EPIDEMIOLOGY

Mycoplasma genitalium: prevalence and behavioural risk factors in the general population

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Background: *Mycoplasma genitalium* has been shown to cause urethritis in men and cervicitis in women and may also be a causative agent in female infertility.

Objective: To estimate the prevalence of urogenital *M genitalium* infection and identify sexual behavioural risk factors in the general population.

Methods: Participating individuals were 731 men and 921 women aged 21–23 years and not seeking the healthcare system because of symptoms. They answered questionnaires on sexual behaviour and provided samples for *M genitalium* testing.

Results: In women aged 21–23 years, the prevalence of infection was 2.3% (21/921) and in men of the same age it was 1.1% (8/731). For both sexes, an increasing number of partners was associated with a greater chance of being infected. Among women a shorter duration of a steady relationship and having a partner with symptoms was associated with being infected, and for men younger age at first intercourse was associated with *M genitalium* infection.

Conclusions: We conclude that the prevalence of infection in the general population is too low for population-based screening. However, the development of test algorithms based on behavioural risk factors is a promising alternative.

Mycoplasma genitalium infection has only recently been established as an infection causing urethritis in men and cervicitis in women.¹ Individuals attending sexually transmitted disease (STD) clinics have been the group most thoroughly studied and those with urethritis/cervicitis have been compared with asymptomatic STD clinic attendees. Men with urethritis had *M genitalium* prevalences of 9.4–29.2%,^{2–7} whereas asymptomatic men in the same high-risk populations had prevalences of 0.8–8.5%.^{2–7} The corresponding figures for women with cervicitis in two major studies were 10–11%^{2,8} and for women without cervicitis it was 3–5%. The concordance rate between partners is high, ranging from 45% to 58% of female partners to male index patients^{2,9} whereas 38% and 56% of male partners to female index patients have been shown to be infected.^{2,10} The optimal treatment for the infection has not yet been established, but azithromycin has shown promising results.¹¹

Although *M genitalium* is now established as a relevant sexually transmitted pathogen for symptomatic men and women, little is known about the infection in the general population. Recent research has shown that *M genitalium* is prevalent in women with pelvic inflammatory disease¹ and acute endometritis¹² and has been shown to be able to ascend the upper genital tract¹³ possibly by attaching to human spermatozoa.¹⁴ A sero-epidemiological case-control study¹⁵ has found an association between antibodies against *M genitalium* in women having tubal factor infertility when compared with women having other kinds of infertility. Thus, several studies indicate that *M genitalium* might be a causative agent in female infertility, and this leads to questions on infection in the general asymptomatic population.

The aim of this study was to estimate the prevalence of *M genitalium* infections and to study associations with sexual behavioural risk factors for men and women in the general population.

MATERIALS AND METHODS

Study population

This study was performed on specimens and questionnaires obtained from a population of 21–24-year-old men and women participating in a population-based screening programme on *Chlamydia trachomatis* in 1997–8.¹⁶ Aarhus county has a population of 631 000 inhabitants (12% of the Danish population), and the second largest city in Denmark (Aarhus city) is situated in Aarhus county. A total of 5 000 men and 4 000 women were invited to participate, and 1 033 men and 1 175 women submitted specimens to be tested for *C trachomatis* infection. The participating individuals were asked whether they would accept the storage of the specimen and questionnaire for future research use. A total of 731 men and 921 women accepted and were included for analysis in this study.

Specimens and storage conditions

Participants obtained the specimens by self-collection at home and mailed them by ordinary mail directly to the laboratory. Men mailed a first-void urine (FVU) specimen in a transport container, whereas women mailed a self-administered vaginal pipette specimen as described previously.¹⁶ The diagnostic efficacy of this method for *C trachomatis* testing was established previously.¹⁷ In the laboratory, the specimens were analysed for *C trachomatis* (see below), the test result were mailed directly to the participating individuals, and in cases of a positive-test result the infected individuals contacted a doctor for treatment and partner notification. Immediately after performing the *C trachomatis* analysis, the specimens were stored at –80°C.

Abbreviations: FVU, first void urine; STD, sexually transmitted disease; STI, sexually transmitted infection

Table 1 Behavioural characteristics and social status of individuals included in the study

Sexual behaviour (% of subjects)	Women (n = 921)	Men (n = 731)
Age at first intercourse		
≥1828.138.9	28.1	38.9
14–17	68.1	58.1
≤133.73	3.7	3
Had intercourse during the past 6 months	94	92
>3 partners in a lifetime	64.7	66.7
>3 partners in the past year	12	12.6
In a steady relationship*	69	64.1
Previously tested positive for <i>C trachomatis</i> infection†	12.9	7.1
Previously diagnosed with an STD, including <i>C trachomatis</i> infection†	29.5	20.7
Reported urogenital complaints†	48.4	5.3
Partner known to have urogenital complaints†	34.5	46.2
Use of oral contraception†	60	48.9
Use of condom during most recent intercourse	26.6	31
Social status†	57.8	52.1
Currently in school	32	41.2
Working	7.4	5.1
Unemployed or not working for other reasons (eg, disabled)	39.5	27.9
Living with a partner†		

STD, sexually transmitted disease.

*p<0.05.

†p<0.001.

Detection of *C trachomatis*

All *C trachomatis* analyses were performed on fresh samples in 1997 and 1998 at the Department of Clinical Microbiology, Aarhus University Hospital, Aarhus, Denmark, using a transcription-mediated amplification assay (Gen-probe, San Diego, USA)¹⁸ according to the manufacturer's instructions. *C trachomatis* positive test results were confirmed.

Detection of *M genitalium*

In 2004 and 2005, the specimens were analysed for *M genitalium* infections at Statens Serum Institut, Copenhagen, Denmark. *M genitalium* was detected by an inhibitor-controlled real-time TaqMan polymerase chain reaction using primers detecting the *M genitalium* MgPa adhesion gene.¹⁹ All positive results were confirmed by a polymerase chain reaction detecting the MgPa adhesion gene.²⁰

Questionnaire

Medline was used to search for published studies describing self-reportable predictors for *C trachomatis* infection, and on the basis of these studies we constructed a questionnaire. The predictor for *C trachomatis* infection, "vaginal douching",²¹ was left out of the questionnaire, as this practice is not performed in Denmark and no Danish word exists for the procedure. Symptoms were described as vaginal discharge, dysuria, intermenstrual or irregular bleeding in women and urethral discharge or dysuria in men. The participants were asked to answer the questions by simply ticking boxes and no open questions were asked. All individuals received a questionnaire together with the *C trachomatis* test results from 1997 to 1998, and the tested individuals were asked to complete and return the questionnaire in a prestamped, preaddressed envelope.

Statistical analyses

The questionnaires were coded using Teleform.²² For analysis of data, the computer program STATA was used. First, the

Table 2 Prevalences of *Mycoplasma genitalium* and *Chlamydia trachomatis* infections

	Women (n = 921)		Men (n = 731)	
	n%	(95% CI)	n%	(95% CI)
<i>Mycoplasma genitalium</i> infection	21	2.3 (1.32 to 3.24)	8	1.1 (0.3 to 1.9)
<i>Chlamydia trachomatis</i> infection	77	8.4 (6.6 to 10.2)	41	5.6 (3.9 to 7.3)

prevalence of *M genitalium* and *C trachomatis* infection with 95% confidence intervals (CI) was calculated. Then we performed a univariate logistic regression analysis with self-reportable characteristics as independent variables and *M genitalium* diagnosis as the dependent variable. For continuous variables, several logistic regression analyses including different cut-off points were analysed and the cut-off points with the lowest p value are reported. Because of a low prevalence of *M genitalium* infection resulting in a low number of cases in the analysis, it was not reasonable to perform a multivariate analysis as planned.

Approvals

The study was approved by the local Ethical Committee in the county of Aarhus and the Danish Data Protection Agency.

RESULTS

Prevalence in the general population

The prevalence of *M genitalium* infection was 2.3% (95% CI 1.32% to 3.24%) in women and 1.1% (95% CI 0.3% to 1.9%) in men and the corresponding prevalences of *C trachomatis* were 8.4% (95% CI 6.6% to 10.2%) and 5.6% (95% CI 3.9% to 7.3%) (table 1), respectively. Only 2 of 21 *M genitalium* infected women had a coinfection with *C trachomatis* and 1 of 8 men had dual infection. Table 2 shows the key characteristics of the study population regarding sexual behaviour and social status.

Risk factors for women

In the univariate analysis of risk factors associated with *M genitalium* in women, we found that especially increasing numbers of partners were associated with a greater risk of being infected (table 3). Thus, women with >3 partners during the past 6 months had an OR of 3.48 (95% CI 0.74 to 16.29) of being infected compared with those having 0–1 partner; women with 4–5 partners during the past year had an OR of 19.83 (95% CI 5.01 to 78.55) compared with those having 0–1 partner; and women with >10 lifetime partners had an OR of 7.33 (95% CI 2.44 to 26.34) compared with those having 1–3 lifetime partners. Also, the duration of a steady relationship influenced the *M genitalium* risk for women and being in a steady relationship for more than a year significantly reduced the risk of the infection compared with those in a steady relationship for <6 months (OR = 0.13; 95% CI 0.03 to 0.52). Use of condoms during last intercourse did not significantly reduce the risk of *M genitalium* infection (OR = 0.45; 95% CI 0.13 to 1.55) and use of oral contraception was not associated with the presence of the infection. Having a partner with urogenital symptoms was significantly associated with the infection (OR = 2.49; 95% CI 1.08 to 6.21), but neither symptoms in the tested women nor having other sexually transmitted infections (STIs) were associated with being infected.

Table 3 Univariate analysis of possible risk factors for *Mycoplasma genitalium* infections

	Women		Men	
	OR (95% CI)	p Value	OR (95% CI)	p Value
Sexual relationships				
Age at first intercourse				
10–13 years	1	0.198	1	0.009**
14–17 years	0.37 (0.08 to 1.69)	0.181	0.10 (0.02 to 0.56)	0.010**
18–23 years	0.32 (0.06 to 1.70)		0.07 (0.01 to 0.54)	
Time since last intercourse				
<1-week ago	1	0.135	1	0.618
Between 1 week and 1 month ago	2.05 (0.80 to 5.28)	0.479	0.58 (0.07 to 4.99)	0.544
Between 1 and 3 months ago	0.47 (0.06 to 3.74)	0.907	1.67 (0.32 to 8.74)	
>3 months ago	0.91 (0.20–4.23)		–	
Number of partners during the past 6 months				
0–1 partner	1	0.022*	1	0.078
2–3 partners	2.95 (1.17 to 7.44)	0.113	4.26 (0.85 to 21.36)	0.008**
>3 partners	3.48 (0.74 to 16.29)		11.91 (1.92 to 73.93)	
Number of partners during the past year				
Partners	1	0.002**	1	0.716
2–3 partners	8.06 (2.20 to 29.55)	0.000**	1.40 (0.23 to 8.42)	0.425
4–5 partners	19.83 (5.01 to 78.55)	0.158	2.53 (0.26 to 24.79)	0.019*
>6 partners	5.19 (0.53 to 51.20)		8.85 (1.43 to 54.92)	
Number of lifetime partners				
1–3 partners	1	0.505	1	0.493
4–10 partners	1.61 (0.40 to 6.48)	0.002**	2.21 (0.23 to 21.39)	0.098
>10 partners	7.33 (2.04 to 26.34)		6.43 (0.71 to 58.05)	
Steady relationship				
No	1	0.104	1	0.524
Yes	0.49 (0.20 to 1.16)		1.69 (0.34 to 8.42)	
Duration of steady relationship	N=633		n=463	
<6 months	1	0.445	1	0.549
Between 6 months and 1 year	0.53 (0.10 to 0.44)	0.004**	0.50 (0.05 to 4.88)	0.148
>1 year	0.13 (0.03 to 0.52)		0.04 (0.04 to 1.60)	
Use of contraception				
Use of condom during last intercourse				
No	1	0.208	1	0.282
Yes	0.45 (0.13 to 1.55)		0.32 (0.04 to 2.48)	
Usually use of condoms during intercourse				
No	1	0.967	1	0.189
Yes	1.02 (0.39 to 2.66)		0.24 (0.03 to 2.00)	
I or my partner use oral contraception				
No	1	0.855	1	0.446
Yes	1.09 (0.45 to 2.65)		1.75 (0.41 to 7.38)	
Urogenital complaints/symptoms				
Urogenital complaints while tested				
No	1	0.606	1	0.245
Yes	0.79 (0.33 to 1.90)		3.63 (0.41 to 7.38)	
Partner with symptoms				
No	1	0.033*	1	0.830
Yes	2.59 (1.08 to 6.21)		1.16 (0.29 to 4.69)	
Other sexually transmitted diseases				
Actual <i>C trachomatis</i> positive test result				
No	1	0.846	1	0.410
Yes	1.16 (0.26 to 5.07)		2.44 (0.29 to 20.31)	
Earlier <i>C trachomatis</i> positive test result				
No	1	0.849	1	0.069
Yes	1.13 (0.33 to 3.89)		4.53 (0.89 to 23.04)	
Earlier STI (including <i>C trachomatis</i>)				
No	1	0.658	1	0.722
Yes	1.23 (0.49 to 3.08)		1.34 (0.27 to 6.71)	

STI, sexually transmitted infection.

Risk factors for men

For men as in women increasing numbers of partners were associated with greater risk of being infected, and, thus, having >3 partners during the past 6 months increased the risk of *M genitalium* infection with an OR of 11.91 (95% CI 1.91 to 73.93)

compared with those having 0–1 partner. Furthermore, men with >6 partners during the past year had an OR of 8.85 (95% CI 1.43 to 54.92) compared with those having 0–1 partner and men with >10 lifetime partners had an OR of 6.43 (95% CI 0.71 to 58.05) of being infected with *M genitalium* compared with

those having 1–3 partners. Among men, a significant reduction in the prevalence of *M genitalium* could be seen if sexual debut was after 18 years compared with a sexual debut at 10–13 years (OR = 0.07; 95% CI 0.01 to 0.54). Neither contraceptive habits, symptoms nor other STIs could be shown to significantly influence the presence of *M genitalium* infection.

DISCUSSION

In this study, we estimated the prevalence of *M genitalium* infection in the general population of 21–23-year-old individuals and identified behavioural risk factors for the infection. Among women, the prevalence of infection was 2.3% (95% CI 1.32% to 3.24%) and the associated risk factors were an increasing number of partners during the past 6 and 12 months, a shorter duration of steady relationship and having a partner with symptoms. The prevalence of infection in men was 1.1% (95% CI 0.3% to 1.9%) and younger age at first intercourse and a higher number of partners during the past 6 and 12 months were significantly associated with being infected.

The establishment of *M genitalium* as a cause of sexually transmitted infections and subsequently the evidence in a seroepidemiological case–control study of an association between *M genitalium* antibodies and tubae factor infertility¹⁵ obviously calls for investigation of the infection in the general population. Thus, this study aims to assist in the development of evidence-based guidelines regarding whether and how the infection should be handled in the general population. To our knowledge, studies on the prevalence in the general population have not been published and a systematic evaluation of behavioural *M genitalium* risk factors has only been published regarding women in a high-risk population.⁸

It might be a limitation of our study that *M genitalium* analysis was performed on specimens that had been stored at –80°C for 7 years. This could potentially decrease the *M genitalium* detection rate compared with the prevalence of true infection in the population, but a recent study¹⁹ showed that freezing of male FVU at –20°C for 1–18 months did not significantly reduce sensitivity. In the same study, a decreased detection rate was observed for female FVU after freezing,¹⁹ but the vaginal pipette specimens used in our study have not been studied regarding stability of *M genitalium* DNA after storage. However, as the prevalence of infection among women in our study corresponds to the expected prevalence of *M genitalium* based on findings in other studies (see below), we believe that loss of *M genitalium* DNA in the stored vaginal pipette specimens is low. Another limitation of our study is related to the analysis of risk factors as the low prevalence of infection, especially among men, resulted in a low number of cases available for the multivariate analysis. However, this is among one of the first studies to evaluate risk factors in the general population and even with a higher number of cases our study would mainly constitute a hypothesis on parameters to select in future research aimed at producing test algorithms for use in the general population. In future research of risk factors in the general population, larger populations will be warranted.

Recently, an abstract was presented by Manhart *et al*²³ showing a prevalence of *M genitalium* of 1.1% in men and 1.4% in women from the general American population aged 18–27 years. In other studies, different populations with an expected low risk of STIs have been defined as control groups. In a Swedish study¹⁰ none of 59 women aged 22–26 years attending a clinic for a pap-smear were infected, in two Japanese studies pregnant women^{24–25} had prevalences of 0% (0/80) and 1% (1/90), and in a study from the United Kingdom²⁶ none of 37 women aged 21–45 years undergoing tubal ligation had *M genitalium*. In asymptomatic women attending STD clinics, the prevalence was 3%² and 5%.⁸ Thus, a prevalence of

Key messages

- *Mycoplasma genitalium* is a sexually transmitted infection.
- The prevalence of *M genitalium* is low in the general population.
- A high-risk population can be identified through sexual behavioural factors.
- Selective screening for *M genitalium* in the general population may be a possibility in the future.

2.3% among women in our study is within the expected range as our population consists of a mixture of high and low-risk individuals, as is seen in the descriptive data of the population (table 1). For males, a low prevalence of 1.1% is also in line with other studies showing a prevalence of 1.1% in men attending a Department of Medicine for check-up²⁷ in Japan and prevalences of 0.8% to 8.5% among asymptomatic men seen in STD clinics.^{2–7} The low coinfection rate with *C trachomatis* among both men and women is somewhat surprising, but in line with results found in other studies.^{2–10} On the basis of the low prevalence in the general population, a widespread screening of the population between 21 and 24 years of age will not be feasible even if *M genitalium* at a later stage shown to be of importance in developing infertility. Thus, the definition of risk factors and definitions of test algorithms might be of major importance in the future.

In our study, we identified several sexual behavioural risk factors significantly associated with *M genitalium* infection, which supports the fact that the mode of transmission is sexual. The risk factors showing the strongest association with *M genitalium* infections in our study were increasing numbers of partners, and this is in line with an earlier study of risk factors among women attending an STD clinic in Seattle.⁸ We also found that condoms were not significantly protective against *M genitalium* infection, and this is in line with the study from Seattle⁸ and with a Chinese study of condom use in commercial sex workers,²⁸ where only an insignificant protection was found. This points to the possibility that condoms might be less protective against *M genitalium* infection than against other STIs such as *C trachomatis*.²⁹

We conclude that the prevalence of *M genitalium* infection in the general population is low. However, based on our results we also hypothesise that it will be possible in the future to define test algorithms based on behavioural risk factors to select individuals with a higher prevalence of infection. With the current knowledge, it is not known whether such selective screening will be relevant to prevent urogenital and reproductive complications, and further research in long-term complications related to *M genitalium* infections is urgently needed.

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