

ORIGINAL ARTICLE

Symptomatic urethritis is more prevalent in men infected with *Mycoplasma genitalium* than with *Chlamydia trachomatis*

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Objectives: To study the prevalence, symptoms, and signs of *Mycoplasma genitalium* and *Chlamydia trachomatis* infections in men attending a Swedish STD clinic and to study the criteria for urethritis.

Methods: A cross sectional study among STD clinic attendees in Örebro, Sweden. Attendees were examined for microscopic urethritis and first void urine (FVU) was tested for *M genitalium* and *C trachomatis*.

Results: The prevalence of *M genitalium* and *C trachomatis* was 7% (34/512) and 12% (61/512), respectively. Dual infection was diagnosed in four men. In both infections 90% of the patients had signs of microscopic urethritis. *M genitalium* positive men had symptomatic urethritis significantly more often than those infected with *C trachomatis* (73% v 40%, RR 1.8; 95% CI 1.2 to 2.7). 63% of female partners of men infected with *M genitalium* were infected with *M genitalium* compared with chlamydial infection in 67% of female partners of men infected with *C trachomatis*. Non-chlamydial non-gonococcal urethritis without evidence of *M genitalium* infection was diagnosed in 180 men (35%). Symptoms and/or visible discharge were reported in 49% in this group.

Conclusions: *M genitalium* is a common infection associated with symptomatic urethritis and with a high prevalence of infected sexual partners supporting its role as a sexually transmitted infection.

M*ycoplasma genitalium* was isolated originally from the urethra of two men with non-gonococcal urethritis (NGU) in 1980.^{1, 2} Isolation of this bacterium is very difficult but the use of polymerase chain reaction (PCR) technology has consistently shown *M genitalium* to be a major cause of sexually transmitted non-chlamydial non-gonococcal urethritis (NCGU) among men.^{3–9} There is also increasing evidence suggesting that *M genitalium* causes mucopurulent cervicitis in women¹⁰ and that it may cause endometritis¹¹ and possibly tubal infection with sequelae in the form of ectopic pregnancy or tubal infertility.¹² Thus, the role of *M genitalium* may not significantly differ from that of genital *Chlamydia trachomatis* infection. Most *M genitalium* studies in STD clinic outpatients have focused on symptomatic patients with urethritis and have used non-symptomatic patients as controls. These studies demonstrate that *M genitalium* is detected significantly more frequently among symptomatic patients than among asymptomatic controls, thus indicating that this bacterium is a pathogen of the genital tract.^{3, 6, 13} The role of *C trachomatis* and *Neisseria gonorrhoeae* as pathogens is well established.^{14–17} In contrast with *M genitalium*, the role of *Ureaplasma urealyticum* appears to be less clearly defined.^{7, 18, 19}

The aim of this cross sectional study was to compare *C trachomatis* and *M genitalium* infections in terms of signs and symptoms in male STD clinic attendees and to study the prevalence of the bacteria and the rate of infection among sexual partners. A secondary aim was to study the benefit of microscopic examination of urethral smears.

METHODS

Patients

During a 6 month study period from 1 February 2000 to 31 July 2000, all male attendees at the Örebro University Hospital STD clinic were included. Data were collected on a standard questionnaire regarding the reasons for attendance,

age, symptoms of urethritis (dysuria and discharge), number of sexual partners within the past 6 months, condom use, sexual intercourse with men, history of STIs, probable STIs among the partners, recent or current antibiotic treatment, and other diseases.

Sampling

A total of 519 men between 16 and 67 years of age (median 27 years) were included. Smears were taken with a blunt curette from the distal urethra and stained with methylene blue. The amount of exudate recovered was estimated, using profuse as a definition of discharge (>≈1 cm² of a single cell layer smear on the slide), moderate and poor as a graduation within the normal amount of secretion. During the study period, the patients were seen by seven clinicians, but four of those examined 95% of all patients. All smears were examined microscopically (1000×) with a Nikon Labophot microscope. The definition of urethritis is generally >4 polymorphonuclear leucocytes (PMNL) per high power field (HPF) in more than four high power fields,¹⁷ but in the current study, smears with 5–10 PMNL per HPF were defined as “grey zone urethritis” and >10 PMNL per HPF as urethritis. After urethral smear was sampled the first void urine (FVU) was collected for *C trachomatis* and *M genitalium* tests and distributed in two screw capped 13 ml polypropylene tubes (Sarstedt, Nümbrecht, Germany). Partners, women and men, attending the STD clinic during the study were all examined for *C trachomatis* and *M genitalium*.

Microbiological analysis

One of the tubes containing 5–10 ml of urine was sent the same day to the department of clinical microbiology, Örebro University Hospital, and stored at 2–8°C. The urine samples were tested by the Cobas Amplicor *Chlamydia trachomatis* Test (Roche Diagnostics Systems, Inc, Branchburg, NJ, USA) as described by the manufacturer.

Table 1 Symptoms (self reported dysuria and/or discharge), signs (observed discharge—that is, ≥ 1 cm² smear on the slide and urethritis (>4 PMNL/HPF)), number of partners, and STI history among all male attendees (n = 512)

	Urethral smears (PMNL/HPF)			Symptoms of urethritis			Number of partners				
	Count (%)						Past 6 months		STI history (%)		
	<5	5–10	>10	ND	Count (%)	RR (CI 95%)	Mean	Median (range)	Ng	Ct	NCNGU
<i>C trachomatis</i> pos (n = 57)	4	0	53 (93)	0	23 (40)	1 (reference)	2.8	2 (0–10)	1 (2)	9 (16)	11 (19)
<i>M genitalium</i> pos (n = 30)	3	0	27 (90)	0	22 (73)	1.8 (1.2 to 2.7)	2.1	2 (1–6)	1 (3)	9 (30)	4 (13)
Mg and Ct pos (n = 4)	0	0	4 (100)	0	3 (75)		7.5	3.5 (2–20)	0 (0)	1 (25)	1 (25)
Urethritis, negative tests (n = 180)	0	30	150 (83)	0	49 (27)	0.7 (0.4 to 1.0)	2.1	2 (0–10)	6 (3)	38 (21)	44 (24)
The rest of attendees (n = 241*)	235	0	1 (1)	5	36 (15)	0.4 (0.2 to 0.6)	1.8	2 (0–9)	12†(5)	33†(14)	37†(15)

*One (homosexual) man had a gonococcal urethritis †Data were missing from one patient.

ND = microscopic examination not done; Ng = *Neisseria gonorrhoeae*; Ct = *Chlamydia trachomatis*; Mg = *Mycoplasma genitalium*; NCNGU = non-chlamydial non-gonococcal urethritis.

The other tube containing 5–10 ml FVU was sent the same day by express mail to Statens Serum Institut, Copenhagen, Denmark for *M genitalium* PCR test. *M genitalium* was detected by an inhibitor controlled PCR using primers detecting the *M genitalium* 16S rRNA gene.²⁰ All positive results were confirmed by a PCR detecting the MgPa adhesin gene.²¹ Samples for *Neisseria gonorrhoeae* (culture) were taken from 88 men. Samples were taken selectively on certain indications—that is, unprotected sexual contacts abroad, purulent discharge, unprotected sexual contacts between men, and partner notification because of gonorrhoea, and not as a screening test because of the current low incidence in Sweden (0.7/100 000 inhabitants—that is, 588 cases in 2000).²²

Follow up

All patients infected with *C trachomatis* and/or *M genitalium* were asked to re-attend for a follow up visit 4–5 weeks after commencing antibiotic treatment. All recent partners of *C trachomatis* and *M genitalium* infected patients were notified and asked to attend the STD clinic for *C trachomatis* and *M genitalium* testing and genital examination. Recent partners were defined as all partners during the past 6 months before attendance or at least the two latest partners. As a part of this study the treatment efficacy in *M genitalium* infected patients was evaluated in an open pilot study.²³ The results from that study suggest that tetracyclines are not sufficient to eradicate *M genitalium*, but that azithromycin might be effective.

Statistical analysis

The χ^2 test and Fisher's exact test were used to test for differences in proportions and Mann-Whitney U test and Kruskal-Wallis test for non-parametric comparison of groups. Stata statistical software version 8.0 was used for calculating confidence intervals.

RESULTS

Neisseria gonorrhoeae was isolated from two men. No patient was smear positive and culture negative for *N gonorrhoeae*. Non-gonococcal urethritis (NGU) was detected in 271 men, comprising 61 patients between 16 and 56 years of age (median 23 years) with *C trachomatis* infection and 34 patients between 20 and 55 years of age (median 28 years) with *M genitalium* infection. Four patients with chlamydia had a concurrent *M genitalium* infection and were excluded in the comparison of signs and symptoms between *M genitalium* and *C trachomatis*. Initially, 41 men had positive PCR tests for *M genitalium*, but seven, of which three had a microscopic

urethritis and four not, had tests that were not confirmed and hence they were excluded from the study. Among these seven patients one had symptoms—that is, symptoms of epididymitis, but the remaining six were asymptomatic. The remaining 180 men with *M genitalium* negative NCNGU were between 16 and 54 years old (median 25.5 years). These patients were considered as having non-specific urethritis (NSU). Among the 180 men 42 had a microscopic urethritis, where the physician diagnosed 11 as prostatitis, 22 as genital papillomavirus infection, and nine with genital herpes simplex infection; these diseases themselves might be the cause of urethritis.

The overall prevalence of *M genitalium* was 7% (34/512), and that of *C trachomatis* 12% (61/512) and of NSU 35% (180/512). *M genitalium* infected men had symptoms of urethritis significantly more often than those with chlamydial infection—that is, 73% (22/30) versus 40% (23/57) and with a relative risk (RR) of 1.8 (95% CI 1.2 to 2.7). The *M genitalium* and *C trachomatis* groups were also compared with the NSU group where the *C trachomatis* group was indexed as 1 (table 1). The rate of microscopic signs of urethritis was high in both infections reaching about 90% and no significant difference was found (RR 0.8; 95% CI 0.3 to 2.0).

The number of partners in the *M genitalium* positive NGU, *C trachomatis* positive NGU, and NSU groups was significantly different ($p = 0.03$). The patients with *C trachomatis* NGU reported significantly more partners in the previous 6 months.

There were no significant differences regarding history of previous STI (urethritis) between the different groups (table 1).

The mean duration of symptoms was 4.2 weeks (median 3, range 1–25) for the 21/23 (data missing from two) men with symptomatic *C trachomatis* NGU, 5.7 weeks (median 2, range 1–60) for the 22 men with symptomatic *M genitalium* positive NGU ($p = 0.42$), and 6.5 weeks (median 2.5, range 1–12) for the three men with symptoms and verified infection with both bacteria. Only 27% (49/180) of the men with NSU had symptoms correlated to urethritis compared to 23 (40%) of 57 with chlamydial infection ($p = 0.068$) and 22 (73%) of 30 with *M genitalium* infection ($p < 0.0001$). Of the men with NSU 49% (75/152) had neither symptoms nor visible discharge compared to 20% (10/51) ($p < 0.001$) and 7% (2/27) ($p < 0.001$), respectively, among *C trachomatis* and *M genitalium* positive men (table 2). Men with *C trachomatis* positive or *M genitalium* positive NGU were more likely to have discharge (as a sign) than were those with NSU ($p < 0.001$).

Table 2 Correlation between symptoms (self reported dysuria and/or discharge) and signs (observed discharge—that is, $\geq 1 \text{ cm}^2$ smear on the slide and urethritis ($>4 \text{ PMNL/HPF}$)) among male attendees infected with *C trachomatis* (Ct), *M genitalium* (Mg), or neither organism (NSU)

		Ct (n=57) No (%)	Mg (n=30) No (%)	Mg and Ct (n=4) No (%)	NSU (n=180) No (%)
Discharge and symptoms	Urethritis				
	+	16 (29)	15 (50)	3 (75)	24 (13)
	-	0	0	0	0
Discharge but no symptoms	Urethritis				
	+	21 (37)	4 (13)	1 (25)	40 (22)
	-	1 (2)	2 (7)	0	0
Symptoms but no discharge	Urethritis				
	+	3 (5)	3 (10)	0	13 (7)
	-	0	1 (3)	0	0
Symptoms, discharge not noted	Urethritis				
	+	2 (3)	3 (10)	0	12 (7)
	-	1 (2)	0	0	0
No symptoms, discharge not noted	Urethritis				
	+	2 (3)	0	0	16 (9)
	-	0	0	0	0
No discharge or symptoms	Urethritis				
	+	8 (14)	2 (7)	0	75 (42)
	-	2 (3)	0	0	0
Data lacking					
Urethritis	+	1 (2)	0	0	0
Total count (%)		57 (100)	30 (100)	4 (100)	180 (100)

Altogether, among all 512 attendees in the current study, eight men reported having sex with men, of which four had had only passive anal sex, three both active and passive anal sex, and one only reciprocal oro-genital sex (fellatio). The 34 *M genitalium* infected men were all heterosexual, except one who also had a concurrent *C trachomatis* infection and had had fellatio with a man a year before attendance, but who subsequently had only had sexual intercourse with women (>20 partners). Among the other *C trachomatis* positive men, there were two homosexual men, of which one was notified because of his partner's chlamydial infection (tested at another clinic) and the other had had partners who could not be identified. Also, among the 180 men with a NSU there was one homosexual man, but his partners did not attend the clinic for testing.

Nineteen female partners of 18 men infected with *M genitalium* were examined; 12 (63%) of them were *M genitalium* positive. Four (21%) were *C trachomatis* positive; two of these patients had partners who were infected with both *C trachomatis* and *M genitalium*. One woman had negative tests but a mucopurulent cervicitis. Correspondingly, 39 partners of 28 *C trachomatis* positive men were examined; three men each had three partners and six men each had two partners. Twenty six (67%) of those partners were *C trachomatis* positive and two (5%) were *M genitalium* positive. The *M genitalium* positive women were partners of dual

infected men. Eight (17%) of the 47 examined partners of men with NSU were *C trachomatis* positive, and three (6%) were *M genitalium* positive. Both *C trachomatis* and *M genitalium* were found significantly more often in partners of men with the corresponding infection, than in partners of men with NSU ($p < 0.0001$ for both). The diagnosis and number of partners of each group are shown in table 3.

DISCUSSION

In this cross sectional study, the prevalence of *C trachomatis* infection was higher than that of *M genitalium* infection among male STD clinic attendees (12% versus 7%, respectively). Only four patients were infected with both bacteria. Only a few true cross sectional studies of STD clinic populations have been published.^{3, 24} Most investigations have been case-control studies. The inclusion criterion in the case group has been either symptoms or microscopic signs of urethritis; in the control group, asymptomatic men or men without microscopic signs of urethritis have been included.^{3, 4, 6-8, 25} In a recent Swedish report,²⁴ all attendees were tested for both *M genitalium* and *C trachomatis* and a similar prevalence for *M genitalium* (6%) was found; however, the prevalence of *C trachomatis* was surprisingly low (5%). In patients examined in another Swedish study³ 3 years earlier than those in the present study, a *C trachomatis* prevalence of 19% and an *M genitalium* prevalence of 7% were found. The

Table 3 Clinical findings in female sexual partners of 24 men infected with *C trachomatis* (Ct), 14 infected with *M genitalium* (Mg), and 44 with non-specific urethritis (NSU)—that is, NGU without positive tests for *C trachomatis* and/or *M genitalium*

Male patients' diagnosis	Ct (n=24/57)	Mg (n=14/30)	Mg and Ct (n=4)	NSU (n=44/180)
Female partners' diagnosis*				
<i>C trachomatis</i>	24 (69)	2† (13)	2† (50)	8 (17)
<i>M genitalium</i>	0 (0)	10† (67)	2† (50)	3 (6)
Non-specific infection	7 (20)	1 (7)	0 (0)	17 (36)
Other diagnoses	4 (11)	3 (20)	1 (25)	19 (41)
Total number of partners	35 (100)	15† (100)	4† (100)	47 (100)

*Among the Ct infected patients, 3 had 3 partners, 6 had 2 partners, and 14 had 1 partner examined. Of the 14 Mg infected patients, 1 had 2 partners, and the rest had 1 partner examined at the clinic. Of the 44 patients with NSU, 3 had 2 partners and the rest had 1 partner examined at the clinic.

†One female partner in each group was both *C trachomatis* and *M genitalium* positive.

Non-specific infection indicates urethritis and/or cervicitis with negative test results for *C trachomatis* and *M genitalium*. Percentages given in parentheses

reason for the differences in the *C trachomatis* prevalence in three Swedish STD clinics within a relatively limited time frame is not clear, but may reflect differences in the populations studied. It is surprising though that such differences were not found in the prevalence of *M genitalium*.

In a recent French study,¹⁸ *M genitalium* was found significantly more frequently among a group of men with urethritis and symptoms than in a group of men with symptoms but no urethritis, which was also the case for *C trachomatis* but not for *Ureaplasma urealyticum*, where the same prevalence (26% v 22%) was found in both groups. The criteria for urethral symptoms were wider than in the present study. Also the criterion for urethritis was different, based on examination of FVU and not urethral smears.¹⁸

In the above mentioned case-control studies the proportion of *M genitalium* and *C trachomatis* in the NGU groups was similar to the results in the current study, although the number of patients with NSU in our study was higher than in some reports^{4, 8} and equal to others.^{5, 13, 25, 26}

M genitalium infected men had symptoms of urethritis significantly more often than those infected with *C trachomatis*. This was not reflected in the microscopic signs of urethritis and the reason for this finding is not clear. It could be speculated that the production of hydrogen peroxide by *M genitalium*²⁷ may contribute to the symptoms. Whether the symptoms reflect also a potential for deeper invasion such as is seen in a chimpanzee model, where two of the 10 inoculated animals had *M genitalium* isolated from the blood stream²⁸ is not clear. The number of patients in the present study was limited and therefore our findings have to be verified in future studies. There is evidence that *M genitalium* may cause endometritis, PID, and sexually acquired reactive arthritis.^{2, 11, 29} Most published studies have focused on symptomatic patients comparing signs and symptoms of infected people with the two organisms. In this study there was no difference in microscopic signs between the bacteria. The high proportion of urethritis in men infected with *M genitalium* and the low rate of mixed infections support the conclusion made by others that *M genitalium* is a pathogen.^{2, 9, 13, 24–26, 30} The present study also showed that *M genitalium* infection seems to have a high prevalence in the society and therefore might be considered for screening purposes at STD clinics.

In this study, 12 of 19 (63%) female partners of men infected with *M genitalium* tested at the STD clinic also had a *M genitalium* infection compared with 26 of 39 (67%) chlamydial infected female partners of men with *C trachomatis* infection. These data emphasise the role of *M genitalium* as a sexually transmitted pathogen, since only three of 47 partners of men with NSU were *M genitalium* positive. *C trachomatis* is a notifiable infection including mandatory partner notification, but partners can attend any clinic, which might explain the rather low number of partners per man infected with *C trachomatis* (39/61) who were examined at our STD clinic. Since legislation regarding *M genitalium* infection does not exist, often only current partners attended the STD clinic. This might explain the rather low attendance rate among partners. For NSU cases some patients attended because of one of their female partners had *C trachomatis* infection, but had negative test results. This might explain the high prevalence of *C trachomatis* among the partners of men with NSU.

The men with chlamydial infection reported a higher number of recent partners than the other groups, including the *M genitalium* positive group. Such a difference has not been demonstrated in other studies.^{4, 8} *M genitalium* positive men were older than those with *C trachomatis* infection. Whether the *M genitalium* positive men have carried the infection for a longer period of time is not known.

Unfortunately, we do not have data on the number of lifetime partners, which may have provided an explanation for this difference; however, no difference in the duration of symptoms was seen. Only eight men reported sexual contact with other men, although three had a *C trachomatis* infection, and one of those was also infected with *M genitalium*. It is not possible from this study to draw any conclusions as to whether *M genitalium* is more or less prevalent among men who have sex with men than among heterosexual men.

In the large NSU group comprising 180 men with urethritis without recognised cause (66% of NGU), significantly more patients were asymptomatic compared with both the *M genitalium* and *C trachomatis* groups, 27% versus 73% and 40% respectively. This presents a serious dilemma in daily clinical work. Is the inflammation caused by a bacterial infection, and are these patients in need of treatment? Most *C trachomatis* infected men are asymptomatic and they should therefore not have been treated if both symptoms and microscopic signs were set as criteria for treatment. The sensitivity of *C trachomatis* PCR tests is high but less than 95% and possibly even lower for *M genitalium* PCR tests, so patients with false negative test results may benefit from treatment. Horner and co-workers³¹ have suggested that treatment guidelines should be revised, and propose that asymptomatic men without discharge should not receive antibiotic treatment. In the current study we attempted to set more objective criteria for the measurement of discharge, and experienced clinicians examined most patients (approximately >85%). The high rate of patients without discharge in combination with lack of symptoms in the NSU group (49%) and the corresponding high rate of discharge and symptoms in *C trachomatis* and *M genitalium* infected men support the proposal by Horner *et al*, although some patients would have been missed at the examination. In these patients, treatment would have been delayed with the risk for further transmission of the infections and possible risk for sequelae.

The most widely accepted and used criterion for urethritis is >4 PMNL/HPF which was established in the late 1970s.¹⁷ The result of this study calls for a reconsideration of this criterion, since none of the patients with *C trachomatis* or *M genitalium* infection had fewer than 10 PMNL/HPF. Only among the men with NSU was “grey zone urethritis” found—in 29 patients. Obviously, the interpretation of the smear depends on several variables: the instrument for sampling, the standard of the microscope and how it is used,

Key messages

- *Mycoplasma genitalium* is an important and frequent cause of male urethritis and this cross sectional study indicates that *M genitalium* even more often than *C trachomatis* gives symptoms of urethritis among male STD attendees
- There are no significant differences in microscopic signs between both bacteria.
- Partners of men infected with *M genitalium* were most often infected with *M genitalium* and to the same extent partners of men infected with *C trachomatis* were infected with *C trachomatis* (67%) supporting the role of the bacteria as pathogens and sexually transmitted infections
- More studies are needed to examine the diagnostic tools for determining the clinically relevant definition of urethritis

the experience of the clinician, and the interpretation of discharge and smears. This subjective procedure can never be strictly scientifically standardised. We believe that more studies are required to examine the diagnostic tools for determining the clinically relevant definition of urethritis.

In summary, *M. genitalium* was strongly associated with symptomatic urethritis and men infected with both *C. trachomatis* and *M. genitalium* transmitted the infections to a large proportion (two thirds) of their sexual partners. More studies are needed to determine the potential sequelae of *M. genitalium* infection in men as well as in women.

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CONTRIBUTORS

LF initiated the study, examined and sampled most of the patients, collected all data, and wrote the first draft of the manuscript; HF was responsible for the *N. gonorrhoeae* and *C. trachomatis* tests, contributed to the design of the study and analysis of the data; JSJ was responsible for the *M. genitalium* tests and provided major contributions to the design of the study and analysis of the data.

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