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Risk factors for *Mycoplasma genitalium* endometritis and incident infection: a secondary data analysis of the T cell Response Against Chlamydia (TRAC) Study

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Abstract

Objective—Assess risk factors for incident and endometrial *Mycoplasma genitalium* infection and determine if *M. genitalium* is associated with histological endometritis, an indicator of pelvic inflammatory disease.

Methods—This study was a secondary data analysis within the T cell Response Against Chlamydia (TRAC) Study, a prospective evaluation of 246 women with or at risk for *Chlamydia trachomatis* from urban outpatient clinics, who were followed quarterly for 12 months. Risk factors for incident *M. genitalium* infection were determined by Cox regression. Relative risks were estimated by Poisson regression with robust error measurements for models examining the association between *M. genitalium* and endometritis (histological evidence of plasma cells in endometrial stroma and neutrophils in the endometrial epithelium) and for models examining risk factors for detection of endometrial *M. genitalium* infection.

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Contributors

All authors were involved in the concept of this study. TD, HCW and SLH were involved in the design of the TRAC (parent) cohort and subsequent data collection. TD, HCW and SLH also assisted with data interpretation and manuscript revisions. BDT completed statistical analyses, interpreted the data and wrote the manuscript. CMO contributed to laboratory assays, data interpretation and manuscript revisions. XZ assisted with statistical analyses, data interpretation and manuscript revisions. DA contributed to the strain typing of MG samples, detection of drug resistance alleles and write-up for the methods and results. All authors approved the final version of this manuscript and agree to be accountable for all aspects of their contributions.

Competing interests SLH received funding from Becton Dickinson and Cepheid, which market products for the detection of STIs. All other authors have no conflicts to disclose.

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Data sharing statement

Any request for data should be made to TD, University of North Carolina Chapel Hill.

Results—*M. genitalium* prevalence was 16.7%, incidence was 25.3 per 100 person-years and 23% had repeated positive tests. Black race (non-black HR_{adj} 0.4, 95% CI 0.2 to 0.9), less education (HR_{adj} 2.4, 95% CI 1.2 to 5.1) and a new sexual partner (HR_{adj} 3.1, 95% CI 1.7 to 5.4) were associated with incident *M. genitalium*. *M. genitalium* was associated with endometritis (RR_{adj} 2.0, 95% CI 1.1 to 3.7). *Trichomonas vaginalis* (RR_{adj} 2.0, 95% CI 1.2 to 3.4) and endometrial *C. trachomatis* (RR_{adj} 1.7, 95% CI 1.1 to 2.8) were associated with endometrial *M. genitalium*.

Conclusions—*M. genitalium* is prevalent in women at high risk for *C. trachomatis*, persists over multiple follow-up visits and is associated with histological endometritis. Studies are needed to determine if screening for *M. genitalium* will improve reproductive outcomes.

INTRODUCTION

Mycoplasma genitalium is an emerging STI linked to reproductive morbidities.¹ Urethral *M. genitalium* is a cause of non-gonococcal urethritis in men and 30% of persistent or recurrent male urethritis.² However, the role of *M. genitalium* in female reproductive tract disease needs further research.

Inoculation of female mice³ and non-human primates⁴ with *M. genitalium* leads to endometritis and salpingitis. A recent meta-analysis reported that *M. genitalium* was associated with a twofold increased risk of clinical pelvic inflammatory disease (PID).¹ However, coinfection with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* is common¹ complicating interpretation of the independent role of *M. genitalium* in PID. Of the 10 studies that examined PID in the meta-analysis, four adjusted for coinfection.^{5–8} Only one of four studies examined the endometrium for evidence of infection and inflammation.⁷ Thus, unanswered questions regarding the epidemiology and pathogenesis of *M. genitalium* in women limit formulation of clear guidelines for screening and treatment.⁹

From a public health standpoint, it is important to understand if *M. genitalium* causes upper genital tract pathology. *M. genitalium* is not susceptible to standard antibiotics for syndromic treatment of cervicitis and clinical PID.^{7,10} Macrolide resistance rates for *M. genitalium* are high (60%–80%).^{11,12} Thus, *M. genitalium* may persist in the genital tract after treatment and clearance of *C. trachomatis* and *N. gonorrhoeae*. We examined the association between *M. genitalium* and histologically confirmed endometritis controlling for other STIs. Additionally, we identified factors associated with endometrial and incident *M. genitalium*.

MATERIALS AND METHODS

Patient population

This study was a secondary data analysis within the T cell Response Against Chlamydia (TRAC) Study. TRAC identified risk factors for *C. trachomatis* and evaluated serum antichlamydia antibodies in association with chlamydial bacterial burden and incident infection.¹³ The data from the current study evaluated risk factors for *M. genitalium* and have not been presented previously. TRAC was a longitudinal cohort study of 246 women aged 15–35 years with STIs or who were at risk for STIs. All 246 women were included in

the current analysis. Women were enrolled in the study from three urban sites in Pittsburgh, Pennsylvania, during 2011–2015. Eligibility criteria included mucopurulent cervicitis, diagnosis of chlamydial infection prior to treatment or reported sexual contact with an individual with a recent diagnosis of chlamydial urethritis or non-gonococcal urethritis. Women with PID according to the Centers for Disease Control and Prevention guidelines⁹ were excluded. All study participants provided informed written consent for infection testing and genetic analysis at enrolment and for follow-up visits. The University of Pittsburgh Institutional Review Board (MOD10010159–12/PRO10010159) and the University of North Carolina Institutional Review Board (13–3074) approved the study.

Data collection

At enrolment, study personnel obtained demographic and medical history data, participants completed a self-reported questionnaire and cervical, vaginal and endometrial swab samples were obtained.¹³ Gram staining was used for bacterial vaginosis (BV) scoring by Amsel criteria.¹⁴ Molecular testing for *Trichomonas vaginalis* (APTIMA TV, Gen-Probe, San Diego, California, USA). Nucleic acid amplification tests (NAAT) detected *C. trachomatis*, *N. gonorrhoeae* (APTIMA Combo 2, Gen-Probe) and *M. genitalium* (APTIMA MG, Gen-Probe).

Endometrial biopsies from study participants were obtained as follows: after cleansing the cervix with betadine, a sterile sheath-enveloped endometrial sampler (Unimar Pipelle de Cornier, CooperSurgical, Shelton, Connecticut, USA) was placed into the endometrial cavity, a tissue sample was aspirated into the cannula and the cannula was pulled back into the sheath prior to removal. Tissue was discharged into a sterile petri dish. Tissue proximal to the sampling portal of the cannula was placed into 10% formalin fixative for histology, and distal tissue was swabbed for molecular testing. Endometritis was determined by two pathologists who independently scored H&E stained sections of endometrial biopsy tissue according to published Kiviat criteria¹⁵ (five or more neutrophils per $\times 400$ field in surface epithelium and/or one or more plasma cells per $\times 120$ field in endometrial stroma). Discrepant evaluations were resolved after rereview and discussion.

A standardised self-reported questionnaire was administered at 1, 4, 8 and 12 months, addressing the participant's interim medical and sexual history. Vaginal fluid and vaginal and cervical swabs were collected at each follow-up visit for STI and BV diagnosis.

All participants at enrolment received single-dose agents for gonorrhoea (ceftriaxone, 250 mg intramuscularly) and chlamydia (azithromycin, 1 g orally). At enrolment, women diagnosed with BV and/or *T. vaginalis* received metronidazole, 2 g orally. No treatment was administered to women with *M. genitalium* infection alone, since none of the women in the cohort had clinical PID.⁹

Among women positive for *M. genitalium* by Aptima testing, DNA was extracted using a Quick-DNA Universal Kit (Zymo Research, Irvine, California, USA) from 100 μ L to 300 μ L of reserved cervical swab eluates to quantify *M. genitalium* burden, strain typing and detection of 23S rRNA alleles. The abundance of *M. genitalium* DNA was determined by quantitative PCR with SsoAdvanced SYBR mix (Bio-Rad) using primers directed against

the highly conserved MG190 locus¹⁶ using a CFX iCycler (Bio-Rad) in duplicate. Primer design (*M. genitalium* 190 Forward: GAGA TCAG GATA GAAC TGAG GAGTA, *M. genitalium* 190 Reverse: TGAT CGCT CCAC TTGC ATTAT) was based on the genome sequence of *M. genitalium* strain G37, GenBank Accession #NC_000908.2. Nested PCR amplified a fragment of *mgbB* for genotyping and region V of the 23S rRNA locus where macrolide resistance mutations accumulate.¹⁷ PCR cycling parameters were: one cycle of 98°C for 3 min, 38 cycles of 98°C for 30 s, 58°C for 30 s and 72°C for 30 s, followed by one cycle of 72°C for 3 min using Phusion Flash High-Fidelity PCR master mix (Thermo Scientific, Waltham, Massachusetts, USA). The resultant PCR products were cleaned and concentrated using a 'DNA Clean and Concentrator-5' kit (Zymo Research) prior to sequencing. Sequencing results were analysed using Clone Manager (Scientific & Educational Software, Denver, Colorado, USA) and Geneious (Auckland, New Zealand).

Statistical analyses

Incident *M. genitalium* infection was any positive *M. genitalium* test during follow-up in women who were negative for *M. genitalium* at their prior visit. The incidence rate was the number of incident infections per person-years at risk. Risk factors for incident infection were determined with Cox regression, with the Wei, Lin and Weissfeld method for within patient correlation,¹⁸ to calculate hazard ratios (HR) and 95% CIs.

Associations between baseline *M. genitalium* (positive at the cervix and/or endometrium) and endometritis were examined. Among *M. genitalium*-positive women with data on endometrial *M. genitalium* infection (39/41), we compared cervical burden between women with cervical *M. genitalium* infection only and women with cervical and endometrial *M. genitalium* infection. We also identified demographic (age, race, ethnicity, marital status, education and insurance), reproductive (pregnancies, sexual behaviour and history of STIs), contraceptive, behavioural and microbial factors associated with endometrial *M. genitalium* infection. Modified Poisson regression with robust error estimates was used to calculate relative risk (RR) and 95% CIs.¹⁹ A stepwise approach was used to build all multivariable models. Variables with P 0.2 in univariable analyses were considered for multivariable analysis, whereas those with P 0.1 were retained in the final model. All analyses were conducted using SAS V.9.2.

RESULTS

M. genitalium prevalence was 16.7% (41/246; 95% CI 13.1% to 20.9%), and 39/41 women were tested for *M. genitalium* endometrial infection with 48.7% testing positive (19/39). Among *M. genitalium*-positive women, 70.7% (29/41; 95% CI 58.1% to 80.8%) had *C. trachomatis*, 73.2% (30/41; 95% CI 60.6% to 82.9%) had BV; 26.8% (11/41; 95% CI 17.2% to 39.4%) had *T. vaginalis*, and 12.2% (5/41; 95% CI 6.1% to 23.0%) had *N. gonorrhoeae*. Women with *M. genitalium* had a mean age of 21±2.4 years, the majority were of black race (70.7%), single (90.2%) and on Medicaid (57.5%). Thirty per cent were uninsured, 41.4% had at least a high school education, 63.4% smoked and the majority denied drug use (65.7%). These characteristics represent TRAC overall, where the median age was 21, 89%

were single, 66% were of black race, 36% had a high school education and 47% were on Medicaid.

Women with *M. genitalium* mono-infection were more likely to report pain during intercourse (5/10; 50%) compared with women with *M. genitalium* co-infection (6/30; 20%), women with chlamydia and/or gonorrhoea (15/109; 13.8%) and uninfected women (11/69; 15.9%), χ^2 $P=0.02$). Urinary symptoms and abnormal vaginal discharge did not significantly differ between groups. There were no differences in reported symptoms between women with cervical *M. genitalium* only compared with those with *M. genitalium* endometrial infection.

Incidence of *M. genitalium* was 25.3 per 100 person-years (95% CI 15.3 to 28.4). Among these women, 43.6% had BV, 13.6% had *T. vaginalis*, 6.8% had *C. trachomatis* and none had *N. gonorrhoeae*. Incident *M. genitalium* mono-infection was accompanied by a similar frequency of urinary symptoms (12%) compared with *M. genitalium* co-infection (10.5%) and no infection (7%; $P=0.45$). Results were similar for urinary frequency (12%, 10.5% and 6%; $P=0.30$), abnormal vaginal discharge (8%, 5% and 8%; $P=0.70$) and vaginal bleeding (12%, 11% and 9%; $P=0.77$). Overall, 28% of *M. genitalium* mono-infections were accompanied by any symptom, compared with 31.6% for co-infection and 28% for women without STIs or BV.

In multivariable models, non-black race (HR 0.4, 95% CI 0.2 to 0.9), less than a high school education (HR 2.4, 95% CI 1.2 to 5.1) and reporting a new sexual partner at follow-up (HR 3.1, 95% CI 1.7 to 5.4) were associated with incident *M. genitalium* infection (table 1).

A total of 72 women tested positive for *M. genitalium* at least once during the study. Among these women, 31 (43%) had two or more positive *M. genitalium* tests and 22 (31%) tested positive for *M. genitalium* during two or more consecutive visits. This is higher than for *C. trachomatis*, where 23% (40/171) had two or more positive tests and 6% (11/171) tested positive during consecutive visits and for *N. gonorrhoeae* where 25% (7/28) had two or more positive tests and 14% (4/28) tested positive at consecutive visits. Results for *T. vaginalis* were similar to *M. genitalium*, 45% (29/65) had two or more positive tests and 34% (22/65) tested positive at consecutive visits.

We investigated azithromycin treatment and resolution of infection retrospectively. Of the 41 women with *M. genitalium* at enrolment, eight were lost to follow-up, 23 were *M. genitalium* negative by their first follow-up visit and 10 tested positive. We were unable to evaluate the genotype or 23S rRNA for 5 of 10 positive women. We identified mutant alleles associated with azithromycin resistance (2058G or 2059G) for three women. One additional individual acquired a new isolate, as their *mgpB* gene sequences amplified from enrolment and follow-up specimens differed. Another carried an identical azithromycin-sensitive wild-type 23S rRNA allele at enrolment and follow-up, consistent with reinfection. Sequence analysis of 9 of 10 samples from *M. genitalium* negative women at follow-up confirmed a wild type, drug-sensitive region V, while one specimen carried the 2058G mutation, indicating this patient's immune response led to effective clearance or follow-up infection was missed.

A total of 135 participants had evaluable endometrial histo-logical data, and 46 were positive for endometritis. The frequency of missing endometritis data did not differ between *M. genitalium* positive (46.3%) and negative women (44.8%). A total of 15 *M. genitalium* positive women had endometritis where 10 had *M. genitalium* endometrial infection and 5 had cervical *M. genitalium* only. Thirty-one women with endometritis were *M. genitalium* negative, 23 were positive for BV, 18 had *C. trachomatis*, 7 had *T. vaginalis* and 2 had *N. gonorrhoeae*. *M. genitalium* positivity (cervical or endometrial) was associated with endometritis (RR 2.0, 95% CI 1.1 to 3.7; table 2) after adjustment for race, *C. trachomatis*, *N. gonorrhoeae* and BV. A total of 90% of women with *M. genitalium* endometrial infection had histological evidence of endometritis compared with only 50% of women with cervical infection only (RR 1.8, 95% CI 1.0 to 5.6). Among women with *M. genitalium* monoinfection, 3 of 5 had endometritis. Among those with *M. genitalium* who also had chlamydia or gonorrhoea, 70.6% had endometritis. Two burden distributions were found, 26 out of 40 women had high cervical burden ($>10^4$ genome copies per swab) and 12 had low cervical burden (<500 genome copies per swab). High *M. genitalium* cervical burden ($>10^4$ genome copies per swab) was not significantly associated with endometritis (RR 1.4, 95% CI 0.6 to 3.2).

Table 3 displays factors associated with *M. genitalium* endometrial infection compared with cervical infection only. In the multivariable model, oral sex was associated with reduced risk of endometrial *M. genitalium* (RR 0.6, 95% CI 0.3 to 0.9) and vaginal *T. vaginalis* with enhanced risk (RR 2.0, 95% CI 1.2 to 3.4). Women who had *C. trachomatis* detected at the cervix and endometrium had an increased risk of endometrial *M. genitalium* (RR 1.7, 95% CI 1.1 to 2.8). High cervical burden ($>10^4$ genome copies per swab) was marginally associated with *M. genitalium* endometrial infection (RR 2.3, 95% CI 1.0 to 5.2).

DISCUSSION

Among women at high risk for chlamydia, *M. genitalium* prevalence was 16.7% and incidence was 25.3 per 100 person-years. This is higher than a population of female university students in the UK²⁰ but comparable with populations of women at risk for STIs.¹⁰²¹ Baseline *C. trachomatis* coinfection with *M. genitalium* was common in our study. This is due to recruitment of women with or at high risk for *C. trachomatis* within a single geographic location. In a general population of low-risk and high-risk clinics/hospitals, Getman *et al*¹⁰ reported that 68.7% of females with *M. genitalium* had monoinfection (absence of *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis*). After treatment, the *C. trachomatis* coinfection rate for incident *M. genitalium* was 6.8%.

Examination of data at follow-up revealed that non-black race reduced the risk for *M. genitalium*, while reporting a new sexual partner and lesser education increased risk. Sexual behaviour is linked to *M. genitalium*.²²²³ Race is also a risk factor for *M. genitalium*.²² Age was not associated with *M. genitalium*, but 80% of cohort was <25 years of age.

Only 28% of women with *M. genitalium* incident infections reported symptoms, which is consistent with reports that *M. genitalium* is asymptomatic in 75% of infected females.¹ Despite the asymptomatic nature, 48.7% of *M. genitalium*-positive women had endometrial

infection. Furthermore, 23% of *M. genitalium*-positive women had positive tests at two or more consecutive visits. Women who tested positive for *M. genitalium* following receipt of 1 g orally of azithromycin were either reinfected by their original partner or from a new partner or failed therapy. Azithromycin treatment failure is common with *M. genitalium*,⁷¹⁰ and macrolide resistance rates are high, 60%–80% in men and women.¹¹¹² Although we cannot confirm azithromycin treatment failure, we detected the 2058G mutation in three of five women with testable DNA who were positive for infection 1-month post-treatment. Symptoms between women with repeated positive tests compared with those who cleared infection did not differ. We do not know if women testing positive at follow-up had endometritis, because endometrial biopsies were not obtained at follow-up.

At baseline, *M. genitalium* was associated with histologically confirmed endometritis independent of *C. trachomatis* and *N. gonorrhoeae*. Although, *M. genitalium* is linked to clinical PID, few studies have examined *M. genitalium* and endometritis or salpingitis, which are more specific indicators of true upper genital tract infection and inflammation.¹ Only one study, of 662 women with clinical PID, has adjusted for *C. trachomatis* and *N. gonorrhoeae*⁷ reporting an adjusted OR for endometritis of 3.0 (95% CI 1.5 to 6.1). Cervical *M. genitalium* is associated with proinflammatory cytokine production and neutrophil influx.²⁴ Thus, it is perhaps not surprising that endometrial infection with *M. genitalium* may lead to endometrial inflammation. Although data suggest *M. genitalium* is associated with infertility, the relationship has not been fully established and is limited primarily by the use of serology.¹ Thus, it is important to evaluate the role of *M. genitalium* in upper genital tract inflammation. Our results coupled with existing data that treatment for PID do not eradicate *M. genitalium*⁷ may suggest that the association between *M. genitalium* and endometritis could be influenced by inappropriate treatment.

Screening for *M. genitalium* is not currently recommended, although may be potentially beneficial for those at high risk for STIs.¹ However, risk factors to identify women at enhanced risk for *M. genitalium* (particularly ascending infection) are not elucidated. Women with endometrial *M. genitalium* were more likely to have *T. vaginalis* and endometrial *C. trachomatis*. The fact that endometrial *C. trachomatis* and *M. genitalium* infections tended to cluster may suggest that those women were immunologically susceptible to ascending infections. Women with endometrial *M. genitalium* had elevated cervical burden. Few studies have examined *M. genitalium* burden and reproductive morbidity. In males, *M. genitalium* burden varies, and high burden may increase treatment failure.²⁵ Oral sex reduced the risk of endometrial infection. Oral sex has been suggested to prime the immune system against infection and decreased endometritis in the PID Evaluation and Clinical Health (PEACH) study.²⁶

We used prospective data to examine risk factors for *M. genitalium* in a population of women at risk for chlamydia and other STIs. This is one of only a handful of studies that examined the association between *M. genitalium* and histologically confirmed endometritis while controlling for coinfection. The use of the APTIMA NAAT is a strength over studies that have relied on serology. Our sample size limited our power for some analyses, especially for endometrial infection. Furthermore, not all women had data on endometritis, which may lead to bias. However, the proportion of missing data for endometritis was

similar between *M. genitalium*-positive and *M. genitalium*-negative women, reducing the potential for bias. The results from this study have narrow generalisability as TRAC recruited women were at high risk for *C. trachomatis*. This also limited our ability to examine *M. genitalium* monoinfection. Although we controlled for coinfection in multivariable analysis, we were unable to examine monoinfection and coinfection separately.

We found that *M. genitalium* was prevalent in women at high risk for STIs. *M. genitalium* may lead to upper genital tract inflammation independent of *C. trachomatis* and *N. gonorrhoeae*. Repeated positive tests for *M. genitalium* were common during follow-up, indicating possible reinfection or chronic infection. Taken together, these data indicate a need for studies to determine if *M. genitalium* monoinfection or coinfection leads to upper genital tract infection and inflammation and, if so, clinical trials are needed to identify antibiotics that are effective at eradicating *M. genitalium* to prevent potential long-term reproductive sequelae.

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Key messages

- *Mycoplasma genitalium* was prevalent in women at high risk for sexually transmitted infections.
- *M. genitalium* was associated with histological endometritis independent of chlamydia and gonorrhoea.
- Further studies are needed to determine if screening for *M. genitalium* will improve reproductive outcomes in women at high risk for STIs.

Table 1

Factors associated with incident *Mycoplasma genitalium* infection

Variable	HR (95%CI)	P values	HR _{adj} (95%CI) *	P values
Demographics and behaviour				
Age	0.9 (0.7 to 0.9)	0.04	0.9 (0.8 to 1.0) †	0.05
Non-black race	0.4 (0.2 to 0.8)	0.007	0.4 (0.2 to 0.9) †	0.03
Uninsured	Reference	Reference		
Private insurance	1.1 (0.5 to 2.6)	0.80	NA	NA
Medicaid	1.4 (0.6 to 2.9)	0.39	NA	NA
Posthigh school education	Reference	Reference		
Less than high school education	2.7 (1.3 to 5.8)	0.009	1.6 (0.8 to 5.1) †	0.02
High school education	2.0 (1.0 to 3.7)	0.05	1.6 (0.8 to 3.1) †	0.16
Married	0.6 (0.2 to 1.7)	0.31	NA	NA
Non-smoker	0.6 (0.3 to 1.0)	0.06	0.7 (0.4 to 1.4) †	0.38
Drug use	1.5 (0.9 to 2.7)	0.15	1.0 (0.6 to 1.9) †	0.90
Alcohol use	1.2 (0.7 to 2.0)	0.62	NA	NA
Douching past 30 days	1.1 (0.4 to 3.3)	0.81	NA	NA
Reproductive history at enrolment, sexual behaviour and contraception use at follow-up				
History of <i>Chlamydia trachomatis</i>	2.1 (1.1 to 3.9)	0.03	1.7 (0.9 to 3.3) †	0.09
History of <i>Neisseria gonorrhoeae</i>	1.4 (0.7 to 2.6)	0.33	NA	NA
History of <i>Trichomonas vaginalis</i>	1.1 (0.6 to 2.1)	0.71	NA	NA
History of BV	1.3 (0.7 to 2.2)	0.40	NA	NA
Infected partner	1.5 (0.4 to 5.4)	0.53	NA	NA
Multiple partners	2.4 (1.2 to 4.6)	0.009	1.1 (0.5 to 2.6) †	0.78
New sexual partner	2.9 (1.6 to 5.3)	0.0003	3.1 (1.7 to 5.4) †	0.0001
Uncircumcised partner	1.1 (0.3 to 4.2)	0.93	NA	NA
Oral sex	1.7 (0.9 to 3.1)	0.08	1.4 (0.7 to 2.6) †	0.29
Anal sex	1.5 (0.5 to 4.6)	0.51	NA	NA
Consistent condom use	Reference	Reference	Reference	

Variable	HR (95%CI)	P values	HR _{adj} [*] (95%CI)	P values
Rarely or never use condoms	1.6 (0.9 to 3.3)	0.11	1.7 (0.9 to 3.1) [‡]	NA
Inconsistent condom use	1.1 (0.5 to 2.4)	0.80	NA	NA
Pills	0.8 (0.4 to 1.9)	0.65	NA	NA
Depo-Provera injections	0.9 (0.4 to 2.1)	0.77	NA	NA
Withdrawal	1.4 (0.7 to 2.8)	0.33	NA	NA
None	0.5 (0.2 to 1.6)	0.23	NA	NA
Intrauterine device	1.1 (0.5 to 2.4)	0.74	NA	NA
Microbiology at enrolment and follow-up				
Baseline <i>C. trachomatis</i>	1.5 (0.8 to 3.1)	0.20	1.5 (0.8 to 2.8) [‡]	0.26
Baseline <i>N. gonorrhoeae</i>	1.8 (0.8 to 4.3)	0.18	1.2 (0.5 to 2.9) [‡]	0.62
Baseline <i>M. genitalium</i>	1.9 (1.0 to 3.8)	0.07	1.8 (0.8 to 3.7) [‡]	0.14
Baseline <i>T. vaginalis</i>	1.8 (1.0 to 3.4)	0.05	1.5 (0.8 to 2.9) [‡]	0.22
Baseline bacterial vaginosis	1.3 (0.6 to 2.6)	0.45	NA	NA
<i>C. trachomatis</i> at follow-up	0.8 (0.3 to 2.6)	0.72	NA	NA
<i>T. vaginalis</i> at follow-up	1.3 (0.6 to 2.9)	0.51	NA	NA
Bacterial vaginosis at follow-up	1.0 (0.7 to 1.4)	0.99	NA	NA

* P 0.2 in univariable analyses were tested in the multivariable analysis, where variables with a P 0.1 were retained.

[‡]Not retained in the final model.

[‡]Included in final model.

BV, bacterial vaginosis.

Table 2

Association between *Mycoplasma genitalium* and endometritis

<i>M. genitalium</i> diagnostic test result	Endometritis*		Univariable models		Multivariable models [†]	
	No n=89	Yes n=46	RR (95%CI)	P values	RR (95%CI)	P values
Results based on a positive <i>M. genitalium</i> test [‡]						
<i>M. genitalium</i> negative	82 (92.1)	31 (67.4)	Reference		Reference	
<i>M. genitalium</i> positive [‡]	7 (7.9)	15 (32.6)	2.5 (1.3 to 4.6)	0.004	2.0 (1.1 to 3.7)	0.04
Results based on <i>M. genitalium</i> monoinfection or coinfection [§]						
<i>M. genitalium</i> negative	82 (92.1)	31 (67.4)	Reference		Reference	
<i>M. genitalium</i> monoinfection	2 (2.2)	3 (6.5)	2.2 (1.0 to 4.8)	0.05	1.8 (0.9 to 3.5)	0.07
<i>M. genitalium</i> coinfection [‡]	5 (5.6)	12 (26.1)	2.5 (1.2 to 4.6)	0.004	1.9 (1.1 to 3.6)	0.04

* A total of 135 women had evaluable endometrial histological data.

[†] Model for *M. genitalium* positive women was adjusted for race, chlamydia, gonorrhoea and bacterial vaginosis. The models for *M. genitalium* monoinfection or coinfection were adjusted for race and bacterial vaginosis.[‡] *M. genitalium* negative includes all women who tested negative for *M. genitalium* at the cervix or endometrium. *M. genitalium* positive includes all women who tested positive for *M. genitalium* at the cervix or endometrium.[§] *M. genitalium* mono (no coinfection with chlamydia or gonorrhoea) or coinfection with chlamydia or gonorrhoea.

Table 3

Factors associated with endometrial *Mycoplasma genitalium* infection

Risk factor *	Cervix only n=20	Endometrial n=19	RR (95%CI)	P values	RR _{adj} (95%CI) [†]	P values
Demographics and behaviour						
Age [‡]	21±3.1	21±2.4	1.0 (0.9 to 1.1)	0.85	NA	NA
Non-black race	9 (45.0)	3 (15.8)	0.4 (0.2 to 1.2)	0.10	0.7 (0.3 to 1.4) [§]	0.31
Uninsured	Reference	Reference	Reference			
Private insurance	2 (10.0)	3 (15.7)	0.9 (0.4 to 2.1)	0.89	NA	NA
Medicaid	12 (60.0)	11 (57.9)	1.2 (0.5 to 2.8)	0.57	NA	NA
Posthigh school education	Reference	Reference	Reference		NA	NA
<High school education	4 (20.0)	5 (26.3)	1.0 (0.5 to 2.6)	0.96	NA	NA
High school education	9 (45.0)	8 (42.1)	1.2 (0.5 to 2.6)	0.66		
Married	2 (10.0)	2 (10.5)	1.0 (0.4 to 2.9)	0.97	NA	NA
Non-smoker	10 (50.0)	5 (26.3)	0.6 (0.3 to 1.3)	0.16	0.7 (0.4 to 1.4) [§]	0.34
Drug user	5 (25.0)	9 (47.4)	1.6 (0.9 to 3.0)	0.13	1.0 (0.4 to 2.5) [¶]	0.97
Douching	9 (45.0)	9 (47.3)	1.1 (0.5 to 1.9)	0.88	NA	NA
Reproductive history, sexual activity and contraception						
History of <i>Chlamydia trachomatis</i>	11 (55.0)	5 (26.3)	0.5 (0.2 to 1.1)	0.10	0.8 (0.4 to 1.7) [¶]	0.59
History of <i>Neisseria gonorrhoeae</i>	7 (35.0)	4 (21.0)	0.7 (0.3 to 1.6)	0.37	NA	NA
History of <i>Trichomonas vaginalis</i>	4 (20.0)	2 (10.5)	0.5 (0.1 to 3.1)	0.48	NA	NA
History of BV	10 (50.0)	7 (36.8)	0.6 (0.2 to 2.2)	0.43	NA	NA
Female partner	3 (15.0)	6 (31.5)	1.6 (0.9 to 3.0)	0.11	1.2 (0.6 to 2.2) [¶]	0.60
Infected partner	6 (30.0)	5 (26.3)	0.8 (0.2 to 3.4)	0.81	NA	NA
Multiple partners	3 (15.0)	3 (16.7)	1.1 (0.2 to 6.0)	0.94	NA	NA
New partner 30d	5 (25.0)	4 (21.1)	0.8 (0.2 to 3.7)	0.79	NA	NA
Oral sex	15 (65.2)	10 (52.6)	0.6 (0.3 to 1.2)	0.13	0.6 (0.3 to 0.9) [§]	0.04
Consistent condom use	Reference	Reference	Reference		NA	NA
Rarely/never use condoms	5 (25.0)	3 (16.7)	0.5 (0.1 to 2.5)	0.38		
Inconsistent condom use	7 (35.0)	5 (26.3)	0.5 (0.1 to 2.3)	0.41		
Depo-Provera injections	3 (15.0)	4 (21.1)	1.2 (0.6 to 2.5)	0.59	NA	NA

Risk factor [*]	Cervix only n=20	Endometrial n=19	RR (95%CI)	P values	RR _{adj} (95%CI) [†]	P values
Withdrawal	8 (40.0)	6 (31.5)	0.7 (0.2 to 2.6)	0.61	NA	NA
None	3 (15.0)	3 (16.7)	1.1 (0.2 to 6.0)	0.95	NA	NA
Microbiology						
<i>C. trachomatis</i>						
None	Reference	Reference	Reference			
Cervical	16 (80.0)	13 (68.4)	0.7 (0.4 to 1.4)	0.38	NA	NA
Endometrial	2 (10.0)	8 (42.1)	2.1 (1.2 to 3.7)	0.009	1.7 (1.1 to 2.8) [§]	0.02
<i>N. gonorrhoeae</i>	3 (15.0)	2 (11.1)	0.7 (0.1 to 4.8)	0.73	NA	NA
<i>BV</i>	15 (75.0)	13 (68.4)	2.8 (0.6 to 12.3)	0.17	1.5 (0.7 to 2.8) [¶]	0.27
<i>T. vaginalis</i>	2 (10.0)	8 (42.1)	2.1 (1.2 to 3.7)	0.009	2.0 (1.2 to 3.4) [§]	0.01
High bacterial burden ^{**}	10 (50.0)	16 (84.2)	2.4 (0.9 to 6.8)	0.08	2.3 (1.0 to 5.2) [§]	0.05

^{*} Factors with cell size <2 were not analysed. This included some contraception methods (pills and IUD) and sexual activity variables (uncircumcised partner, anal sex, etc and so on).

[†] P 0.2 in univariable analyses were tested in the multivariable analysis, where variables with a P 0.1 were retained.

[‡] Age is a continuous variable, values listed in table are mean±SD. All others are binary or categorical and listed as n (%).

[§] Included in final model.

[¶] Not retained in the final model.

^{**} High burden >10⁴ DNA copies/swab.

BV, bacterial vaginosis; IUD, intrauterine device.