1%), smoking (56.5%), and multiple partners (63.0%) in males were significantly higher than for the females (p = 0.001, p = 0.001, and p = 0.001, respectively). Unprotected sex was reported by 92.0% of the females. Dysuria and pruritus were significantly higher in female patients than in male patients (98.0% vs. x%, p = 0.003, and 72.0% vs. x%, p = 0.001, respectively; Table 1). Similar findings were noted in 8.3% (n = 48) of the females patients’ partners and in 17.4% (n = 46) of the male patients’ partners. A history of infertility was reported in 27 (58.7%) of the male patients, whereas none of the female patients reported infertility.

Of the cervical and urethral swab samples, 49.0% (47/96) were positive for at least 1 causative agent. *U. urealyticum* was detected in 28 (29.1%) of the samples, *M. hominis* in 10 (10.4%), *C. trachomatis* in 8 (8.3%), *N. gonorrhoeae* in 7 (7.3%), *M. genitalium* in 6 (6.3%), and *T. vaginalis* in 4 (4.2%). *U. urealyticum* was detected as the sole pathogen in 10 (10.4%) of the patients, but in 1 patient, it was detected in 2 of them, but in 1 patient, it was detected as the sole pathogen in the second. The multiplex PCR results were negative in all *Candida* spp. positive patients. The remaining 2 patients (4%) were pre-diagnosed with cervicitis, and coinfection with *U. urealyticum* and *M. hominis* were determined in both of them.

On the basis of the results, the patients were classified into 3 groups: i) positive results for only *U. urealyticum*, ii) positive results for *N. gonorrhoeae* and/or *C. trachomatis* and/or *M. genitalium* and/or *T. vaginalis*, and iii) negative results. Patients with *U. urealyticum* positivity together with other organisms were excluded from the analysis. The patients detected to have only *M. hominis* were recorded as negative and any agent detected together with *M. hominis* was accepted as a single agent, as *M. hominis* has not been accepted as a causative agent for urethritis or cervicitis. Additionally, patients positive for *Candida* spp. were excluded from the analysis. Table 3 shows the distribution of the results for female and male patients, according to their demographic characteristics and signs and symptoms. In men, there was no statistical difference for the presence of discharge (p = 0.089), dysuria (p = 0.167), pruritus (p = 0.741), and infertility (p = 0.450) between the 3 groups.

A history of unprotected sexual intercourse was reported in 66.7% of the patients with *U. urealyticum*, in 80% of the patients with *N. gonorrhoeae* and/or *C. trachomatis* and/or *M. genitalium* and/or *T. vaginalis*, and in 76.9% of those with negative findings (p = 0.683). Among patients with multiple partners, these values were determined as 46.7%, 73.3%, and 69.2%, respectively (p = 0.271). Eight patients stated a history of painful ejaculation, *U. urealyticum* was solely detected in 2 of them, but in 1 patient, it was detected along with other agents (*N. gonorrhoeae*, *M. hominis*, *M. genitalium*, and *T. vaginalis*).

Table 1. Distribution of some demographic data, signs and symptoms according to gender

| Characteristic                  | Female (n = 50) | Male (n = 46) | p value | OR 95% CI |
|---------------------------------|----------------|--------------|---------|-----------|
| Demographics                    |                |              |         |           |
| Lower income                   | 3 (6.0)        | 18 (39.1)    | 0.001   | 10.07 2.72-37.28 |
| Illiterate                      | 4 (8.0)        | 1 (2.2)      | 0.364   | ND        |
| Smoking                         | 7 (14.0)       | 26 (56.5)    | 0.001   | 7.99 2.97-21.47 |
| Multiple partner                | 0 (—)          | 29 (63.0)    | 0.001   | ND        |
| Unprotected sex                 | 46 (92.0)      | 35 (76.0)    | 0.048   | 0.28 0.08-0.94 |
| Signs and symptoms              |                |              |         |           |
| Discharge                       | 45 (90.0)      | 44 (95.7)    | 0.438   | 0.41 0.75-2.22 |
| Dysuria                         | 49 (98.0)      | 36 (78.3)    | 0.003   | 13.61 1.67-111.17 |
| Pruritus                        | 36 (72.0)      | 13 (28.3)    | 0.001   | 6.53 2.68-15.90 |
| Dyspareuia                      | 44 (91.7)      |              |         |           |
| Bleeding during sexual intercourse | 11 (22.9)  |              |         |           |
| Lower abdominal pain            | 48 (96.0)      |              |         |           |

1: <1,000 TRY/monthly.
2: Unprotected sex: condom usage.
3: Fisher’s exact test.
OR, odds ratio; CI, confidence interval; ND, not determined.

Table 2. Distribution of organisms according to gender

| Organism                  | Female | Male | Total |
|---------------------------|--------|------|-------|
|                           | N %    | N %  | N %   |
| Negatives                 | 37     | 74.0 | 12    | 26.0 | 49 | 51.0 |
| Single                    |        |      |       |      |    |     |
| *U. urealyticum*          | 5      | 10.0 | 13    | 28.3 | 18 | 18.8 |
| *N. gonorrhoeae*          | 0      | —    | 4     | 8.7  | 4  | 4.2  |
| *C. trachomatis*          | 0      | —    | 4     | 8.7  | 4  | 4.2  |
| *M. genitalium*           | 0      | —    | 3     | 6.5  | 3  | 3.1  |
| *T. vaginalis*            | 0      | —    | 2     | 4.3  | 2  | 2.1  |
| *M. hominis*              | 1      | 2.0  | 1     | 2.2  | 2  | 2.1  |
| Multiple                  |        |      |       |      |    |     |
| *U. urealyticum, M. hominis* | 3     | 6.0  | 2     | 4.3  | 5  | 5.3  |
| *U. urealyticum, C. trachomatis* | 1 | 2.0  | 1     | 2.2  | 2  | 2.1  |
| *U. urealyticum, N. gonorrhoeae* | 0  | —    | 1     | 2.2  | 1  | 1.0  |
| *U. urealyticum, M. genitalium, N. gonorrhoeae* | 0 | —    | 1     | 2.2  | 1  | 1.0  |
| *U. urealyticum, M. genitalium, M. hominis* | 1 | 2.0  | 0    | —    | 1  | 1.0  |
| *N. gonorrhoea, C. trachomatis* | 0  | —    | 1     | 2.2  | 1  | 1.0  |
| *T. vaginalis, M. hominis* | 2      | 4.0  | 0     | —    | 2  | 2.1  |
| *C. trachomatis, M. genitalium* | 0  | —    | 1     | 2.2  | 1  | 1.0  |
| Total                     | 50     | 100  | 46    | 100  | 96 | 100 |

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rhoeae and/or N. gonorrhoeae or bacterial vaginosis. Although mainly urethral discharge in male patients (agents for STIs within the scope of this study are M. hominis and/or C. trachomatis) and/or non-gonococcal/non-chlamydial pathogens such as M. genitalium and T. vaginalis) and abnormal vaginal discharge (T. vaginalis, C. albicans, or bacterial vaginosis, N. gonorrhoeae and/or C. trachomatis) in females. Dysuria, pruritus, dyspareunia, bleeding after sexual intercourse, and lower abdominal pain are the most frequent accompanying symptoms of these STI syndromes (1,11). In this study, a case definition comprising the symptoms of urethritis (urethral discharge, dysuria, or pruritus) and cervicitis and/or vaginitis (vaginal discharge, dysuria, pruritus, bleeding after sexual intercourse, or dyspareunia) was used. Lower abdominal pain and painful ejaculation were also included. In both female and male patients, discharge was a common symptom. On the other hand, dysuria was found to be 13.61 times higher (95% CI 1.67–111.17) in female patients than in males. Pruritus was 6.53 times higher (95% CI 1.67–111.17) in female patients than in males. Dysuria was found to be 13.61 times higher (95% CI 1.67–111.17) in female patients than in males. Pruritus was 6.53 times higher (95% CI 1.67–111.17) in female patients than in males.

Abnormal vaginal discharge may appear frequently due to vaginitis caused by T. vaginalis and C. albicans, or bacterial vaginosis. Although N. gonorrhoeae and/or C. trachomatis generally lead to cervicitis that is usually asymptomatic, in some women abnormal vaginal discharge, pain, and bleeding subsequent to intercourse may be detected (1). Therefore, it is mostly not possible to distinguish vaginitis or bacterial vaginosis and cervicitis based on signs and symptoms; moreover, multiple infections are prevalent. In this study, 37 (74%) of the female patients were negative for all of the pathogens investigated; however, U. urealyticum and M. hominis were detected either alone or together with mixed pathogens in 20% and 12% of the female patients, respectively. In Turkey, Sarsar et al. reported that the prevalence of U. urealyticum and M. hominis was 50.0% and 6.6%, respectively, in cases with sterile pyuria, using the PCR method (12). The reason for the low prevalence might be due to the fact that in this study, most of the patients were pre-diagnosed with vaginitis or bacterial vaginosis (96.0%) and only 2 of them were pre-diagnosed with cervicitis. Additionally, in the 10 symptomatic, but multiplex PCR-negative patients, Candida vaginitis was detected. On the other hand, among the U. urealyticum-positive patients only 2 were confirmed by the laboratory to have bacterial vaginosis; hence, it is difficult to explain the signs and symptoms as vaginitis. The first patient was positive for C. trachomatis and the second was positive for M. hominis, in addition to U. urealyticum. A previous study has shown that M. hominis and less likely, Ureaplasma spp. are among the causes of bacterial vaginosis (13). Cedillo-Ramirez et al. reported that among women with symptomatic vaginosis, M. hominis was isolated in 17% and Ureaplasma types were isolated in 52%, whereas the corresponding prevalence in healthy women was 2% and 13%, respectively (14). Although these results are remarkable, the number is inadequate to state a clear association of bacterial vaginosis with U. urealyticum and M. hominis.

Although the findings are not strong enough for Ureaplasma spp. and M. hominis to be pathogenic agents, it has been previously reported that Ureaplasma spp. can be associated with urethral syndrome in females (2,6,15). Moreover, the prevalence of U. urealyticum can differ according to sexual activity and hormone profile; therefore, it is noteworthy that the detection ratio may be differentiated by various age groups and societies, as well (16). Baczynska et al. showed that M. hominis can be an infectious cause of infertility (17). However, the relation of Ureaplasma species to infertility is currently not proven (2).
infertility was noted among the female patients of this study.

The presence of urethral Ureaplasma infection in healthy men suggests the persistence of the organisms in untreated asymptomatic subjects and/or the role of only certain serovars in the emergence of the disease (7). In this study, U. urealyticum was solely detected in 28.3% of the symptomatic male patients diagnosed with urethritis or urethral syndrome. As a limitation, there were no healthy controls in this study; however, high positivity of U. urealyticum in this group of symptomatic patients is striking and indicates that it can be a considered a cause of urethritis in men. In fact, Wetmore et al. detected U. urealyticum in 24% of cases with urethral discharge and inflammation symptoms in a case-control study that compared the demographic, behavioral, and clinical characteristics in men with NGU (5).

There was no statistical difference in the symptoms of discharge, dysuria, and pruritus between the patients with negative findings, the patients with N. gonorhoeae and/or C. trachomatis and/or M. genitalium and/or T. vaginalis, and the patients with U. urealyticum. Nevertheless, the patients with U. urealyticum and N. gonorhoeae and/or C. trachomatis and/or M. genitalium and/or T. vaginalis had similar percentages of discharge and pruritus other than dysuria (Table 3).

It was reported that the presence of U. urealyticum was associated with urethral discharge in the multivariable analysis performed by Wetmore et al (5).

There were no statistically significant differences between U. urealyticum, other causative agents, and negatives in the presence of multiple partners (p = 0.271) and unprotected sexual intercourse (p = 0.683).

Although the association of U. urealyticum with prostate infection is controversial, Jalil et al. isolated the agent from the urethras and the epididymal aspirates of patients with non-gonococcal and non-chlamydial acute epididymo-orchitis and showed the specific antibody response and the response to tetracycline (18). In this study, 8 subjects had painful ejaculation, and U. urealyticum was detected alone or together with other pathogens in 5 of them.

Although there are very recent reports concerning changes in sperm motility caused by Ureaplasma spp., the impact of these organisms on infertility is still controversial (6). In this study, the ratio of infertility was found to be higher in patients with N. gonorhoeae, C. trachomatis, M. genitalium, and T. vaginalis infections (73.3%) than that in patients with either U. urealyticum infection (53.3%) or with negative findings (53.8%), but the statistical differences were not significant.

As a result, the role of U. urealyticum in lower genital tract infections as a single causative agent is still controversial. In a meta-analysis, Zhang et al. reported that the prevalence of U. urealyticum was significantly higher in patients with NGU (18.3%) than that in a control group (13.7%); moreover, U. urealyticum was reported as a causative agent of NGU (6). Limitations of this study include the lack of a control group and the detection method. Determination of U. urealyticum alone or together with other pathogens in 20% of symptomatic female patients and 39.1% of symptomatic male patients is an important finding. Although these findings suggest that U. urealyticum might have a role as a causative agent in lower genital tract infections, particularly in men, a number of other risk factors should be considered, such as sexual behavior, number of partners, and recurrent urethritis.

Conflict of interest None to declare.

REFERENCES
1. Ndowa F, Ballard RC. Syndromic management. In: Morse SA, Holmes KK, Ballard RC, editors. Atlas of Sexually Transmitted Diseases and AIDS. 4th ed. Amsterdam: Elsevier; 2010. p. 337-44.
2. Kimberly AW, Gail AB; Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep. 2015;64 (RR-03): 1-137.
3. Gites KB, Taylor-Robinson D. Mycoplasma and ureaplasma. In: Versalovic J, Carroll KC, Funke G, et al., editors. Manual of Clinical Microbiology. 10th ed. Washington DC: ASM Press; 2011. p. 970-85.
4. Choe HS, Lee DS, Lee SJ, et al. Performance of Anyplex™ II multiplex real-time PCR for the diagnosis of seven sexually transmitted infections: comparison with currently available methods. Int J Infect Dis. 2013;17:e1134-40.
5. Wetmore CM, Manhart LE, Lowens MS, et al. Demographic, behavioral, and clinical characteristics of men with nongonococcal urethritis differ by etiology: a case-comparison study. Sex Transm Dis. 2011;38:180-6.
6. Zhang N, Wang R, Li X, et al. Are Ureaplasma spp. a cause of nongonococcal urethritis? A systematic review and meta-analysis. PLoS One. 2014;9:e113771.
7. Lee SJ, Park DC, Lee DS, et al. Evaluation of Seeplex STD6 ACE detection kit for the diagnosis of six bacterial sexually transmitted infections. J Infect Chemother. 2012;18:494-500.
8. Samra Z, Rosenberg S, Madar-Shapiro L. Direct simultaneous detection of 6 sexually transmitted pathogens from clinical specimens by multiplex polymerase chain reaction and auto-capillary electrophoresis. Diagn Micro Infec Dis. 2011;70:17-21.
9. Vića ML, Junie LM, Tătaru A, et al. Simultaneous PCR-based detection of six pathogens inducing sexually transmitted diseases J Clin Lab Invest. 2011;3:11-6.
10. Horii T, Ohitsu K, Osaki M, et al. Use of a dual priming oligonucleotide system to detect multiplysexually transmitted pathogens in clinical specimens. Lett Appl Microbiol. 2009;49:46-52.