ORIGINAL ARTICLE

MJS PUBLISHING

Prevalence of *Mycoplasma Penetrans* in Urethral and Rectal Specimens from Men Attending an STI Clinic

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***Mycoplasma penetrans* has gained attention in relation to non-gonococcal urethritis (NGU), a syndrome where a significant proportion of patients have no identified aetiological agent. In this study, we estimated the prevalence of urethral and rectal *M. penetrans* in a male population attending a sexually transmitted infection (STI) clinic in Copenhagen, Denmark. Specimens were assessed using *M. penetrans*-specific real-time polymerase chain reaction (PCR). Demographic characteristics and clinical symptoms were extracted from patient records. A total of 704 urethral specimens and 223 rectal specimens from 721 individual male patients were included. The prevalence of urethral and rectal *M. penetrans* was 0.3% and 2.7%, respectively. Overall, the prevalence among men who have sex with men (MSM) was 3.6%, significantly higher than in men who have sex with women ($p < 0.001$). Among MSM, a higher number of partners was associated with *M. penetrans* infection ($p = 0.008$). No association was found between the presence of *M. penetrans* and clinical symptom status, and its pathogenic potential remains unclear.**

Key words: men who have sex with men; *Mycoplasma penetrans*; nongonococcal urethritis; rectum; sexually transmitted infections.

Submitted Mar 22, 2025. Accepted Mar 31, 2025

Published May 5, 2025. DOI: 10.2340/actadv.v105.43443

Acta Derm Venereol 2025; 105: adv43443.

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SIGNIFICANCE

A significant proportion of patients with non-gonococcal urethritis have no identified aetiological agent, which challenges both correct treatment and unnecessary use of antibiotics. A possible role of *Mycoplasma penetrans* in non-gonococcal urethritis has been suggested in the literature. In this study, we estimated the prevalence of *Mycoplasma penetrans* in a male population attending a sexually transmitted infection clinic. No association was found between *Mycoplasma penetrans* and symptoms at site of infection. The presence of *Mycoplasma penetrans* appears to be associated with men who have sex with men and number of partners was identified as a risk factor for infection.

Sexually transmitted infections (STIs) are a major and increasing health concern worldwide with an estimated number of more than 1 million newly infected individuals every day (1). Sexually transmitted infections can cause urethritis and cervicitis, and left untreated may lead to complications such as pelvic inflammatory disease (PID), infertility, ectopic pregnancy, miscarriage, congenital infections, and foetal death (2).

Nongonococcal urethritis (NGU) is a condition characterized by the absence of *Neisseria gonorrhoeae* as well as the presence of urethral symptoms including dysuria, pruritus, urethral discharge, and elevated polymorphonuclear leukocytes (3). Most commonly, NGU is caused by *Chlamydia trachomatis* and *Mycoplasma genitalium* (4, 5). Other less frequent pathogens associated with NGU are *Haemophilus influenza*, *Trichomonas vaginalis*, herpes simplex virus, and adenovirus (4, 6, 7). Despite advances in molecular methods, a significant proportion of patients with urethritis have no identified aetiological agent, leading to challenges regarding both correct antibiotic treatment and surveillance (8). Therefore, it is of great interest to identify other potential urogenital pathogens. Different aetiologies of NGU have been proposed in men who have sex with men (MSM) and men who have sex with women (MSW), respectively. Research has suggested an association between *M. penetrans* and NGU, primarily in MSM (9). However, in a recent study, the same association could not be found, questioning the potential role of *M. penetrans* in NGU (10). The present study aimed to determine the prevalence of *M. penetrans* in urethral and rectal specimens from a male population attending an STI clinic in Copenhagen, Denmark.

METHODS

Patients

From March to June 2016, 1,500 patients were included in the Nordic Aptima MG Evaluation (NAME) study at the STI clinic at Bispebjerg Hospital, Copenhagen University Hospital, Denmark. All patients ≥ 18 years with a Danish Personal Identification Number were eligible, and only patients who declined to participate were excluded (11). Specimens were collected according to clinical indication and risk profile. For the present

study, urethral and rectal swabs from all male patients were included. The patient records were reviewed, and the following data were extracted: symptoms of an STI, sociodemographic information, sexual orientation (e.g., MSM status), HIV status, and sexual risk behaviour. Patients were categorized as symptomatic if they reported rectal itching or discharge, urethral itching or discharge, or pain/discomfort during urination.

Statistical analysis

Categorical data were compared using Fisher's exact test and continuous data with the Wilcoxon rank sum test. Statistical analyses were performed using R (version 4.1.3; RStudio; R Foundation for Statistical Computing, Vienna, Austria) (12).

Ethics

Patients provided written informed consent to participate in the NAME study and permission for future research related to sexually transmitted infections on remaining biological material. The study was approved by the Danish Data Protection Agency (journal number P-2022-805) and the Committee on Health Research Ethics (journal number H-15018251).

Sample preparation and real-time PCR (qPCR)

DNA was extracted by processing 200 µl of the sample using the Small Volume Universal Pathogen Extraction protocol in a MagNA Pure 96 instrument (Roche Diagnostics, Hvidovre, Denmark) and was eluted into 100 µl. *M. penetrans* was detected by qPCR as previously described (9, 13).

RESULTS

A total of 704 urethral specimens and 223 rectal specimens from 721 individual male patients were available for qPCR; 206 patients had both urethral and rectal specimens available. The number of *M. penetrans*-positive specimens was 2 (0.3%, (95% confidence interval [CI]: 0.0–1.0) and 6 (2.7%, 95% CI: 1.0–5.8) among urethral and rectal specimens, respectively (**Tables I** and **II**). None of the patients were positive at both anatomical sites. Median *M. penetrans* DNA concentrations were 22.8 genome equivalents (GEQ)/µl for urethral specimens and 4.5 GEQ/µl for rectal specimens. Among the 2 patients with *M. penetrans*-positive urethral specimens, 1 patient presented with urethral symptoms (Table I). Rectal symptoms were not reported by any patient with *M. penetrans*-positive rectal specimens (Table I). No patients with urethral *M. penetrans* were coinfected with urethral *N. gonorrhoeae*, *C. trachomatis*, or *M. genitalium* (Table I). Rectal coinfection was observed in 2 patients positive for rectal *M. penetrans*: 1 patient was coinfected with rectal *N. gonorrhoeae* and another was coinfected with rectal *C. trachomatis* (Table I). Overall, coinfection with *N. gonorrhoeae, C. trachomatis* and/or *M. genitalium* from rectal and/or urethral sites was observed in 4 *M. penetrans*-positive patients (50%, 95% CI: 15.7–84.3).

Table I. Study characteristics of the 721 male patients

	Urethral specimens				Rectal specimens			
Characteristics	Total *n* = 704	*M. penetrans*-positive *n* = 2	*M. penetrans*-negative *n* = 702	*p*-value[a]	Total *n* = 223	*M. penetrans*-positive *n* = 6	*M. penetrans*-negative *n* = 217	*p*-value[a]
Age, median (range)	32 (18–70)	43.5 (35–52)	32 (18–70)	0.225[b]	37 (18–69)	39.5 (33–52)	37 (18–69)	0.411[b]
Symptoms, *n* (%)								
Urethral symptoms[c]	241	1 (50.0)	240 (34.2)	1	66	3 (50.0)	63 (29.0)	
Rectal symptoms[d]	15	0 (0)	15 (2.1)		13	0 (0)	13 (6.0)	1
Asymptomatic	450	1 (50.0)	449 (64.0)		146	3 (50.0)	143 (65.9)	
Sexual orientation								
MSM, *n* (%)	210	2 (100)	208 (30.4)	0.093[e]	205	6 (100)	199 (93.4)	1[e]
Sex partners past 6 months, median (IQR)		55 (33–78)	5 (2–10)	0.067[b]		10 (10–15)	4 (2–10)	0.051[b]
MSW, *n* (%)	477	0 (0)	477 (69.6)		14	0 (0)	14 (6.6)	
Sex partners past 6 months, median (IQR)		–	2 (2–4)		14	–	3 (1–4)	
Known HIV-positive, *n* (%)	38	1 (50.0)	37 (5.3)	0.105	38	0 (0)	38 (17.5)	0.593
Positive for other STIs, *n* (%)								
Urethra								
Chlamydia trachomatis, *n* (%)	65 (696[f])	0 (0)	65 (9.4)	1	15 (218[f])	1 (16.7)	14 (6.6)	0.351
Mycoplasma genitalium, *n* (%)	34 (696 [f])	0 (0)	34 (4.9)	1	16 (212[f])	1 (16.7)	15 (7.3)	0.379
Neisseria gonorrhoeae, *n* (%)	28 (693 [f])	0 (0)	28 (4.1)	1	18 (216 [f])	1 (16.7)	17 (8.1)	0.411
Rectum								
Chlamydia trachomatis, *n* (%)	10 (220[f])	0 (0)	10 (4.6)	1	10 (223 [f])	1(16.7)	9 (4.1)	0.243
Mycoplasma genitalium, *n* (%)	13 (214[f])	0 (0)	13 (6.1)	1	8 (223 [f])	0 (0)	8 (3.7)	1
Neisseria gonorrhoeae, *n* (%)	33 (220[f])	1 (50.0)	32 (14.7)	0.278	34 (223 [f])	1 (16.7)	33 (15.2)	1
Antibiotics past 4 weeks	42 (679[g])	0 (0)	42 (6.2)	1	10 (215[g])	0 (0)	10 (4.8)	1

[a]Fisher's exact test unless otherwise specified. [b]Wilcoxon rank sum test. [c]Urethral symptoms were defined as urethral itching, discharge, or pain/discomfort during urination. [d]Rectal symptoms were defined as rectal itching or discharge. [e]*P*-value calculated for MSM compared with MSW. [f]Total number of patients tested. [g]Total number of patients with available information.
MSM: men who have sex with men; MSW: men who have sex with women only; IQR: interquartile range; HIV: human immunodeficiency virus; STI: sexually transmitted infection.

Table II. Distribution of *Mycoplasma penetrans* in the studied population and subpopulations

Population	Prevalence of *M. penetrans*, urethra, %	(95% CI)[a]	Prevalence of *M. penetrans*, rectum, %	(95% CI)[a]
Study population	0.3 (2/704)	(0.0–1.0)	2.7 (6/223)	(1.0–5.8)
Men who have sex with men	1.0 (2/210)	(0.1–3.4)	2.9 (6/205)	(1.1–6.3)
Men who have sex with women only	0 (0/477)		0 (0/14)	
Known HIV-positive	2.6 (1/38)	(0.1–13.8)	0 (0/38)	
Positive for other STIs, urethra				
Chlamydia trachomatis	0 (0/65)		6.7 (1/15)	(0.2–32.0)
Mycoplasma genitalium	0 (0/34)		6.3 (1/16)	(0.2–30.2)
Neisseria gonorrhoeae	0 (0/28)		5.6 (1/18)	(0.1–27.3)
Positive for other STIs, rectum				
Chlamydia trachomatis	0 (0/10)		10.0 (1/10)	(0.3–44.5)
Mycoplasma genitalium	0 (0/13)		0 (0/8)	
Neisseria gonorrhoeae	3.0 (1/33)	(0.1–15.8)	2.9 (1/34)	(0.1–15.3)

[a]Exact 95% CI calculated using R.
CI: confidence interval; HIV: human immunodeficiency virus; STI: sexually transmitted infection.

All patients with *M. penetrans*-positive specimens were MSM (Table I). Among MSM the prevalence of urethral *M. penetrans* was 1.0% (95% CI: 0.1–3.4) and the prevalence of rectal *M. penetrans* was 2.9% (95% CI: 1.1–6.3) (Table II). The overall prevalence of *M. penetrans* among MSM was 3.6% (95% CI: 1.6–6.9), which was significantly higher when compared with MSW ($p<0.001$). One subject with urethral *M. penetrans* was HIV-positive ($p=0.105$), while no patients with rectal *M. penetrans* were HIV-positive (Table I). The median number of partners in the past 6 months among urethral *M. penetrans*-positive and -negative MSM was 55 and 5, respectively ($p=0.067$) (Table I). For rectal *M. penetrans*-positive and -negative MSM the median number of partners was 10 and 4 ($p=0.051$), respectively (Table I). Among MSM positive for *M. penetrans* from any site compared with *M. penetrans*-negative MSM, the median (IQR) number of partners in the past 6 months was 10 (10–15) and 4 (2–10), respectively, which was significantly higher among *M. penetrans*-positive MSM ($p=0.008$).

DISCUSSION

The present study investigated the prevalence of urethral and rectal *M. penetrans* in a male population attending an STI clinic, finding that all patients positive for *M. penetrans* were MSM. Higher prevalence in MSM is in accordance with what has previously been described in the literature (9, 10, 14). Previous studies have reported a prevalence of urethral *M. penetrans* among MSM between 3.5% and 9.0%, which is higher than the 1.0% observed in the present study (9, 10, 14, 15). For *M. genitalium*, higher prevalence in rectal swabs compared with urine samples has been observed, suggesting that the rectum could be the favoured location for infection (16). Our findings suggest that the same may apply to *M. penetrans*, as a higher prevalence was observed in rectal specimens. This could, at least partly, explain the more frequent detection among MSM.

M. penetrans was initially detected in the urine of HIV-positive MSM and was suggested to be uniquely associated with HIV (17, 18). Later studies, like the present, have detected *M. penetrans* in HIV-negative subjects, discarding this theory. Nevertheless, an association between *M. penetrans* and HIV has been proposed, observing a higher prevalence in HIV-positive compared with HIV-negative individuals (10, 19). However, these studies did not adjust for the number of partners. Despite this, the association with HIV is inconsistent, as a study examining the prevalence of *M. penetrans* in 910 individuals, including 210 HIV-positive patients, did not find any significant association with positive HIV status (20). The latter is in accordance with the present study, where we observed no significant difference in the prevalence of *M. penetrans* when considering HIV status.

In the present study, no significant association was found between the presence of *M. penetrans* and clinical symptom status. However, bacterial loads were low, which potentially could explain the absence of symptoms. A possible role of *M. penetrans* in NGU has been discussed in the literature. An association between *M. penetrans* and NGU in MSM was proposed by Srinivasan et al., finding a prevalence of 13% among MSM with NGU compared with 1% in MSM with no NGU (9). The same association could not be found in MSW. An earlier study investigated the presence of *M. penetrans* in 28 MSM with and without NGU, finding a prevalence of 10% vs zero positive urethral swabs, respectively (15). Furthermore, the study found a prevalence of rectal *M. penetrans* of 10% among patients with NGU, compared with 5.6% among patients without NGU. Rectal symptoms were not investigated. In contrast, a study from 1996 examining urethral specimens from 158 Japanese males with and without NGU failed to detect *M. penetrans* in any of the patients (21). Additionally, a recent study found no association between the presence of *M. penetrans* and urethritis or urogenital symptoms on examining urogenital samples from 429 men screened for *C. trachomatis* and *N. gonorrhoea* (10).

The presence of *M. penetrans* has also been assessed in 231 *C. trachomatis*-positive rectal samples from males submitted for lymphogranuloma venereum (LGV) typing, observing a prevalence of 13% (13). These results

suggested that *M. penetrans* may cause coinfection with *C. trachomatis*. In our study, 1 patient positive for rectal *M. penetrans* was coinfected with *C. trachomatis* and no significant association between rectal *M. penetrans* and coinfection with *C. trachomatis* was detected. However, we observed that the median number of partners in the past 6 months was significantly higher among *M. penetrans*-positive MSM compared with *M. penetrans*-negative MSM. A study conducted in Massachusetts, USA found that a small subset of the population accounted for a significant proportion of reported bacterial STIs (22). Hence, the discrepancy in the observed prevalence of rectal *M. penetrans* could be attributed to variations in the study populations, as patients tested for LGV might represent a high-risk population in contrast to the present study population (13).

Strengths and limitations

This study has several strengths including access to medical records from all study subjects. Moreover, testing was conducted from both urethral and rectal sites according to risk profile. Our study also had limitations. The prevalence of *M. penetrans* was low and numbers were small when investigating specific characteristics. Hence, certain associations may have gone undetected. Additionally, information regarding sexual partners and HIV status was incomplete in many records and therefore missing in a proportion of the study population. Further, the study participants were exclusively included from a single STI clinic in Copenhagen, Denmark and outcomes may therefore not apply to other demographic groups.

Conclusion

In this study, we found a prevalence of urethral and rectal *M. penetrans* of 0.3% and 2.7%, respectively, with the number of partners being a risk factor for *M. penetrans* infection. As suggested in prior research, the presence of *M. penetrans* appears to be strongly linked to MSM. No significant association between the presence of *M. penetrans* and symptoms at the site of infection was observed, and its proposed role in NGU remains unclear. Future research with larger cohorts is required to fully clarify the significance of *M. penetrans*.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to their colleagues at Statens Serum Institut, Copenhagen, Denmark. In particular, they would like to thank research laboratory scientist Mette Holm for excellent technical assistance.

Potential conflicts of interest: JSJ reports grants, personal fees, and non-financial support from Hologic, grants and personal fees from Nabriva, personal fees from Leo Pharma, all outside the submitted work. KS-R reports financial support from Hologic outside the submitted work. The remaining authors declare no potential conflicts of interest.

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