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Prevalence of *Mycoplasma genitalium* and macrolide resistance in rectal and urine samples among men who have sex with men in Sweden

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ABSTRACT

Objectives While *Mycoplasma genitalium* is reported as a common rectal infection among men who have sex with men (MSM), published data refer predominantly to urethral infections. Currently, most guidelines recommend *M. genitalium* testing from urine in men with symptomatic, non-gonococcal urethritis. Macrolide resistance-associated mutations (MRMs) among *M. genitalium* have increased during the last decade especially among MSM. We aim to demonstrate the prevalence and anatomical distribution of *M. genitalium* infection and MRM in urine and rectal specimens among MSM in Sweden.

Methods In this cross-sectional study in 2019, paired urine and rectal samples from symptomatic and asymptomatic MSM attending a sexually transmitted infection clinic in the south of Sweden were screened for *M. genitalium*, presence of MRM, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, HIV and syphilis.

Results The overall prevalence of *M. genitalium* was 10.5% (64 of 609), rectal samples 7.6% (46 of 609) and urine samples 3.9% (24 of 609) (p=0.007). Among *M. genitalium*-positive cases, single rectal and single urethral infection was detected in 62.5% (40 of 64) and 28.1% (18 of 64), respectively (p<0.0001). Infection at both sites was seen in 9.4% (6 of 64). The prevalence of MRM was 67.9% (19 of 28). *M. genitalium* was significantly associated with HIV (OR 2.60, 95% CI 1.14 to 5.88, p=0.02). Among the MSM, 7.4% (45 of 609) were infected with *N. gonorrhoeae,* 6.7% (41 of 609) with *C. trachomatis*, 7.1% (43 of 609) with HIV and 0.7% (4 of 609) with syphilis.

Conclusions In this study, among MSM, most infections with *M. genitalium* were detected as rectal mono infections. The prevalence of *M. genitalium* among MSM was almost twofold higher in rectal samples (7.6%) compared with urine samples (3.9%). The prevalence of macrolide resistance was high with no difference between urine and rectal samples.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ *Mycoplasma genitalium* is a common infection among both men and women and has been shown to be widespread in men who have sex with men (MSM). Studies have reported the infection to also exist in the rectal site, containing high levels of macrolide resistance, but published data refer predominantly to urethral infections and most guidelines recommend testing from urine in men with symptomatic, non-gonococcal urethritis.

WHAT THIS STUDY ADDS

⇒ This study demonstrates the prevalence of *M. genitalium* infection and macrolide resistance in urine and rectal specimens, among 609 MSM in Sweden. This has not been shown in Sweden previously.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The study shows a 10.5% prevalence of *M. genitalium* with most infections at the rectal site with a high level of macrolide resistance. Testing MSM with urine samples only would leave two-thirds of all *M. genitalium* infections undetected. This has implications on perspectives on clinical management and rectal testing of MSM.

INTRODUCTION

Mycoplasma genitalium has, since its discovery in the early 1980s, been demonstrated as an important sexually transmitted infection (STI) causing genital infections in both men and women.[1 2] *M. genitalium* is a common cause of non-gonococcal urethritis (NGU) in men and most published data include urethral infections in men who have sex with women (MSW). Reports on prevalence of urethral and particularly rectal infections in men who have sex with men (MSM) are less common.[3]

In a systematic review and meta-analysis using data from 1981 up to 2018, the mean prevalence of *M. genitalium* infection in MSM was 5.0% (95% CI 3.5% to 6.8%) in urethra and 6.2% (95% CI 4.6% to 8.1%) in rectum.[3] Studies including more recent data report higher prevalence rates in rectal infections ranging from 7.0% to 13.4%, with 7.0% among 1001 asymptomatic MSM in Melbourne, Australia to 13.4% among a population including both asymptomatic and symptomatic MSM in Germany.[4–8]

Rectal infection is reported to be more common than urethral infection in MSM. In 2018, an Australian study on a population including both asymptomatic and symptomatic MSM showed a prevalence of rectal infection at 8.9% (45 of 505), almost double that of urethral infection (4.7%; 24 of 508); and in 2019, in another study from the

same country in asymptomatic MSM, rectal *M. genitalium* infection was almost three times more common than urethral infection (7.0% vs 2.7%).[4 7]

M. genitalium is generally treated with the macrolide antibiotic azithromycin. Resistance to macrolides is common and the prevalence of macrolide resistance-associated mutations (MRMs) has increased steeply during the last decade. In a recent review, the mean global macrolide resistance was estimated to be 51.4% in 2017.[6] Several studies have reported a higher rate of macrolide resistance in MSM than in MSW. In an Australian study from 2013 to 2015, MSM were twice as likely to harbour MRM as MSW[7] and in 2019, McIver *et al* reported an overall macrolide resistance in men of 70.7% with significantly more MRMs in MSM (89.7%) than MSW (50.0%).[9]

There is some controversy whether *M. genitalium* causes proctitis or not. In most previous studies, no association has been shown between rectal *M. genitalium* infection and clinical symptoms and the rectal site has been described as a potential reservoir for asymptomatic infections,[9–11] but two studies which included a comparison group have reported an association between *M. genitalium* and proctitis.[12 13]

Considering a rapidly increasing antimicrobial resistance (AMR) and inconsistent data on clinical relevant rectal infection in men, most current guidelines recommend *M. genitalium* testing from urine samples in men with symptomatic NGU with no recommendation for testing rectal specimens in MSM.[14–16] European guidelines recommend rectal testing only in case of proctitis that is negative for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.[14]

In this study, we aim to demonstrate the prevalence and distribution of *M. genitalium* infection and macrolide-resistant strains in urethra and rectum among a population of MSM in the south of Sweden. We also report on the prevalence of coinfections with *C. trachomatis*, *N. gonorrhoeae,* HIV and syphilis.

MATERIAL AND METHODS

Study population/study design

This study is an epidemiological cross-sectional study. For 8 months, from 1 January 2019 to 31 August 2019, all visitors at the Centre of Sexual Health (CSH), Skane University Hospital, Malmö, Sweden who were undergoing routine screening for *C. trachomatis* and *N. gonorrhoeae* were also offered screening for *M. genitalium*.

CSH is a sexual health clinic in the south of Sweden serving a population of 348 000. The clinic receives around 16 000 visits yearly from about 8400 individuals.

MSM, defined by themselves as having sex with men, were included consecutively, and provided both urine and rectal samples.

Laboratory procedures

All specimens were placed into Aptima tubes containing specimen transport medium and transferred at room temperature within 1 day to the regional Clinical Microbiology Laboratory in Lund, Sweden. Samples were analysed for *M. genitalium* by transcription-mediated amplification (TMA) using APTIMA *M. genitalium* assay, which detects *M. genitalium* 16S rRNA, on the Hologic Panther System. The samples were also tested by the APTIMA Combo 2 Assay for *C. trachomatis* and *N. gonorrhoeae* using the Hologic Panther System.

HIV screening was performed by VITROS HIV Combo Test (Ortho Clinical Diagnostics, Rochester, USA). Screening for syphilis was performed by VITROS syphilis *Treponema pallidum* agglutination assay (Ortho Clinical Diagnostics, Rochester, USA).

Regarding macrolide resistance assay, performed on each sample positive for *M. genitalium,* the *M. genitalium* 23S rRNA gene of region V was amplified by reverse transcription-PCR with TAQ-Man probes for detection of wild-type *M. genitalium* and mutants with G at position 2071 or at 2072 (online supplemental file 1).

Statistical analysis

Data were analysed using IBM SPSS Statistics V.25.0. Frequencies of categorical variables such as *C. trachomatis, N. gonorrhoeae*, HIV, symptoms and macrolide resistance were stratified by *M. genitalium* infection, rectal and/or urethral using Pearson's X^2 test. Fisher's exact test was used in cases of small numbers (less than five in one cell). Binary logistic regression with ORs, 95% CI and p>0.05 was used for associations in significant variables, adjusting for possible confounders such as age, HIV and other STIs.

RESULTS

Study population

In 2019, for 8 months, 3667 men and 2503 women were screened for *M. genitalium* of whom 609 consecutively tested MSM were included.

The mean age of all included MSM was 36.1 years, ranging from 19 to 81 years. Among MSM cases positive for *M. genitalium*, the mean age was 36.4 years, with a range of 21–63 years, and in *M. genitalium* negative, 36.0. years, with a range of 19–81 years.

Prevalence and distribution of urethral and rectal *M. genitalium* infection

From 609 participants paired specimens, one urine and one rectal specimen, a total of 1218 specimens were obtained for testing. Overall, prevalence of *M. genitalium* infection was 10.5% (64 of 609). In rectal and urine specimens, *M. genitalium* positivity was 7.6% (46 of 609) and 3.9% (24 of 609), respectively (p=0.007).

Among *M. genitalium*-positive cases, single rectal and single urethral infection was detected in 62.5% (40 of 64) and 28.1% (18 of 64), respectively (p<0.0001). Rectal infection was detected in 71.9% (46 of 64) and urethral infection in 37.5% (24 of 64) of positive cases (p<0.0001). A simultaneous rectal and urethral infection was seen in 9.4% (6 of 64) (table 1).

Prevalence of *N. gonorrhoeae, C. trachomatis*, HIV, syphilis and coinfection with *M. genitalium*

Overall, 7.4% (45 of 609) of included subjects were infected with *N. gonorrhoeae,* 6.7% (41 of 609) with *C. trachomatis*, 7.1% (43 of 609) with HIV and 0.7% (4 of 609) with syphilis (table 1).

Infection with *M. genitalium* was more common than infection with both *C. trachomatis* (p=0.004) and *N. gonorrhoeae* (p=0.05).

Total coinfection rate (syphilis and HIV excluded) in *M. genitalium*-positive MSM was 14.1% (9 of 64), including 7.8% (5 of 64) coinfected with *N. gonorrhoeae*, 4.7% (3 of 64) with *C. trachomatis* and 1.6% (1 of 64) with both *N. gonorrhoeae* and *C. trachomatis*. For syphilis and HIV, coinfection rate was 3.1% (2 of 64) and 14.1% (9 of 64), respectively (table 2).

One man with *M. genitalium* infection was coinfected with both *N. gonorrhoeae* and *C. trachomatis* (1.6%; 1 of 64). Mono infection with *M. genitalium* was detected in 9.0% (55 of 609).

Table 1 Prevalence and anatomical distribution of *Mycoplasma genitalium* (Mg), *Chlamydia trachomatis* (Ct), *Neisseria gonorrhoea* (Ng), HIV and syphilis among 609 MSM

	Overall N=609 (%)	Rectal (%)	Urethral (%)	Rectal vs urethral P value	Rectal mono infection (%)	Urethral mono infection (%)	Rectal vs urethral mono infection P value	Pharyngeal (%)	Pharyngeal mono infection (%)
Mg	64 (10.5)	46/64 (71.9)	24/64 (37.5)	<0.0001	40/64 (62.5)	18/64 (28.1)	<0.0001	–	–
Ng	45 (7.4)	31/45 (68.9)	4/45 (8.9)	<0.0001	13/45 (28.9)	0/45 (0)	0.001	30/45 (66.7)	14/45 (31.1)
Ct	41 (6.7)	31/41 (75.6)	14/41 (34.1)	0.002	24/41 (58.5)	8/41 (19.5)	0.0003	5/41 (12.2)	2/41 (4.9)
HIV	43 (7.1)								
Syphilis	4 (0.7)								

MSM, men who have sex with men.

There were no differences in the prevalence of infections between *M. genitalium*-positive cases and *M. genitalium*-negative cases regarding *N. gonorrhoeae* (7.8% (5 of 64) vs 7.3% (40 of 545)), *C. trachomatis* (6.3% (4 of 64) vs 6.8% (37 of 545)) or syphilis (3.1% (2 of 64) vs 0.4% (2 of 545)) (table 2). Concurrent infection with *C. trachomatis* and *N. gonorrhoeae* without *M. genitalium* infection was detected in 1.0% (6 of 609) of cases.

M. genitalium was more common in MSM living with HIV than in HIV-negative men (20.9% (9 of 43) vs 9.7% (55 of 566); p=0.03), and logistic regression analysis showed an independent association with *M. genitalium* (OR 2.60, 95% CI 1.14 to 5.88, p=0.02) when adjusting for age, *N. gonorrhoeae*, *C. trachomatis* and syphilis. There was no association between HIV and any other STIs including *N. gonorrhoeae*, *C. trachomatis* and syphilis (table 3).

Macrolide resistance

To receive a result regarding macrolide resistance, a sample should contain at least 15 copies of *M. genitalium* nucleic acid/µL. In 60.0% (42 of 70) of the *M. genitalium*-positive samples, the bacterial load was below 15 copies/µL hampering the analysis of MRM. These samples were classified as containing undetermined strains. The distribution of MRM, wild-type *M. genitalium* strains and undetermined strains is shown in table 4. The proportion of undetermined strains was higher in rectal than in urine specimens (64.3% (27 of 42) vs 35.7% (15 of 42); p=0.008).

Among 28 specimens with valid macrolide resistance assay, the prevalence of MRM was higher (67.9%; 19 of 28) than wild type (32.1%; 9 of 28) (p=0.008). There was no significant difference between rectal and urine specimens regarding MRM, which was found in 39.3% (11 of 28) and 33.3% (8 of 24), respectively (table 4).

Among 64 *M. genitalium*-positive cases, there were 9 HIV-positive and 55 HIV-negative individuals. There was a non-significant trend that MRM was more common in men living with HIV (100.0%; 5 of 5) than among men with a negative HIV test (52.6%; 10 of 19) (p=0.07). Undetermined strains were equally common in men living with HIV (44.4%; 4 of 9) as in HIV-negative men (65.5%; 36 of 55) (table 4).

DISCUSSION

To the best of our knowledge, this is the first study in Sweden that informs on the prevalence of both rectal and urethral *M. genitalium* infection in a population of MSM. In this study among 609 MSM, the prevalence of *M. genitalium* (10.5%) was higher than both *N. gonorrhoeae* (7.4%) and *C. trachomatis* (6.7%). Almost two-thirds of *M. genitalium* infections were rectal mono infections. We also found a high prevalence of MRM strains. In the 40% of samples which could yield resistance data, macrolide resistance was 68%. Infection with *M. genitalium* was strongly associated with being HIV positive.

Earlier data on the prevalence of *M. genitalium* have shown a lower prevalence in MSM than in MSW. In 2019, McIver *et al* showed a steep increase, during the last decade, and reported the same level as in MSW in Sydney.[9] This is similar to the findings in the present study, in which we found the prevalence in MSM to be about the same (10.5%) as in MSW (8.5%).

Rectal and urethral *M. genitalium* prevalence in MSM with and without symptoms has been reported from Australia in 2018 to be 8.9% and 4.7%, respectively, which is in agreement with our result (7.6% vs 3.9%).[7]

In the present population, a majority of the *M. genitalium* infections were single rectal infections and rectal infection was detected in 71.9% of all *M. genitalium*-positive individuals. This concurs with previous studies that have shown rectal infection to be more common than urethral infection. In a study from Australia in 2019, Read *et al* reported rectal infection to be almost three times as common as urethral infection which is at the same level as in the present study, where rectal infection was two and a half times as common as urethral infection.[4]

In this population of high-risk individuals, STIs other than *M. genitalium* were also common with *N. gonorrhoeae*, *C.*

Table 2 Coinfection and comparison of prevalence of *Chlamydia trachomatis* (Ct), *Neisseria gonorrhoeae* (Ng), HIV and syphilis in 609 *Mycoplasma genitalium* (Mg)-positive and Mg-negative MSM

	Mg positive	Mg negative	P value
Ct	6.3% (4/64)	6.8% (37/545)	0.92*
Ng	7.8% (5/64)	7.3% (40/545)	0.91*
HIV	14.1% (9/64)	6.2% (34/545)	0.03
Syphilis	3.1% (2/64)	0.4% (2/545)	0.06*

P values are calculated by Pearson's X^2 test.
*Cells <5 are calculated using Fisher's exact test.
MSM, men who have sex with men.

Table 3 Associations between HIV positive and *Mycoplasma genitalium* (Mg), *Chlamydia trachomatis* (Ct) and *Neisseria gonorrhoeae* (Ng) in 609 MSM by logistic regression

HIV positive	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Mg	2.46 (1.12 to 5.40)	0.02	2.60 (1.14 to 5.88)	0.02
Ng	1.31 (0.45 to 3.86)	0.62	1.24 (0.40 to 3.80)	0.71
Ct	1.51 (0.51 to 4.46)	0.46	0.92 (0.32 to 2.80)	0.93

Adjusted OR=adjusted for age, Mg, Ng, Ct and syphilis infection.
MSM, men who have sex with men.

Table 4 Distribution of macrolide resistance-associated mutations (MRMs), wild-type *Mycoplasma genitalium* (Mg) strains and undetermined strains in 46 rectal and 24 urine Mg specimens and among 9 HIV-positive and 55 HIV-negative MSM

	Mg all specimens (%)	Mg rectal specimens (%)	Mg urine specimens (%)	P value Mg rectal vs Mg urine	HIV positive (%)	HIV negative (%)	P value HIV positive vs HIV negative
MRMs	19/70 (27.1)	11/28 (39.3)	8/24 (33.3)	0.57*	5/5 (100)	10/19 (52.6)	0.07*
Wild type	9/70 (12.9)	8/19 (42.1)	1/9 (11.1)	0.23*	0/5 (0.0)	9/19 (47.4)	0.07*
Undetermined strains	42/70 (60.0)	27/42 (64.3)	15/42 (35.7)	0.008	4/9 (44.4)	36/55 (65.5)	0.20*

Undetermined strains were not included when calculating the proportion between MRMs and wild-type strains and when comparing the prevalence in rectal and urine specimens. MRMs and wild-type strains were not included when calculating the proportion and prevalence between rectal and urine specimens. P values are calculated by Pearson's X^2 test.
*Cells <5 calculated using Fisher's exact test.
MSM, men who have sex with men.

trachomatis and HIV, all showing a prevalence around 7%. Newly detected syphilis was uncommon with a prevalence less than 1%. The prevalence of *M. genitalium* was higher than both *N. gonorrhoeae* and *C. trachomatis* and one probable reason is that screening and treatment is routinely performed for *N. gonorrhoeae* and *C. trachomatis* among visitors to the CSH.

Coinfection rate with either *N. gonorrhoeae* or *C. trachomatis* was high (17.6%) in this group of *M. genitalium*-positive cases and was at the same level as reported by Read *et al* in 2019.[4] In the present study, there was no significant difference between proportions of coinfection with *N. gonorrhoeae* or *C. trachomatis*; this result is corroborated by a study from 2020 by Latimer *et al* who found coinfection with *N. gonorrhoeae* and *C. trachomatis* at the same level in rectal specimens.[17]

M. genitalium, but neither *C. trachomatis* nor *N. gonorrhoeae,* was strongly associated with HIV (OR of 2.60). This has also been reported in earlier studies.[18–20] In women, temporal studies have shown that *M. genitalium* infection increases the risk of acquiring HIV, but no such studies on men are available so far.[21–23]

The prevalence of MRM has been increasing steeply during the last decade and a review and meta-analysis from 2020 shows that the summary global prevalence has increased from 10% in 2010 to 51% in 2016–2017.[6] Geographical differences are reported with a higher rate of MRM in Western Pacific countries than in European countries.[6] In the present population, the MRM was 68% in the 40% of samples that could yield result. Similar prevalence has also been reported in other studies among MSM, such as 70.7% in 2019 by McIver *et al* and 79.4% in 2018 by Couldwell *et al* in Australia.[7 9] This is in large contrast to the rate of MRM among men and women in Sweden where the MRM rate has been reported in the range of 13–21%.[24 25] This difference could be due to a higher rate of testing and use of macrolide antibiotic among MSM in our population, of which a substantial part is using pre-exposure prophylaxis to prevent HIV infection and is tested on a regular basis.

The frequency of MRM showed no difference between rectal and urine samples, which is on par with another study in 2019.[4]

In the light of the rapid spread of AMR,[6] neither Swedish nor European or US guidelines are currently recommending *M. genitalium* testing of rectal specimens in MSM. Urine sampling is recommended in case of urethral symptoms among men in general. European guidelines recommend rectal testing only in case of proctitis when infection with *C. trachomatis* and *N. gonorrhoeae* is excluded.[14]

Using only urine specimens in *M. genitalium* testing in MSM will leave most rectal infections undetected, which may contribute to transmission of especially macrolide-resistant strains. However, detecting and treating rectal *M. genitalium* infections in MSM will increase the development of both MRM and fluoroquinolone resistance and it is necessary to weigh benefits against harms when testing. The benefits of screening for *M. genitalium* are now under debate as evidence of adverse sequelae in men is lacking and effective treatment might not be available for all infected patients. In women, data on the risk of sequelae are still limited.[26]

However, when testing MSM with symptoms of urethritis, an additional rectal test could be considered to interrupt a transmission chain. Diagnostic testing in MSM with symptoms without waiting for results from *C. trachomatis* and *N. gonorrhoeae* would shorten the time to diagnosis and correct treatment. This could decrease the transmission of macrolide-resistant strains and the risk of transmission to women from bisexual men.

Strengths of this study are the large number of paired samples from MSM and that during the study time, all visitors to the CSH were screened which provide data on both asymptomatic and symptomatic participants.

There are several limitations to this study. The ratio of successful MRM analyses was 40.0%. This is likely due to the higher sensitivity of the nucleic acid amplification testing used in this study (APTIMA *M. genitalium* assay) as the macrolide resistance assay had a lower sensitivity, requiring at least 15 copies of *M. genitalium* nucleic acid/µL to yield a result. This suggests that a high proportion of the screened MSM cohort in this study contained a low amount of *M. genitalium* considering that 60% of the positive APTIMA TMA samples did not yield macrolide resistance result, whereas the corresponding rate among a mixed population of visitors to the CSH during 2021–2022 was limited to 20% (502 of 2505). This result likely reflects a high proportion of MSM harbouring presumably very low level of the bacteria and it brings into question the clinical relevance of these low-level infections.

CONCLUSION

This study found a total prevalence of *M. genitalium* of 10.5% among MSM visiting a sexual health clinic. The prevalence was similar to earlier results for MSW in this population. A majority of the infections were rectal mono infections. The proportion of MRMs was high and similar in both urine and rectal samples. Testing this population with urine samples only would leave two-thirds of all *M. genitalium* infections undetected.

A high proportion of samples from this population of MSM could not yield a macrolide resistance result, suggesting a low level of *M. genitalium*. If this low level of *M. genitalium* has any

impact on cure, transmission or symptoms remain to be seen, and future research to answer this question is warranted.

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Contributors All the authors have been contributing to the manuscript. CB—is the guarantor and came up with the idea, inclusion of patients, statistics, analysis and writing. OF—idea, analysis of samples, statistics, analysis and writing. RK—statistics and writing. AJ—idea, inclusion of patients, analysis and writing. SK—inclusion of patients and writing.

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