

Etiologies of Nongonococcal Urethritis: Bacteria, Viruses, and the Association with Orogenital Exposure

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(See the editorial commentary by Handsfield, on pages 333–5.)

Background. The purpose of the present study was to determine pathogens and behaviors associated with nongonococcal urethritis (NGU) and the usefulness of the urethral smear in predicting the presence of pathogens.

Methods. We conducted a case-control study of men with and without symptoms of NGU. Sexual practices were measured by questionnaire. First-stream urine was tested for *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Ureaplasma parvum*, *U. urealyticum*, herpes simplex virus (HSV)–1, HSV-2, adenoviruses, and *Gardnerella vaginalis* by polymerase chain reaction.

Results. *C. trachomatis* (20%), *M. genitalium* (9%), adenoviruses (4%), and HSV-1 (2%) were more common in cases with NGU ($n = 329$) after age and sexual risk were adjusted for ($P \leq .01$); *U. urealyticum*, *U. parvum*, and *G. vaginalis* were not. Infection with adenoviruses or HSV-1 was associated with distinct clinical features, oral sex, and male partners, whereas infection with *M. genitalium* or *C. trachomatis* was associated with unprotected vaginal sex. Oral sex was associated with NGU in which no pathogen was detected ($P \leq .001$). Fewer than 5 polymorphonuclear leukocytes (PMNLs) per high-power field (HPF) on urethral smear were present in 32%, 37%, 38%, and 44% of cases with *C. trachomatis*, *M. genitalium*, adenoviruses, and HSV, respectively.

Conclusion. We identified adenoviruses and HSV-1 as significant causes of NGU with distinct clinical and behavioral characteristics and highlighted the association between insertive oral sex and NGU. A urethral PMNL count of ≥ 5 PMNLs/HPF is not sufficiently sensitive to exclude pathogens in men with urethral symptoms.

Acute nongonococcal urethritis (NGU) is one of the commonest sexually transmitted infections affecting men, yet a pathogen is not identified in a significant proportion of cases (20%–50%). *Chlamydia trachomatis* typically accounts for 30%–50% of cases of NGU [1, 2], and *Mycoplasma genitalium* accounts for 10%–30% [2, 3]; *Ureaplasma urealyticum*, *Haemophilus* species, *Streptococcus* species, and *Gardnerella vaginalis* have been associated with NGU, but their role is un-

proven [4, 5]. Few studies have investigated potential viral causes of acute NGU, such as herpes simplex virus (HSV) [6–8] and adenoviruses [9–12].

The diagnosis of NGU has traditionally required microscopic evidence of urethritis, defined as ≥ 5 polymorphonuclear leukocytes (PMNLs) per high-power field (HPF) ($\times 1000$ magnification) in ≥ 5 fields of a urethral Gram stain. Urethral pathogens, however, are often detected in symptomatic men with < 5 PMNLs/HPF [13–16], particularly when sensitive nucleic acid amplification tests are used. Studies of NGU frequently restrict enrollment to men with urethral discharge or dysuria and ≥ 5 PMNLs/HPF, and the prevalence of urethral pathogens in such populations has been well described. However, a significant proportion of men with urethral symptoms do not have microscopic evidence of urethritis or experience other urethral symptoms, including burning or irritation. We conducted a case-control study of men with symptoms of NGU—regardless of urethral PMNL count—and asymptomatic

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control subjects, to establish the prevalence of known and suspected bacterial and viral pathogens, to examine their associations with sexual practices, and to investigate the usefulness of the urethral smear cutoff of ≥ 5 PMNLs/HPF in predicting the presence of pathogens.

SUBJECTS AND METHODS

Study population. Men presenting to Melbourne Sexual Health Centre (MSHC), Australia, between March 2004 and March 2005 were eligible for enrollment. MSHC, the principle public sexual health service in Melbourne, provided 10,122 consultations to men during this period. Cases included men with urethral symptoms (discharge, dysuria, and urethral burning or irritation) for ≤ 1 month, and controls included men with no current urethral symptoms. Men with urethral gonorrhoea (gram-negative intracellular diplococci on urethral Gram stain and/or *N. gonorrhoeae* on culture) or genital herpes with lesions were excluded at enrollment or on chart review. Clinicians were asked to recruit all eligible cases, starting in March 2004; triage nurses marked files of eligible patients; and a computerized study reminder activated with the diagnosis of acute NGU. There was no electronic reminder for controls, but enrollment was coordinated to match recruitment of cases with monthly e-mail reminders to the staff.

Clinical and laboratory methods. Participants completed a questionnaire regarding symptoms (rating scale, 1–5) and sexual and behavioral practices and underwent genital examination. Clinicians recorded clinical and laboratory findings on standardized data-collection sheets. Cases underwent a urethral smear, but controls were not subject to a urethral smear, because of poor acceptability of the procedure in asymptomatic men. All participants provided a first-stream urine specimen and recorded time since last voiding. Two experienced members of the on-site laboratory staff evaluated urethral Gram-stained smears from cases for PMNLs in 5 oil-immersion fields ($\times 1000$ magnification). Cases were treated presumptively with 1 g of oral azithromycin.

First-stream urine from all participants was tested for *C. trachomatis* by strand-displacement amplification (ProbeTec-ETCT-Amplified DNA-Assay; Becton Dickinson) and for *M. genitalium*, HSV-1 and -2, *Trichomonas vaginalis*, *U. urealyticum*, *U. parvum*, *G. vaginalis*, and adenoviruses by polymerase chain reaction (PCR). One milliliter of urine was centrifuged, and the pellet was extracted using the automated MagNA Pure LC (Roche) with the DNA Isolation Kit I protocol. Extracted DNA was amplified by PCR for 7 targets (5- μ L aliquots of DNA for each reaction). Amplification and detection was performed for the β -globin gene (as a positive control) [17], *M. genitalium* [18], *T. vaginalis* [19], *U. urealyticum* and *U. parvum* [20], *G. vaginalis* [21], adenoviruses, and HSV, using an adaptation of a previously described assay targeting a 239-bp

sequence of the glycoprotein D gene and a 206-bp sequence of the hexon gene [22, 23]. All PCR assays have been shown to be highly specific for the target amplified, with analytical sensitivity of 10 copies/reaction (200 copies/mL of urine). Adenovirus serovars were determined by sequence comparison between amplicons from positive samples and known nucleotide sequences in the GenBank database, using the BLAST search algorithm.

Cases were tested for *Neisseria gonorrhoeae* by culture in modified Thayer-Martin medium. Controls were not screened for urethral *N. gonorrhoeae*, because of the very low prevalence of *N. gonorrhoeae* in asymptomatic men [24]. The Human Research and Ethics Committee of the Alfred Hospital, Victoria, approved this study.

Statistical analysis. Data were entered and stored in Microsoft Access and analyzed using SPSS (version 12; SPSS). Clinical, behavioral, and laboratory findings in cases were compared with those in controls. Proportions were compared using χ^2 and Fisher's exact tests; unpaired *t* tests, analysis of variance, and nonparametric tests (Mann-Whitney *U* test and Kruskal-Wallis test) were used for continuous variables, where appropriate. Univariate analysis was used to calculate crude odds ratios (ORs) and 95% confidence intervals (CIs). Logistic regression was used to control for confounding factors. Variables included in the model were those found to be significant in the univariate analysis ($P < .05$) and those considered to be important on the basis of published literature; variables were examined for multicollinearity. Patients were excluded from the analysis when clinical information or specimens were not available. With 300 cases and controls, this study has 80% power to detect an OR of ≥ 1.7 for factors present in 20% of controls.

RESULTS

Characteristics of the study population. Six hundred and thirty-six heterosexual men and men who have sex with men (MSMs) with a mean \pm SD age of 32.3 ± 9.1 years were enrolled; 329 had urethral symptoms (cases) and 307 did not (controls). During the study period, 479 men with acute NGU attended MSHC, 69% of whom were enrolled; those not enrolled included men who were not eligible, declined, or were not asked to participate. Controls were enrolled during the same period as cases; however, we could not determine their participation rate, because the study definition of a control (male without urethral symptoms) is not captured in the clinic database.

Clinical features in cases included a history of acute urethral discharge (62%), dysuria (73%), urethral itch (56%), and urethral burning (62%), and, on examination, urethral discharge (57%), meatitis (meatal inflammation and/or edema) (38%), and macroscopically evident mucous threads in first-stream urine (34%). The mean \pm SD time since last urine passage for the

study population was 2.6 ± 2.5 h; there was no difference between cases and controls ($P = .85$). Table 1 describes the demographic and behavioral characteristics of the study population.

Urethral organisms associated with nongonococcal urethritis.

C. trachomatis, *M. genitalium*, HSV-1, and adenoviruses were detected more frequently in cases than controls ($P < .01$; table 2). *C. trachomatis* occurred in 20% (95% CI, 15%–24%) of cases, and *M. genitalium* occurred in 9% (95% CI, 7%–13%), whereas adenoviruses (4% [95% CI, 2%–6%]) and HSV-1 and -2 (3% [95% CI, 1%–5%]) were significant but less common causes of NGU. *T. vaginalis* occurred in only 1 case. Herpes serologic testing was not performed; 1 case with HSV-2 had a history of genital herpes. One case was coinfecting with *C. trachomatis* and adenovirus, but no other participant had >1 urethral pathogen. *G. vaginalis*, *U. parvum*, and *U. urealyticum* (biovar 2) were detected more frequently in controls, although this difference was significant only for *G. vaginalis* and *U. parvum* ($P < .01$). Associations between organisms and NGU remained unchanged after age and unprotected vaginal, oral, and anal sex with a casual partner in the past month were adjusted for, with the exception of *U. parvum*, which no longer remained associated with being a control. Serotypes of 9 adenovirus samples were available: 6 were subgenus D (3 serotype 9 and 3 serotype 37), 2 were subgenus B2 (serotype 35), and 1 was subgenus E (serotype 4).

Clinical characteristics of cases with and cases without urethral pathogens. Table 3 shows the clinical characteristics of cases infected with specific urethral pathogens (hereafter, the wording “with a pathogen” indicates infection with *C. trachomatis*, *M. genitalium*, *T. vaginalis*, HSV-1 or -2, or adenoviruses but not with *G. vaginalis* or ureaplasmas). Self-reported urethral discharge, itch, burning, balanitis, epididymitis, or ≥ 5 PMNLs/HPF on urethral Gram stain were not associated with specific pathogens. Cases with *C. trachomatis* infection were more likely to have macroscopically evident mucous threads in first-stream urine than were other cases ($P < .001$).

Cases with HSV or adenoviruses were significantly more likely than other cases to present with meatitis and moderate to severe dysuria: 20 (91%) had meatitis, 16 (73%) had moderate to severe dysuria, and 15 (68%) had both findings, in contrast to 33%, 30%, and 11% of cases without viral pathogens, respectively ($P < .001$). Meatitis was a sensitive indicator of the presence of viral pathogens in NGU (91%), with a high negative predictive value (99%), but was not specific (67%). Moderate to severe dysuria had a high negative predictive value (97%) but was not specific (71%) or sensitive (73%). The combination of both clinical characteristics had a high specificity (89%) and negative predictive value (98%) for the presence of viral pathogens but was not sensitive (68%), and it had a positive predictive value of 31%. Conjunctivitis was reported in 4 cases (39%) with adenovirus but not in cases with other path-

Table 1. Demographic and behavioral characteristics of the study population.

Characteristic	No. (%) of		Crude OR (95% CI)
	Controls (n = 307)	Cases (n = 329)	
Age >31 years			
No	175 (57)	141 (43)	1.0
Yes	132 (43)	188 (57)	1.8 (1.3–2.4)
NSP in past month			
No	154 (50)	115 (35)	1.0
Yes	152 (50)	214 (65)	1.9 (1.4–2.6)
RSP in past month			
No	141 (46)	166 (50)	1.0
Yes	165 (54)	163 (50)	0.84 (0.6–1.1)
>1 FSP in past month			
No	241 (79)	223 (68)	1.0
Yes	65 (21)	105 (32)	1.7 (1.2–2.5)
≥ 1 MSP in past month			
No	232 (76)	220 (67)	1.0
Yes	74 (24)	107 (33)	1.5 (1.1–2.2)
UPVS with CSP in past month			
No	234 (77)	197 (62)	1.0
Yes	69 (23)	123 (38)	2.1 (1.5–3.0)
IOS with CSP in past month			
No	164 (54)	107 (33)	1.0
Yes	140 (46)	220 (67)	2.4 (1.7–3.3)
UPAS with CSP in past month			
No	278 (91)	283 (88)	1.0
Yes	26 (9)	38 (12)	1.4 (0.9–2.4)

NOTE. CI, confidence interval; CSP, casual sex partner; FSP, female sex partner; IOS, <100% condom use for insertive oral sex; MSP, male sex partner; NSP, new sex partner; OR, odds ratio; RSP, regular sex partner; UPAS, <100% condom use for insertive anal sex; UPVS, <100% condom use for vaginal sex. “Past month” denotes the month before the onset of symptoms.

ogens. Adenovirus infections were seasonally clustered; 8 occurred in autumn (comprising 8% of all cases in autumn), 4 occurred in winter/early spring, and 1 occurred in summer ($P < .01$).

Relationship between urethral PMNL count and detection of urethral pathogens. Microscopic evidence of urethritis was related to the presence of urethral discharge on history (OR, 3.6 [95% CI, 2.2–6.0]) and examination (OR, 11.3 [95% CI, 6.3–20.4]) but was not associated with other clinical characteristics. A urethral PMNL count of ≥ 5 PMNLs/HPF was present in 133 cases (42% [95% CI, 36%–47%]), including 41 (68%) cases with *C. trachomatis*, 17 (63%) with *M. genitalium*, 8 (62%) with adenoviruses, and 5 (56%) with HSV. *C. trachomatis* (OR, 3.9 [95% CI, 2.1–7.1]) and *M. genitalium* (OR, 2.6 [95% CI, 1.1–5.8]) were significantly associated with ≥ 5 PMNLs/HPF on urethral Gram stain. A urethral pathogen (*C. trachomatis*, *M. genitalium*, adenoviruses, or HSV) was detected in 20% (95% CI, 15%–27%) of men with urethral symptoms but <5 PMNLs/HPF, which was significantly more common than in asymptomatic controls (4% [95% CI, 2%–7%]) ($P < .001$).

Table 2. Urethral organisms associated with nongonococcal urethritis.

Organism	No. (%) of		OR (95% CI)	
	Controls (n = 307)	Cases (n = 329)	Crude	Adjusted
<i>Chlamydia trachomatis</i>				
Negative	299 (97)	265 (80)	1.0	1.0
Positive	8 (3)	64 (20) ^a	9.0 (4.2–19.2)	12.3 (5.4–28.1)
<i>Mycoplasma genitalium</i>				
Negative	303 (99)	298 (91)	1.0	1.0
Positive	3 (1)	31 (9)	10.5 (3.2–34.7)	14.7 (4.3–50.6)
HSV-1				
Negative	306 (100)	322 (98)		
Positive	0	7 (2) ^b	S ^c	...
HSV-2				
Negative	306 (100)	327 (99)		
Positive	0	2 (1) ^b	NS ^d	...
<i>Trichomonas vaginalis</i>				
Negative	306 (100)	328 (99.7)		
Positive	0	1 (0.3)	NS ^e	...
Adenoviruses				
Negative	306 (99.7)	316 (96)	1.0	1.0
Positive	1 (0.3)	13 (4) ^a	12.6 (1.6–98.8)	12.6 (1.4–109.7)
<i>Ureaplasma parvum</i>				
Negative	263 (86)	306 (93)	1.0	1.0
Positive	44 (14)	23 (7)	0.4 (0.3–0.8)	0.7 (0.4–1.4)
<i>Ureaplasma urealyticum</i>				
Negative	253 (82)	286 (87)	1.0	
Positive	54 (18)	43 (13)	0.7 (0.5–1.1)	...
<i>Gardnerella vaginalis</i>				
Negative	215 (70)	277 (84)	1.0	1.0
Positive	92 (30)	52 (16)	0.4 (0.3–0.6)	0.5 (0.3–0.8)

NOTE. Variables in the adjusted analysis included age; unprotected vaginal sex, oral sex, and anal sex with a casual sex partner in the past month; and infection with *C. trachomatis*, *M. genitalium*, adenoviruses, *U. parvum*, or *G. vaginalis*. Data on 617 patients included 19 missing from the multivariable model. One case and 3 controls were coinfecting with *U. parvum* and *U. urealyticum*. Coinfection with adenoviruses, *C. trachomatis*, *M. genitalium*, and HSV-1 occurred in 15 cases with *U. urealyticum* and 3 cases with *U. parvum*. CI, confidence interval; HSV, herpes simplex virus; NS, not significant; OR, odds ratio; S, significant.

^a One case was coinfecting with *C. trachomatis* and adenoviruses.

^b Men with visible lesions consistent with genital herpes were excluded from the study.

^c *P* = .01.

^d *P* = .17.

^e *P* = .33.

Behavioral associations with urethral organisms. Table 4 shows the prevalence of urethral organisms in participants, according to sexual preference. Cases with HSV-1 or adenoviruses were more likely to have had male sex partners during the past month (*P* = .03), whereas cases with *C. trachomatis* or *M. genitalium* were more likely to be heterosexual (*P* = .07); *U. parvum* and *G. vaginalis* were also significantly more common in heterosexual cases and controls (*P* ≤ .02).

To determine whether urethral pathogens were associated with specific sexual practices, cases with each pathogen were compared with controls without the pathogen. Unprotected vaginal sex with a casual partner was associated with *C. trachomatis* (OR, 2.6 [95% CI, 1.5–4.6]) and *M. genitalium* (OR, 2.5 [95% CI, 1.1–5.3]),

insertive oral sex with a casual partner was associated with adenoviruses (OR, 3.9 [95% CI, 1.1–14.6]) and *C. trachomatis* (OR, 2.1 [95% CI, 1.2–3.6]) and was more common in men with HSV-1 (71% vs. 46%; *P* = .18), and anal sex with a casual partner (OR, 3.1 [95% CI, 1.0–9.8]) was associated with adenoviruses. After age and unprotected oral, vaginal, and anal sex with a casual partner were adjusted for, *C. trachomatis* (adjusted OR, 2.3 [95% CI, 1.2–4.3]) and *M. genitalium* (adjusted OR, 2.4 [95% CI, 1.1–5.2]) remained associated with unprotected vaginal sex with a casual partner. An adjusted analysis was performed for each viral pathogen, but numbers were small, and behavioral associations were not significant.

Table 5 shows the behavioral associations in pathogen-pos-

Table 3. Clinical features associated with specific organisms in men with nongonococcal urethritis (n = 328).

Feature	<i>Chlamydia trachomatis</i> (n = 63) ^a	<i>Mycoplasma genitalium</i> (n = 31)	Adenoviruses (n = 13) ^a	HSV-1 and -2 (n = 9)	Pathogen negative ^b (n = 212)	P
Age >31 years	26 (41)	20 (65)	7 (54)	3 (33)	131 (62)	.03
Self-reported dysuria						.006
None	15 (24)	12 (40)	2 (15)	1 (11)	57 (27)	
Mild	30 (48)	12 (40)	2 (16)	1 (11)	86 (41)	
Moderate to severe	18 (28)	6 (20)	9 (69)	7 (78)	65 (31)	
Self-reported urethral discharge	40 (64)	20 (67)	9 (69)	6 (67)	125 (60)	.89
Urethral discharge on examination	47 (75)	19 (61)	10 (77)	7 (78)	102 (48)	<.001
Nature of urethral discharge						.007
None/normal	17 (27)	12 (39)	3 (23)	2 (22)	110 (52)	
Mucoid	39 (62)	15 (48)	10 (77)	6 (67)	81 (38)	
Mucopurulent	7 (11)	4 (13)	0	1 (11)	21 (10)	
Meatitis	20 (33)	8 (26)	12 (92)	8 (89)	75 (35)	<.001
Urine threads/cloudy	38 (63)	10 (32)	6 (46)	2 (25)	53 (25)	<.001
≥5 PMNLs/HPF on urethral Gram stain	40 (68)	17 (63)	8 (62)	5 (56)	62 (29)	<.001

NOTE. Data are no. (%) of cases, unless otherwise indicated. HPF, high-power field; HSV, herpes simplex virus; PMNLs, polymorphonuclear leukocytes.

^a One case with adenovirus was coinfecting with *C. trachomatis* and is included only in the adenoviruses column.

^b "Pathogen-negative" means that cases with *C. trachomatis*, *M. genitalium*, adenoviruses, or HSVs were excluded from the analysis.

itive and pathogen-negative cases compared with pathogen-negative controls. Pathogen-positive and pathogen-negative cases were significantly more likely to have engaged in unprotected vaginal and insertive oral sex with a casual partner than were pathogen-negative controls. After age and unprotected vaginal, oral, and anal sex with a casual partner were adjusted for, casual unprotected vaginal sex remained associated with pathogen-positive cases, whereas insertive oral sex and, to a lesser extent, unprotected vaginal sex with a casual partner were associated with pathogen-negative cases. Exclusion of men who practiced unprotected vaginal or anal sex from the analysis strengthened the association between pathogen-negative NGU and casual insertive oral sex (OR, 8.5 [95% CI, 3.6–20.5]).

To examine behavioral associations with organisms not associated with NGU, controls with *G. vaginalis* were compared with controls without *G. vaginalis*. Controls with *G. vaginalis* were more likely to have had unprotected vaginal sex within 14 days (median, 14 days), to have had a greater number of female partners, and to have *U. parvum* and *U. urealyticum* (table 6). *U. parvum* had similar behavioral associations but was negatively associated with *U. urealyticum*. In the adjusted analyses, both *G. vaginalis* and *U. parvum* remained more likely to be detected in the urine of asymptomatic men within 14 days of unprotected vaginal sex.

DISCUSSION

In this study of men with urethral symptoms, adenoviruses and HSV-1 were found to be significantly associated with NGU and to have different clinical and behavioral associations with *C. trachomatis* and *M. genitalium*. Adenoviruses and HSV-1 were associated with sex with men and with insertive oral sex, whereas

C. trachomatis and *M. genitalium* were associated with sex with women and unprotected vaginal sex. Insertive oral sex was significantly associated with NGU in which no pathogen was detected. *T. vaginalis* and HSV-2 were uncommon causes of NGU in this population, and *U. urealyticum*, *U. parvum*, and *G. vaginalis* were not associated with NGU. To our knowledge, this is the first case-control study of NGU to examine a broad range of viral and bacterial pathogens in heterosexual men and MSMs by use of sensitive molecular techniques and to demonstrate a significant association of HSV-1 and adenoviruses with NGU.

Few studies have examined the prevalence and role of adenoviruses as causative agents of urethritis. Subgenus D adenoviruses have been reported to manifest an affinity for the eye and genital tract [25] and are an established cause of keratoconjunctivitis [12, 26–29]. We previously reported a series of NGU cases associated with marked dysuria, meatitis, conjunctivitis, and constitutional symptoms, in which adenoviruses were isolated [9]. Insertive oral sex preceded onset of symptoms in all cases, in 4 cases the adenoviruses were serotyped and found to be subgenus D, and there was seasonal clustering (autumn to spring). Azariah and Reid reported similar associations in 6 MSMs who had adenovirus-associated urethritis [10], and Harnett et al. [11] and Swenson et al. [12] reported adenoviruses to be uncommon causes of urethritis and to be associated with conjunctivitis. These studies used viral cultures, and the majority of the adenoviruses were subgenus D serotypes. Our data indicate an association between adenoviruses and insertive oral sex and, possibly, between adenoviruses and insertive anal sex, although the latter rarely occurred in the absence of unprotected oral sex. Adenoviruses have uncom-

Table 4. Prevalence of organisms in cases and controls (n = 635).

Organism	Cases			Controls		
	Heterosexual (n = 220)	MSM (n = 109)	P	Heterosexual (n = 231)	MSM (n = 75)	P
HSV-1	2 (1)	5 (5)	.04	0	0	1.00
HSV-2	2 (1)	0	.55	0	0	1.00
Adenoviruses	7 (3)	6 (6)	.37	0	1 (1)	.24
<i>Mycoplasma genitalium</i>	23 (11)	8 (7)	.36	3 (1)	0	1.00
<i>Chlamydia trachomatis</i>	48 (22)	16 (15)	.12	6 (3)	1 (1)	1.00
<i>Trichomonas vaginalis</i>	1 (1)	0	1.00	0	0	1.00
<i>Ureaplasma parvum</i>	22 (10)	1 (1)	<.01	43 (19)	1 (1)	<.01
<i>U. urealyticum</i>	26 (12)	17 (16)	.34	43 (19)	11 (15)	.44
<i>Gardnerella vaginalis</i>	48 (22)	4 (4)	<.01	78 (34)	14 (19)	.01

NOTE. Data are no. (%) of subjects, unless otherwise indicated. HSV, herpes simplex virus; MSM, men who have sex with men.

monly been isolated from the genital tract in women [12, 29–31], indicating that vaginal transmission could occur. Despite limited recognition in the past, adenoviruses—particularly those of subgenus D—appear able to be sexually transmitted, associated with insertive oral sex, and capable of causing a distinct clinical syndrome in men with NGU [9, 10].

Our data suggest that HSVs are a cause of NGU in the absence of visible herpetic lesions and that HSV-1 may be a more common cause of NGU than HSV-2. Both viruses can cause urethritis during an initial episode of genital herpes. However, HSV-2 has infrequently been reported as a cause of NGU without lesions [6–8], and there have been few studies of HSV-1 in NGU [7]. It is possible that we detected shedding of HSV in the presence of another urethral pathogen; however, the strong association with symptoms of urethritis, the similarity of clinical characteristics with those in cases with adenoviruses, and the absence of other known coinfections and of HSV in asymptomatic controls make it probable that HSV was the cause of urethral symptoms in these cases. The association between HSV-1, insertive oral sex, and sex with men may exist because oral sex is a particularly common exposure in MSMs [32]. As HSV-1 becomes responsible for an increasing proportion of genital herpes [33, 34], it may become a more significant cause of NGU in populations in which oral sex is commonly practiced. The characteristic clinical features of NGU found to be caused by viruses in the present study may mean that these findings could be used clinically to distinguish viral from bacterial causes of urethritis, although the predictive value of these signs is dependent on the prevalence of viral pathogens in the population.

Unprotected insertive oral and vaginal sex with a casual partner was associated with pathogen-negative NGU, and the association between pathogen-negative NGU and insertive oral sex was greater in men not practicing unprotected vaginal or anal sex. Unprotected anal sex was not a risk factor for pathogen-negative NGU in this study. Few studies have addressed

the role of insertive oral sex in NGU. Insertive oral sex has been found to be a risk factor in several [35–37], but not all, studies that have examined the association between oral sex and NGU [38]. Insertive oral sex is recognized as a mode of transmission for *N. gonorrhoeae*, *Streptococcus pneumoniae* [39] and *N. meningitis* [40–43] have been isolated in patients with NGU after oral sex, and *Haemophilus influenzae* and *H. parainfluenzae* have been cultured in patients with NGU [44]. These findings support transmission of oropharyngeal pathogens or commensal oral flora via oral sex to the urethra. Such organisms may be responsible for a greater proportion of urethral symptoms than has been previously recognized. Our findings regarding both adenoviruses and HSV-1 support this premise and highlight the need for further studies of oropharyngeal viruses and bacteria in NGU.

U. urealyticum (biovar 2), *U. parvum*, and *G. vaginalis* was not found to be associated with NGU in the present study. Human and animal inoculation studies and some case-control studies [45, 46] have reported an association between *U. urealyticum* and NGU, with biovar 2 most recently being implicated [47, 48]. However, *U. urealyticum* is common in the genital tract of sexually active men and women and is associated with increased numbers of partners [49], which poses difficulties for determining its role in NGU. It has been proposed that symptoms are related to higher bacterial loads [50, 51], specific serotypes of biovar 2 [52, 53], or initial exposure [54] but subside with colonization. Although we found no association between biovar 2 and NGU, we did not perform serotyping or quantitation.

Neither *U. parvum* nor *G. vaginalis* were found to be associated with urethral symptoms in the present study but were more likely to be detected within 14 days of unprotected vaginal sex. Because both organisms form part of the normal vaginal flora [55], detection may be a consequence of transient colonization of the male urethra after vaginal sex. *G. vaginalis* has been reported as being more common in asymptomatic men

Table 5. Behavioral associations for cases with and without urethral pathogens, compared with controls without urethral pathogens.

Feature	Pathogen-negative ^a controls, no. (%) (n = 294)	Pathogen-positive cases (n = 117)			Pathogen-negative ^a cases (n = 212)		
		No. (%)	Crude OR (95% CI)	Adjusted OR (95% CI)	No. (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
Age >31 years							
No	165 (56)	60 (51)	1.0	1.0	81 (38)	1.0	1.0
Yes	129 (44)	57 (49)	1.2 (0.8–1.9)	1.2 (0.7–1.8)	131 (62)	2.1 (1.4–3.0)	2.0 (1.4–2.9)
>1 FSP in past month							
No	234 (80)	82 (70)	1.0	...	141 (67)	1.0	...
Yes	60 (20)	35 (30)	1.7 (1.0–2.7)	...	70 (33)	1.9 (1.3–2.9)	...
≥1 MSP in past month							
No	223 (76)	82 (70)	1.0	...	138 (66)	1.0	...
Yes	71 (24)	35 (30)	1.3 (0.8–2.2)	...	72 (34)	1.6 (1.1–2.4)	...
RSP in past month							
No	137 (47)	65 (56)	1.0	...	101 (48)	1.0	...
Yes	157 (53)	52 (44)	0.7 (0.5–1.1)	...	111 (52)	1.0 (0.7–1.4)	...
CSP in past month							
No	103 (35)	28 (24)	1.0	...	40 (19)	1.0	...
Yes	189 (65)	89 (76)	1.7 (1.1–2.8)	...	172 (81)	2.3 (1.5–3.6)	...
UPVS with CSP in past month							
No	224 (77)	68 (59)	1.0	1.0	129 (63)	1.0	1.0
Yes	67 (23)	47 (41)	2.3 (1.5–3.7)	2.0 (1.2–3.4)	76 (37)	2.0 (1.3–2.9)	1.6 (1.0–2.4)
IOS with CSP in past month							
No	159 (54)	43 (37)	1.0	1.0	64 (30)	1.0	1.0
Yes	133 (46)	74 (63)	2.1 (1.3–3.2)	1.5 (0.9–2.5)	146 (70)	2.7 (1.9–4.0)	2.3 (1.5–3.4)
UPAS with CSP in past month							
No	266 (91)	101 (89)	1.0	1.0	182 (88)	1.0	1.0
Yes	26 (9)	13 (11)	1.3 (0.7–2.7)	1.1 (0.5–2.4)	25 (12)	1.4 (0.8–2.5)	1.0 (0.5–1.8)

NOTE. Data on as many as 10 subjects were missing in the univariate analysis. Variables in the adjusted analysis included age and unprotected vaginal sex, oral sex, and anal sex with a casual sex partner (CSP). Data on as many as 12 subjects were missing in the adjusted analysis. CI, confidence interval; FSP, female sex partner; IOS, <100% condom use for insertive oral sex; MSP, male sex partner; OR, odds ratio; RSP, regular sex partner; SP, sex partner; UPAS, <100% condom use for insertive anal sex; UPVS, <100% condom use for vaginal sex. "Past month" denotes the month before the onset of symptoms.

^a "Pathogen negative" means that subjects with *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Trichomonas vaginalis*, herpes simplex virus, or adenoviruses were excluded from the analysis.

than in men with NGU [54], but it has also been isolated from men with urethritis [5, 56]. Although *G. vaginalis* is a commensal of the female genital tract, in increased numbers it is associated with bacterial vaginosis (BV), and an association between NGU and BV has been reported [57]. We may have failed to detect an association between *G. vaginalis* and NGU; however, if an association with BV and NGU exists, it is also possible that it is with another BV-associated organism that is not commonly part of the normal vaginal flora.

Laboratory evidence of urethritis was associated with the presence of urethral discharge and was predictive of the presence of *C. trachomatis* and *M. genitalium*, but using ≥5 PMNLs/HPF as the sole criterion for urethritis in our study would have resulted in a failure to identify a significant proportion of pathogens in cases (*C. trachomatis* [32%], *M. genitalium* [37%], adenoviruses [38%], and HSV [44%]). Previous studies have also reported that a significant proportion of men with NGU and urethral pathogens have <5 PMNLs/HPF [13–16]. Although ≥5 PMNLs/HPF on urethral Gram stain has been shown to be associated with isolation of *C. trachomatis* and *N.*

gonorrhoeae, the sensitivity for *C. trachomatis* in some studies has been as low as 29% [14] to 63% [15]. A urethral PMNL count of ≥5 PMNLs/HPF in our study was not useful for predicting the presence of a pathogen in the absence of urethral discharge, a finding also reported by Janier [14]. Therefore, in studies of NGU in which enrollment is not limited to those with urethral discharge, a weaker association between NGU and the urethral Gram stain may be reported. Although the urethral Gram stain may provide objective evidence of inflammation, it appears that it cannot reliably be used to exclude a urethral pathogen in NGU.

Our study did not have sufficient power to examine all behavioral risk factors for urethral pathogens, and studies with greater numbers would be helpful in exploring these associations. Recruitment on the basis of symptoms may have resulted in the enrollment of men with symptoms that had noninfectious causes, which may have reduced the prevalence of urethral pathogens. However, pathogen-negative cases reported greater sexual risks than did controls, had symptoms similar to those in other cases, and had a significant association with casual

Table 6. Associations with detection of *Gardnerella vaginalis* and *Ureaplasma parvum* in the urine of pathogen-negative controls (*n* = 294).

Feature	<i>G. vaginalis</i>				<i>U. parvum</i>			
	Negative (<i>n</i> = 204)	Positive (<i>n</i> = 90)	Crude OR (95% CI)	Adjusted OR (95% CI)	Negative (<i>n</i> = 251)	Positive (<i>n</i> = 43)	Crude OR (95% CI)	Adjusted OR (95% CI)
Age								
≤31 years	112 (55)	53 (59)	1.0	1.0	140 (56)	25 (58)	1.0	1.0
>31 years	92 (45)	37 (41)	0.9 (0.5–1.4)	1.0 (0.6–1.8)	111 (44)	18 (42)	0.9 (0.5–1.7)	0.9 (0.4–1.8)
Any UPVS in past month								
No	116 (57)	26 (30)	1.0		131 (53)	11 (26)	1.0	
Yes	87 (43)	60 (70)	3.1 (1.8–5.3)	...	115 (47)	32 (74)	3.3 (1.6–6.9)	...
Any UPVS								
≤14 days ago	54 (26)	48 (54)	1.0	1.0	80 (32)	22 (51)	1.0	1.0
>14 days ago	150 (74)	41 (46)	0.3 (0.2–0.5)	0.4 (0.2–0.8)	170 (68)	21 (49)	0.4 (0.2–0.9)	0.5 (0.2–1.0)
Any oral sex in past month								
No	48 (24)	12 (14)	1.0	1.0	51 (21)	9 (21)	1.0	1.0
Yes	155 (76)	77 (86)	2.0 (1.0–4.0)	1.6 (0.7–3.6)	198 (80)	34 (79)	1.0 (0.4–2.1)	0.7 (0.3–1.8)
Any UPAS in past month								
No	163 (81)	73 (82)	1.0	1.0	201 (81)	35 (83)	1.0	1.0
Yes	39 (19)	16 (18)	0.9 (0.5–1.7)	0.7 (0.4–1.6)	48 (19)	7 (17)	0.8 (0.3–2.0)	0.9 (0.4–2.4)
<i>U. parvum</i>								
Negative	185 (91)	66 (73)	1.0	1.0
Positive	19 (9)	24 (27)	3.5 (1.8–6.9)	5.0 (2.4–10.6)
<i>G. vaginalis</i>								
Negative	185 (74)	19 (44)	1.0	1.0
Positive	66 (26)	24 (56)	3.5 (1.8–6.9)	5.1 (2.4–10.7)
<i>U. urealyticum</i> (biovar 2)								
Negative	186 (91)	57 (63)	1.0	1.0	203 (81)	40 (93)	1.0	1.0
Positive	18 (9)	33 (37)	6.0 (3.1–11.4)	7.6 (3.7–15.6)	48 (19)	3 (7)	0.3 (0.1–1.0)	0.1 (0–0.5)

NOTE. CI, confidence interval; OR, odds ratio; UPAS, <100% condom use for insertive anal sex; UPVS, <100% condom use for vaginal sex. "Past month" denotes the month before the onset of symptoms. "Pathogen negative" means that controls with *C. trachomatis*, *M. genitalium*, or adenoviruses (*n* = 12) were excluded from the analysis. Median time since last UPVS in controls, 14 days. Data on as many as 5 subjects were missing in the univariate analysis. Variables in the adjusted analysis included age, UPVS with any partner within 14 days, any oral sex, any UPAS in past month, and *U. urealyticum*. *U. parvum* was included in the analysis for *G. vaginalis*. Data on 290 subjects were included in the adjusted analysis, and data on 4 were incomplete and, therefore, excluded.

unprotected oral and vaginal sex, suggesting that infection with potential pathogens not identified in this study is likely. Although cases represented 69% of men with acute NGU who attended MSHC, we were not able to determine the enrollment rate for controls. We consider it unlikely that there was a significant selection bias, but it is possible that controls were not representative of all eligible men attending MSHC. A urethral Gram stain was not performed in controls, because of the poor acceptance of the urethral smear in asymptomatic men. A study of NGU at MSHC in 2003 (*n* = 160) [56] showed that 18% of asymptomatic controls had microscopic evidence of urethritis, which was not associated with the detection of urethral pathogens. It is possible that the inclusion of controls with asymptomatic urethritis may have reduced our ability to detect clinical and behavioral associations with NGU.

In conclusion, we have identified adenoviruses and HSV-1 as significant causes of NGU, in addition to *C. trachomatis* and *M. genitalium*. We found that distinctive clinical features were associated with viral pathogens and that insertive oral sex and male partners were associated with adenoviruses and HSV-1 in

the crude analysis. Insertive oral sex was also a significant risk factor in pathogen-negative NGU. These findings raise the potential importance of the oropharynx as a significant source of bacterial and viral pathogens and indicate that we should broaden our search for pathogens in NGU. A urethral PMNL count of ≥5 PMNLs/HPF does not appear to be sufficiently sensitive to exclude urethral infection in NGU. Our data suggest that treatment decisions are best based on clinical features of urethritis and not solely on microscopic assessment.

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