Nongonococcal Urethritis: New Views through the Prism of Modern Molecular Microbiology

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The rapidly advancing technology of modern molecular microbiology has greatly improved our understanding of the epidemiology of sexually transmitted infections and the etiology and pathogenesis of the diseases they cause. It is now clear that *Mycoplasma genitalium* accounts for a significant proportion of nonchlamydial nongonococcal urethritis (NGU) cases. DNA sequencing of *Ureaplasma* spp has revealed a new species, *Ureaplasma parvum*. This organism may account for much of the colonization observed in asymptomatic men, which confounded many past studies of the role of *Ureaplasma* spp in NGU. At long last, we can say that *Ureaplasma urealyticum* is a true pathogen. The use of polymerase chain reaction technology has shown that *Trichomonas vaginalis* occurs more frequently in men with NGU than had been thought; however, such studies also have demonstrated that it is more prevalent in asymptomatic men. Finally, recent studies have shown that adenovirus should be added to the list of viral causes of NGU.

Introduction

Since the early 1990s, our understanding of the causes of nongonococcal urethritis (NGU) has expanded greatly, in large part due to tremendous advances in molecular microbiology and the resulting development of many new nucleic acid amplification tests (NAATs) for the diagnosis of sexually transmitted infections (STIs) and infectious diseases in general. Molecular methods such as NAATs have enabled the discovery of etiologic agents and have greatly advanced the epidemiologic study of infections caused by organisms that previously could not be studied adequately because of their poor growth in vitro. In the field of STIs, *Ureaplasma parvum* is an example of the former and *Mycoplasma genitalium* of the latter. This review focuses on recent advances in our understanding of the etiology and epidemiology of NGU.

Table 1 lists the organisms currently implicated as causes of NGU and provides estimates of the proportion of cases caused by each. These prevalence estimates may vary widely depending on an individual patient's STI risk factors and prevalence of the pathogens in different populations. Before NAAT testing, a certain proportion of *Chlamydia trachomatis*–negative NGU cases was suspected to be due to the relatively low sensitivity of culture for this organism. More recent studies based on chlamydia-specific NAATs have provided greater certainty as to the true proportion of NGU cases caused by this organism, as reflected in Table 1. Over the last several years, relatively little has changed regarding our understanding of the role of *C. trachomatis* in NGU, and so this infection is not discussed further here.

A New Species of *Ureaplasma*

Four *Mycoplasma* spp commonly inhabit the human genitourinary tract: *Mycoplasma hominis*, *M. genitalium*, *U. parvum*, and *U. urealyticum*. The latter two were included previously in the single species *U. urealyticum*. Recent genetic studies have shown that the genus actually is comprised of the two species, a finding that could help clarify the confusion surrounding the role of these organisms in NGU [1]. Whenever *U. urealyticum* is mentioned in the older literature, it is important to remember that the authors are really talking about a mixture of the two *Ureaplasma* spp. *M. hominis* does not appear to have a role in NGU; in numerous studies, researchers found it to be equally present in men with and without disease.

The best early evidence for a pathogenic role for *Ureaplasma* spp in NGU came from a series of studies conducted by Dr. William Bowie in Seattle. The first of these was a study of relatively sexually inexperienced men having their first urethritis episodes. In these young men,

Table 1. Etiology of nongonococcal urethritis in 2008

Chlamydia trachomatis, 15%–40%
Mycoplasma genitalium, 15%–25%
Other
Trichomonas vaginalis, 10%–20%
Ureaplasma urealyticum, 10%–20%
Herpes simplex virus, 2%–3% (in the absence of skin lesions)
Adenovirus, 2%–4% (seasonal, associated with receptive oral sex)
Haemophilus spp, *Neisseria meningitidis**, rare
Unknown, 25%–40%

*Rare cases of *Neisseria meningitidis* urethritis presenting like gonococcal urethritis have been reported in Europe.

the rate of isolation of and—perhaps more importantly—the concentration of *Ureaplasma* spp in first-voided urine were significantly greater in those with *C. trachomatis*–negative NGU than in those with *C. trachomatis*–positive NGU and in those in a comparison group without urethritis [2•]. Importantly, in this study, the asymptomatic comparison group was matched to the cases for sexual activity. The second line of evidence supporting a role of *Ureaplasma* spp in NGU came from antibiotic probe studies of selective eradication of *Ureaplasma* spp and *C. trachomatis*. Sulfonamides and rifampin were known to be active against *C. trachomatis* but not the mycoplasmas, whereas spectinomycin and streptomycin had the opposite activity. The symptoms of men whose cultures were *C. trachomatis*–negative but *Ureaplasma* spp–positive responded poorly to sulfonamides and rifampin. In contrast, men responded well to streptomycin or spectinomycin if *Ureaplasma* spp were eradicated, but not when the organism persisted [2•]. In another study, NGU persisted 6 to 12 days after onset of minocycline therapy significantly more often in men infected with tetracycline-resistant *Ureaplasma* spp than in those infected with tetracycline-sensitive strains, and persistent NGU correlated with persistence of the tetracycline-resistant strains [2•]. Finally, intraurethral inoculation of *Ureaplasma* spp in two men and several nonhuman primates resulted in disease, which was associated with culture evidence for the organism's proliferation in vivo, thus fulfilling Koch's postulates [2•].

Despite the evidence cited above, confusion has persisted concerning the role of *Ureaplasma* spp in NGU, largely due to the finding in more recent NGU survey studies among high-risk men that *Ureaplasma* spp are consistently present as often in controls as in cases. Among asymptomatic men at high risk for STIs, *Ureaplasma* spp commonly colonize the male urethra, and this finding strongly correlated with the total number of recent and lifetime female sex partners. With the notable exception of the Seattle studies cited above, many of the early studies of NGU in relatively low-risk populations failed to take this issue into account [2•]. These issues, the ubiquity of the organism, and the lack of evidence that it causes serious morbidity, caused a considerable slip in interest in *Ureaplasma* spp as STI pathogens over the years.

However, as noted above, recent molecular studies have revealed that the genus *Ureaplasma* actually contains two species, *U. urealyticum* and *U. parvum*. The latter is named "parvum" as its genome size is significantly smaller that of *U. urealyticum*. Furthermore, recent studies have suggested that *U. urealyticum* is associated with NGU, but *U. parvum* is not. Deguchi et al. [3] found *U. urealyticum* in 18% of men with chlamydia-negative NGU compared with 7.3% of controls, whereas *U. parvum* prevalence was 9.9% and 13.2%, respectively. Povlsen et al. [4] used a different polymerase chain reaction (PCR) assay and found that *U. urealyticum* was associated with NGU, but after multiple regression analysis, the *P* value was only 0.15. A quantitative study showed that although the concentrations of *U. parvum* did not differ between men with NGU and normal controls, *U. urealyticum* was significantly higher in the former group than in the latter [5]. Another study using molecular genotyping suggested that only a subpopulation of *U. urealyticum* is actually associated with NGU [6].

These very interesting data have provided new insight into the role of *Ureaplasma* spp in NGU, but many unanswered questions remain. Studies thus far have been carried out in populations at relatively low risk for STIs, and they must be repeated in high-risk populations. Cases and controls must be carefully matched for sexual behavioral risk factors to avoid past mistakes. Future studies of NGU must take into account not only *C. trachomatis* but also the role of *M. genitalium*. Careful quality controls must be built into these studies to assure high specificity of the molecular assays and to avoid confounding the data through cross-reactions with other organisms. If *U. urealyticum* is indeed a true NGU pathogen, future investigations should readdress its role in genital tract disease in women. Finally, in addition to further molecular research, culture-based studies are needed to determine the current antibiotic susceptibility profile of this resurrected STI.

Mycoplasma genitalium

The greatest advance in our understanding of the etiology of urethritis over the last 15 years has been the unequivocal establishment of the important role of *M. genitalium* in NGU. *M. genitalium* was first implicated as a cause of NGU in 1981 when it was isolated from 2 of 13 men with NGU after 50 days of incubation of a primary culture [7]. As shown in this initial work, *M. genitalium* is a very fastidious organism, and at best, it adapts very slowly to in vitro growth conditions. In fact, efforts to repeat this initial success over the next decade met with failure. Only after the development of specific PCR assays

did knowledge of the organism's true role in NGU begin to grow. Early studies showed that *M. genitalium* is present in as many as 25% of men with NGU but only in 6% of normal men [2•]. These data have withstood the test of time, and a number of studies supporting these early observations have been reported over the past 10 years, as reviewed by Jensen [8•]. Combining the data from 19 studies, the percentage of patients with NGU harboring *M. genitalium* is 21.1% compared with 6.7% of controls without NGU (OR 3.8, 95% CI, 3–4.9). For combined chlamydia-negative NGU cases, the *M. genitalium* prevalence is 21.7% compared with 6% (OR 5.15, 95% CI, 3.6–7.4). Clearly, *M. genitalium* is a cause of a significant number of NGU cases, previously referred to as "etiology unknown." Additionally, a recent publication suggests *M. genitalium* is a cause of postgonococcal urethritis, as is *U. urealyticum* [9]. Relatively few studies have provided detailed clinical descriptions of *M. genitalium*–associated urethritis. Based on the available data, however, this organism seems to cause relatively mild disease and is indistinguishable from chlamydial urethritis [10–12]. Finally, based on genotyping analyses of concurrently infected couples, unequivocal evidence now exists that *M. genitalium* is sexually transmitted [13].

At least in part, *M. genitalium* may explain why nonchlamydial NGU cases seem to have a poorer response to treatment than do cases caused by *C. trachomatis*. Preliminary data suggest that *M. genitalium* is relatively resistant to the tetracycline class of antibiotics [14,15] and that some strains may be resistant to the erythromycin class [16]. In an as yet unpublished randomized treatment study, we found that a 7-day course of doxycycline failed to eradicate *M. genitalium* in 20 of 31 (64%) men with NGU who were examined at least once following treatment compared with 4 of 25 (16%) men treated with a single dose of azithromycin. Currently, several ongoing treatment trials of NGU include diagnostic testing for *M. genitalium*; therefore, data could soon be available to help determine if NGU treatment recommendations should be revised.

Trichomonas vaginalis

Most early studies of the role of *T. vaginalis* in NGU relied on microscopic examination of the first voided urine. From such studies, the notion arose that *T. vaginalis* was an uncommon cause of the disease. However, in a large study in Seattle based on culture of both urethral secretions and urine using well-standardized Diamond's medium, researchers found the organism in 18% of cases, compared with 8% of controls [2•]. In another relatively recent culture-based study, investigators found that age was a factor: the older the NGU patient, the more likely it was that *T. vaginalis* may have caused the problem [17]. However, use of the more sensitive PCR assays to diagnose *T. vaginalis* infection has shown that the organism is found more frequently in the urethra of asymptomatic men, which raises the question of how many NGU cases truly are attributable to *T. vaginalis*. Schwebke and Hook [18] found *T. vaginalis* in 17% and 13% of men with urethral symptoms and urethral inflammation, respectively, but also in 14% of asymptomatic men and 11% of men without Gram stain evidence of inflammation. Wendel et al. [19] found *T. vaginalis* in 17% of urethritis cases and 12% of those without evidence of disease. The differences between cases and controls were not significant in both studies.

In summary, although it has become increasingly clear that *T. vaginalis* is a cause of urethritis in men, the relative contribution of the organism to urethritis-associated morbidity is difficult to estimate, especially now that recent studies have found high carriage rates in men without urethritis. Additionally, these studies have shown high coinfection rates between *T. vaginalis* and the other known urethral pathogens, further complicating the task of estimating the contribution of *T. vaginalis* to incident urethritis case rates. Nonetheless, empiric treatment of men who fail the standard NGU treatment regimens should include a single 2-gram dose of metronidazole, as *T. vaginalis* infection is more likely the cause of the problem in these cases than it is in men with NGU at first presentation [20].

Investigators have conducted detailed comparisons of the clinical manifestations of gonococcal, chlamydial, and trichomonal urethritis [2•]. Only 55% of men with trichomoniasis have a discharge on examination, compared with 82% of men with chlamydial infection and 93% of those with gonorrhea. The discharge caused by *Neisseria gonorrhoeae* is almost always moderate to large in amount and is purulent in nearly 80% of cases. In contrast, the discharges associated with chlamydial and trichomonal infections are virtually indistinguishable; almost all patients have small or moderate amounts that are either clear or mucoid in character.

Viral Causes of Nongonococcal Urethritis

Only two viruses have been implicated as causes of male urethritis. Urethritis occurs in approximately 30% of men with primary genital herpes simplex virus (HSV) infection and in a much lower percentage of men with recurrent genital HSV infection [2•]. Most but not all such patients have penile lesions. In two older studies, HSV was not isolated more often from the urethras of men with NGU than from those of controls [2•]. Two recent studies using PCR assays were similar in that HSV was detected in 2% to 3% of NGU cases [11,21•]. However, only the Bradshaw et al. [21•] study had sufficient numbers of patients enrolled to demonstrate a significant difference between cases and controls. In this study, HSV-1 was more common than HSV-2, and unprotected oral sex was a major risk factor [21•].

Adenoviruses were first reported in men with urethritis in Perth, Australia. Subsequently, researchers reported isolating adenovirus from 0.3% of 7000 patients attending the Seattle sexually transmitted disease clinic and other health department clinics [2•]. Of 20 infected men, 75% had urethritis [2•]. Six additional cases were reported more recently from New Zealand [22]. Bradshaw et al. [21•] studied 329 NGU cases and compared them to 307 controls in the Melbourne, Australia, Sexual Health Center. Adenovirus was identified in 4% of cases and only 0.3% of controls, thus providing strong support for the addition of this virus to the list of urethral pathogens in men [21]. Evidence that adenovirus-associated NGU is sexually transmitted is incomplete at this point, but its strong association with receptive oral sex supports this hypothesis. In summary, HSV (especially type 1) and adenovirus appear to cause NGU, though the proportion of cases is small.

Clinically, urethritis caused by HSV and adenovirus results in dysuria that is usually severe, significantly more so than that reported on average by men with bacterial NGU [21,23,24]. I have seen a case of HSV urethritis with such severe dysuria that the patient developed urinary retention requiring insertion of an indwelling catheter. Despite the severity of dysuria, discharge is scant and mucoid or clear rather than purulent. Most men with viral urethritis also present with meatitis, which is seen in a minority of cases caused by bacteria. O'Mahony [25•] published an excellent color photo of adenovirus-associated meatitis. Adenovirus cases tend to occur in the fall and winter and are often associated with conjunctivitis [21–23]. Regional lymphadenopathy and constitutional symptoms may be seen with primary HSV urethritis [24].

Viral urethritis is self-limited and usually does not require treatment. Effective antiadenoviral drugs are not available. If HSV infection is suspected, acyclovir, 400 mg, three times daily until symptoms subside might be helpful in speeding recovery, but there are no data to confirm this. Valacyclovir and penciclovir would also be effective but are more expensive.

Other Causes of Nongonococcal Urethritis

As noted, despite recent advances in our understanding of NGU etiology, it is unknown in a significant proportion of urethritis cases in heterosexual men and in men who have sex with men. Early studies suggested a possible role for *Bacteroides ureolyticus*, but more recent work has not found this organism more frequently among men with NGU than among normal men [2•]. *Haemophilus influenzae* and *Haemophilus parainfluenzae* cause urethritis infrequently. Similarly, coliforms may cause a few cases of urethritis in men who have sex with men [2•]. *N. meningitidis* has been reported as a cause of urethritis relatively more often in areas such as Western Europe where the incidence of gonorrhea has dropped to very low levels. Today, men in these countries with urethral smears positive for gram-negative diplococci may be relatively likely to have an infection caused by this organism in view of the rarity of *N. gonorrhoeae* [2•]. Oral-genital sex is the presumed mode of transmission. This hypothesis was strengthened recently by the isolation of a *N. meningitidis* strain from from the pharynx of a woman, which matched the strain isolated from the urethra of her symptomatic male partner by pulsed field gel electrophoresis analysis [26].

Recently, molecular approaches to the study of vaginal flora have revealed a number of previously unknown organisms (eg, *Atopobium vaginae*), which are important members of the genital tract bacterial ecosystem [27,28]. Many of these organisms are most closely related to strict anaerobes, and some have never been cultivated. Keane et al. [29] showed an association between NGU in males and bacterial vaginosis in their partners, suggesting the possibility that one or a combination of the organisms associated with this syndrome could infect their male partners, resulting in urethritis. Molecular methods have been applied to a few patients with NGU and controls, and indeed, at least one unknown organism is present in symptomatic men and not in controls [30]. More extensive investigations focusing specifically on the organisms now known to be associated with bacterial vaginosis likely will advance our understanding of nonchlamydial NGU.

Conclusions

The application of the rapidly advancing technology of modern molecular microbiology to studies of sexually transmitted organisms has greatly improved our understanding of their epidemiology and the etiology and pathogenesis of the diseases that they cause. It is now clear that *M. genitalium* accounts for a significant proportion of nonchlamydial NGU cases. As this organism may be more resistant to treatment with recommended antibiotic treatment regimens for NGU, current management guidelines likely will change over the next several years. In the meantime, the institution of screening programs for *M. genitalium* awaits solid data implicating it in serious upper tract disease in women. DNA sequencing of *Ureaplasma* spp has revealed a new species, *U. parvum*. This organism may account for much of the colonization observed in asymptomatic men that has confounded many of the past studies of the role of *Ureaplasma* spp in NGU. It now appears that the second species, *U. urealyticum*, is the true pathogen; however, estimating the number of cases attributable to this organism presents a challenge, as *U. urealyticum* also occurs in asymptomatic men though in lower numbers than *U. parvum*. The issue of tetracycline resistance in *U. urealyticum* will be important to revisit as we consider changing treatment guidelines. PCR has shown that *T. vaginalis* occurs more frequently in men with NGU than previously thought; however,

these same types of studies have also demonstrated that it is more prevalent in asymptomatic men than previously recognized. Adenovirus now joins HSV as a viral cause of NGU. As many as 4% of NGU cases may be caused by the former, especially during the respiratory virus infection season. Clinically, viral NGU should be suspected in men presenting with relatively severe dysuria but who have little or no discharge. Finally, it appears that some causes of NGU are yet to be discovered: even with modern molecular techniques at our disposal, 25% to 40% of NGU cases are not associated with any known STIs.

Disclosure

The author has reported no potential conflicts of interest relevant to this article.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Robertson JA, Stemke GW, Davis JW Jr, et al.: **Proposal of Ureaplasma parvum sp. nov. and emended description of Ureaplasma urealyticum (Shepard et al. 1974) Robertson et al. 2001.** *Int J Syst Evol Microbiol* 2002, **52:**587–597.
2. • Martin DH: **Urethritis in males.** In *Sexually Transmitted Diseases*. 4th ed. Edited by Holmes KK, Sparling PF, Stamm W, et al. New York: McGraw-Hill; 2007.

Detailed discussions of the older literature frequently alluded to in this review. All the pertinent older literature is in the bibliography of this chapter.

3. Deguchi T, Yoshida T, Miyazawa T, et al.: **Association of Ureaplasma urealyticum (biovar 2) with nongonococcal urethritis.** *Sex Transm Dis* 2004, **31:**192–195.
4. Povlsen K, Bjornelius E, Lidbrink P, et al.: **Relationship of Ureaplasma urealyticum biovar 2 to nongonococcal urethritis.** *Eur J Clin Microbiol Infect Dis* 2002, **21:**97–101.
5. Yoshida T, Deguchi T, Meda S, et al.: **Quantitative detection of Ureaplasma parvum (biovar 1) and Ureaplasma urealyticum (biovar 2) in urine specimens from men with and without urethritis by real-time polymerase chain reaction.** *Sex Transm Dis* 2007, **34:**416–419.
6. Yoshida T, Ishiko H, Yasuda M, 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:**454–457.
7. Tully JG, Taylor-Robinson D, Cole RM, et al.: **A newly discovered mycoplasma in the human urogenital tract.** *Lancet* 1981, **1:**1288–1291.
8. • Jensen JS: **Mycoplasma genitalium: the aetiological agent of urethritis and other sexually transmitted diseases.** *J Eur Acad Dermatol Venereol* 2004, **18:**1–11.

Summarizes the literature showing that *M. genitalium* is unequivocally the cause of NGU.

9. Yokoi S, Maeda S, Kubota Y, et al.: **The role of Mycoplasma genitalium and Ureaplasma urealyticum biovar 2 in postgonococcal urethritis.** *Clin Infect Dis* 2007, **45:**866–871.
10. Mena L, Wang X, Mroczkowski TF, et al.: **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.
11. Totten PA, Schwartz MA, Sjostrom KE, et al.: **Association of Mycoplasma genitalium with nongonococcal urethritis in heterosexual men.** *J Infect Dis* 2001, **183:**269–276.
12. Morency P, Dubois MJ, Gresenguet G, et al.: **Aetiology of urethral discharge in Bangui, Central African Republic.** *Sex Transm Infect* 2001, **77:**125–129.
13. Hjorth SV, Bjornelius E, Lidbrink P, et al.: **Sequence-based typing of Mycoplasma genitalium reveals sexual transmission.** *J Clin Microbiol* 2006, **44:**2078–2083.
14. Falk L, Fredlund H, Jensen JS: **Tetracycline treatment does not eradicate Mycoplasma genitalium.** *Sex Transm Infect* 2003, **79:**318–319.
15. Wikstrom A, Jensen JS: **Mycoplasma genitalium: a common cause of persistent urethritis among men treated with doxycycline.** *Sex Transm Infect* 2006, **82:**276–279.
16. Bradshaw CS, Jensen JS, Tabrizi SN, et al.: **Azithromycin failure in Mycoplasma genitalium urethritis.** *Emerg Infect Dis* 2006, **12:**1149–1152.
17. Joyner JL, Douglas JM Jr, Ragsdale S, et al.: **Comparative prevalence of infection with Trichomonas vaginalis among men attending a sexually transmitted diseases clinic.** *Sex Transm Dis* 2000, **27:**236–240.
18. Schwebke JR, Hook EW 3rd: **High rates of Trichomonas vaginalis among men attending a sexually transmitted diseases clinic: implications for screening and urethritis management.** *J Infect Dis* 2003, **188:**465–468.
19. Wendel KA, Erbelding EJ, Gaydos CA, et al.: **Use of urine polymerase chain reaction to define the prevalence and clinical presentation of Trichomonas vaginalis in men attending an STD clinic.** *Sex Transm Infect* 2003, **79:**151–153.
20. US Centers for Disease Control and Prevention: **Sexually Transmitted Diseases Treatment Guidelines, 2006.** *MMWR Morb Mortal Wkly Rep* 2006, **55(RR-11):**1–94.
21. • Bradshaw CS, Tabrizi SN, Read TR, et al.: **Etiologies of nongonococcal urethritis: bacteria, viruses, and the association with orogenital exposure.** *J Infect Dis* 2006, **193:**336–345.

Excellent clinical study of the etiology of NGU utilizing modern molecular diagnostic methods. As such, it is a model for future NGU studies.

22. Azariah S, Reid M: **Adenovirus and non-gonococcal urethritis.** *Int J STD AIDS* 2000, **11:**548–550.
23. Bradshaw CS, Denham IM, Fairley CK: **Characteristics of adenovirus associated urethritis.** *Sex Transm Infect* 2002, **78:**445–447.
24. Lautenschlager S, Eichmann A: **Urethritis: an underestimated clinical variant of genital herpes in men?** *J Am Acad Dermatol* 2002, **46:**307–308.
25. • O'Mahony C: **Adenoviral non-gonococcal urethritis.** *Int J STD AIDS* 2006, **17:**203–204.

Contains excellent color photographs of a patient with adenoviral urethritis.

26. Urra E, Alkorta M, Sota M, et al.: **Orogenital transmission of Neisseria meningitidis serogroup C confirmed by genotyping techniques.** *Eur J Clin Microbiol Infect Dis* 2005, **24:**51–53.
27. Ferris MJ, Masztal A, Aldridge KE, et al.: **Association of Atopobium vaginae, a recently described metronidazole resistant anaerobe, with bacterial vaginosis.** *BMC Infect Dis* 2004, **4:**5.
28. Fredricks DN, Fiedler TL, Marrazzo JM: **Molecular identification of bacteria associated with bacterial vaginosis.** *N Engl J Med* 2005, **353:**1899–1911.
29. Keane FE, Thomas BJ, Whitaker L, et al.: **An association between non-gonococcal urethritis and bacterial vaginosis and the implications for patients and their sexual partners.** *Genitourin Med* 1997, **73:**373–377.
30. Riemersma WA, van der Schee CJ, van der Meijden WI, et al.: **Microbial population diversity in the urethras of healthy males and males suffering from nonchlamydial, nongonococcal urethritis.** *J Clin Microbiol* 2003, **41:**1977–1986.