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MAJOR ARTICLE

Mycoplasma genitalium Incidence, Organism Load, and Treatment Failure in a Cohort of Young Australian Women

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Background. *Mycoplasma genitalium* (MG) is an emerging sexually transmitted infection (STI) that is potentially associated with reproductive tract sequelae in women. This study aimed to estimate MG incidence and treatment failure and provide estimates of organism load in infection.

Methods. 1110 women aged 16–25 years were recruited from primary care clinics in Australia. Women were tested for MG at baseline, 6 months, and 12 months, and MG organism load was measured by quantitative polymerase chain reaction (PCR). MG-positive cases were screened for MG 23S ribosomal RNA (rRNA) gene point mutations shown to confer azithromycin resistance using high-resolution melt following PCR.

Results. MG incidence rate was 1.3 per 100 person-years (n = 14; 95% confidence interval [CI], .8–2.3); women reporting 3 or more sex partners in the last 12 months had an increased rate of incident infection (rate ratio [RR], 5.1; 95% CI, 1.3–19.6]). There were 3 cases of MG reinfection (0.8 per 100 person-years [95% CI, .1–.9]. Organism load was higher for prevalent than incident infection ($P = .04$). There were 3 cases of treatment failure (9.4% [95% CI, 2.0–25.0]); organism load was higher in cases with treatment failure than in successfully treated cases ($P < .01$). An MG 23S rRNA mutation was detected in 5 cases (3 cases of treatment failure and 2 successfully treated).

Conclusions. Although MG incidence was relatively low, testing should be recommended for women considered to be at increased risk based on sexual history. Our results also suggest that organism load might be important in azithromycin treatment failure.

Keywords. *Mycoplasma genitalium*; STI epidemiology; organism load; young women; treatment failure.

Mycoplasma genitalium (MG) is an emerging sexually transmitted infection (STI) that is associated with acute and chronic urethritis in men. Data on infection in women are more limited and inconsistent, but do suggest that MG is also associated with urethritis, cervicitis [1–4], pelvic inflammatory disease (PID) [5], and possible infertility in women [6, 7].

To date, there have been few population-based studies of MG infection, and prevalence estimates vary

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widely. MG prevalence has been estimated to be 2.4% (95% confidence interval [CI], 1.5–3.3) in 16- to 25-year-old women in Australia [8], 0.8% (95% CI, .4–1.6) among 18- to 27-year-old sexually active women in the United States [9], 2.3% (95% CI, 1.3–3.2) [10] in 21- to 23-year-old women in Denmark, and 3.3% (95% CI, 2.6–4.1) in sexually active students in the United Kingdom [11]. The same UK study reported a relatively low MG incidence of 0.9 (95% CI, .5%–1.6%) per 100 woman-years [11], but a cohort study of Kenyan sex workers has reported an MG incidence of as high as 22.7 per 100 woman-years [12].

We conducted a cohort study to determine the incidence of chlamydia and MG among young Australian women. Given that commercial assays for MG are not available in Australia, with only large sexual health services and tertiary public hospitals having access to validated in-house assays, this study was designed to provide evidence to indicate whether MG testing should be more widely available. This paper presents the results of the MG analysis, providing MG incidence data as well as the clinical and epidemiologic characteristics associated with incident MG infection. We also present novel MG organism load data, comparing organism load between prevalent and incident infection, and provide estimates of azithromycin treatment failure.

METHODS

Recruitment

Young women were recruited as part of the Chlamydia Incidence and Re-infection Rates Study, whose methods have been published in detail elsewhere [13]. This was a 12-month longitudinal study that aimed to measure chlamydia incidence in Australian women aged 16–25 years recruited from primary care clinics. A secondary aim of the study was to measure MG incidence. In brief, consecutive women were recruited from 29 primary health clinics (including general practice, sexual health, and family planning clinics) in the Australian states and territories of New South Wales, Victoria, and the Australian Capital Territory between May 2007 and August 2008. Research assistants were employed to approach women in these clinics to determine their eligibility and obtain informed consent for their participation in the study. Women aged 16–25 years who were not pregnant at the time of recruitment, had ever had vaginal sex with a male, were competent in written English, and were able to be contacted by post within Australia during the 12-month study were eligible for participation. Follow-up was completed in December 2009.

Testing

Women provided a vaginal self-collected flocked swab (www.copanitalia.com) at baseline, 6 months, and 12 months for MG testing. In addition, those women who tested positive at any stage during the study provided a specimen for a test-of-cure 4 weeks after their treatment. Swabs collected at baseline were given to the research assistant at the clinic and all follow-up swabs were sent through the postal service. Swabs were tested for MG in real time and results provided to the participants. All testing was done at the Royal Women's Hospital, Melbourne, Victoria, and if the swab tested positive, further analyses were performed including organism quantification and macrolide resistance.

MG testing was conducted by rotating the vaginal swab in 400 μL of phosphate-buffered saline and 200 μL was used for DNA extraction, with detection of MG amplified by polymerase chain reaction (PCR) targeting a 517-bp region of the 16S ribosomal RNA (rRNA) gene [14]. Any remaining specimen was stored at −80°C.

Organism Load

The MG quantification of each sample was performed using a quantitative polymerase chain reaction (qPCR; TaqMan MGB Probe) assay targeting the *MgPa* gene [15, 16] by comparing the crossing points of each sample to a standard curve constructed by amplifying a range of known copy numbers of the target gene.

Azithromycin Resistance

All MG-positive cases were also screened for MG 23S rRNA gene point mutations shown to confer azithromycin resistance using high-resolution melt following PCR as described previously by Twin et al [17].

Management of Participants

All women who tested positive for MG were managed by the research team, who organized a telephone consultation with a sexual health physician who discussed symptoms and explained treatment. One gram of azithromycin was prescribed [18] and provided free of charge along with resources supporting notification of sex partners regarding MG. If the woman reported any symptoms suggestive of PID or there were any other clinical concerns, a face-to-face consultation with a clinician and further clinical assessment were arranged.

A self-collection specimen kit for a test-of-cure was sent 4 weeks after treatment. Women with a positive test-of-cure had another telephone consultation with a sexual health physician who determined whether the positive result was likely to be due to treatment failure or MG reinfection. Likely treatment failure was treated with 400 mg moxifloxacin daily for 10 days [19, 20], and reinfections were treated with a repeat 1-g dose of azithromycin [20]. A second test-of-cure was offered a further 4 weeks after the second treatment.

Definitions for Prevalent Infection, Incident Infection, Treatment Failure, Reinfection, Persistent Infection

A *prevalent infection* was a positive MG test result at the time of recruitment (baseline). An *incident infection* was defined as any new infection acquired during the study period following either a previous negative MG test result or a negative test-of-cure result. *Treatment failures* were defined as a positive test-of-cure following treatment with azithromycin in women who also reported either having either no sexual intercourse after treatment or consistently used condoms after treatment. An MG *reinfection* was defined as a positive MG test result following either a negative test-of-cure result or unprotected sex with an untreated sexual partner. A patient's history of unprotected sex was ascertained from 2 sources: (1) the woman's self-completed questionnaire and (2) a telephone or face-to-face consultation with a sexual health physician prior to prescribing treatment. Reinfections were also incident infections. An infection was defined as *persistent* if the woman had 2 consecutive positive MG test results and was not treated following the first positive result (Figure 1). These definitions were based on a modified version of the chlamydia reinfection algorithm developed by Batteiger et al [21]. An infection was considered successfully treated if the result of the test-of-cure was negative.

Data Collection

Women completed a self-administered questionnaire at baseline and at 3-monthly intervals during the 12-month study period. The questionnaire collected demographic data, sexual behavior data, recent antibiotic and contraceptive use, any pregnancies including termination and/or miscarriages, and genital symptoms that might have occurred since the previous questionnaire.

Statistical Methods

Power calculations suggested that a sample size of 1000 would be sufficient to generate a standard error of 1.0% for incidence estimates of 2%. MG incidence rates and 95% CIs based on the estimation of robust standard errors were calculated using Poisson regression. The rate of reinfection was calculated using only data from those who tested positive at least once during the study and had 2 or more tests. The association between demographic, behavioral, and clinical factors and the incidence of MG was investigated using a discrete-time version of the proportional-hazards regression model described by Carlin et al [22], from which rate ratios (RRs) and robust standard errors were generated. Rate ratios were adjusted for clinic type from which the woman was recruited to take any potential confounding by clinic type into consideration. Further adjustment was limited by the small number of incident MG cases.

MG organism load values were logarithm ($\log_{10}$) transformed for the purpose of analysis. Box plots were used to display the range of organism load values by infection type (prevalent, incident). The *t* test or Mann-Whitney *U* test was used to compare organism load between groups where appropriate. Data were analyzed using Stata software, version 11.1 [23].

Ethics approval to conduct this study was obtained from 10 human research ethics committees throughout Australia. Informed consent was obtained from every participant prior to enrollment in the study.

Results

Sample Characteristics

A total of 1110 women participated in the study with a response rate of 66%. A total of 735 women (66%) were recruited from general practice clinics and 375 (34%) were recruited from sexual health and family planning clinics. Overall, 877 participants (79%) provided a specimen at the study end, providing 1056.34 person-years of follow-up. A total of 27 women tested positive for MG at baseline (prevalence: 2.4% [95% CI, 1.5–3.3]) and all women with baseline infection received treatment. The MG prevalence results have been reported in detail elsewhere [8].

Incident Infection

A total of 14 incident infections were identified, yielding an incidence rate of 1.3 per 100 person-years (95% CI, .8–2.3). Univariate analysis found that MG incidence was higher in women recruited from sexual health and family planning clinics compared with women recruited from general practice and increased with an increasing number of new sex partners in the last 12 months. This persisted once adjusted for type of clinic, with women reporting 3 or more sex partners in the last 12 months having more than a 5 times greater rate of incident MG, compared with women reporting 1 or no new sex partners (RR = 5.1 [95% CI, 1.3–19.6]). No other factors were associated with incident MG (Table 1).

Reinfection Rate and Persistent Infection

Three reinfections were diagnosed, giving a reinfection rate of 0.8 per 100 person-years (95% CI,.1–.9) and a cumulative risk of reinfection of 11.1% (3 of 27; 95% CI, 2.4%–29.2%). Two of these reinfections were diagnosed 6 months after a negative test-of-cure result and 1 infection was diagnosed at 4 weeks after treatment (but the participant reported unprotected sex with an untreated partner). There were no persistent infections diagnosed during the study.

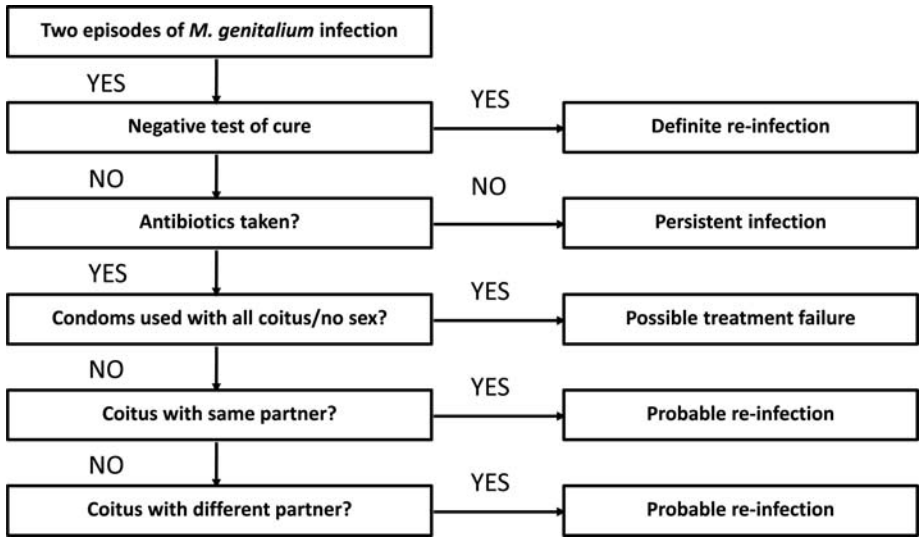

Figure 1. Algorithm to differentiate *Mycoplasma genitalium* reinfection and persistent infection adapted from Batteiger et al [21].

Treatment Failure

Of the 41 positive tests, 32 women provided test-of-cure specimens. Of these 32, 3 had a positive test-of-cure (9.4% [95% CI, 2.0–25.0]) and 29 had a negative test-of-cure. The 3 positive test-of-cure results were categorized as treatment failure with azithromycin on the basis that all 3 women had adhered to treatment and had not had sex with an untreated sex partner. All 3 cases were successfully treated with 400 mg moxifloxacin daily for 10 days and had a negative test-of-cure 4 weeks following treatment.

MG 23S rRNA Gene Point Mutations

Both the pretreatment and the test-of-cure specimens for the 3 women classified as treatment failure were analyzed for MG 23S rRNA gene point mutations. All 3 were found to carry a wild-type 23S rRNA gene sequence prior to treatment with 1 g azithromycin and then had a relevant macrolide resistance mutation detected in their test-of-cure sample.

Of the 29 negative tests-of-cure, 27 (93.1%) had pretreatment specimens available for analysis for MG 23S rRNA gene point mutations and 2 of the 29 (7.4%) were identified as carrying 23S RNA mutations. Nine participants who tested MG positive did not provide a test-of-cure after treatment with azithromycin and could not be included in this analysis.

Organism Load

Organism load could be measured for 22 of the 27 (81.5%) prevalent infections and 13 of the 14 (92.9%) incident infections (including the 3 reinfections). Organism load per swab was significantly higher in the 22 prevalent (mean = 4.7 [SD = 0.9], median = 4.7 copies per swab [$\log_{10}$]) than the 13 incident infections (mean = 4.0 [SD = 1.1], median = 3.8 copies per swab [$\log_{10}$]) ($P = .04$; Figure 2).

Organism load per swab was also significantly higher in pretreatment swabs from the 3 women (mean = 6.1 [SD = 0.3], median = 6.0 copies per swab [$\log_{10}$]) who experienced treatment failure, compared with pretreatment swabs from the 22 women (mean = 4.5 [SD = 0.9], median = 4.5 copies per swab [$\log_{10}$]) who were successfully treated with azithromycin and for whom organism load was measurable ($P = .01$; Figure 3).

Five women had a 23S rRNA mutation detected, 3 of whom experienced treatment failure and 2 who were successfully treated. The organism load was higher in the pretreatment swab for those who had treatment failure (3 women) compared with those who were successfully treated (2 women; $P < .01$).

Clinical Presentation of Those Diagnosed With Incident MG

When the 14 women with incident infection were telephoned with their positive result, 3 were asymptomatic and the remaining 11 reported a number of symptoms including vaginal discharge (n = 3), vaginal odor (n = 2), burning on urination (n = 2), intermenstrual spotting (n = 5), abdominal pain (n = 2), or dyspareunia (n = 1). One of these was diagnosed with clinical PID (7.1% [95% CI, .2%–33.9%]) and in addition to azithromycin, received treatment with doxycycline and metronidazole. No further clinical information was available.

DISCUSSION

The results of Australia's first MG incidence study show an incidence rate of 1.3 per 100 person-years, a risk of reinfection of 11.1%, and an azithromycin treatment failure of 9.4%.

Table 1. Demographic and Behavioral Factors Associated With Incident *Mycoplasma genitalium* Infections Among Women (n = 14)

Variable	Incidence (95% CI)	Unadjusted RR (95% CI)	Adjusted RR[a](95% CI)
Age			
<21 y	2.1 (1.0–4.9)	1	1
21–25 y	.8 (.4–1.8)	.4 (.1–1.1)	.4 (.2–1.2)
Area of residence			
Rural	1.8 (.9–3.9)	1	1
Metropolitan	1.0 (.4–3.5)	.5 (.2–1.5)	.7 (.3–1.3)
Education			
Secondary school only	1.7 (.9–3.8)	1	1
Tertiary or further education	.9 (.4–2.3)	.5 (.2–1.4)	.5 (.2–1.5)
Employment			
Unemployed/not working	1.3 (.5–3.6)	1	1
Employed	1.4 (.9–2.5)	1.1 (.4–2.8)	1.0 (.4–2.4)
No. of new partners during study period			
0/1	.8 (.4–2.6)	1	1
2	1.6 (.4–1.2)	1.9 (.2–19.6)	1.5 (.1–12.2)
3+	6.4 (2.0–27.9)	7.6 (1.5–37.9)	5.1 (1.3–19.6)
Recent antibiotic use			
No	1.0 (.4–2.9)	1	1
Yes	1.9 (.5–9.9)	1.9 (.4–9.2)	2.0 (.4–9.9)
Type of clinic			
Sexual health/ family planning	3.0 (2.2–4.1)	1	N/A
General practice	.6 (.2–1.5)	.2 (.1–.4)	

Abbreviations: CI, confidence interval; N/A, not applicable; RR, rate ratio.

[a] Adjusted for clinic type (general practice or sexual health/family planning clinic).

We provide the first published quantitative data of MG organism load and its association with prevalent as well as incident MG infection. We found that organism load was higher in prevalent than incident infections, suggesting that either organism load may increase over time or that there may be more rapid host clearance of lower organism load infections or "less fit" organisms, compared with higher load infections. We also found that women with treatment failure were more likely to have a higher organism load and 23S rRNA gene mutations, a finding that has not previously been reported. We also found that 2 of the 29 women who were successfully treated with 1 g of azithromycin had a 23S rRNA gene mutation detected. The organism load for these 2 women was significantly lower than the organism load for the 3 women who also had a 23S rRNA gene mutation and had treatment failure. Although this finding is limited by the small number of cases, it is a novel finding and raises questions of whether this could represent heterotypic resistance that is only manifested at higher organism loads. The phenomenon of heterotypic resistance has been described in relation to chlamydia infection and our study raises the question of whether this is also pertinent to MG [24].

Our incidence estimates are similar to those reported in a recent study conducted in the United Kingdom, which reported an incidence of 0.9% in young sexually active female

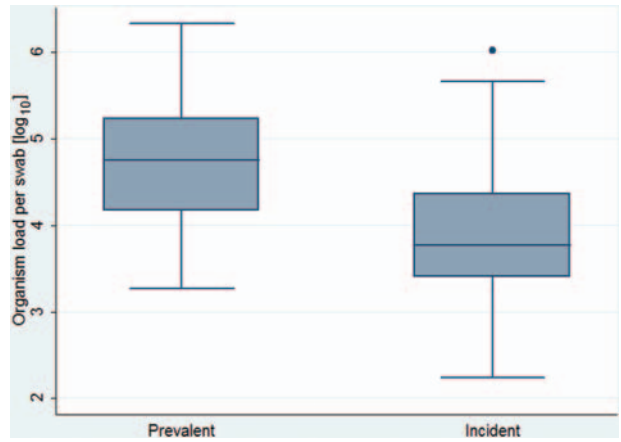

Figure 2. The differences in *Mycoplasma genitalium* organism load per swab (log_{10}) between prevalent infections and incident infections in a cohort of young Australian women.

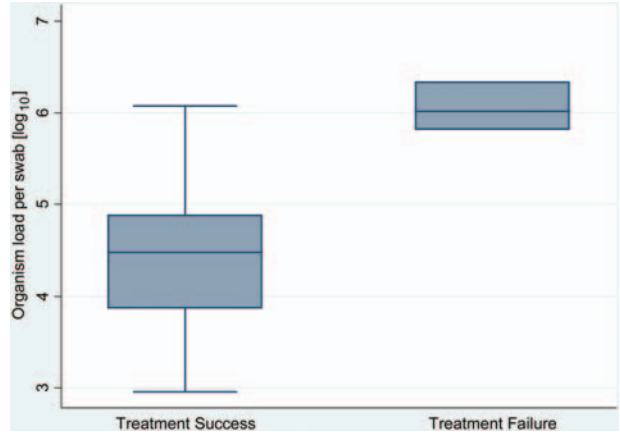

Figure 3. The differences in *Mycoplasma genitalium* organism load per swab (log_{10}) between infections that were not effectively treated with 1 g azithromycin and those infections that were treated effectively by azithromycin in a cohort of young Australian women.

university students [11]. They also found that the incidence was higher with an increasing number of new sexual partners. We did find a high risk of MG reinfection (11%), which highlights the importance of partner notification, treatment, and retesting following a positive diagnosis.

Overall our finding of 9.4% treatment failure in this study is slightly less than findings of other Australian and international studies which have found azithromycin to be effective in approximately 85% of cases [19, 20, 25, 26]. However, these studies had a high proportion of symptomatic participants who are more likely to have a high organism load [27] which, as our findings suggest, may be associated with treatment failure.

We did find an incidence of PID of 7.1%, which is comparable to recent PID estimates of 9.5% following chlamydial infection obtained from a cohort of women in the United Kingdom [28].

There were a number of limitations to our study. First, as we have previously reported [13], our sample had a higher proportion of well-educated women who reported a greater number of sexual partners in the last 12 months than the general population in Australia of the same age [29, 30]. Given that incident MG was associated with an increasing number of sex partners in the past 12 months, it is possible that we have overestimated the incidence of MG. However, it is common that well-educated, more sexually active women tend to be oversampled in similar sexual health research [31, 32]. Also, we were limited by the small number of incident cases (n = 14) in our analysis. There are potential limitations with the organism load analysis because we were unable to analyze organism load on all MG-positive swabs. Reasons for missing organism load data included degraded DNA, loss of specimen, and reduced sensitivity of qPCR at low organism loads [18]. However, there were no differences in participant characteristics including age or number of partners between those who had measurable organism load and those who did not. In terms of quantifying organism load, equal efficiency of self-collected sampling could not be assured and positive samples were subjected to a number of assays depleting the sample with each extraction. Nevertheless, this study did have important strengths—it had a large sample size, including a greater proportion of women from general practice rather than sexual health clinics, and a high retention rate (79%) with negligible loss to follow-up bias [13].

MG is emerging as an important STI, yet currently there is no available commercial assay and the majority of MG infections worldwide in women go undiagnosed and untreated. We believe that MG testing should be more widely available to clinicians who manage patients with genital symptoms. However, at this stage it is too early to determine if screening asymptomatic patients for MG can be justified.

Treatment failure of MG infections following 1 g of azithromycin is common and has been reported to often be due to detection of macrolide resistance mutations after exposure to the antibiotic [19, 20, 25, 26]. Our data suggest that effective treatment for MG might also be complicated by variable organism load, and while moxifloxacin appears to be highly effective, worryingly, a small number of cases of moxifloxacin failure have recently been reported in Scandinavia and Australia (C. Bradshaw, oral communication, December 2010). Whether a higher dose or more prolonged treatment with azithromycin [33] would be more effective for higher organism load cases warrants further investigation.

CONCLUSIONS

These are the first MG incidence data for young Australian women and our results confirm that MG is not uncommon in young women. The lack of widespread availability of a commercial assay and limited knowledge of this infection in the medical and general community mean that the majority of women infected with MG internationally currently remain undiagnosed and untreated. Clearly, more research is needed to determine the potential effect of organism load on treatment efficacy and to determine the contribution of macrolide resistance to treatment failure. Studies with larger populations will provide more information about transmission dynamics and duration of infection, both of which are important factors in understanding the management of MG in the population.

Notes

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Potential conflicts of interest. B. D. has received honoraria from CSL Biotherapies, Sanofi Pasteur, MSD, and Merck; and S. M. G. has received honoraria from Merck, CSL, and GSK for human papillomavirus–related research and education. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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