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CLINICAL REPORT

Urethritis-associated Pathogens in Urine from Men with Non-gonococcal Urethritis: A Case-control Study

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The aetiology of non-gonococcal urethritis (NGU) remains unexplained in 30–40% of patients. Urine samples from men attending Swedish sexually transmitted disease clinics were examined by species-specific quantitative PCRs for *Chlamydia trachomatis, Mycoplasma genitalium, Trichomonas vaginalis, Ureaplasma urealyticum, U. parvum*, adenovirus, herpes simplex virus, *Neisseria meningitidis, Haemophilus influenzae, Moraxella catarrhalis* and *Streptococcus pneumoniae*. A total of 187 men with acute NGU (symptoms ≤30 days) and 24 with chronic NGU (symptoms >30 days) were cases, and 73 men without NGU were controls. Number of lifetime sexual partners was negatively associated with *U. urealyticum* bacterial load. *C. trachomatis* and *M. genitalium* were associated with NGU, as was *U. urealyticum*, with bacterial loads $\geq 1.3 \times 10^3$ genome equivalents/ml urine. Virus and *H. influenzae* might explain a few NGU cases, but the aetiology in at least 24% of patients with acute NGU was unexplained. In multivariate analysis, detection of *U. urealyticum* was significantly more common in acute NGU (20%) compared with controls (11%). *Key words: non-gonococcal urethritis; qPCR;* ureaplasma urealyticum*;* Mycoplasma genitalium.

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Non-gonococcal urethritis (NGU) is one of the most common sexually transmitted diseases (STDs) in men. NGU is characterized by inflammation of the urethra and is often associated with symptoms such as discharge, urethral itching and dysuria. *Chlamydia trachomatis* and *Mycoplasma genitalium* infections account for approximately half of NGU cases. *Trichomonas vaginalis* is a common NGU pathogen in some parts of the world, but appears to be rare in Northern Europe (1, 2). Other less common aetiologies are herpes simplex virus type 1 and 2, and human adenovirus (3–5). The role of ureaplasmas in male NGU has been examined in several studies (6–8), but since ureaplasmas are part of the urethral flora in more than 40% of healthy, sexually active men (9), their importance remains controversial. Using molecular biology techniques, the ureaplasmas have been divided into 2 separate species, *Ureaplasma urealyticum* and *U. parvum* (10, 11). In some studies *U. urealyticum*, but not *U. parvum*, appears to be associated with NGU (12, 13), but simple qualitative detection of *U. urealyticum* has not uniformly been associated with urethritis (4). Recently, a high bacterial load of *U. urealyticum* in first-void urine (FVU) has been associated with male urethritis (14, 15). On the other hand, it has been shown that repeated exposure to *U. urealyticum* leads to diminished urethritis signs and symptoms (16), and that the bacterium is more strongly associated with NGU in men with fewer lifetime sexual partners (17). Thus, there is a need for further studies on the role of the ureaplasmas.

After excluding known NGU pathogens, more than one-third of NGU cases remain unexplained. Over the years, a number of other pathogens, such as *Haemophilus* sp. (18, 19), *Neisseria meningitidis* (20–22), *Moraxella catarrhalis* (23, 24) and *Streptococcus pneumoniae* (25, 26), have been associated with male NGU, but few controlled studies have been undertaken.

In this study, FVU samples from male patients with and without NGU were analysed by quantitative PCRs (qPCRs) specific for 13 known or putative urethritis pathogens to study their association with urethritis.

METHODS

Patients and specimens

In this case-control study of aetiologies of NGU, male patients attending 3 urban STD clinics in Stockholm, Sweden between June 2008 and April 2012 were enrolled after providing informed consent. The Regional Ethics Committee for Stockholm approved the study (Protocol: 2008/695-31/2; approval date May 4, 2008).

Patients were enrolled as cases if they had dysuria, self-reported or observed urethral discharge and a urethral smear showing ≥10 polymorphonuclear leukocytes (PMNL) per high-power (×1,000) microscopic field (hpf), and as controls if no urethritis symptoms were reported and <5 PMNLs/hpf were found by microscopy. The controls were recruited among men attending the clinic for dermatological problems, such as eczema, as venereologists in Sweden see these patients, and among men attending for condyloma treatment. The cases

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were classified as acute NGU if they had symptoms lasting for 1–30 days and as chronic NGU if their symptoms had lasted for more than 30 days. Exclusion criteria were: suspicion of gonorrhoea by microscopy, age below 18 years, sexual contact with a partner with known *C. trachomatis, N. gonorrhoeae* or *M. genitalium* infection, antibiotic treatment within the last 3 months, or voiding < 2 h before the examination. All participants were seen by the same 2 clinicians (PL and AW).

The type and duration of symptoms, number of sexual partners within the last 6 months and during their lifetime, information about sexual preference, and unprotected anal or oral sex within the last 6 months, were recorded.

After the clinical examination, approximately 20 ml of FVU was collected, and 7–13 ml was mailed to Copenhagen. Details of the microbiological methods are given in Appendix S1[1].

Statistical analysis

Fisher's exact test and odds-ratios (OR) with 95% confidence intervals (CI) were used for dichotomous variables. The Mann-Whitney test was used for continuous variables when comparing 2 groups. Where more than 2 groups were compared, the Kruskal–Wallis test was used. All were calculated in StatsDirect version 2.7.8 (StatsDirect Ltd, Cheshire, UK). Significance level was 5% and 2-sided results were used throughout.

Receiver operating characteristic (ROC) curve analysis was used to determine optimal cut-off for the *U. urealyticum* bacterial load in genome equivalents (geq)/ml of the original urine sample in controls and NGU patients. Simple regressions were calculated in StatsDirect and visualized using GraphPad Prism 6 Software, Inc. (La Jolla, CA, USA). Tests for linearity, variance homogeneity and normal distribution of data were carried out for all simple regressions. Logistic regressions were calculated in Stata (version 12, StataCorp, TX, USA).

RESULTS

Study population characteristics

A total of 284 patients fulfilled the inclusion criteria and had FVU samples available for analysis. Of those, 211 were NGU patients and 73 were asymptomatic controls. Of the 211 NGU patients, 187 had acute NGU and 24 had chronic NGU. The median age was 28 years (age range 19–64 years) in the control group, and 28 (range 19–66) and 27 (range 20–43) years in the patients with acute and chronic NGU, respectively, and did not differ between the groups ($p = 0.61$) (Table I). The median number of sexual partners within the past 6 months was 2 in all groups (mean 2.7, 3.4 and 2.2, respectively), but patients with acute NGU statistically had a significantly higher number of partners than men with chronic NGU ($p = 0.004$, Kruskal–Wallis; $p = 0.006$, Mann–Whitney between acute and chronic NGU) (Table I). The median number of lifetime sexual partners was 25 in the controls, 30 in the patients with acute NGU, and 15 in the group with chronic NGU ($p = 0.03$, Kruskal–Wallis). With pairwise comparison, both controls and acute NGU patients had had more lifetime sexual partners than had the patients with chronic NGU ($p = 0.03$ and $p = 0.01$, respectively, Mann-Whitney) (Table I).

Table I. *Patient characteristics*

Characteristics	Asymptomatic controls ($n = 73$)	Non-gonococcal urethritis (NGU)		
		Acute symptomatic[a] ($n = 187$)	Chronic symptomatic[b] ($n = 24$)	p-value
Age, years	28 (19–64)	28 (19–66)	27 (20–43)	0.61
Number of of sexual partners				
<6 months	2 (0–15)	2 (1–24)[c]	2 (1–9)	0.004
Lifetime partners	25 (2–150)[d]	30 (1–200)[e]	15 (2–55)[f]	0.03
Symptoms				
Dysuria	–	156 (83)	23 (96)	–
Objective discharge	–	167 (89)	16 (54)	–
Subjective discharge	–	121 (65)	14 (58)	–
Oral sex (yes/no)[g]	27/6 (82)	54/17 (76)	7/0 (100)	0.43
Anal sex (yes/no)[g]	5/28 (15)	9/59 (13)	3/3 (50)	0.08
MSM	1 (1)	4 (2)	0	>0.99
Previous sexually transmitted disease				
C. trachomatis	14 (29)	61 (33)	4 (17)	0.05
M. genitalium	2 (3)	10 (5)	2 (8)	0.40
N. gonorrhoeae	1 (1)	4 (2)	1 (4)	0.62
HSV	3 (4)	8 (4)	0	0.79
Condyloma	31 (43)	25 (14)	3 (13)	<0.0001
Other NGU	4 (6)	29 (16)	2 (8)	0.07
Never	23 (32)	74 (40)	10 (42)	0.45
Not determined	1 (1)	11 (6)	4 (17)	0.03

Continuous variables are summarized as median (range) and categorical variables (yes/no) as *n* (%). *p*-values are from multiple comparisons between all groups using the non-parametric Kruskal–Wallis test.
[a]NGU symptoms 1–30 days. [b]NGU symptoms >30 days. [c–f]No information for 5, 8, 57 and 8 patients, respectively. [g]Patient information obtained only for the "yes/no".
HSV: herpes simplex virus. MSM: men who have sex with men.

In the group of men with chronic NGU, the reported median duration of symptoms was 60 days (range 35–400).

As patients attending the clinic for condyloma treatment were included as controls, they appeared with a higher frequency in the control group compared with the NGU groups ($p = 0.0001$, Kruskal–Wallis) (Table I). None of the patients were HIV-positive.

Association of C. trachomatis *with NGU*

None of the controls had *C. trachomatis* detected, while 33% of the 187 men with acute NGU were positive ($p < 0.0001$). *C. trachomatis* was also found in 13% of chronic NGU ($p = 0.014$) (Table SI[1]). The proportion of patients with new variant *C. trachomatis* (27) was not statistically different in the patients with acute NGU (13%) and chronic NGU (33%) ($p = 0.40$). No difference in the organism load was found between patients infected with wild-type and new-variant *C. trachomatis* (median bacterial loads of 6.6×10^5 geq/ml and 2.6×10^5 geq/ml, respectively ($p = 0.35$)).

Association of M. genitalium with NGU

M. genitalium was associated with acute and chronic NGU (both $p < 0.0001$) in univariate analysis (Table

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SI[1]). Patients with symptomatic NGU (acute and chronic) had higher bacterial loads (median 1.9×10^4 geq/ml in acute NGU and 1.6×10^4 in chronic NGU) than the controls (median 44 geq/ml) ($p = 0.007$ and $p = 0.04$, Mann-Whitney) (Table SI[1]). Only 2 (3%) *M. genitalium* positive patients carried *M. genitalium* with macrolide resistance mediating mutations.

Association of U. urealyticum *and* U. parvum *with NGU*

U. urealyticum was detected with a similar prevalence in the controls (11%), acute NGU (20%), and chronic NGU (13%) (not significant [NS]) as was *U. parvum* (18%, 16%, and 25%, respectively; NS) (Table SI[1]).

In a multivariate model adjusting for all tested pathogens *U. urealyticum* was associated with acute NGU with adjusted odds ratios (ORadj) 3.5 ($p = 0.01$ (95% CI 1.3–8.9)), but not with chronic NGU. *U. parvum* was found more often in chronic NGU with ORadj 5.4 in the multivariate model ($p = 0.039$ (95% CI 1.09–27.1)) (Table II).

Table II. *Adjusted odds ratios (ORadj) with 95% confidence intervals (CI) and* p*-values in acute and chronic non-gonococcal urethritis (NGU) after multivariate logistic regression analysis for 10 possible NGU pathogens*[a]

	Acute NGU[b]			Chronic NGU[c]		
	ORadj	95% CI	*p*-value	ORadj	95% CI	*p*-value
M. genitalium	33	7.6–144	<0.0001	91	14–589	<0.0001
U. urealyticum	3.5	1.34–8.9	0.01	2.5	0.3–21	0.41
U. parvum	1.6	0.66–3.9	0.3	5.4	1.1–27	0.04
HSV 1+2	8.8	1.0–76	0.049	[d]	[d]	[d]
S. pneumoniae	0.4	0.04–3.4	0.37	0.6	0.02–14	0.72

[a]*C. trachomatis, T. vaginalis, H. influenzae, M. catharralis, N. meningitidis* and adenovirus were excluded from the model as none of the controls were positive and therefore predicted success perfectly. [b]NGU symptoms 1–30 days. [c]NGU symptoms > 30 days. [d]Herpes simplex virus (HSV) was excluded from the analysis as none of the chronic NGU patients were positive and therefore predicted success perfectly.

Quantitative detection of U. urealyticum *in patients with NGU*

Bacterial loads of *U. urealyticum* were higher in both acute NGU (median 6.7×10^3 geq/ml) and chronic NGU (median 5.5×10^4 geq/ml) when compared with the control group (median 313 geq/ml) ($p = 0.002$ and $p = 0.02$, respectively), whereas the bacterial load of *U. parvum* was similar in the NGU groups and the control group. (Table SI[1]). As *U. urealyticum* was found to be associated with acute NGU in multivariate analysis, ROC curve analysis was used to determine the cut-off for the *U. urealyticum* bacterial load optimally predicting acute NGU. The optimal cut-off was 1.3×10^3 geq/ml urine. The area under the curve (AUC) was 84%, suggesting a relatively good discriminatory power. With the cut-off applied, 1 control (1%) and 30 patients (16%) with acute NGU were positive for *U. urealyticum* ($p = 0.0004$ (OR14 (95% CI 2.2–569)) (Table III). In the multivariate model a *U. urealyticum* load ≥ 1,325 geq/ml was associated with acute NGU ($p = 0.006$ ORadj 18.6 (95% CI 2.3–150))) (Table IV).

In men with chronic NGU, high loads of *U. urealyticum* were found in 3 patients (13%), ($p = 0.049$ (OR 10 (95% CI 0.7–532))) (Table II). In the multivariate model a *U. urealyticum* load ≥ 1,325 geq/ml was associated with chronic NGU ($p = 0.048$ (ORadj 19 (95% CI 1–368)), as was *M. genitalium* ($p < 0.0001$), and *U. parvum* ($p = 0.03$ (ORadj 5.4 (95% CI 1.1–27))) (Table IV).

U. urealyticum *bacterial load, age and number of lifetime sexual partners*

Forty-nine (23%) of the 211 men included in the study were *U. urealyticum* positive and had a median age of 25 years (range 19–57 years). There was no linear association between age and *U. urealyticum* bacterial load ($p = 0.89$) in the 49 men. *U. urealyticum* positive NGU patients were significantly younger (median 25 years) than *U. urealyticum* negative NGU patients (median 29 years) ($p = 0.0005$). Also, they were younger than the *C. trachomatis* positive patients (median 27 years) ($p = 0.04$). The number of lifetime sexual partners did not differ between *U. urealyticum* positive and negative NGU patients.

Information about the number of lifetime sexual partners was available for 36 *U. urealyticum* positive patients. Three men having sex with men were excluded from the analysis, leaving 33 heterosexual men (median 30 lifetime sexual partners, range 2–150). There was a significant negative linear association between the number of lifetime sexual partners and the *U. urealyticum* bacterial load ($p = 0.037$). The predicted *U. urealyticum* bacterial load decreased with 2.2% for each additional sexual partner (Fig. 1).

Table III. *Acute[a] and chronic[b] non-gonococcal urethritis (NGU) patients compared with the controls after applying a receiver operating characteristic (ROC) cut-off to* U. urealyticum *positives at ≥1,325 geq/ml*

	Control ($n = 73$)	Acute NGU ($n = 187$)	OR (95% CI)	*p*-value	Chronic NGU ($n = 24$)	OR (95% CI)	*p*-value
U. urealyticum	1 (1)	30 (16)	14 (2.2–569)	0.0004	3 (13)	10 (0.7–532)	0.049
Geq/ml[c]	30,800	22,463	–	NS	55,450	–	NS

[a]NGU symptoms 1–30 days. [b]NGU symptoms >30 days. [c]Median organism load (geq/ml urine) for positive qPCR result.
Continuous variables are summarized as median and categorical variables (positive/negative) as number and (%).
OR: odds ratio; CI: confidence interval; Geq: genome equivalents; NS: not significant.

Table IV. *Adjusted odds ratios (ORadj) with 95% confidence intervals (95% CI) and* p*-values in acute and chronic non-gonococcal urethritis (NGU) after multivariate logistic regression analysis for 10 possible NGU pathogens[a] and a receiver operating characteristic (ROC) cut-off at ≥1,325 geq/ml urine for* U. urealyticum *positive*

	Acute NGU[b]			Chronic NGU[c]		
	ORadj	95% CI	*p*-value	ORadj	95% CI	*p*-value
M. genitalium	31	7.1–135	<0.0001	91	14–620	<0.0001
U. urealyticum ≥1,325 geq/ml	18.6	2.3–150	0.006	19	1–368	0.048
U. parvum	1.6	0.7–3.9	0.3	5.4	1.1–27	0.03
HSV 1+2	8.6	0.99–74	0.052	[d]	[d]	[d]
S. pneumoniae	0.3	0.03–3.3	0.36	0.6	0.03–14	0.75

[a]*C. trachomatis, T. vaginalis, H. influenzae, M. catharralis, N. meningitidis* and adenovirus were excluded from the analysis as none of the controls were positive and therefore predicted success perfectly. [b]NGU symptoms 1–30 days. [c]NGU symptoms >30 days. [d]Herpes simplex virus (HSV) was excluded from the analysis as none of the chronic NGU patients were positive and therefore predicted success perfectly.

T. vaginalis

T. vaginalis was not detected in controls, but in 2 (1%) men with acute NGU (NS). One was co-infected with *M. genitalium* and the other with *C. trachomatis* (Table SI[1]).

HSV-1 and -2 and adenovirus

One control patient with external penile lesions on clinical examination and with known recurrent genital herpes was positive for HSV-2 (Table SI[1]). HSV was detected in 8 (4%) of men with acute NGU (Table SI[1]). Three of those had external lesions. Logistic regression showed an association of HSV with acute NGU (ORadj 9.1 (95% CI 1.1–77) ($p = 0.04$)) (Table II).

Adenovirus was not detected in any of the 73 controls, but in 5 (3%) of men with acute NGU (NS) (Table SI[1]). Four were adenovirus D and 1 adenovirus B. All cases were detected in mid-August to late-March.

S. pneumoniae, H. influenzae, N. meningitidis *and* M. catarrhalis *in NGU*

S. pneumoniae was detected more frequently in control patients than in patients with acute NGU (Table SI[1]). A trend towards a higher *H. influenzae* detection rate was found in acute NGU (5%) compared with controls (0%) ($p = 0.07$). *H. influenzae* was not detected in men with chronic NGU. Of the 9 *H. influenzae* positive patients, 1 was co-infected with *M. genitalium* (25 geq/ml) and 2 with adenovirus (1 type B and 1 type D). The adenovirus type D positive patient was further co-infected with *U. urealyticum* (7.1×10^4 geq/ml), making the main aetiology difficult to determine. *N. meningitidis* was detected in one patient (0.5%) with acute NGU and in none of the other groups (NS); this patient also had *U. urealyticum* detected (5.5×10^2 geq/ml).

M. catarrhalis was detected in 3 patients (2%) with acute NGU and 1 (4%) with chronic NGU, which did not differ from the controls (0%) (Table SI[1]).

Pathogen-negative NGU

Pathogen-negative cases, i.e. patients negative for *C. trachomatis, M. genitalium, U. urealyticum, T. vaginalis*, HSV-1 and -2, and adenovirus, accounted for 24% (45 of 187) and 33% (8 of 24) in the groups with acute and chronic NGU, respectively (NS).

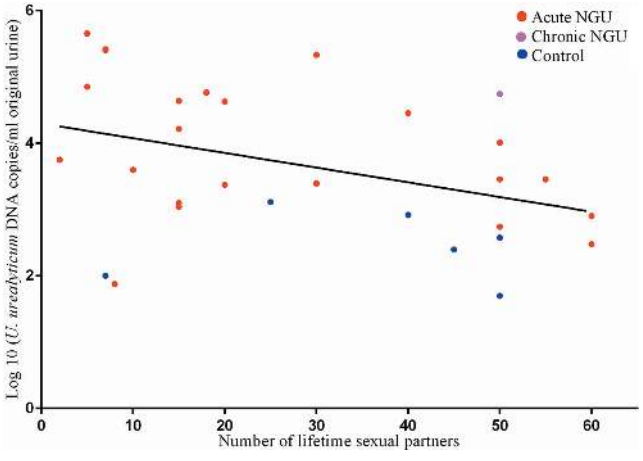

Fig. 1. Scatterplot showing number of lifetime sexual partners in 30 men infected with *U. urealyticum* with acute NGU (*red*), chronic NGU (*pink*) and controls (*blue*), and the *U. urealyticum* bacterial load/ml. The regression line is depicted ($p = 0.037$). Three outliers, with 100, 120 and 150 lifetime sexual partners, were excluded from the analysis.

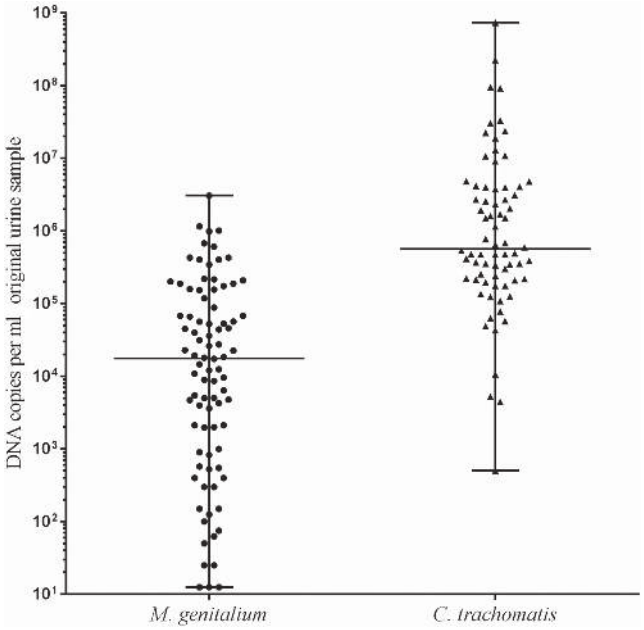

Fig. 2. Scatterplot showing *C. trachomatis* and *M. genitalium* log10 bacterial load per ml first-void urine from men. Medians are depicted by a horizontal bar ($p < 0.0001$ by Mann–Whitney).

Diagnostic consequences of differences in bacterial load between C. trachomatis *and* M. genitalium

C. trachomatis was detected with a 42-fold higher median bacterial load than *M. genitalium* in all patient groups combined; 5.9×10^5 (range 5.0×10^2–7.4×10^8) geq/ml and 1.4×10^4 (range 1.3×10^1–3.1×10^6) geq/ml ($p < 0.0001$) (Fig. 2). With a limit of detection at 1×10^3 geq/ml urine, corresponding to testing unconcentrated urine, 99% (68 of 69) of *C. trachomatis* infections would be diagnosed, compared with only 74% (61 of 82) of *M. genitalium* infections ($p < 0.0001$ (OR 23 (95% CI 3.5–982))).

DISCUSSION

In this case-control study, we screened for 13 different putative NGU pathogens by PCR to determine the relevance of the organisms in men with and without urethritis.

Strengths of the present study are the well-characterized cases and controls according to symptoms, and a highly standardized smear evaluation with the exclusion of low-grade NGU (5–9 PMNLs/hpf), which ensure a reliable distinction between NGU cases and asymptomatic controls. Two experienced clinicians evaluated the urethral smears, ensuring low observer variability. Patients with low-grade urethritis were not included, as they may be at higher risk of being misclassified. However, this also points to a weakness of the study, as this approach may introduce selection bias. We feel, however, that for a study aiming to elucidate the role of new putative pathogens, the lowered risk of misclassification outweighs the risk of selection bias.

As expected, both *C. trachomatis* and *M. genitalium* were strongly associated with urethritis, but in contrast to several other studies, *M. genitalium* was as common as *C. trachomatis* in the group with acute NGU. This may, to some extent, be explained by the exclusion of men attending the clinic for partner tracing or with a known *C. trachomatis* positive contact.

Patients infected with *M. genitalium* carrying macrolide resistance mediating mutations in the 23S rRNA gene were very few in this study (3%). The low prevalence of resistance most likely reflects the Swedish standard treatment recommendations for NGU, where doxycycline is used as first-line treatment instead of the widely used single 1 g dose of azithromycin (28, 29), which has been suggested to select for macrolide-resistant strains (28–31). In striking contrast, neighbouring Denmark, where azithromycin is used as first-line therapy, has 40% resistance (32).

C. trachomatis was detected with a 42-fold higher median bacterial load than *M. genitalium*, confirming previous studies (33, 34) and emphasizing the importance of *M. genitalium* diagnostic assays with a very low limit of detection. It is likely that, even with well-optimized assays, false-negative tests will occur, explaining occasional findings of eradication of macrolide-resistant *M. genitalium* with azithromycin. New variant *C. trachomatis* accounted for 13% of the acute chlamydial infections. In these patients, urethritis symptoms, as well as organism load, did not differ from infections with wild-type *C. trachomatis*.

Recent studies have suggested that *U. urealyticum* is present in larger quantities in men with NGU (14, 15), and these findings were further supported by the present study. In multivariate analysis, the mere detection of *U. urealyticum* was significantly associated with acute NGU, but not with chronic NGU. Since *U. urealyticum* is also common in men without NGU, we speculated, that introducing an objectively determined cut-off by ROC curve analysis would increase the specificity of detection of *U. urealyticum* in a clinical setting. According to the ROC curve analysis, the detection of $\geq 1.3 \times 10^3$ geq/ml urine of *U. urealyticum* should be considered clinically relevant in patients with acute and chronic NGU with ORadj 18.6 and 19, respectively. Interestingly, the cut-off at approximately 1×10^3 bacteria/ml of urine corresponds well with earlier suggestions based on culture of undifferentiated ureaplasmas (7, 35), and we have recently documented the qPCR assay for ureaplasma to be accurate compared with culture of ureaplasmas (36).

We also found that the *U. urealyticum* bacterial load in urine from men declined significantly with increasing numbers of lifetime sexual partners, and that men with *U. urealyticum* positive NGU were younger than those with NGU of other aetiologies. This provides support for the hypothesis that adaptive immunity reduces the clinical symptoms, and may explain the high number of colonized individuals (7, 17).

Surprisingly, a high load of *U. parvum* was found to be associated with chronic NGU. A similar observation was made by Deguchi et al. (37) in a study using flow cytometry to quantify leukocytes more precisely. This could suggest that even though *U. parvum* colonization in most men is asymptomatic, high loads may occasionally lead to urethral inflammation. It could also be speculated, however, that *U. parvum* was only a surrogate marker for other bacterial vaginosis-associated bacteria that may cause urethritis, such as suggested by Manhart et al. (38). This merits further studies, preferably including more patients.

Human adenovirus was detected only in acute NGU cases and not in controls. We detected subgenus D and B in 3% of acute NGU, which agrees well with the findings of Tabrizi et al. (5). All adenovirus cases occurred from mid-August to late-March, which corresponds to autumn to early-spring in Sweden; an earlier study has shown adenovirus in NGU to have seasonal variation (39).

A trend was found towards association of *H. influenzae* with acute NGU possibly accounting for 5% of

cases. Interestingly, empirical antibiotic treatment with doxycycline might have a better effect than azithromycin for this aetiology, as susceptibility to tetracyclines is much higher than for macrolides (40).

There was no association between NGU and the respiratory pathogens *S. pneumoniae, M. catarrhalis* and *N. meningitidis*, but only very few positives were detected and, therefore, this study might not have enough power to show such association. Most of the cases described in the literature, associating respiratory bacteria with NGU by culture, have not been examined for well-known NGU aetiologies, such as *C. trachomatis* and *M. genitalium*. Larger studies are needed to clarify the role of respiratory bacteria in NGU; however, according to our findings, the diagnostic yield of screening would be low.

Using PCR analysis for multiple pathogens, Manhart et al. found *Leptotrichia/Sneathia* to be associated with idiopathic NGU, although their exact role remains to be determined (38). We did not examine for bacterial vaginosis-associated bacteria in this study; this area requires further elucidation. Finally, inflammation of the urethra may not always be directly mediated by infection (41).

In the group with chronic NGU as many as 50% of patients were *M. genitalium* positive. Although the patients had not received antibiotics within the previous 3 months, it cannot be excluded that some had received doxycycline for a suspected chlamydial infection previously, or, alternatively, had been seen by physicians only testing for *C. trachomatis*, thus delaying the test result for *M. genitalium*.

In this study, 24% of the 187 men with acute NGU were negative for *C. trachomatis*, *M. genitalium*, *U. urealyticum, T. vaginalis*, HSV-1 and -2, and adenovirus, and rightly deserve the classification of idiopathic urethritis. Although new technologies, such as microbiome studies, will probably not provide all the answers, they could be a tool in the search for infectious causes of idiopathic urethritis, leading to better treatment and less frustration for patients.

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