Published in final edited form as:
*J Adolesc Health*. 2007 May ; 40(5): 412–417.

# *Mycoplasma genitalium* among adolescent women and their partners

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## Abstract

**Purpose—***Mycoplasma genitalium* is a possible sexually transmitted pathogen and its study among the adolescent age group has been limited. In this longitudinal study, the epidemiology, natural history, and associated clinical findings of *M. genitalium* among adolescents in a primary care setting were explored.

**Methods—**A sample of 383 young women (14 -17 years of age) and 117 male partners provided sexual behavior data and urogenital samples for PCR testing to detect *M. genitalium, Chlamydia trachomatis*, and other sexually transmitted infections. Women were tested quarterly for up to 27 months and, during every other quarter, tested weekly. The presence of any signs or symptoms of infection among the female subjects was also documented.

**Results—**Cumulatively, 13.6% (52/383) of women tested positive for *M. genitalium.* All women with *M. genitalium*, except one, were sexually experienced. *M. genitalium* was associated with number of sexual partners ($p<0.001$) and *C. trachomatis* infection ($p<0.03$). *M. genitalium* was more likely among male partners of *M. genitalium*-positive women ($p<0.02$). 31.3% of untreated *M. genitalium* cases had infection lasting ≥8 weeks. *M. genitalium* was not associated with the presence of clinical signs or symptoms of infection.

**Conclusions—**Findings support sexual transmissibility of *M. genitalium* and add to understanding of *M. genitalium* natural history and clinical findings.

### Keywords

Mycoplasma genitalium; Chlamydia trachomatis; sexually transmitted diseases; epidemiology; adolescent; polymerase chain reaction

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Sources of Support: Research supported in part by the National Institutes of Allergy and Infectious Diseases (U19 AI1131494) and Maternal and Child Health Bureau (T71 00008).

## INTRODUCTION

*Mycoplasma genitalium* is the smallest prokaryote capable of self-replication. It was first isolated in the 1980s from the urethra of two men with non-gonococcal urethritis (NGU) [1]. Later, its genome was the first of any micro-organism to be fully sequenced [2]. Because of its small size and growth requirements, *M. genitalium* is highly fastidious making diagnosis by culture of the organism impractical. With the advent of PCR assays [3,4], detection became much more reliable and led to expanded research of this organism as a possible sexually transmitted pathogen.

Sexual transmission is suggested by demonstration of infection in urogenital sites of both men and women [5,6], and increased infection risk for female partners of infected men [7]. *M. genitalium* is associated with NGU, mucopurulent cervicitis, and other reproductive tract infections [8-11].

Our current knowledge of *M. genitalium* infections is based largely on studies of adult males in sexually transmitted infection (STI) clinic settings. *M. genitalium* as a potential STI among the adolescent age group has not been well described. Understanding the epidemiology of the organism is critical to improving reproductive health among adolescents since they bear the highest burden of curable STI. Additionally, an *M. genitalium* nucleic acid-based assay is now available as a research tool from a diagnostic manufacturer [12]. Although this assay is not yet cleared by FDA, it is a clear indication that there is interest in this organism and it is relevant to assess the epidemiology of *M. genitalium* in a population of high-risk adolescents. We describe the epidemiology, natural history, and associated clinical findings of *M. genitalium* in a longitudinal study of adolescent women and their sexual partners in a primary care setting.

## METHODS

### Participants and Procedures

Women aged 14-17 years were enrolled from one of three participating urban primary health care clinics located in a large, Midwestern city from 1999 to 2006. Young women attending the clinics were approached in waiting areas and informed consent was obtained following an explanation of study procedures. Recent male sexual partners of the index women were identified and invited to participate by telephone call or field visit. Informed consent was obtained from those agreeing to the study. Parental permission was obtained for all minors consenting to participate. All research procedures were approved by the local Institutional Review Board.

Data from female participants were collected at enrollment and at quarterly clinic return visits for the following 27 months, accounting for up to nine follow-up clinic visits. Participants completed detailed questionnaires about sexual behaviors at enrollment and at each annual visit. At each quarterly visit, a research nurse practitioner obtained vaginal specimens for STI testing and a structured face to face interview was conducted by a trained research assistant to obtain detailed sexual behavior information for the preceding three months. To maximize the probability of capturing incident STI while also attempting to limit subject burden, vaginal swabs were obtained at quarterly visits and weekly during intensive 12-week collection periods after every other quarterly visit. To examine natural history of *M. genitalium*, all quarterly samples were PCR-tested for *M. genitalium*. Weekly samples following a positive quarterly visit were also tested. An episode of infection was defined for this analysis as beginning with a positive quarterly visit and continuing until two or more consecutive samples were negative. Additional episodes began with the next positive sample. Although the exact onset of infection is unknown, for this analysis, duration was defined by the date of collection of *M. genitalium* positive samples.

Each male partner who agreed to participate completed a detailed questionnaire and structured face-to-face interview (similar to those obtained from index female participants) and provided a urine sample for STI testing. For remuneration, all study subjects, female and male, received $20 for each clinic visit and $5 for each vaginal or urine specimen.

All vaginal and urine samples were tested for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and *M. genitalium* using PCR assays described below. Symptomatic female participants were screened for yeast infection and clue cells using standard wet-mount microscopy and whiff-testing was performed using KOH. Participants testing positive for *N. gonorrhoeae, C. trachomatis, T. vaginalis*, candidiasis, or bacterial vaginosis were treated according to CDC guidelines at the clinic visit or within two weeks of laboratory confirmation of infection. Due to the retrospective nature of the *M. genitalium* assay and the lack of clear guidelines for the treatment of *M. genitalium* infections, participants solely testing positive for *M. genitalium* were not treated.

## STI Diagnosis

Diagnostic assays for *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* were performed in real time, with results available for clinical action within 48 hours of laboratory receipt. Vaginal swabs in dry containers and urine specimens were transported to the laboratory within 8 hours of collection. Swabs were hydrated with 1 ml of sterile, UV-inactivated water. The vaginal samples were vigorously vortexted and the liquid expressed from the swab. The urine specimens were placed in a wash buffer to remove inhibitors before addition of a lysis reagent. *C. trachomatis* and *N. gonorrhoeae* infections were identified using the AMPLICOR® (Roche Diagnostics, Indianapolis, IN) CT/NG polymerase chain reaction (PCR) assay as previously described [13,14]. Following sample elution, testing followed the manufacturer's protocol, including interpretation of results.

*T. vaginalis* PCR was performed using the sample processed for CT/NG PCR. Primers specific for *T. vaginalis* DNA were added to the AMPLICOR® amplification preparation and amplified material was detected using an ELISA format with *T. vaginalis*-specific probe sequences as previously described [15].

*M. genitalium* PCR was performed retrospectively on specimens frozen for up to 48 months at -70°C. Samples previously processed for PCR assays were used in the *M. genitalium* assay described by Dutro et al [16]. Briefly, 50 μl of processed sampled was added to 50 μl of amplification preparation that included 0.1 μM of each botinylated primer, 200 μM dNTPs, 2.5 U *Taq* polymerase, 4 mM $MgCl_2$and 1 U uracyl-N-glycosolase to prevent carry-over contamination. Amplification was performed using the following cycling parameters: strand separation at 94°C for 4 minutes; 35 cycles of 94°C for 10 seconds, 55°C for 50 seconds, 72°C for 45 seconds; final extension at 72°C for 5 minutes. Amplified target sequence was identified using a digoxigenin label probe specific for *M. genitalium* which was detected using a commercially available PCR DIG ELISA kit (Roche Molecular Systems, Indianapolis, IN). Samples were considered positive if the OD at 405 nm was at least 0.110 after background subtraction. All positive samples were repeated and the second assay was also required to give a positive result for the sample to be classified as containing *M. genitalium* DNA.

To obtain an approximation of assay performance, forty selected *M. genitalium* samples were sent to a reference laboratory (Dr. Patricia Totten, University of Washington [UW], Seattle) for comparison testing. Reference laboratory personnel were blinded to the results obtained at Indiana University (IU). Of the 40 samples, 20 were negative in the IU laboratory (OD < .110), 10 were weakly positive (OD = .110 – 1.000), and 10 were strongly positive (OD >1.000). Confirmatory testing showed 88% (35/40) agreement between the UW laboratory and IU laboratory results. The five discordant results were two IU laboratory negatives (with OD just

below diagnostic threshold) identified as positive by the UW laboratory, two IU laboratory positives (with OD just above diagnostic threshold) called negative by the UW lab, and one sample with OD well above diagnostic threshold called positive by the IU lab and negative by the UW lab. Therefore 4/5 discordant results were near the cut-off separating negative from positive results and may have been a reflection of assay sensitivity. The IU laboratory positive that was negative at the UW laboratory was excluded from analysis. The remaining four samples were analyzed according to the IU laboratory results.

### Analysis of Signs and Symptoms of *M. genitalium*

At each quarterly visit, female participants were screened for clinical signs and symptoms. Signs were defined as vaginal erythema, vulvar erythema, and vaginal discharge; symptoms were vaginal itching, vaginal burning, and dyspareunia. Observations (visits) were excluded from this analysis if no data for signs and symptoms were available. To avoid confounders that could contribute to the presence of signs and symptoms, observations were also excluded if the women tested positive for *N. gonorrhoeae* or *T. vaginalis*, had microscopic findings of yeast or clue cells, or had a positive whiff test.

### Statistical Analysis

Analyses were performed for women who reported at least one episode of vaginal intercourse. Logistic regression models with generalized estimating equations (GEE) to adjust for correlated observations within people were used to examine associations between *M. genitalium* and demographic and behavioral variables, other STI, and clinical signs and symptoms. In the latter analysis, the presence of signs or symptoms was the dependent variable and the infection was the independent (predictive) variable. Those factors that were associated ($p<0.05$) with *M. genitalium* detection were then included in a multiple logistic regression model. For male partner infectivity data, a logistic regression model was used with GEE to adjust for multiple partners for some of the women. All statistical tests were performed using a two-sided 5% significance level.

## RESULTS

A total of 383 women (90% African-American) were enrolled and mean age at enrollment was 15.8 years. Fifty-five women eventually dropped out of the study (24 due to reasons initiated by the subject [most frequently other time commitments] and 31 due to repeated missed visits). Therefore, the discontinuation rate for our study was 14.4% (55/383).

At enrollment, about 83% of the participants reported at least one experience of vaginal intercourse and mean age of first intercourse was 13.5 years. At enrollment, the point prevalence for *M. genitalium* was 3/383 (0.8%). For comparison, point prevalences at enrollment for *C. trachomatis, N. gonorrhoeae,* and *T. vaginalis* were 10.2%, 3.8%, and 5.9%, respectively. Examining each of the quarterly visits after enrollment, point prevalence ranges for *M. genitalium, C. trachomatis, N. gonorrhoeae*,and *T. vaginalis* were 0.8-4.1%; 7.8%-11.9%, 1.7-7.1%; and 1.7-8.4%, respectively.

*M. genitalium* DNA was detected in 78/3110 (2.5%) samples from 52/383 women (13.6% cumulative prevalence). *M. genitalium* was identified in one woman reporting no lifetime sexual activity. 49/52 *M. genitalium* positive participants had incident detection during the course of the study. Sixteen women had *M. genitalium* positive samples at multiple visits. Cumulative prevalence rates were 41.8%, 19.3% and 21.4% for *C. trachomatis, N. gonorrhoeae, and T. vaginalis*, respectively over the 27 months of the study.

As no differences were found between the results of the female subjects at-large [data not shown] and those who reported sexual experience, reporting of statistical analysis is limited to participants reporting at least one episode of vaginal intercourse. No differences in age, African-American race, age at first intercourse, condom use at most recent coitus, coital frequency in the past three months, or cunnilingus in the past three months were seen for those with and without at least one *M. genitalium* positive sample (Table 1). *M. genitalium* detection was not associated with *N. gonorrhoeae* or *T. vaginalis* infection. Participants testing positive for *M. genitalium* had more sexual partners in the past three months compared to those testing negative ($p<0.001$) and were more likely to have *C. trachomatis* infection ($p<0.02$). Among the number of samples testing positive for *C. trachomatis* (n=289), 15 (5.2%) also had concurrent *M. genitalium* detection. Both recent sexual partners (OR=1.43; 95% CI=1.17, 1.74; $p<0.001$) and *C. trachomatis* infection (OR=1.89; 95% CI=1.07, 3.34; $p<0.03$) remained significant in the multiple logistic regression model.

Out of an estimated 291 possible male sexual partners, 117 urine samples were available for testing. Of these specimens, eight were from male sexual partners of women testing positive for *M. genitalium* and 109 were from partners of *M. genitalium*-negative women. Two (25%) of the eight partners to infected women were positive for *M. genitalium* within three months of the index female partners' positive visit, while the remaining six males tested negative. Three (2.8%) of the 109 partners of uninfected women tested positive for *M. genitalium*. The difference in male *M. genitalium* detection rates between those with and without *M. genitalium*-positive female partners was significant ($p<.02$).

Natural history was approximated by testing available weekly vaginal samples after a positive *M. genitalium* quarterly sample. Data from 27 12-week collection periods were available from 23 subjects who were *M. genitalium* positive at the preceding quarterly visit. Twenty women were positive at only one quarterly visit and contributed one 12-week data set each, while the remaining three women were *M. genitalium* positive at multiple quarterly visits. Thirty-five episodes of *M. genitalium* detection were observed in 27 12-week periods. A single episode of *M. genitalium* was observed in 19/27 (70.4%) of weekly collection periods, while two episodes with intervening negative visits were observed in the remaining eight (29.6%) collection periods. *M. genitalium* shedding occurred for >8 weeks in 10/32 (31.3%) collection periods and for ≥ 12 weeks in 7/32 (21.9%) collection periods.

Among all subjects testing positive for *M. genitalium* for whom clinical data was available, the most common signs reported were vaginal erythema (13/58) and vaginal discharge (12/56). A comparison of *M. genitalium* and *C. trachomatis* infection status and presence of symptoms or signs is presented in Table 2, excluding those women positive for *N. gonorrhoeae*, *T. vaginalis*, yeast, clue cells, or whiff test. Detection of *C. trachomatis* was significantly associated with presence of signs ($p<.001$) but not symptoms. Detection of *M. genitalium* was associated with neither clinical signs nor symptoms.

## DISCUSSION

The cumulative prevalence of *M. genitalium* among urban adolescent women in this study was 13.6%, suggesting that it is a relatively common organism in this population. Point prevalence ranged from 0.8-4.1%. Previous cross-sectional studies that included some adolescents found a point prevalence between 3.5-7% [10,17,18]. The difference in point prevalence found here may be attributable to the target population for this project as the young women were not recruited from STD or family planning clinics. In one study, the average age among *M. genitalium* infected women was 20 years, compared to 23 years among uninfected women [10]. This suggests that risk for *M. genitalium* detection, as with other STI, is increased for adolescent women. Furthermore, the prevalence of *M. genitalium* in our sample indicates that

it is relatively common for the organism to be found in adolescent women and justifies further epidemiologic study.

Our data offer four pieces of indirect support for the assumed but unproven sexual transmissibility of *M. genitalium.* First, with only one exception, *M. genitalium* was identified solely among women reporting experience with vaginal intercourse. Second, we demonstrated that women testing positive for *M. genitalium* had significantly more sexual partners in the past three months than women testing negative. For each additional sexual partner, the risk of detecting *M. genitalium* increased by nearly 45%, a clinically significant finding. Third, *M. genitalium* detection was more likely among the male partners of infected compared to uninfected women. Finally, this study demonstrated a statistically significant association between *M. genitalium* detection and *C. trachomatis* infection. The implications for management of *M. genitalium* and *C. trachomatis* infections warrant further study into this association. While others [10] have not shown an association between these two organisms, the difference in study findings may be explained by our sample population of adolescents in whom STI infection is highly prevalent.

The data also demonstrate the capacity of *M. genitalium* to cause either prolonged or repeated infections among adolescent women. We found that untreated *M. genitalium* shedding may persist longer than 8-12 weeks among some adolescents. *M. genitalium* infection over prolonged periods have also been demonstrated in a longitudinal study of older women [19]. Further research is recommended to further elucidate the natural history and reoccurrence of *M. genitalium* infections.

The most common symptoms among those with *M. genitalium* were vaginal discharge and erythema, however there was no significant association of *M. genitalium* detection with presence of clinical signs or symptoms. There is disagreement in the literature about the presence of clinical signs and symptoms in women testing positive for *M. genitalium*. A prevalence study using STI clinic patients failed to find an association with clinical symptoms, but did find that microscopic evidence of infection was associated with *M. genitalium* detection [20]. A study using female subjects with active STI did find an association between *M. genitalium* detection and symptoms [21]; while, no association between microscopic signs and *M. genitalium* was found in a cross-sectional study using female STI clinic subjects [17]. Our data is among the first to present *M. genitalium* signs and symptoms data using a primary care, non-STI clinic, patient population.

Several limitations exist in the study design. The generalizability of our findings to the adolescent population at-large is not possible because the study subjects were predominantly racial minorities with high STI prevalence. However, since no data has previously been available on the epidemiology of *M. genitalium* exclusively among adolescents, using a high-risk urban primary-care clinic population allows for increased probability of finding differences among those with and without *M. genitalium* as well determining *M. genitalium* prevalence and natural history. A second limitation is that due to the difficulty of *M. genitalium* culture, there is no reliable gold standard available to assess the sensitivity or specificity of the assay used. However, Dutro et al [16], did find the assay to be highly concordant when compared to a Southern blot-based *M. genitalium* PCR assay. Another limitation was that the study design did not allow for evaluation of antibiotic efficacy for *M. genitalium*. Currently there are no guidelines for treatment of this organism.

In summary, the prevalence of *M. genitalium* in our sample population establishes it as an important potential pathogen in urogenital infections among adolescents. Our findings lend further support for the sexual transmissibility of *M. genitalium* and add to the current knowledge of the natural history and associated clinical symptoms of *M. genitalium*.

**Acknowledgements**

Assistance in data collection and data analysis by Patricia Brooks,Les Meyer, and the Young Women's Project staff is deeply appreciated.

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**Table 1**

Associations of selected demographic, behavioral and other STI variables with *M. genitalium* (MG) detection status for the sexually experienced population

| | Simple Logistic Regression | | | Multiple Logistic Regression | | |
|---|---|---|---|---|---|---|
| | **Odds Ratio** | **95% CI** | **P-value** | **Odds Ratio** | **95% CI** | **P-value** |
| Age | 0.998 | (0.86, 1.16) | 0.98 | | | |
| African-American | 0.64 | (0.24, 1.66) | 0.36 | | | |
| Age at 1st sex | 1.10 | (0.88, 1.37) | 0.40 | | | |
| Number of recent partners | **1.45** | **(1.18, 1.78)** | **<0.001** | **1.43** | **(1.17, 1.74)** | **<0.001** |
| Condom use, most recent coitus with each partner | 1.09 | (0.65, 1.83) | 0.74 | | | |
| Coital frequency, past 3 months | 1.003 | (0.99, 1.02) | 0.64 | | | |
| Cunnilingus, past 3 months | 1.50 | (0.85, 2.64) | 0.16 | | | |
| *C. trachomatis* | **2.07** | **(1.18, 3.63)** | **0.012** | **1.89** | **(1.07, 3.34)** | **0.029** |
| *N. gonorrhoeae* | 0.95 | (0.30, 3.05) | 0.94 | | | |
| *T. vaginalis* | 0.99 | (0.38, 2.60) | 0.99 | | | |

**Table 2**

Associations of clinical signs or symptoms with *M. genitalium* (MG) and *C. trachomatis* (CT) detection

| Infection status | Symptoms[1] | p- value | Signs[2] | p- value | Symptoms or Signs | p- value |
|---|---|---|---|---|---|---|
| CT- MG- | 3.6%(41/1132) | | 16.3%(184/1126) | | 17.5% (184/1049) | |
| CT- MG+[3] | 6.1% (2/33) | 0.33 | 23.5% (8/34) | 0.35 | 21.9% (7/32) | 0.63 |
| CT+ MG-[3] | 1.8% (2/111) | 0.27 | 28% (30/107) | **0.001** | 27.9 (29/104) | **0.005** |
| CT+ MG+[4] | 0 (0/7) | ------ | 0 (0/6) | ------ | 0 (06) | ------ |

[1] Symptoms were self-report of vaginal itching, vaginal burning, or dyspareunia

[2] Signs were presence of vaginal erythema, vulvar erythema, or vaginal discharge

[3] p-values in comparison to the CT- MG- group

[4] As no subjects in the CT+ MG+ group had signs or symptoms, no p-values are derived