

Infection/Inflammation

# The Prevalence and Clinical Significance of Urethritis and Cervicitis in Asymptomatic People by Use of Multiplex Polymerase Chain Reaction

Suk-Ju Kim, Dong Sup Lee<sup>1</sup>, Seung-Ju Lee<sup>1</sup>

Department of Urology, Uijeongbu St. Mary's Hospital, The Catholic University of Korea College of Medicine, Uijeongbu, <sup>1</sup>St. Vincent's Hospital, The Catholic University of Korea College of Medicine, Suwon, Korea

**Purpose:** Our purpose was to conduct a screening test for urethritis or cervicitis as a sexually transmitted disease (STD) by using multiplex polymerase chain reaction (PCR) and to determine the prevalence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Trichomonas vaginalis* in asymptomatic people.

**Materials and Methods:** From July 2010 to December 2010, 709 persons who came to the hospital for a general checkup were tested. Multiplex PCR assays were done with first voided urine samples or endocervical swabs by use of the Seeplex<sup>®</sup> STD6 ACE Detection kit.

**Results:** The mean age in this study was 45.4±8.1 years. Among the 709 persons, 229 (32.3%) had a positive result for at least one microorganism, 48 (6.8%) had two different species, 6 (0.8%) had three different species, and 1 person had four different species. The overall prevalence of asymptomatic STDs such as urethritis or cervicitis was 7.1% (50/709). The prevalence rates of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Trichomonas vaginalis* infection in asymptomatic persons were 5.6% (40/709), 0.4% (3/709), 0.3% (2/709), 22.1% (157/709), 11.6% (82/709), and 1.1% (8/709), respectively.

**Conclusions:** With only a single sample, we could identify the prevalence rates of six microorganisms and the overall proportion of urethritis or cervicitis in asymptomatic people. This proportion cannot be neglected; therefore, screening tests for sexually transmitted diseases such as urethritis or cervicitis should be recommended to asymptomatic people.

**Key Words:** *Chlamydia*; *Mycoplasma*; *Polymerase chain reaction*; *Sexually transmitted diseases*; *Ureaplasma*

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Article History:**

received 24 May, 2011  
accepted 22 August, 2011

**Corresponding Author:**

Seung-Ju Lee  
Department of Urology, St. Vincent's Hospital, The Catholic University of Korea College of Medicine, 93-6, Ji-dong, Paldal-gu, Suwon 442-723, Korea  
TEL: +82-31-249-8305  
FAX: +82-31-253-0949  
E-mail: lee.seungju@gmail.com

## INTRODUCTION

*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis* are known pathogens in sexually transmitted infection (STI). However, most of those species present atypical symptoms and some patients have no symptoms [1-3]. Therefore, physicians sometimes have difficulty in making a differential

diagnosis. Moreover, except for *Chlamydia trachomatis* and *Mycoplasma genitalium*, the treatment regimens between the species differ.

In addition, routine bacterial culture may give negatives result for commercial sexual workers or asymptomatic people who recently experienced unprotected sexual contact and have acquired an STI [1-3]. Thus, there are neglected asymptomatic patients in the community who can serve as

a reservoir of the STI [1-4].

There are limited studies about *Ureaplasma urealyticum* and *Mycoplasma hominis* in asymptomatic people. Generally, *Ureaplasma urealyticum* is not known as a clinical pathogen but it can be a cause of urethritis, especially in patients resistant to routine treatment [5]. *Mycoplasma hominis* also does not play a great role in the pathogenesis of urethritis, but it can be a heavy pathogen in immunocompromised patients [6]. Recently, many reports have revealed that microorganisms such as *Ureaplasma urealyticum* and *Mycoplasma hominis* can contribute not only to lower genitourinary infection but also to infertility [7].

Therefore, identification of the incidence of asymptomatic STI such as urethritis or cervicitis and determination of the prevalence of these 6 species in asymptomatic people is very important. Accordingly, we conducted a screening test for STI by using multiplex polymerase chain reaction (PCR) in asymptomatic people.

## MATERIALS AND METHODS

This study was a point prevalence study. The institutional review board of The Catholic University of Korea, College of Medicine, approved the study protocol, and all patients provided written informed consent to participate in the study (IRB no: SC10SNSI0091).

### 1. Sample collection

From July 2010 to December 2010, 802 persons who came to the hospital for a general checkup participated voluntarily and a brief questionnaire was obtained. Sexually active, asymptomatic people aged between 20 and 60 years were enrolled. We defined 'sexually active' as having had sexual intercourse in the preceding 3 months. People who had a recently cured genitourinary infectious disease within 3 months were excluded. People with genitourinary symptoms such as urethral or cervical discharge, dysuria, or itching in the genital area were also excluded. Finally, 709 persons were assigned to be tested. Among these, 430 were men and 279 were women. In men, first-voided urine specimens were collected in sterile 50 ml screw-cap plastic bottles. In women, specimens were obtained via endocervical swabs by one gynecologist.

### 2. Pretreatment of clinical specimens and DNA extraction

The specimens from the 709 men and women were equilibrated to room temperature and centrifuged at 5000xg for 15 minutes. The supernatant was discarded, and the pellet was resuspended in 1 ml 1xPBS before DNA extraction. Genomic DNA was extracted from the pretreated specimens (swab or urine) by using the QIAamp<sup>®</sup> DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

### 3. Multiplex polymerase chain reaction assay

PCR amplification was performed with the Seeplex<sup>®</sup> STD6

ACE detection kit (Seegene, Seoul, Korea) according to the manufacturer's instructions. The kit contains sets of primers that were specifically designed from highly conserved regions of genetic sequences for the 6 organisms (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Trichomonas vaginalis*) by use of Seegene DPO technology [8]. An internal control was included in the PCR mix to detect the presence of PCR inhibitors. For the negative control, sterile deionized water was used as the PCR template instead of nucleic acid. The positive control contained in the kit was assayed to check the integrity of the primers used in the PCR assay. Amplified fragments were separated by use of the LabChip<sup>®</sup> DX Seeplex<sup>®</sup> Assay system (Caliper, Hopkinton, MA, USA).

### 4. LabChip<sup>®</sup> DX Seeplex<sup>®</sup> assay system

Amplified PCR products were separated and detected by automated gel electrophoresis with the LabChip<sup>®</sup> DX Seeplex<sup>®</sup> assay system (Caliper, Hopkinton, MA, USA). The PCR products from each sample (20 µl) were transferred to 96-well plates and placed in the LabChip<sup>®</sup> DX instrument. The samples were loaded automatically on the Seeplex<sup>®</sup> Chip and detected sequentially according to the sample order. Analysis was performed with designated software (Seegene viewer) that presents each of the samples and identifies the fragments that yield a positive readout for the bands of interest in the presented results. A tabulated matching matrix provides a simple readout, identifying matching bands to the type of STI pathogens. A text file is automatically generated and saved for each tape.

## RESULTS

The mean age in this study was 45.4±8.1 years. Among the 709 persons, 229 (32.3%) had at least one microorganism in his or her genitourinary tract. The prevalences of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Trichomonas vaginalis* in asymptomatic people were 5.6% (40/709), 0.4% (3/709), 0.3% (2/709), 22.1% (157/709), 11.6% (82/709), and 1.1% (8/709) respectively (Table 1). Among all persons, 50 (7.1%) had pathogens of STI in their urinary tract (Table 2). Of these patients, the number of patients with a single pathogen was 47 (6.6%), for which the number with isolated *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, or *Trichomonas vaginalis* was 37, 1, 1, and 8, respectively. Three double-infections, i.e., samples containing 2 or more pathogens, were identified, and each sample had *Chlamydia trachomatis*. When *Trichomonas vaginalis* was identified, it was either isolated alone or co-infected with *Mycoplasma hominis* only and no other species. The total number of people who had at least two microorganisms in their genitourinary tract was 55 (7.5%). Among these 55 persons, 48 (6.8%) had two different species whether they were pathogens or not, 6 (0.8%) persons

had three different species, and 1 patient had four different species.

The prevalences of *Ureaplasma urealyticum* and *Mycoplasma hominis* were 22.1% and 11.6%, respectively. In people who were infected with *Chlamydia trachomatis*, the prevalences of *Ureaplasma urealyticum* (35.0%) and *Mycoplasma hominis* (22.5%) were higher than in those not infected with *Chlamydia trachomatis* (Table 1, 2).

## DISCUSSION

*Chlamydia trachomatis* infection is the most prevalent

**TABLE 1.** Species isolated from an asymptomatic general population

Species in 709 samples	Total no.	Men (430)	Women (279)
Free of microorganisms	480 (67.7)	282 (65.6)	198 (71.0)
<i>C. trachomatis</i> <sup>a</sup>	40 (5.6)	29 (6.7)	11 (3.9)
<i>N. gonorrhoeae</i> <sup>a</sup>	3 (0.4)	2 (0.5)	1 (0.4)
<i>M. genitalium</i> <sup>a</sup>	2 (0.3)	1 (0.2)	1 (0.4)
<i>U. urealyticum</i> <sup>a</sup>	157 (22.1)	112 (26.0)	45 (16.1)
<i>M. hominis</i> <sup>a</sup>	82 (11.6)	39 (9.1)	43 (15.4)
<i>T. vaginalis</i> <sup>a</sup>	8 (1.1)	1 (0.2)	7 (1.0)

Values are presented as number (%), <sup>a</sup>: regardless of the existence of other species

sexually transmitted bacterial infection among women and men worldwide. *Chlamydia trachomatis* is usually described as an obligate intracellular pathogen and accounts for 30% to 40% of the etiopathogenesis of urethritis [9]. However, about half of infected subjects are asymptomatic. Thus, there are many silent infections in the community. Mason et al showed that the prevalence of *Chlamydia trachomatis* in asymptomatic men was 4% (14/349) [10]. Takahashi et al reported that *Chlamydia trachomatis* was detected in 6% of samples from healthy people [11]. Similarly, in our study, *Chlamydia trachomatis* was observed in 40 persons (5.6%). Unfortunately, it is usually not detected by routine microbiological diagnosis [1,12]. Thus, most asymptomatic patients are neglected unless they visit the clinic to be evaluated for STI by chance or face complications such as pelvic inflammatory disease.

*Neisseria* are fastidious Gram-negative cocci that require nutrient supplementation to grow in laboratory cultures. Although gonorrhea can be diagnosed by inspection of the yellowish discharge from the urethra, many patients infected with *Neisseria gonorrhoeae* have no discharge. Furthermore, it is known that 10% of infected males and 50% of infected females are asymptomatic. Hein et al studied 2672 sexually active adolescents and reported that prevalence rates of asymptomatic gonorrhea were 1.9% among boys and 7.0% among female adolescents [13]. Regardless of age group, a surveillance study reported prevalence rates of gonorrhea in healthy people of 0.06%

**TABLE 2.** Samples containing clinical pathogens

Combinations with <i>C. trachomatis</i>	Total no. (40)	Men (29)	Women (11)
<i>C. trachomatis</i> only	22 (55.0)	18 (62.1)	4 (36.4)
<i>C. trachomatis</i> + <i>U. urealyticum</i> <sup>a</sup>	7 (17.5)	4 (13.8)	3 (27.3)
<i>C. trachomatis</i> + <i>M. hominis</i> <sup>a</sup>	2 (5.0)	0	2 (18.2)
<i>C. trachomatis</i> + <i>U. urealyticum</i> + <i>M. hominis</i> <sup>a</sup>	6 (15.0)	4 (13.8)	2 (18.2)
<i>C. trachomatis</i> <sup>b</sup>	37 (92.5)	26 (90.0)	11 (100)
<i>C. trachomatis</i> + <i>N. gonorrhoeae</i> <sup>a</sup>	2 (5.0)	2 (6.9)	0
<i>C. trachomatis</i> + <i>M. genitalium</i> + <i>U. urealyticum</i> + <i>M. hominis</i>	1 (2.5)	1 (3.4)	0
<i>C. trachomatis</i> + <i>U. urealyticum</i> <sup>c</sup>	14 (35.0)	9 (31.0)	5 (45.5)
<i>C. trachomatis</i> + <i>M. hominis</i> <sup>c</sup>	9 (22.5)	5 (17.2)	4 (36.4)
Combinations with <i>N. gonorrhoeae</i>	Total no. (3)	Men (2)	Women (1)
<i>N. gonorrhoeae</i> only	1 (33.3)	0	1 (100)
<i>N. gonorrhoeae</i> + <i>C. trachomatis</i>	2 (66.7)	2 (100)	0
Combinations with <i>M. genitalium</i>	Total no. (2)	Men (1)	Women (1)
<i>M. genitalium</i> + <i>U. urealyticum</i> + <i>M. hominis</i>	1 (50.0)	0	1 (100)
<i>M. genitalium</i> + <i>C. trachomatis</i> + <i>U. urealyticum</i> + <i>M. hominis</i>	1 (50.0)	1 (100)	0
Combinations with <i>T. vaginalis</i>	Total no. (8)	Men (1)	Women (7)
<i>T. vaginalis</i> only	6 (75.0)	0	6 (85.7)
<i>T. vaginalis</i> + <i>M. hominis</i> <sup>a</sup>	2 (25.0)	1 (100)	1 (14.3)

Values are presented as number (%), *C. trachomatis*: *Chlamydia trachomatis*, *U. urealyticum*: *Ureaplasma urealyticum*, *M. hominis*: *Mycoplasma hominis*, *N. gonorrhoeae*: *Neisseria gonorrhoeae*, *M. genitalium*: *Mycoplasma genitalium*, *T. vaginalis*: *Trichomonas vaginalis*, <sup>a</sup>: samples not containing other species, <sup>b</sup>: samples not containing other pathogens, regardless of the existence of commensal microorganisms, <sup>c</sup>: regardless of the existence of other species

to 0.18% [14]. In our study, the prevalence in asymptomatic people was 0.4%, and the result was easily obtained by PCR test with a urine or endocervical swab sample.

*Mycoplasma genitalium* was first isolated in 1981 from two men with nongonococcal urethritis (NGU) [15]. Nowadays, *Mycoplasma genitalium* is known as a causative pathogen of NGU. Results from a meta-analysis of 19 studies on patients with NGU showed that *Mycoplasma genitalium* was found in 21% compared with 7% of patients without NGU [16]. Ross et al reported a detection rate of 0.6% for *Mycoplasma genitalium* in asymptomatic British persons [17]. In Japan, the detection rate of this organism was 1% [11]. Our study showed a prevalence of this mycoplasma of 0.3% in asymptomatic people. *Mycoplasma genitalium* is difficult to identify by routine culture. Currently, nucleic acid amplification tests (NAATs) are usually recommended to detect this pathogen [1]. Wikström and Jensen reported that *Mycoplasma genitalium* is a common cause of persistent or recurrent urethritis among men treated with doxycycline, and erythromycin appears to be less efficient than azithromycin in eradicating the infection [18]. Therefore, when physicians encounter patients who complain of continuous symptoms of urethritis, they should consider drug-refractory urethritis such as that caused by *Mycoplasma genitalium*.

*Trichomonas vaginalis* is an anaerobic flagellated protozoan. It is another pathogen of STI of the urogenital tract, and men with this infection rarely exhibit symptoms [19]. In women, greenish-yellow frothy vaginal discharge and itching can develop. Trichomoniasis is treated and cured with metronidazole or tinidazole, which should be prescribed to any sexual partners as well because they may be asymptomatic carriers. Sutton et al reported that the prevalence of *Trichomonas vaginalis* in reproductive-aged women was 3.1% [20]. In our study, the total prevalence rate was 1.1% in asymptomatic people, and prevalence rates in asymptomatic men and women were 0.2% and 2.5%, respectively.

Screening for these pathogens is important not only to identify infected symptomatic individuals for the diagnosis and management of their infections but also to identify asymptomatic individuals who serve as reservoirs for infection [1-4]. In our study, there were 50 (7.1%) infected people among 709 asymptomatic people; thus, this proportion should not be neglected. Among the 50 infected patients, 37 with *Chlamydia trachomatis*, 1 with *Neisseria gonorrhoeae*, 1 with *Mycoplasma genitalium*, 8 with *Trichomonas vaginalis*, 2 with *Chlamydia trachomatis* with *Neisseria gonorrhoeae*, and 1 with *Chlamydia trachomatis* with *Mycoplasma genitalium* were confirmed. Thus, regardless of *Chlamydia trachomatis*, 13 patients should be considered as candidates for antimicrobial treatment. Moreover, there were 3 double-infections among the 709 asymptomatic people. These facts suggest the possibility of prescribing the wrong medicines if practitioners give symptomatic patients some regimens empirically. Therefore, the need to detect multiple species at once should be

raised. In this sense, use of the multiplex PCR assay to screen asymptomatic people may be important because the treatment regimen could be optimized for people with positive results, especially for those with multiple organisms. This concept can be also applied to symptomatic patients. *Ureaplasma urealyticum* has been recognized as a pathogen for NGU since the 1950s [21]. However, *Ureaplasma urealyticum* is frequently isolated from the urethra of healthy men, and some studies have reported that there is no significant difference in its prevalence between men with NGU and men without NGU [22]. Nevertheless, there are some reports that *Ureaplasma urealyticum* serves as a cause of persistent NGU [23,24]. For that reason, *Ureaplasma urealyticum* is sometimes recognized as a pathogen and sometimes as a commensal organism. Therefore, *Ureaplasma* infection must be considered in patients with treatment failure for NGU or patients with multiple sexual partners. If no species except *Ureaplasma* are detected, symptoms and leukocyte numbers in first voided urine samples are helpful for clarifying the diagnosis of *Ureaplasma* infection [25].

*Mycoplasma hominis* is frequently identified from the genitourinary tract. In general, it is known as a commensal species but it can work as a pathogen in special conditions such as in an immunocompromised state [6]. Generally, the rate of colonization of *Mycoplasma hominis* in the urogenital tract was reported to be between 4% and 13% in men and between 21% and 54% in women [26]. Our study showed that the incidence of this species was 9.1% in men and 15.4% in women.

In our study, the incidences of *Ureaplasma urealyticum* and *Mycoplasma hominis* were 22.1% and 11.6%. Nevertheless, those incidences were increased up to 35.0% and 22.5% when the samples had *Chlamydia trachomatis*. The reason for this increase with chlamydial infection is not clear, and a limitation of our study is that we did not include people who had symptoms. Therefore, comparison of the prevalence of these species in symptomatic urethritis patients is necessary.

As described previously, most pathogens causing STI as well as commensal microorganisms are difficult to cultivate by routine microbiological diagnosis. However, NAATs, such as PCR, are useful for the identification of microorganisms that are difficult to cultivate and for those that grow slowly [25]. In another recent study in Korea, the multiplex PCR kit (Seegene Inc., Seoul, Korea) was used to detect causative microorganisms of STI in patients with chronic prostatitis and vaginitis [27]. In multiplex PCR, more than one target sequence can be amplified by including more than one pair of primers in the reaction. Multiplex PCR has the potential to produce considerable savings of time and effort within the laboratory without compromising test utility. Furthermore, when the clinical sample amount is limited, multiplexing allows more targets to be analyzed by using a single aliquot of sample material [28]. Although there are some worries that multiplex PCR in the clinical setting may have difficulties such as poor sensi-

tivity or specificity, false-negative results, and nonspecific interactions, Horii et al showed that the multiplex PCR assay had an overall sensitivity of 96% and specificity of 100% compared with uniplex PCR assays. Furthermore, there was no cross-reaction with other microorganisms [29].

The multiplex PCR assay has recently made it convenient for clinicians to test for causative organisms simultaneously from many clinical fields [30]. Similarly, we succeeded in easily generating results by use of multiplex PCR with only a single sample per person.

Asymptomatic individuals play a role as reservoirs for STI. In our study, there were 50 (7.1%) infected people among 709 asymptomatic people; therefore, this proportion should not be neglected. An STI screening test should be considered as a part of a general checkup, especially in high-risk groups, e.g., commercial sexual workers, people who have multiple sexual partners, and immunocompromised persons [1-3,6,25]. By use of multiplex PCR, physicians can easily identify many microorganisms at once, and treating asymptomatic persons contributes to the prevention of STI in the community.

## CONCLUSIONS

The prevalences of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Trichomonas vaginalis* in asymptomatic people were 5.6%, 0.4%, 0.3%, 22.1%, 11.6%, and 1.1%, respectively. We hope that these results will be used as baseline data for future studies.

In our study, about 32.3% of asymptomatic people had at least one microorganism, and excluding *Ureaplasma urealyticum* and *Mycoplasma hominis* from the count, the prevalence rate of silent STI was 7.1% with multiplex PCR. These asymptomatic people would be a source of STI. Therefore, screening for STI should be considered as a part of a general checkup, especially in high-risk groups.

## Conflicts of Interest

The authors have nothing to disclose.

## REFERENCES

- Centers for Disease Control and Prevention, Workowski KA, Berman SM. Sexually transmitted disease treatment guidelines 2006. MMWR Recomm Rep 2006;55:1-94.
- Hamasuna R, Tsukino H. Urethritis. In: Naber KG, Schaeffer AJ, Heyns CF, Matsumoto T, Shoskes DA, Bjerklund Johansen TE, editors. Urogenital Infections. 1st ed. Arnhem, the Netherlands: European Association of Urology-International Consultation on Urological Diseases; 2010:777-803.
- Martin DH. Urethritis in males. In: Homes KK, Sparling Pf, Stamm WE, Piot P, Wasserheit JN, Corey L, et al editors. Sexually Transmitted Diseases. 4th ed. New York: McGraw Hill; 2008: 1107-26.
- Leblanc MM. When to refer an infertile male to a theriogenologist. Theriogenology 2008;70:421-9.
- Horner P, Thomas B, Gilroy CB, Egger M, Taylor-Robinson D. Role of *Mycoplasma genitalium* and *Ureaplasma urealyticum* in acute and chronic nongonococcal urethritis. Clin Infect Dis 2001;32:995-1003.
- Alados JC, Cobo F, Jiménez MD, Jurado M, de Cueto M, Miranda C, et al. Catheter infection by *Mycoplasma hominis* in a patient with acute lymphoblastic leukemia. Enferm Infecc Microbiol Clin 1998;16:252.
- Gdoura R, Kchaou W, Chaari C, Znazen A, Keskes L, Rebal T, et al. *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis* and *Mycoplasma genitalium* infections and semen quality of infertile men. BMC Infect Dis 2007;7:129.
- Chun JY, Kim KJ, Hwang LT, Kim YJ, Lee DH, Kim JK, et al. Dual priming oligonucleotide system for the multiplex detection of respiratory viruses and SNP genotyping of CYP2C19 gene. Nucleic Acids Res 2007;35:e40.
- Keane FE, Thomas BJ, Gilroy CB, Renton A, Taylor-tobinson 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: 435-9.
- Mason PR, Gwanzura L, Gregson S, Katzenstein DA. *Chlamydia trachomatis* in symptomatic and asymptomatic men: detection in urine by enzyme immunoassay. Cent Afr J Med 2000;46:62-5.
- Takahashi S, Takeyama K, Miyamoto S, Ichihara K, Maeda T, Kunishima Y, et al. Detection of *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum* DNAs in urine from asymptomatic healthy young Japanese men. J Infect Chemother 2006;12:269-71.
- Piémont Y, Jaulhac B. Value of molecular biology methods for diagnosis in bacteriology. Ann Dermatol Venereol 1995;122:206-12.
- Hein K, Marks A, Cohen MI. Asymptomatic gonorrhea: prevalence in a population of urban adolescents. J Pediatr 1977;90: 634-5.
- Centers for Disease Control and Prevention. Gonorrhea. Transmitted Disease Surveillance 2007. Atlanta: Division of STD prevention; 2008;17-32.
- Tully JG, Taylor-Robinson D, Cole RM, Rose DL. A newly discovered mycoplasma in the human urogenital tract. Lancet 1981;1(8233):1288-91.
- Jensen JS. *Mycoplasma genitalium*: the aetiological agent of urethritis and other sexually transmitted diseases. J Eur Acad Dermatol Venereol 2004;18:1-11.
- Ross JD, Brown L, Saunders P, Alexander S. *Mycoplasma genitalium* in asymptomatic patients: implications for screening. Sex Transm Infect 2009;85:436-7.
- Wikström A, Jensen JS. *Mycoplasma genitalium*: a common cause of persistent urethritis among men treated with doxycycline. Sex Transm Infect 2006;82:276-9.
- Jackson DJ, Rakwar JP, Chohan B, Mandaliya K, Bwayo JJ, Ndinya-Achola JO, et al. Urethral infection in a workplace population of East African men: evaluation of strategies for screening and management. J Infect Dis 1997;175:833-8.
- Sutton M, Sternberg M, Koumans EH, McQuillan G, Berman S, Markowitz L. The prevalence of *Trichomonas vaginalis* infection among reproductive-age women in the United States, 2001-2004. Clin Infect Dis 2007;45:1319-26.
- Taylor-Robinson D, Furr PM. Update on sexually transmitted mycoplasmas. Lancet 1998;351(Suppl 3):12-5.
- Hooton TM, Roberts MC, Roberts PL, Holmes KK, Stamm WE, Kenny GE. Prevalence of *Mycoplasma genitalium* determined by DNA probe in men with urethritis. Lancet 1988;1:266-8.

23. Yoshida T, Ishiko H, Yasuda M, Takahashi Y, Nomura Y, Kubota Y, et al. Polymerase chain reaction-based subtyping of *Ureaplasma parvum* and *Ureaplasma urealyticum* in first-pass urine samples from men with or without urethritis. *Sex Transm Dis* 2005; 32:454-7.
24. Stimson JB, Hale J, Bowie WR, Holmes KK. Tetracycline-resistant *Ureaplasma urealyticum*: a cause of persistent non-gonococcal urethritis. *Ann Intern Med* 1981;94:192-4.
25. Daxboeck F, Zitta S, Stadler M, Iro E, Krause R. *Mycoplasma hominis* and *Ureaplasma urealyticum* in patients with sterile pyuria. *J Infect* 2005;51:54-8.
26. Kilic D, Basar MM, Kaygusuz S, Yilmaz E, Basar H, Batislam E. Prevalence and treatment of *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Mycoplasma hominis* in patients with non-gonococcal urethritis. *Jpn J Infect Dis* 2004;57:17-20.
27. Kim TH, Kim HR, Myung SC. Detection of nanobacteria in patients with chronic prostatitis and vaginitis by reverse transcriptase polymerase chain reaction. *Korean J Urol* 2011;52: 194-9.
28. Henegariu O, Heerema NA, Dlouhy SR, Vance GH, Vogt PH. Multiplex PCR: Critical parameters and step-by-step protocol. *Biotechniques* 1997;21:504-11.
29. 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.
30. Dierkes C, Ehrenstein B, Siebig S, Linde HJ, Reischl U, Salzberger B. Clinical impact of a commercially available multiplex PCR system for rapid detection of pathogens in patients with presumed sepsis. *BMC Infect Dis* 2009;9:126.