Time to eradication of *Mycoplasma genitalium* after antibiotic treatment in men and women

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**Objectives:** The objectives of this study were to evaluate the time to a *Mycoplasma genitalium*-negative test after start of treatment and to monitor if and when antibiotic resistance developed.

**Methods:** Sexually transmitted disease (STD) clinic attendees with suspected or verified *M. genitalium* infection were treated with azithromycin (5 days, 1.5 g; *n* = 85) or moxifloxacin (*n* = 5). Subjects with symptomatic urethritis or cervicitis of unknown aetiology were randomized to either doxycycline (*n* = 49) or 1 g of azithromycin as a single dose (*n* = 51). Women collected vaginal specimens and men collected first-catch urine 12 times during 4 weeks. Specimens were tested for *M. genitalium* with a quantitative MgPa PCR and for macrolide resistance-mediating mutations with a PCR targeting 23S rRNA. Clinical Trials Registration: NCT01661985.

**Results:** Ninety *M. genitalium* cases were enrolled. Of 56 patients with macrolide-susceptible strains before treatment with azithromycin (1.5 g, *n* = 46; 1 g single oral dose, *n* = 10), 54 (96%) had a negative PCR test within 8 days. In four patients, *M. genitalium* converted from macrolide susceptible to resistant after a 10 day lag time with negative tests (azithromycin 1.5 g, *n* = 3; 1 g single oral dose, *n* = 1). Moxifloxacin-treated subjects (*n* = 4) were PCR negative within 1 week. Six of eight (75%) remained positive despite doxycycline treatment.

**Conclusions:** PCR for *M. genitalium* rapidly became negative after azithromycin treatment. Macrolide-resistant strains were detected after initially negative tests. Test of cure should be recommended no earlier than 3–4 weeks.

**Introduction**

*Mycoplasma genitalium* is a common pathogen causing sexually transmitted infections (STIs). In contrast to *Chlamydia trachomatis*, antibiotic resistance is common. Despite reports of excellent eradication rates by azithromycin treatment (85%–95%) in studies recruiting patients before 2005, there is now growing evidence of decreased susceptibility to macrolides.4–8 Macrolide resistance following a single 1 g dose of azithromycin has been frequently reported and has been suspected to be the major cause of resistance in the community.4,9–11 The resistance is caused by single-base mutations in region V of the 23S rRNA gene.12 In most STI treatment guidelines, doxycycline or 1 g of azithromycin as a single oral dose is the recommended treatment for chlamydial infections as well as for non-gonococcal urethritis and cervicitis of unknown aetiology.13,14 First-line treatment of *M. genitalium* in Scandinavia is an extended azithromycin course (500 mg on the first day followed by 250 mg on days 2–5). If macrolide resistance occurs, the currently accepted effective option is moxifloxacin treatment with 400 mg once daily for 7–10 days. Due to emerging resistance, a test of cure is recommended. There is, however, no available information regarding how long it takes until *M. genitalium* is cleared after antibiotic treatment has started. The aims of this prospective trial were to evaluate the time after initiation of treatment to a negative test, and to monitor if and when antibiotic resistance develops.

**Methods**

**Trial design**

The study was a prospective longitudinal cohort study comprising an observational study and a randomized treatment trial. From a research point of view, a randomized trial, preferably comprising four arms...
Laboratory analyses

M. genitalium was detected by a quantitative PCR amplifying a part of the MgPa gene as previously described. In brief, 1.8 mL of the urine sample was concentrated by centrifugation and DNA was released from the pellet by boiling in 300 μL of a 20% (w/v) Chelex 100 slurry (Bio-Rad, Richmond, CA, USA) in TE buffer [10 mM Tris–HCl (pH 8.0)/1 mM EDTA]. Similarly, 100 μL of the vaginal swab sample was added to 300 μL of Chelex slurry. All positive results were confirmed in a conventional PCR amplifying the 235 rRNA gene and amplicons were subjected to sequencing on a PyroMark Q96 (Qiagen, Hilden, Germany) sequencing platform. Strain typing from selected M. genitalium-positive samples was carried out using an MgPa typing assay.

Statistical analyses

All data were registered in an Excel book using a coded identity for all participants. Kaplan–Meier survival plots were generated and log-rank analysis was performed in GraphPad Prism v6.03.

Results

Of the 191 enrolled subjects, 100 were allocated to randomization (Group 2) and 22 (22%) of these were M. genitalium positive. The remaining 91 (68 (75%) M. genitalium positive) were allocated to either the extended azithromycin or the moxifloxacin treatment arm (Group 1). A flow chart of allocation and treatment is presented in Figure 1. Twenty-five men and 37 women were treated with extended azithromycin, 3 men and 2 women were treated with moxifloxacin, 10 men and 5 women were allocated to 1 g of azithromycin treatment as a single dose, and 6 men and 2 women were treated with doxycycline. Demographic data are presented in Table 1. All 12 follow-up specimens were collected and analysed from 37 (80%) women and 16 (36%) men. More than six specimens were submitted by a further five women (11%) and 14 men (32%). Among the 28 men harbouring a macrolide-susceptible M. genitalium strain, 13 submitted all samples and 7 submitted at least eight samples (mean 9.3), with the last sample taken between days 17 and 26.

Observational study of M. genitalium–infected subjects

Fifty-three M. genitalium–infected patients, 18 men and 35 women, treated with extended azithromycin (1.5 g), were evaluable, and, of these, 33 (62%) had negative tests by day 3 after starting treatment (pattern A, Figure S1, available as Supplementary data at JAC Online) and remained negative in the remaining tests during the study period of 26 days. Of 10 patients with macrolide-susceptible M. genitalium infections treated with 1 g of azithromycin as a single dose, 9 cleared the infection before day 8. There was no gender difference in the clearance rate.

The four patients treated with moxifloxacin cleared their infections before day 3, whereas among the seven evaluable subjects treated with doxycycline, three became M. genitalium negative within 1 week. In one of these (a man) symptoms recurred and the tests became M. genitalium positive on day 13.

Comparison of outcomes between doxycycline and azithromycin

For subjects without pre-existing macrolide resistance there was no difference in time to eradication between 1 g of azithromycin and extended azithromycin (1.5 g) treatment, whereas the difference between azithromycin and doxycycline was statistically significant (P=0.03 compared with 1 g and P=0.006 compared with extended azithromycin) (Figure 2). When all subjects regardless of pre-existing macrolide resistance were compared, only extended azithromycin had a shorter time to eradication than doxycycline (P=0.04).
Emergence of macrolide resistance following treatment with azithromycin

Two women treated with extended azithromycin and one woman treated with a single dose of 1 g of azithromycin and with macrolide-susceptible strains cleared their infections within 5 days, but after 2 weeks sporadic specimens that were positive, with a few DNA copies of a macrolide-resistant strain (A2058G mutation), began to appear until M. genitalium was persistently detected with an increasing organism load with the A2058G mutation in consecutive samples (Table 2) (pattern D, Figure S1). One symptomatic man with a high M. genitalium load at inclusion converted from susceptible to macrolide resistant (A2058G) on day 5 (pattern B, Figure S1). All four subjects denied sexual intercourse during the study period and all MgPn strain types for each subject were identical. There was no difference in the M. genitalium load in the pre-treatment samples between patients developing resistance and those who cleared the infection, with a mean pre-treatment bacterial load of 9786 copies (per 5 µL of extracted DNA) and 7355 copies, respectively.

Discussion

The present prospective observational study showed that M. genitalium was eradicated within a few days after antibiotic treatment. For some patients, however, even with extended azithromycin treatment conversion from macrolide-susceptible to macrolide-resistant strains occurred and in most cases it occurred after consecutive negative samples. Among the few patients treated with doxycycline, the clearance rate was low and in line with other studies.1–5,10

Previous studies have reported a slow clearance of C. trachomatis with persistent positive nucleic acid amplification tests for 3 weeks.17,18 M. genitalium DNA would be expected, consequently, to be detectable for weeks, but this was clearly not the case. One explanation could be that the organism load...
among M. genitalium–infected patients is 100-fold lower than in C. trachomatis–infected patients. Despite the low M. genitalium DNA load, however, the proportion of symptomatic patients was similar to that reported for patients with chlamydia (Table 1). One man treated with doxycycline had a temporary clearance of M. genitalium DNA, but a subsequent relapse. This was probably due to a temporary suppression of the infection with DNA shedding below the limit of detection. This is in good agreement with previous observations of a temporary effect of tetracyclines and subsequent relapse. There is little evidence from in vitro studies that tetracycline resistance is common despite frequent treatment failure.

In four azithromycin-treated subjects, macrolide resistance developed during or after treatment. Obviously, it is impossible to exclude the possibility of re-infection from an untreated partner, but DNA typing clearly suggested that it was the same strain, and the participants denied any risk of a new infection during the study period. In contrast to other studies, we did not find a higher M. genitalium organism load in patients developing resistance compared with those who cleared the infection, but the number of patients developing resistance was small. Many studies have shown an overall good response to azithromycin treatment, but there have been reports of resistance emerging after single-dose azithromycin treatment. Development of resistance in 3 (5%) of 53 patients treated with extended 1.5 g azithromycin is in good agreement with previous experience of treatment failure in 5% of patients treated with this dosage. However, it also stresses that the extended dosage does not preclude resistance development. With only a few patients treated with a single dose of 1 g of azithromycin, the figures are too small to allow an estimation of differences in the risk for development of resistance between the two regimens. A study by Anagrius et al., however, suggested that single-dose azithromycin was more likely to select for macrolide resistance than the extended dosage scheme and no development of resistance among 77 patients receiving extended azithromycin was observed. However, 52 of these patients had been treated with doxycycline prior to the...
Emerging macrolide resistance has been reported in Sweden, from 0% in 2006 to 20% in 2011. In Sweden, doxycycline is recommended as first-line treatment for chlamydia and the adherence to this recommendation is very high. Azithromycin is in general only prescribed when there is a verified or high suspicion of M. genitalium infection. The easy access to diagnosis of M. genitalium infection in Sweden and the increasing awareness of the infection means that previously undetected asymptomatic cases now are treated. This might increase the risk of development of antibiotic resistance, especially since most laboratories do not test for macrolide resistance–mediating mutations. Consequently, resistant strains may have a higher risk of being transmitted in the community.

Spontaneous eradication probably occurred in several patients in the present study. Whether such clearance improves the immune response and diminishes the risk of re-infection, as has been suggested in women treated for chlamydia, is unclear and not studied. Apart from treatment of symptoms, the reason for treatment is to avoid sexual transmission and prevent salpingitis. Most treatment regimens in pelvic inflammatory disease treatment guidelines do not cover M. genitalium, and this may increase the risk of tubal factor infertility or extra-uterine pregnancies. Although spontaneous eradication may occur, studies have suggested that M. genitalium may persist for more than a year. Consequently, efficient treatment is essential to prevent spread of the infection.

Among the subjects in the present study, there was a very low proportion (11%) not collecting samples. Although the subjects were informed about the importance of not having any unprotected sex during the sampling period, it cannot be ruled out that the adherence was <100%. We did not have any control of adherence to antibiotic intake (except for those prescribed 1 g of azithromycin as a single dose) and there may be participants not completing the treatment. A few individuals had a few sporadic positive tests, but in these cases the remaining tests were negative. Whether such clearance improves the immune response and diminishes the risk of re-infection, as has been suggested in women treated for chlamydia, is unclear and not studied. Apart from treatment of symptoms, the reason for treatment is to avoid sexual transmission and prevent salpingitis. Most treatment regimens in pelvic inflammatory disease treatment guidelines do not cover M. genitalium, and this may increase the risk of tubal factor infertility or extra-uterine pregnancies. Although spontaneous eradication may occur, studies have suggested that M. genitalium may persist for more than a year. Consequently, efficient treatment is essential to prevent spread of the infection.

In conclusion, the present study indicated that the commonly used recommendation of test of cure 3–4 weeks after the start of treatment can still be used. This is in accordance with a Japanese study where the optimal time for test of cure was found to be 20 days after the start of treatment. The present study raises concern that first-line treatment with extended azithromycin for 5 days (1.5 g), as commonly recommended, may not be optimal. With the increasing level of pre-existing macrolide resistance in most settings, treatment failure will be common. Moxifloxacin treatment was effective and may still be an alternative for second-line treatment of macrolide-resistant infections. However, the emergence of moxifloxacin-resistant strains may also threaten the use of this drug. In the absence of routine

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</table>

**Table 2. Details of Four Treatment Failures with a Conversion from Macrolide-susceptible (WT) to Macrolide-resistant M. genitalium Strains after Azithromycin Treatment**

1. AZM: 1 g of azithromycin as a single dose; extended AZM: 500 mg of azithromycin on day 0 followed by 250 mg on days 1–4 (1.5 g); PNNL, polymorphonuclear leukocytes; hpf, high-power field (×1000); NA, not applicable.

2. A cervicitis (intermenstrual bleeding), asynomatic symptoms and signs, discharge, dysuria. Bacterial vaginosis.

3. An extended azithromycin course, which may partly explain this discrepancy in the risk of macrolide resistance development in two populations that would be considered very similar.
testing for macrolide resistance-mediating mutations, a test of cure 3–4 weeks after treatment is highly recommended.

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Transparency declarations
None to declare.

Author contributions
L. F. initiated the study, examined and sampled most of the patients in Norrköping, collected all data and wrote the first draft of the manuscript. M. E. examined patients in Västervik and contributed to amendments to the manuscript. J. S. J. was responsible for the laboratory tests performed at SSI in Copenhagen and contributed to the design of the study, analysis of the data and amendments to the manuscript.

Supplementary data
Figure S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References

