

BMJ Open A cross-sectional study of *Mycoplasma genitalium* infection and correlates in women undergoing population-based screening or clinic-based testing for *Chlamydia* infection in London

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ABSTRACT

Objective: To determine *Mycoplasma genitalium* infection and correlates among young women undergoing population-based screening or clinic-based testing for *Chlamydia* infection.

Design: Cross-sectional study.

Setting: National Chlamydia Screening Programme (NCSP) and two London sexually transmitted infection (STI) clinics.

Participants: 2441 women aged 15–64 years who participated in the NCSP and 2172 women who attended two London STI clinics over a 4-month period in 2009.

Outcome measures: (1) *M genitalium* prevalence in defined populations (%). (2) Age-adjusted ORs (aORs) for correlates of *M genitalium* infection.

Results: The overall frequency of *M genitalium* and *Chlamydia trachomatis* was 3% and 5.4%, respectively. Co-infection was relatively uncommon (0.5% of all women); however 9% of women with *C trachomatis* also had *M genitalium* infection. *M genitalium* was more frequently detected in swab than urine samples (3.9 vs 1.3%, $p < 0.001$) with a significantly higher mean bacterial load ($p \leq 0.001$). Among NCSP participants, *M genitalium* was significantly more likely to be diagnosed in women of black/black British ethnicity (aOR 2.3, 95% CI 1.2 to 4.5, $p = 0.01$). *M genitalium* and *C trachomatis* were both significantly associated with multiple sexual partners in the past year (aOR 2.4, 95% CI 1.3 to 4.4, $p = 0.01$ and aOR 2.0, 95% CI 1.4 to 2.8, $p < 0.01$). Among STI clinic attendees, *M genitalium* was more common in women who were less than 25 years in age.

Conclusions: *M genitalium* is a relatively common infection among young women in London. It is significantly more likely to be detected in vulvovaginal swabs than in urine samples. Co-infection with *Chlamydia* is uncommon. The clinical effectiveness of testing and treatment strategies for *M genitalium* needs further investigation.

INTRODUCTION

Pelvic inflammatory disease (PID) and its sequelae (chronic pelvic pain, ectopic pregnancy and tubal infertility) are major causes

Strengths and limitations of this study

- This is the largest UK based-cross-sectional study to date to provide estimates of *Mycoplasma genitalium* prevalence in both community and sexually transmitted infection clinic-based populations.
- *M genitalium* PCR results were confirmed positive by genotype sequencing.
- Our analysis of potential correlates for *M genitalium* and *Chlamydia trachomatis* is limited by the availability of data.

of morbidity in women in developed and developing countries.¹ In the USA more than \$10 billion is spent annually in treating these conditions.² *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, two sexually transmitted infections (STIs) are known causes of PID. However, in up to 70% of PID cases no cause is found³ and there is increasing evidence that *Mycoplasma genitalium* might be a cause of PID.^{4–8}

There is also strong evidence that it is sexually transmitted.^{5–6} It is significantly associated with endometritis and⁹ tubal factor infertility¹⁰ although the association with cervicitis is complex.^{11–12} As with *C trachomatis* it can be asymptomatic, acting as a reservoir for further spread.¹³ It may also be associated with HIV acquisition.¹⁴

Although at present *M genitalium* is not routinely tested for in most countries, there is interest in introducing testing and treatment. However, before this is done there is a need to gain a better understanding of the infection to avoid repeating the problems encountered with *C trachomatis* screening.¹⁵ In the UK there are few data on the frequency of *M genitalium* infection in different population groups of women. Oakeshott *et al*¹⁶ found that *M genitalium*

prevalence was 3.3% among young women in a community based sample who took part in a *C trachomatis* screening trial in the UK. Estimates from studies in other countries indicate that levels of *M genitalium* are 40–60% lower than *C trachomatis*, with little co-infection.^{17 18} The recommended treatment for uncomplicated Chlamydia infection is a single dose of azithromycin 1 g stat. There is growing evidence of considerably lower *M genitalium* cure rates with this dose of azithromycin compared with *C trachomatis* (79–87% vs 92–97%, respectively).^{19–21} Resistance has been shown to develop following 1 g of azithromycin and macrolide resistance is endemic in some populations.^{22–24}

We investigated *M genitalium* infection by real-time PCR and determined its correlates in the largest cross-sectional study of *M genitalium* among women screened for *C trachomatis* in the National Chlamydia Screening Programme (NCSP) and STI clinics in the UK.

METHODS

Patients and specimens

We used an unlinked anonymised method to test routinely collected and stored cervical swabs, self-taken vaginal swabs and first catch urine samples for *M genitalium*. The samples were from 2180 women aged 15–64 years who had *C trachomatis* screening when they attended two STI clinics in central and North London and 2455 women aged 15–24 years who participated in the NCSP in London in a 4-month period in 2009. Each clinic offers comprehensive STI screening, treatment and partner notification services to symptomatic and asymptomatic women and men, irrespective of age. Samples from all female clinic attendees were eligible for the study. The NCSP is a national screening programme for Chlamydia in the UK among women and men who are under 25 years in age. The NCSP samples were from a variety of low and high STI risk settings within two London boroughs. In 2009 the majority of participating sites from which the samples were tested were family planning clinics (47%), universities (17%) and general practices (16%). Other testing sites included pharmacies, abortion services, outreach, young persons' services, schools and postal testing (Tina Sharp, NCSP Chlamydia Coordinator, personal communication).

The samples were originally collected from the NCSP and clinics and transported to the Microbiology Laboratory at University College London Hospital in 3 mL (self-taken vaginal and cervical swabs) or 4 mL (urine samples diluted 1:1) of APTIMA transport medium (Gen-Probe Inc, San Diego, USA) for routine *C trachomatis* testing. After *C trachomatis* testing the negative samples were stored for 6 weeks at –20°C and positive samples were stored for 3 months at –20°C before they were released for testing as part of this study. Available demographic, sexual behaviour, clinical PID diagnosis and STIs data were recorded before samples were unlinked from all personal identifiers prior to *M genitalium* testing.

M genitalium testing

Samples were thawed and DNA from 200 µL of the APTIMA transport medium was purified by BioRobot 9604 automated workstation using the QIAamp Virus BioRobot 9604 Kit (QIAGEN, Hilden, Germany). Before freezing and storing the eluate at –20°C it was tested by quantitative PCR (qPCR) adapted from a method by Jensen *et al.*^{17 25} The qPCR targeted the MgPa adhesion gene (MG191) using MgPa-355FW and MgPa-432R primers and MgPa-380 MGB probe (primers and probes were provided by Applied Biosystems, Warrington, UK). Pilot laboratory work showed no difference in APTIMA Urine Specimen Transport Tubes and APTIMA Vaginal Swab Specimen Collection Kit transport medium and phosphate-buffered saline (PBS) spiked with *M genitalium* DNA in different concentrations.

We introduced a degenerate oligonucleotide ('wobble') in the forward primer to account for a frequent detected base substitution that has previously been shown to be successful in another study by Chalker *et al.*²⁶ As an internal control for PCR inhibition we used murine cytomegalovirus (mCMV) and primers mCMVTAQ1 (forward primer) and mCMVTAQ2 (reverse primer) and mCMVTAQPR probe labelled with JOE (Primers and probe were provided by Eurofins MWG Operon) designed by Garson *et al.*²⁷ The qPCR assays were performed in 25 µL volumes; comprising 1× EXPRESS qPCR Supermix (Universal, Invitrogen, Life technologies Ltd Paisley, UK), 0.4 µM forward and reverse primers, 0.2 µM probes and 7.5 µL of samples and nuclease-free water (Promega UK Ltd, Southampton, UK).

Thermal cycling was performed on an ABI 7500 Real-time PCR instrument using the following conditions: hotstart at 95°C for 2 min and 1 cycle, denaturation at 95°C for 15 s, annealing and extension at 60°C for 1 min and 45 cycles. The data was analysed using Sequence Detection Software (SDS) V.1.4 with manual baseline/threshold settings to estimate quantification cycle.

Positive samples were re-extracted and retested by qPCR. If these tested negative the samples were re-extracted and tested by qPCR a third time. If negative again the sample was considered equivocal and was excluded from the analysis.

M genitalium genotyping

M genitalium PCR-positive samples were sequenced by MgPa1–3 typing assay according to Hjort *et al.*²⁸ The assay was modified with respect to PCR reagents and PCR conditions. In a total volume of 50 µL the following were mixed: 25 µL of Taq PCR Master Mix kit (QIAGEN, Hilden, Germany), 0.4 µM of mgpa-1 and mgpa-3 primers, 5 µL of template and nuclease-free water. To increase the sensitivity of the assay 10 µL of the template was used in cases where the bacterial load was less than 1 genome copy/µL.

The PCR was performed on an ABI9700 instrument and in three-step cycling conditions: denaturation at 94°C

C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min and 50 cycles.

The amplified product were purified manually by QIAquick PCR purification kit (QIAGEN, Hilden, Germany) and sent to the UCL sequencing service for sequencing of both the forward and reverse strand.

Statistical analysis

We have only included data from women who are at least 15 years old in the analysis. Data were analysed using SPSS V.14.0 for Windows. Paired sample t test was used to compare the difference of mean values. Multiple logistic regression analysis was used to investigate the relationship between *M genitalium* or *C trachomatis* infection and demographic and sexual behaviour characteristics in women attending NCSP or STI clinics.

Categorical variables in the NCSP model included participant age, specimen type, a new sexual partner within 3 months, more than one partner within 12 months and ethnicity. The categorical variables included in the STI model were participant age, specimen type, current STI infections and ethnicity. Frequency, ORs adjusted for age (aOR) and 95% CIs were calculated and values of $p < 0.05$ were considered statistically significant.

RESULTS

Of 4635 samples, we excluded 21 samples for which the *M genitalium* test result was equivocal and included 4613 samples in our analysis (figure 1).

NCSP participants were aged 15–24 years whereas STI clinic attendees were aged 15–64 years. Women attending the two clinics had significantly different mean ages (20.1 years, SD 2.5 vs 27.8 years, SD 7.6 years, $p < 0.0001$). The highest prevalence of *M genitalium* and *C trachomatis* was in age groups 15–24 years in NCSP and the STI clinics. As we only had ethnicity data for 39% (851/2172) of the STI clinic attendees, we did not compare ethnicity across the clinics.

M genitalium and *C trachomatis* infection

As shown in table 1, the overall frequency of *M genitalium* and *C trachomatis* was 3% (138/4613, 95% CI 2.5% to 3.5%) and 5.4% (249/4613, 95% CI 4.8% to 6.1%), respectively. The overall co-infection rate was 0.5% (23/

4613, 95% CI 0.3% to 0.7%). Of 249 women with *C trachomatis*, 23 (9%) women had *M genitalium* infection.

Among NCSP participants, *M genitalium* and *C trachomatis* frequency were 2.3% (57/2441, 95% CI 1.7% to 2.9%) and 6.8% (166/2441, 95% CI 5.8% to 7.8%), respectively.

M genitalium infection significantly differed between the two clinics (5.3%, 95% CI 3.7% to 7.0% and 3.0%, 95% CI 2.1% to 3.8%, $p < 0.01$) but the difference was not significant after adjusting for age ($p = 0.16$). *C trachomatis* did not differ significantly between the two clinics (3.2%, 95% CI 1.9% to 4.5% and 4.1%, 95% CI 3.1% to 5.1%, $p = 0.30$).

Association of *M genitalium* and *C trachomatis* with sexual behaviour and demographic characteristics of participants in the NCSP

Table 2 shows the association of *M genitalium* and *C trachomatis* with sexual behaviour and demographic characteristics among NCSP participants. *M genitalium* was less frequently detected than *C trachomatis* in both age groups (15–19 years old 2.8%, 29/1045 vs 8.3%, 83/1045 and 20–24 years old 2.0%, 28/1396 vs 5.7%, 79/1396, respectively). When adjusted for age *M genitalium* was significantly more common in black/black British women compared with white women (aOR 2.3, 95% CI 1.2 to 4.5, $p = 0.01$). Women who reported multiple sexual partners in the past 12 months were twice as likely to have both *M genitalium* and *C trachomatis* infections compared with women who reported only one partner (aOR 2.4, 95% CI 1.3 to 4.4, $p = 0.01$) and (aOR 2.0, 95% CI 1.4 to 2.8, $p < 0.01$), respectively. Women who reported new sexual partners in the previous 3 months were also more likely to have *C trachomatis* infection (aOR 1.6, 95% CI 1.1 to 2.3, $p = 0.01$). Those who did not self-identify as white, black/black British, Asian/Asian British or mixed ethnicity were less likely to be infected with *C trachomatis* compared with white women (aOR 0.6, 95% CI 0.4 to 0.9, $p = 0.01$).

Association of *M genitalium* and *C trachomatis* with sexual behaviour and demographic characteristics of STI clinic attendees

Table 3 shows the association of *M genitalium* and *C trachomatis* with sexual behaviour and demographic characteristics among STI clinic attendees. The age

Figure 1 Of 4635 samples, 21 samples were excluded for which the *M genitalium* test result was equivocal and 4613 samples were included in the analysis.

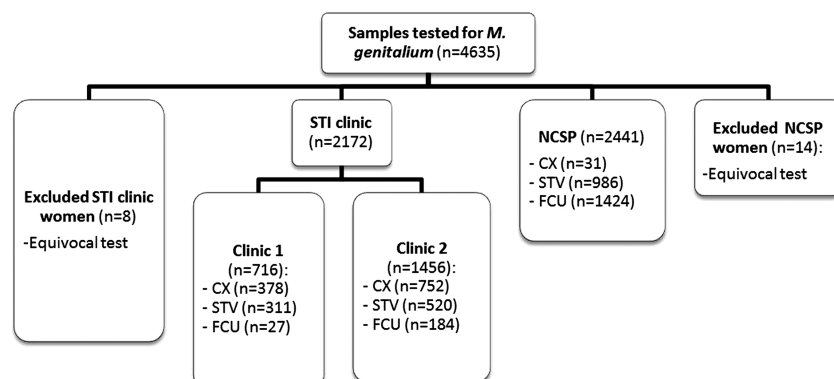


Table 1 *Mycoplasma genitalium* and *Chlamydia trachomatis* infection among NCSP and STI clinic attendees

Infection	Clinic 2 N=716 N (%; 95% CI)	Clinic 1 N=1456 N (%; 95% CI)	NCSP N=2441 N (%; 95% CI)	Total N=4613 N (%; 95% CI)
<i>M genitalium</i> and <i>C trachomatis</i>	3 (0.4, 0 to 0.9)	4 (0.3, 0 to 0.6)	16 (0.7, 0.4 to 1.0)	23 (0.5, 0.3 to 0.7)
Total <i>M genitalium</i>	38 (5.3, 3.7 to 7.0)	43 (3.0, 2.0 to 3.9)	57 (2.3, 1.7 to 2.9)	138 (3.0, 2.5 to 3.5)
<i>M genitalium</i> only	35 (4.9, 3.3 to 6.5)	41 (2.8, 2.0 to 3.7)	39 (1.6, 1.1 to 2.1)	115 (2.5, 2.0 to 2.9)
Total <i>C trachomatis</i>	23 (3.2, 1.9 to 4.5)	60 (4.1, 3.1 to 5.1)	166 (6.8, 5.8 to 7.8)	249 (5.4, 4.8 to 6.1)
<i>C trachomatis</i> only	20 (2.8, 1.6 to 4.0)	56 (3.8, 2.9 to 4.8)	150 (6.1, 5.2 to 7.1)	226 (4.9, 4.3 to 5.5)

NCSP, National Chlamydia Screening Programme; *M genitalium*, *Mycoplasma genitalium*; *C trachomatis*, *Chlamydia trachomatis*; STI, sexually transmitted infection.

distribution for both *M genitalium* and *C trachomatis* was similar with infections more frequently detected in younger women (15–19 years 9.7%, 18/186 vs 6.4%, 12/186, respectively and 20–24 years 6.2%, 41/665 vs 6.0%, 40/665) than other age groups. *M genitalium* was more frequently detected in 15–19 year-old women than *C trachomatis* although this was not statistically significant ($p=0.28$).

Specimen type and bacterial load

Overall *M genitalium* was detected in 3.7% (43/1161), 4.0% (74/1817) and 1.3% (21/1635) of cervical swabs, self-taken vulval swabs and first-void urine samples, respectively. Since *M genitalium* frequency in cervical and self-taken swabs was similar ($p=0.86$), the results for the two groups of swabs were merged and tested against first-void urine samples in the statistical model. *M genitalium* was

Table 2 Association of characteristics with *Mycoplasma genitalium* and *Chlamydia trachomatis* in NCSP attendees

Characteristic	(N=2441) Per centage of women with characteristic	<i>M genitalium</i> (%) (proportion of women)	aOR* (95% CI)	p Value	<i>C trachomatis</i> (%) (proportion of women)	aOR* (95% CI)	p Value
Age							
15–19	41.6	2.8 (29/1045)			8.3 (87/1045)		
20–24	56.5	2.0 (28/1396)			5.7 (79/1396)		
Ethnicity							
White	46.6	2.0 (23/1138)	1		7.4 (84/1138)	1	
Black or black British	12.8	4.8 (15/314)	2.3 (1.2 to 4.5)	0.01	8.3 (26/314)	1.1 (0.7 to 1.7)	0.83
Asian or Asian British	4.4	1.9 (2/108)	0.9 (0.2 to 4.0)	0.93	6.2 (5/108)	0.6 (0.3 to 1.6)	0.33
Mixed	7.7	3.7 (7/187)	1.8 (0.8 to 4.3)	0.18	10.2 (19/187)	1.3 (0.8 to 2.3)	0.29
Other ethnic groups	28.4	1.4 (10/694)	0.7 (0.3 to 1.5)	0.35	4.6 (32/694)	0.6 (0.4 to 0.9)	0.01
New sexual partner in previous 3 months							
Yes	31.5	3.2 (25/770)	1.5 (0.8 to 2.6)	0.20	9.2 (71/770)	1.6 (1.1 to 2.3)	0.01
No	39.3	2.2 (21/959)	1		5.8 (56/959)	1	
Do not want to answer	0.2	0.0 (0/6)	–	–	0.0 (0/6)	–	–
Not filled in	28.9	1.6 (11/706)	0.7 (0.3 to 1.4)	0.33	5.5 (39/706)	0.9 (0.6 to 1.4)	0.69
Sex with >1 partner within 12 months							
Yes	30.8	3.9 (29/751)	2.4 (1.3 to 4.4)	0.01	10.0 (75/751)	2.0 (1.4 to 2.8)	<0.01
No	39.5	1.7 (16/963)	1		5.4 (52/963)	1	
Do not want to answer	0.3	0.0 (0/8)	–	–	0.0 (0/8)	–	–
Not filled in	29.5	1.7 (12/719)	1.0 (0.5 to 2.1)	0.99	5.4 (39/719)	1.0 (0.6 to 1.5)	0.99
Specimen							
Cervical/ Self-taken vaginal	1.3	3.2 (1/31)	3.3 (0.4 to 25.8)	0.26	9.7 (3/31)	2.0 (0.6 to 7.4)	0.21
First catch urine	40.4	4.2 (41/986)	4.2 (2.3 to 7.6)	<0.001	9.3 (92/986)	2.0 (1.5 to 2.8)	<0.001
	58.3	1.0 (15/1424)	1		5.0 (71/1424)	1	

*aOR, ORs adjusted for age only.

Table 3 Association of characteristics with *Mycoplasma genitalium* and *Chlamydia trachomatis* in women attending two London STI clinics

Characteristic	(N=2172) Percentage of women with characteristic	<i>M genitalium</i> (%) (proportion of women)	aOR* (95% CI)	p Value	<i>C trachomatis</i> proportion of women)	aOR* (95% CI)	p Value
Age							
15–19	8.6	9.7 (18/186)			6.4 (12/186)		
20–24	30.6	6.2 (41/665)			6.0 (40/665)		
25–29	28.6	1.6 (10/621)			2.9 (18/621)		
30–34	15.6	2.3 (9/339)			3.2 (11/339)		
35–64	16.6	0.8 (3/361)			0.6 (2/361)		
Ethnicity							
White	23.0	6.0 (30/499)	1		7.0 (35/499)	1	
Black or black British	6.9	7.4 (11/149)	1.2 (0.6 to 2.5)	0.60	4.0 (6/149)	0.5 (0.2 to 1.3)	0.54
Asian or Asian British	1.7	17.6 (6/36)	3.1 (1.2 to 8.1)	0.19	5.6 (2/36)	0.8 (0.2 to 3.4)	0.73
Mixed	3.9	4.8 (4/84)	0.7 (0.2 to 2.1)	0.54	7.1 (6/84)	0.9 (0.4 to 2.3)	0.91
Other Ethnic groups	3.9	9.5 (8/83)	1.6 (0.7 to 3.7)	0.24	3.6 (3/83)	0.5 (0.1 to 1.6)	0.49
Unknown	60.8	1.7 (22/1321)	0.5 (0.2 to 1.1)	0.09	2.3 (31/1321)	0.7 (0.3 to 1.4)	0.66
Specimen							
Cervical/ Self-taken	90.3	3.8 (75/1961)	1.4 (0.6 to 3.2)	0.48	3.4 (38/1130)	0.7 (0.4 to 1.6)	0.44
vaginal					4.3 (36/831)	0.9 (0.4 to 2.0)	0.83
First catch urine	9.7	2.8 (6/211)	1		4.3 (9/211)	1	

*aOR, ORs adjusted for age only.
STI, sexually transmitted infection.

significantly more likely to be detected in swabs compared with urine specimens (3.9 vs 1.3%, $p<0.001$).

The overall frequency of *C trachomatis* in cervical swabs, self-taken vulval swabs and first-void urine samples was 3.5% (41/1161), 7.0% (128/1817) and 4.9% (80/1635), respectively. *C trachomatis* significantly differed between cervical and self-taken swabs ($p<0.001$) and the two groups were separately tested against the urine samples in the statistical model.

The majority (58%, 1424/2441) of specimens provided by the women in NCSP were urine samples. However, swab samples were almost four times more likely to test positive for *M genitalium* compared with urine samples (aOR 3.6, 95% CI 1.9 to 6.7, $p<0.001$) and *C trachomatis* infection was almost twice as high among swabs compared with urine samples (aOR 1.8, 95% CI 1.2 to 2.4 $p=0.001$). Conversely the majority (90.3%, 1961/2172) of clinic specimens were swabs. *M genitalium* and *C trachomatis* in the clinic swab and urine specimens also differed (*M genitalium* 3.8%, 75/1961 vs 2.8%, 6/211 and *C trachomatis* 3.8%, 74/1961 vs 4.3%, 9/211, respectively).

In quantitative analysis of *M genitalium* positive specimens, mean *M genitalium* bacterial load in swab and urine samples did not significantly differ between the clinics or NCSP. Clinical data were therefore combined for comparison of the mean bacterial load in different specimen types. There was no difference in overall cervical and self-taken vaginal swab bacterial loads (3.72 (CI

3.39 to 4.05) vs 3.91 (CI 3.66 to 4.17) $\log_{10}$ genome copies/mL, equivalent to geometric means of 5218 (CI 2438 to 11 171) and 8192 (CI 4575 to 14 669) organisms/mL, respectively; $p=0.349$). The overall mean bacterial load in swabs 3.84 (CI 3.52 to 4.11) equivalent to 6705 (CI 3506 to 12 920) organisms/mL was significantly higher than in first-void urine samples (3.14 (CI 2.87 to 3.41) equivalent to 1386 (CI 740 to 2597) organisms/mL) ($p<0.0001$, equal variances not assumed).

Genetic diversity

The absence of false-positive results was confirmed by the presence of 57 different genotypes by sequence analysis of 127 *M genitalium*-positive specimens and 13 sequences from previously isolated strains (figure 2). The discriminatory index by Hunter and Gaston²⁹ was calculated to be 0.94 both with and without inclusion of the previously isolated strain sequences. None of the sequenced samples were identical with the type strain G37 used as a PCR standard control. Genetic diversity data are available in FASTA format for download in the online supplementary material.

DISCUSSION

Overall *M genitalium* was relatively common at 3% among NCSP participants and STI clinical attendees. *M genitalium* was more likely to be found in swabs

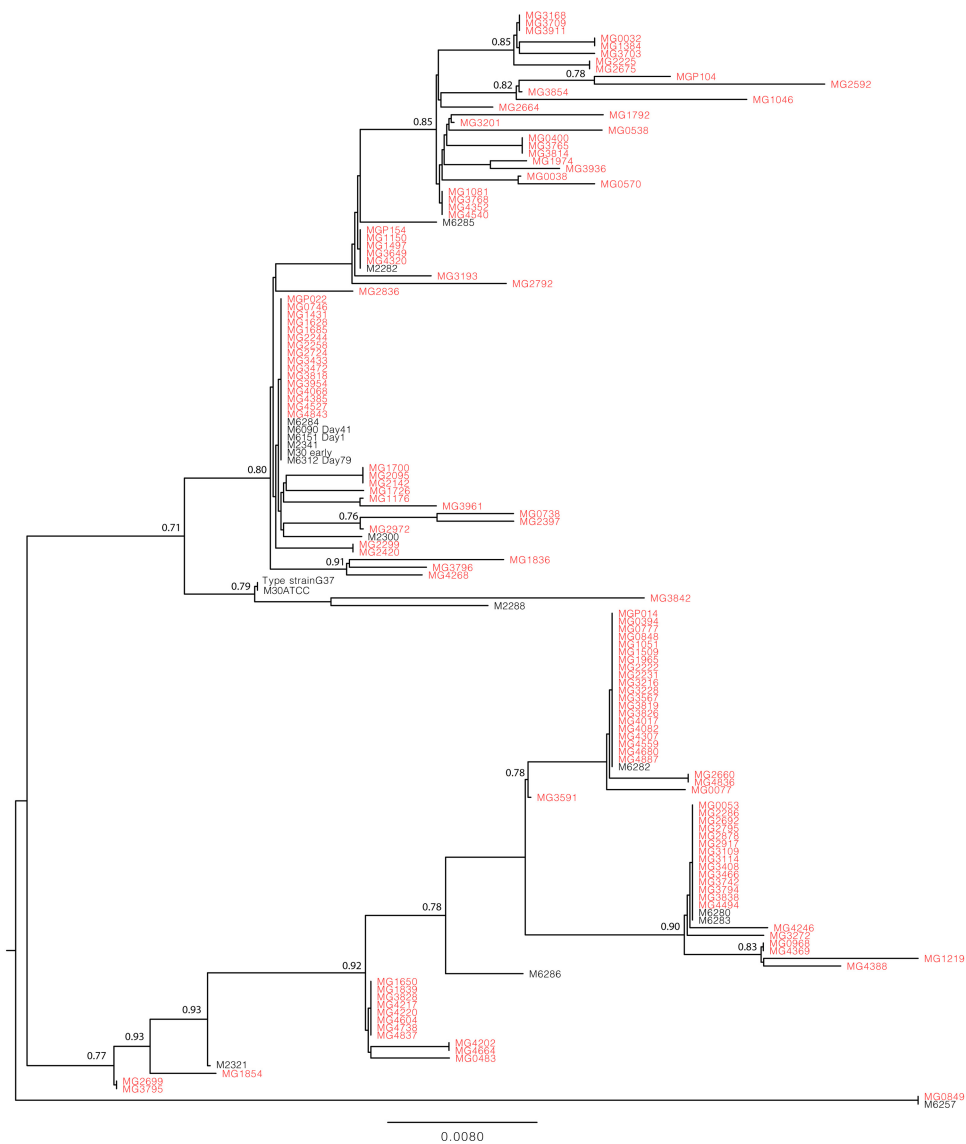


Figure 2 Phylogenetic tree showing clustering of 127 DNA sequences from the *Mycoplasma genitalium*-positive specimens of the study (marked with grey font) and 13 DNA sequences from *M genitalium* strain from patients with no known sexual relationship (marked with black font).

compared with urine samples (3.9% vs 1.3%, respectively) and the mean bacterial load was also much higher (6705 (CI 3506 to 12 920) organisms/mL vs 1386 (CI 740 to 2597) organisms/mL, respectively).

Only 0.5% of all the women had both *C trachomatis* and *M genitalium* infections. Among women who had *C trachomatis*, 9% were coinfectd with *M genitalium* compared with <5% in population-based studies.^{16 18 30 31} Among NCSP participants the age-adjusted odds of detecting *M genitalium* were twice as high among women of black/black British ethnicity (aOR 2.3) and those reporting multiple sexual partners in the past year (aOR 2.4) compared with women of white ethnicity or those who reported only one partner, respectively. After adjusting for age, *C trachomatis* was also significantly more likely to be diagnosed in women with multiple partners (aOR 2.0) and new sexual partners in the previous 3 months (aOR 1.6) but was less likely to be detected in

women who did not give a self-identified ethnic group (aOR 0.6) compared with reporting only one partner, not reporting new partners or being of white ethnicity, respectively. No significant associations were observed for either infection among STI clinical attendees.

This is the largest UK-based *M genitalium* study to date to provide estimates of infection among both community and STI clinic-based populations. Transport media may affect the sensitivity of DNA-based PCR tests. The study samples were originally collected in APTIMA medium. We, therefore, tested APTIMA and PBS media with *M genitalium* DNA and did not find any differences. We confirmed positive *M genitalium* PCR results by genotype sequencing. Our analysis of *M genitalium* and *C trachomatis* correlates is limited by availability of data: only age and ethnicity were available for both clinical and NCSP datasets and ethnicity data was missing for 61% of STI clinical attendees. There is also a possibility that

some young women may have had Chlamydia tests through both the NCSP and the STI clinics during the sample collection period. It is not possible to quantify this although we speculate that the numbers are likely to be low given the relatively short time frame.

Our STI clinical *M genitalium* frequency is similar to that found in several studies of female STI clinical attendees (4.5–7%)^{32 33} although other studies have reported much higher frequencies (19.3–38.2%).^{12 34} In lower risk non-STI clinical attendees such as college students infection has been shown to range from <1% to 5%^{5 35} which is in keeping with our estimate in the Chlamydia screening population. The higher frequency of *M genitalium* in women attending clinics than the NCSP (3–5.3% vs 2.3%, respectively) may in part reflect the higher proportion of swabs taken in clinics than in NCSP settings. Urine samples have been shown to be less sensitive for *M genitalium* diagnosis than swabs (61–65% compared with 74–91%).^{36 37} It is therefore likely that our NCSP *M genitalium* frequency is an underestimation. Although urine sample sensitivity may be increased by up-concentrating the samples by centrifugation this is not a practical step for large scale testing. A higher bacterial load may be associated with symptoms as has been shown for men.²⁵ This may also explain the difference in infection between the two populations since STI clinical attendees are more likely to be symptomatic than NCSP participants. The association of *M genitalium* with multiple sexual partners and black ethnicity has been previously observed.^{16 31} Additional risk factors include younger age as observed in our STI clinical attendees, bacterial vaginosis, being symptomatic, cervicitis, douching, smoking, prior miscarriage, menstrual cycle, social class and marital status.^{12 16 31 38–40}

M genitalium appears to be a relatively common infection among women in London. The low level of *M genitalium* and *C trachomatis* co-infection (0.5%) suggests that diagnosing and treating Chlamydia will have little impact on *M genitalium*. However, azithromycin 1 g used to treat uncomplicated *C trachomatis* infection appears to be sub-optimal for *M genitalium* treatment.²⁴ This treatment dose has also been associated with the development of *M genitalium* macrolide resistance in some studies of predominantly symptomatic men.^{22 24} The risk of inadvertent *M genitalium* antibiotic resistance in coinfecting women who are treated for Chlamydia with 1 g of azithromycin is therefore potentially a cause for concern although further research is required to confirm this.

To avoid the problems encountered with *C trachomatis* screening and *M genitalium* antimicrobial resistance, prior to introducing routine testing for *M genitalium*, further research is needed to better understand its natural history, the role of asymptomatic and symptomatic *M genitalium* in PID and determine optimum treatment guidelines.

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Contributors HFS, SSD, CC, PG, SM-J, MK and JMS contributed to conception and design of the study and/or to acquisition of data. HFS performed the experiments. HFS, SSD and JS drafted the paper and all authors contributed to critical revision of the paper.

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Competing interests None.

Patient consent None.

Ethics approval On the advice of the chair of the local ethics committee, ethical approval was not required since the study team received anonymised samples for testing in the study from the laboratory and no other identifiable data were available.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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