REVIEW ARTICLE

Background review for the 2016 European guideline on *Mycoplasma genitalium* infections

J.S. Jensen,[1,*] M. Cusini,[2] M. Gomberg,[3] H. Moi[4]

[1]Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark
[2]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[3]Moscow Scientific and Practical Centre of Dermatovenereology and Cosmetology, Moscow, Russia
[4]Olafia Clinic, Oslo University Hospital, Institute of Medicine, University of Oslo, Oslo, Norway
*Correspondence: J.S. Jensen. E-mail: jsj@ssi.dk

Abstract

Mycoplasma genitalium is a cause of 10–35% of non-chlamydial non-gonococcal urethritis in men and in women, and is associated with cervicitis and pelvic inflammatory disease (PID). Transmission of *M. genitalium* occurs through direct mucosal contact. In women, symptoms include vaginal discharge, dysuria or symptoms of PID – abdominal pain and dyspareunia. In men, urethritis, dysuria and discharge predominates. Asymptomatic infections are frequent. In this review, we present the evidence base for the recommendations in the 2016 European guideline on *M. genitalium* infections and describe indications for testing, recommended diagnostic methods, treatment and patient management. The guideline was prepared on behalf of the European branch of The International Union against Sexually Transmitted Infections; the European Academy of Dermatology and Venereology; the European Dermatology Forum; the European Society of Clinical Microbiology and Infectious Diseases; the Union of European Medical Specialists. The European Centre for Disease Prevention and Control and the European Office of the World Health Organisation also contributed to their development.

Received: 3 March 2016; Accepted: 23 June 2016

Conflicts of interest

None declared.

Funding sources

None declared.

Introduction

Mycoplasmas, the trivial name for members of the class Mollicutes, are the smallest free-living micro-organisms. They lack the rigid cell wall of other bacteria so that they resist penicillins and other β-lactams.[1] The mycoplasmas isolated commonly from humans belong to the family Mycoplasmataceae. This family comprises the genus *Mycoplasma*, and the genus *Ureaplasma*, which hydrolyses urea. In the urogenital tract, the relevant species are *M. genitalium, U. urealyticum, U. parvum* and *M. hominis. M. hominis* and the ureaplasmas will not be dealt with in the present guideline.

Mycoplasma genitalium was first isolated in 1980 from two of 13 men with non-gonococcal urethritis (NGU).[2] It is an extremely slow-growing and fastidious bacterium, and its role as a pathogen in human disease was not established until the first diagnostic polymerase chain reaction assays (PCRs) were developed in the early 1990s.[3,4]

Male NGU was the first syndrome unequivocally associated with *M. genitalium* infection[5,6] and in a meta-analysis including 37 studies up to 2010,[7] *M. genitalium* was associated with a pooled OR of 5.5 (95% CI:4.4–7.0) for NGU. In the 29 studies where information on chlamydial infection was available, *M. genitalium* was associated with a pooled OR of 7.6 (95% CI:5.5–10.5) for non-chlamydial non-gonococcal urethritis (NCNGU). The prevalence of *M. genitalium* in men with NCNGU ranges from 10% to 35%;[7] thus, contributing significantly to the overall burden of disease. In comparison, *M. genitalium* is detected in only 1% to 3.3% of men and women in the general population.[8–11] In women, several studies have demonstrated the association between *M. genitalium* and urethritis, cervicitis, endometritis and pelvic inflammatory disease (PID).[12–16] In a recent meta-analysis,[17] significant associations were found between *M. genitalium* and cervicitis [pooled odds ratio (OR) 1.66 (95% CI:1.4–2.0)], and PID [pooled OR 2.14 (95% CI:1.3–3.5)]. While there are less data in pregnancy, *M. genitalium* has been associated with preterm birth [pooled OR 1.89 (95% CI:1.3–2.9)] and spontaneous abortion [pooled OR 1.82 (95% CI:1.1–3.0)], but the prevalence of *M. genitalium*

in pregnant women has generally been low in many European settings[18,19] and therefore, the relative importance of *M. genitalium* as a cause of adverse pregnancy outcome in Europe is probably rather small. Serological studies and studies based on detection of *M. genitalium* using nucleic acid amplification tests (NAATs) have also shown an association with increased risk of tubal factor infertility [pooled OR 2.43 (95% CI:0.9–6.3)]. In sub-analyses that accounted for co-infections, Lis *et al.* found these associations to be stronger and more statistically significant [pooled OR 3.27 (95% CI: 1.3–8.6)].[17]

Persistence of *M. genitalium* after treatment is associated with recurrent or persistent NGU. In men with persistent NCNGU after doxycycline therapy, as many as 41% were found to be *M. genitalium* positive,[20] and 91% of patients with persistent *M. genitalium* infection experienced persistent urethral symptoms compared to 17% of patients in whom *M. genitalium* was eradicated.[21] A recent meta-analysis included 21 studies on treatment efficacy of *M. genitalium*-positive urethritis. These studies presented data on the presence of urethritis in patients where antibiotic treatment failed to eradicate the infection.[22] In the 19 studies where data on men with persistent and eradicated *M. genitalium* infection could be evaluated, 220 (77%) of the 285 patients with persistent *M. genitalium* infection had persistent urethritis, compared to only 78 (16%) of the 499 patients where *M. genitalium* was successfully eradicated ($P < 0.0001$). Persistent *M. genitalium* was associated with a pooled odds ratio of 26 (95% CI: 11–57) for persistent urethritis (signs and/or symptoms). This analysis clearly demonstrates that failure to eradicate *M. genitalium* leads to persistent or recurrent signs and symptoms of urethritis in the vast majority of men with persistent infection and that diagnosis and optimal treatment is extremely important. *M. genitalium* has been shown to facilitate HIV transmission, in particular, in studies from Sub-Saharan Africa.[23–25] If eradication fails due to inappropriate treatment, this may have particularly important implications for increased risk of HIV transmission.

Transmission

Transmission is primarily by direct genital–genital mucosal contact with inoculation of infected secretions as illustrated by a high concordance rate of identical DNA types in sexual partners.[26] *M. genitalium* has been detected in anorectal samples by culture and NAATs,[27,28] and transmission from penile–anal sexual contact has been established.[29] Orogenital contact is less likely to contribute to any significant extent, as carriage of *M. genitalium* in the oropharynx is low.[30,31] Mother-to-child transmission at birth has not been systematically studied, but *M. genitalium* has been detected in the respiratory tract of newborn children.[32] The risk of contracting *M. genitalium* per sexual encounter has not been determined, but because *M. genitalium* is present in lower concentration in genital tract specimens than *C. trachomatis*,[33] it could be considered slightly less contagious than chlamydia.

There are no estimates of the global burden of disease. Prevalence estimates are variable as a wide variation in the sensitivity of detection assays is present and there is no agreed gold standard. In sexually transmitted infection (STI) patients, the prevalence is usually from 60% to 85% of that of *C. trachomatis*, but in the general population, the ratio is generally significantly lower.[8,10]

Compared to *C. trachomatis*, the prevalence of *M. genitalium*-infected patients appear to peak approximately 5 years later for both men and women and to remain higher in the older age-groups.[34,35]

Clinical features

Urogenital infections

Symptoms and signs in women

- Among sexually transmitted diseases (STD) clinic attendees, 40–75% are asymptomatic.[15,16]
- Symptoms are related to cervical and urethral infection and include increased or altered vaginal discharge (<50%), dysuria or urgency (30%) and occasionally, intermenstrual or post-coital bleeding or menorrhagia.[15,16,36]
- Cervicitis.
- Rectal and pharyngeal infections are usually asymptomatic.
- Lower abdominal pain (<20%) should raise suspicion of PID.

Complications in women[17]

- PID (endometritis, salpingitis).
- Tubal factor infertility (probably).
- Sexually acquired reactive arthritis (SARA) may occur.[37]

Symptoms and signs in men[7]

- 70% symptomatic in some STD clinic settings.[38]
- Urethritis (acute, persistent, and recurrent).
- Dysuria.
- Urethral discharge.
- Proctitis.
- Balanoposthitis has been associated with *M. genitalium* infection in one study.[39]

Complications in men

- SARA may occur.[37]
- Epididymitis may occur.

Ocular infections

Ocular infections can result in conjunctivitis in adults[40] but has not been systematically studied. Neonatal conjunctivitis has not been systematically studied.

Indications for laboratory testing [IV; C]

Symptoms

- Symptoms or signs of urethritis in men.
- Mucopurulent cervicitis.
- Cervical or vaginal discharge with risk factor for STI.
- Intermenstrual or post-coital bleeding.
- Acute pelvic pain and/or PID.
- Acute epididymo-orchitis in a male aged <50 years.

Risk factors

- Any of the above symptoms in a regular sexual partner.
- Persons with high-risk sexual behaviour (age < 40 years and >3 new sexual contacts in the last year). However, the public health value of testing asymptomatic persons for *M. genitalium* has not been established and decisions on testing for *M. genitalium* should be informed by local epidemiology when available.
- Sexual contact of persons with an STI or PID, in particular, contacts of *M. genitalium*-infected persons.
- Before termination of pregnancy or other procedures, that breaches the cervical barrier.
- Regular testing of men who have sex with men (MSM), including anal sampling could be considered due to the risk of increased HIV transmission.

Laboratory diagnostics [III; B]

Recommended diagnostic assays

NAATs identifying *M. genitalium*-specific nucleic acid (DNA or RNA) in clinical specimens are the only useful methods for diagnosis, due to the difficulties in isolating *M. genitalium* by culture[41,42] and in the absence of specific and sensitive diagnostic serological assays [III; B][7]. However, at present no commercially available NAAT assays have been evaluated up to the US FDA approval standard, and the tests on the market which have been CE marked to document conformity according to the EU legislation suffer from very limited validation. Consequently, it is extremely important that diagnostic laboratories carefully validate any commercial or in-house assays and participate in external quality assurance assessment (EQA) schemes such as the EQUALIS EQA scheme (http://www.equalis.se/sv/vaar-verksamhet/extern-kvalitetssaekring/kvalitetssaekringsprogram/m-r/mycoplasma-genitalium-nukleinsyra-288-2015/). This EQA scheme has demonstrated substantial differences in the sensitivity of participating laboratories. In Russia, routine diagnostics for *M. genitalium* with commercially available tests manufactured in Russia is widely used. The tests were internationally validated and have sensitivity range from 74% to 100% and 100% specificity for different types of clinical samples obtained from men and women.[43]

With the widespread macrolide resistance in Europe, it is strongly recommended that all positive tests are followed up with an assay capable of detecting macrolide resistance-mediating mutations. A variety of methods are available for this purpose.[35,44–48] The main determinant for the selection of a resistance assay are: (i) its practical implementation in the laboratory, and (ii) its sensitivity (proportion of screening positive tests that can be resistance typed). The latter aspect varies significantly between assays.

Determination of moxifloxacin resistance can also be carried out using molecular methods although the correlation between mutations in parC and *in vitro* moxifloxacin resistance is less clear. The current assays are based on conventional sequencing of a PCR-amplified fragment of parC.[49] At present, detection of moxifloxacin resistance mediating mutations is probably not indicated on a routine basis in Europe, as the level of resistance is low (app 5%)[50] but it may be considered in the Asia-Pacific region where moxifloxacin resistance is more common[51–53] or in patients having acquired the infection in this region.

Specimens

It is difficult to make accurate recommendations regarding the optimal sample type. Provided that the sample extraction procedure includes processing of the urine sample to provide a concentration step, first void urine from men and women provide a good diagnostic specimen which may be self-obtained.[34] No data regarding the importance of holding urine for a certain time are available; so, procedures already in place for *C. trachomatis* sampling can be followed. Vaginal swab (physician or self-collected) also provide an appropriate sensitivity.[54–56] No data are available regarding time after exposure to testing, but again in analogy to *C. trachomatis*, a 2-week period is considered the minimal incubation time. Anal samples are useful in MSM where as many as 70% of the infections will be missed if this site is not sampled,[57] but may also be relevant in women at risk.[28] The association between an anal infection and symptoms is uncertain, but the infection is likely to be transmitted if not detected and treated.

In most settings, it will be appropriate to use the same sampling procedure as for *C. trachomatis* testing. However, some transport media such as the Aptima® (Hologic, Inc., MA, USA) transport medium designed for *C. trachomatis* NAAT will lyse *M. genitalium*, and may provide a poor sensitivity in an in-house assay. This should be carefully evaluated for all in-house assays and even for assays where a validated collection and nucleic acid purification kit is not included [III B].

Management of patients

Information, explanation and advice for the patient

- Patients with *M. genitalium* infection should be advised to abstain from unprotected sexual contact until they and their

partners have completed treatment, their symptoms have resolved and their test of cure is negative [IV; C].
- Patients with *M. genitalium* infection (and their sexual contacts) should be given information about the infection, including details about transmission, prevention and complications. It is recommended that both verbal and written information be provided. Patient information leaflets are available at the International Union against Sexually Transmitted Infections website [IV; C].
- Patients with anal infection including MSM should be informed about the risk of transmission from this site and that the infection may be more difficult to eradicate. Consequently, a test of cure is important.
- Patients with *M. genitalium* infection should be screened for other STIs, including *C. trachomatis*, *N. gonorrhoeae*, syphilis, HIV and *T. vaginalis* where appropriate [IV; C].

Pregnancy

- *M. genitalium* infections during pregnancy may be associated with a modest increase in the risk of spontaneous abortion and preterm birth.[17] In macrolide-susceptible infections, a 5-day course of azithromycin is generally acceptable. The choice of drugs for treatment in macrolide-resistant infections is important and often difficult because of their possible adverse effects on fetal development and pregnancy outcome. In many cases, the risk associated with treatment with the available antibiotics would appear to outweigh the risk of adverse pregnancy outcome, and treatment, especially in women with infection with a macrolide-resistant *M. genitalium* strain, may be considered postponed until after delivery. Pristinamycin is considered safe in pregnancy and may be considered in symptomatic women after consultation with an experienced microbiologist. Although little is known about transmission during birth, the neonate should be observed for signs of infection, primarily conjunctivitis and respiratory tract infection [IV; C].

Indications for therapy [IV; C]

- Detection of *M. genitalium*-specific nucleic acid in a clinical specimen.
- Current partners of *M. genitalium*-positive patients should be treated with the same antimicrobial as the index patient.
- If current partner does not attend for evaluation and testing, epidemiological treatment should be offered with the same regimen as given to the index patient.
- On epidemiological grounds, for recent sexual contacts (previous 3 months). Ideally specimens for *M. genitalium* NAAT should be collected before treatment and treatment should await the result of testing.

Therapy

Treatment of individuals with *M. genitalium* urogenital infection prevents sexual transmission and is likely to reduce the risk of complications, including PID[9] and tubal factor infertility.[17]

M. genitalium has demonstrated a remarkable capability of developing resistance to all antimicrobials used until today. Unfortunately, only few antimicrobial classes have activity against mycoplasmas including tetracyclines, macrolides and fluoroquinolones.

Doxycycline has been shown in several controlled trials to have a poor efficacy in eradicating *M. genitalium*[58–61] with microbiological cure rates between 30% and 40%, whereas azithromycin given as a 1 g single dose generally has proven more effective with cure rates in early studies[58,59] at approximately 85%, but with a declining efficacy to 40% in the most recently conducted trial with inclusion of patients between 2007 and 2011.[61] The declining efficacy is caused by a rapidly increasing prevalence of macrolide resistance, most likely caused by widespread use of azithromycin as a 1 g single dose without test of cure and subsequent spread of resistant strains.

Azithromycin given as an extended regimen with 500 mg on day one followed by 250 mg on days 2–5 (1.5 g total dose) has been recommended as the primary choice of treatment of *M. genitalium* infections in Scandinavia. This is based on the reported effect of extended azithromycin on the closely related *M. pneumoniae*,[62] and approval of this regimen for treatment of pneumonia from the regulatory bodies. In a recent meta-analysis comparing studies with extended and 1 g single dose azithromycin, microbiological cure rates of 88% and 81%, respectively, ($P = 0.026$) were found.[22] It should be noted, however, that a large proportion of the patients receiving extended azithromycin had it as a second-line treatment, most often after doxycycline. Using extended azithromycin or other macrolide antibiotics after failure with the 1 g single-dose regimen or in the presence of pre-existing macrolide resistance-mediating mutations will not eradicate *M. genitalium*.

It has been proposed that azithromycin 1 g single dose may be more likely to be selected for macrolide resistance compared to the extended regimen.[63] An observational study[64] has examined the development of resistance after extended azithromycin. This study found that none of 77 patients treated with extended azithromycin developed resistance. In contrast, 10% of 318 patients treated with a 1 g azithromycin in six studies developed resistance during treatment, lending support to the concept that single-dose therapy appears to be associated with induction of resistance compared to extended regimens. On the other hand, a recent study clearly documented that resistance can be selected also during the extended azithromycin, as three of 46 (6.5%) patients with pre-treatment-susceptible strains developed resistance after treatment, comparable to one of 10 (10%) receiving the 1 g single dose.[65]

Macrolide resistance rates vary significantly geographically, but where azithromycin 1 g single dose is used for treatment of NGU, it is usually found in 30–45% of samples[35,50,53,66] and in Greenland where azithromycin is widely used, a resistance rate of 100% has been reported.[67]

Another macrolide, josamycin, is widely used in Russia for treatment of *M. genitalium*-positive patients as first-line treatment. In a recently published study, josamycin given as 500 mg three times a day for 10 days showed a 93.5% eradication rate in males with urethritis caused by macrolide-susceptible *M. genitalium*.[68] Macrolide resistance to this 16-membered macrolide was reported with approximately the same rate as for azithromycin but the mutation was selected at the A2062G position of the 23S rRNA gene (different from the A2058G/A2059G mutations described for azithromycin). *In vitro*, this mutation resulted in resistance of *M. pneumoniae* to pristinamycin but no cross resistance with azithromycin.[69]

Moxifloxacin is the most commonly used second-line antimicrobial. Moxifloxacin is bactericidal and generally well tolerated, and in early studies, it appeared to have a cure rate approaching 100%.[21,64,70,71] However, a declining cure rate for moxifloxacin has been observed, primarily in patients from the Asia-Pacific region with treatment failures in up to 30%. A significant proportion of the *M. genitalium* strains had concurrent macrolide resistance-mediating mutations leaving very few available treatment options.[52,72–74]

Pristinamycin is the only antimicrobial with documented activity in patients failing both azithromycin and moxifloxacin. Many of these cases additionally failed eradication with extended dosage doxycycline (100 mg twice daily for 14 days).[74] In Europe, it is registered only in France, but can be acquired after special permit in most European countries. It should only be used in the maximal recommended dose of 1 g four times a day for 10 days (oral) as these patients are facing their last known active antimicrobial therapy. A dose reduction is not advisable since some of the multidrug-resistant strains have an elevated minimal inhibitory concentration (MIC) of 0.5 mg/L (Jørgen S. Jensen, unpublished) which may lead to failure with lower doses. Treatment failure has been reported also for pristinamycin, but the influence of compliance in these cases is not fully understood.

Recommended treatment for uncomplicated *M. genitalium* infection in the absence of macrolide resistance-mediating mutations [IIb; B]

- Azithromycin 500 mg on day one, then 250 mg once daily days 2–5 (oral).
- Josamycin 500 mg 3 times daily for 10 days [IV; C].

Recommended treatment for uncomplicated macrolide-resistant *M. genitalium* infection [IIb; B]

- Moxifloxacin 400 mg once daily for 7–10 days (oral). The optimal duration of treatment is uncertain and a few observational studies have found higher cure rate after longer treatment in cervicitis.[72]

Recommended second-line treatment for uncomplicated persistent *M. genitalium* infection [IIb; B]

- Moxifloxacin 400 mg once daily for 7–10 days (oral).

Recommended third-line treatment for persistent *M. genitalium* infection after azithromycin and moxifloxacin [III;B]

- Doxycycline 100 mg two times daily for 14 days can be tried and will eradicate *M. genitalium* in approximately 30% of the patients, but the patient must be informed about the poor eradication rate and accept to comply with advice regarding sexual abstinence or condom use.
- Pristinamycin 1 g four times daily for 10 days (oral). The patient should be informed about the need to comply strictly with the dosage scheme.

Recommended treatment for complicated *M. genitalium* infection (PID, epididymitis) [IV;C]

- Moxifloxacin 400 mg once daily for 14 days (oral).[75]

Partner notification

- Contact notification should be performed and documented by appropriately trained professionals at the time of diagnosis to improve outcome [IV; C].
- Current partner should always be tested and treated with the same antimicrobial as the index patient [IV; C].
- If current partner does not attend evaluation and testing, epidemiological treatment should be offered with the same regimen as given to the index patient [IV; C].
- Recent sexual contacts (previous 3 months) should be contacted and offered testing for *M. genitalium* infection and testing for other STIs [IV; C].

Follow-up and test of cure (TOC)

- A TOC should be routinely performed in all patients due to the high prevalence of macrolide resistance either present pre-treatment or developing during treatment with azithromycin and in the absence of routine testing for fluoroquinolones resistance [III; B]. This recommendation differs from the BASHH and CDC guidelines[76,77] where TOC for asymptomatic cases is not recommended. However, many patients enter a stage of few or no symptoms after treatment, but with persistent carriage and subsequent risk for spread of resistance in the community. Test of cure samples should be collected no earlier than 3 weeks after start of treatment [III, B]. In patients responding to treatment, *M. genitalium* will be undetectable within 1 week in most patients, but tests may become temporarily false negative in patients failing treatment.[65]

Acknowledgements

Paddy Horner, Keith Radcliffe, Henry de Vries, Anne Olsen and Gilbert Donders are thanked for comments to the guideline.

Proposed guideline review date: 2018

Composition of Editorial Board

http://www.iusti.org/regions/Europe/pdf/2013/Editorial_Board.pdf.

List of contributing organizations

http://www.iusti.org/regions/Europe/euroguidelines.htm.

Qualifying statement

Decisions to follow these recommendations must be based on professional clinical judgement, consideration of individual patient circumstances and available resources. All possible care has been undertaken to ensure publication of the correct dosage of medication and route of administration. However, it remains the responsibility of the prescribing clinician to ensure the accuracy and appropriateness of the medication they prescribe.

References

1 Taylor-Robinson D, Gilroy CB, Jensen JS. The biology of *Mycoplasma genitalium*. *Venereology* 2000; **13**: 119–127.
2 Tully JG, Taylor-Robinson D, Cole RM, Rose DL. A newly discovered mycoplasma in the human urogenital tract. *Lancet* 1981; **1**: 1288–1291.
3 Jensen JS, Uldum SA, Søndergård-Andersen J, Vuust J, Lind K. Polymerase chain reaction for detection of *Mycoplasma genitalium* in clinical samples. *J Clin Microbiol* 1991; **29**: 46–50.
4 Palmer HM, Gilroy CB, Furr PM, Taylor-Robinson D. Development and evaluation of the polymerase chain reaction to detect *Mycoplasma genitalium*. *FEMS Microbiol Lett* 1991; **61**: 199–203.
5 Jensen JS, Ørsum R, Dohn B *et al.* *Mycoplasma genitalium*: a cause of male urethritis? *Genitourin Med* 1993; **69**: 265–269.
6 Horner PJ, Gilroy CB, Thomas BJ, Naidoo RO, Taylor-Robinson D. Association of *Mycoplasma genitalium* with acute non-gonococcal urethritis. *Lancet* 1993; **342**: 582–585.
7 Taylor-Robinson D, Jensen JS. *Mycoplasma genitalium*: from Chrysalis to Multicolored Butterfly. *Clin Microbiol Rev* 2011; **24**: 498–514.
8 Andersen B, Sokolowski I, Østergaard L *et al.* *Mycoplasma genitalium*: prevalence and behavioural risk factors in the general population. *Sex Transm Infect* 2007; **83**: 237–241.
9 Oakeshott P, Aghaizu A, Hay P *et al.* Is *Mycoplasma genitalium* in women the "New Chlamydia?" A community-based prospective cohort study. *Clin Infect Dis* 2010; **51**: 1160–1166.
10 Manhart LE, Holmes KK, Hughes JP, Houston LS, Totten PA. *Mycoplasma genitalium* among young adults in the United States: an emerging sexually transmitted infection. *Am J Public Health* 2007; **97**: 1118–1125.
11 Sonnenberg P, Ison CA, Clifton S *et al.* Epidemiology of *Mycoplasma genitalium* in British men and women aged 16-44 years: evidence from the third National Survey of Sexual Attitudes and Lifestyles (Natsal-3). *Int J Epidemiol* 2015; **44**: 1982–1994.
12 Cohen CR, Manhart LE, Bukusi EA *et al.* Association between *Mycoplasma genitalium* and acute endometritis. *Lancet* 2002; **359**: 765–766.
13 Manhart LE, Critchlow CW, Holmes KK *et al.* Mucopurulent cervicitis and *Mycoplasma genitalium*. *J Infect Dis* 2003; **187**: 650–657.
14 Cohen CR, Mugo NR, Astete SG *et al.* Detection of *Mycoplasma genitalium* in women with laparoscopically diagnosed acute salpingitis. *Sex Transm Infect* 2005; **81**: 463–466.
15 Anagrius C, Loré B, Jensen JS. *Mycoplasma genitalium*: prevalence, clinical significance, and transmission. *Sex Transm Infect* 2005; **81**: 458–462.
16 Falk L, Fredlund H, Jensen JS. Signs and symptoms of urethritis and cervicitis among women with or without *Mycoplasma genitalium* or *Chlamydia trachomatis* infection. *Sex Transm Infect* 2005; **81**: 73–78.
17 Lis R, Rowhani-Rahbar A, Manhart LE. *Mycoplasma genitalium* infection and female reproductive tract disease: a meta-analysis. *Clin Infect Dis* 2015; **61**: 418–426.
18 Oakeshott P, Hay P, Taylor-Robinson D *et al.* Prevalence of *Mycoplasma genitalium* in early pregnancy and relationship between its presence and pregnancy outcome. *BJOG* 2004; **111**: 1464–1467.
19 Peuchant O, Le RC, Desveaux C *et al.* Screening for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma genitalium* should it be integrated into routine pregnancy care in French young pregnant women? *Diagn Microbiol Infect Dis* 2015; **82**: 14–19.
20 Wikström A, Jensen JS. *Mycoplasma genitalium*: a common cause of persistent urethritis among men treated with doxycycline. *Sex Transm Infect* 2006; **82**: 276–279.
21 Bradshaw CS, Chen MY, Fairley CK. Persistence of *Mycoplasma genitalium* following azithromycin therapy. *PLoS ONE* 2008; **3**: e3618.
22 Jensen JS, Bradshaw C. Management of *Mycoplasma genitalium* infections - can we hit a moving target? *BMC Infect Dis* 2015; **15**: 343.
23 Vandepitte J, Weiss HA, Bukenya J *et al.* Association between Mycoplasma genitalium infection and HIV acquisition among female sex workers in Uganda: evidence from a nested case-control study. *Sex Transm Infect* 2014; **90**: 545–549.
24 Mavedzenge SN, Van der Pol B, Weiss HA *et al.* The association between *Mycoplasma genitalium* and HIV-1 acquisition among women in Zimbabwe and Uganda. *AIDS* 2012; **26**: 617–624.
25 Manhart LE. Another STI associated with HIV-1 acquisition: now what? *AIDS* 2012; **26**: 635–637.
26 Hjorth SV, Björnelius E, Lidbrink P *et al.* Sequence-based typing of *Mycoplasma genitalium* reveals sexual transmission. *J Clin Microbiol* 2006; **44**: 2078–2083.
27 Soni S, Alexander S, Verlander N *et al.* The prevalence of urethral and rectal *Mycoplasma genitalium* and its associations in men who have sex with men attending a genitourinary medicine clinic. *Sex Transm Infect* 2010; **86**: 21–24.
28 Lillis RA, Nsuami MJ, Myers L, Martin DH. Utility of urine, vaginal, cervical, and rectal specimens for detection of *Mycoplasma genitalium* in women. *J Clin Microbiol* 2011; **49**: 1990–1992.
29 Edlund M, Blaxhult A, Bratt G. The spread of *Mycoplasma genitalium* among men who have sex with men. *Int J STD AIDS* 2012; **23**: 455–456.
30 Bradshaw CS, Fairley CK, Lister NA *et al.* *Mycoplasma genitalium* in men who have sex with men at male-only saunas. *Sex Transm Infect* 2009; **85**: 432–435.
31 Deguchi T, Yasuda M, Yokoi S *et al.* Failure to detect *Mycoplasma genitalium* in the pharynges of female sex workers in Japan. *J Infect Chemother* 2009; **15**: 410–413.
32 Luki N, Lebel P, Boucher M *et al.* Comparison of polymerase chain reaction assay with culture for detection of genital mycoplasmas in perinatal infections. *Eur J Clin Microbiol Infect Dis* 1998; **17**: 255–263.
33 Walker J, Fairley CK, Bradshaw CS *et al.* The difference in determinants of *Chlamydia trachomatis* and *Mycoplasma genitalium* in a sample of young Australian women. *BMC Infect Dis* 2011; **11**: 35.
34 Jensen JS, Björnelius E, Dohn B, Lidbrink P. Comparison of first void urine and urogenital swab specimens for detection of *Mycoplasma genitalium* and *Chlamydia trachomatis* by polymerase chain reaction in patients attending a sexually transmitted disease clinic. *Sex Transm Dis* 2004; **31**: 499–507.

35 Salado-Rasmussen K, Jensen JS. *Mycoplasma genitalium* testing pattern and macrolide resistance: a Danish nationwide retrospective survey. *Clin Infect Dis* 2014; **59**: 24–30.
36 Bjartling C, Osser S, Persson K. *Mycoplasma genitalium* in cervicitis and pelvic inflammatory disease among women at a gynecologic outpatient service. *Am J Obstet Gynecol* 2012; **206**: 476–478.
37 Taylor-Robinson D, Gilroy CB, Horowitz S, Horowitz J. *Mycoplasma genitalium* in the joints of two patients with arthritis. *Eur J Clin Microbiol Infect Dis* 1994; **13**: 1066–1069.
38 Falk L, Fredlund H, Jensen JS. Symptomatic urethritis is more prevalent in men infected with *Mycoplasma genitalium* than with *Chlamydia trachomatis*. *Sex Transm Infect* 2004; **80**: 289–293.
39 Horner PJ, Taylor-Robinson D. Association of *Mycoplasma genitalium* with balanoposthitis in men with non-gonococcal urethritis. *Sex Transm Infect* 2011; **87**: 38–40.
40 Björnelius E, Jensen JS, Lidbrink P. Conjunctivitis Associated with *Mycoplasma genitalium* Infection. *Clin Infect Dis* 2004; **39**: e67–e69.
41 Hamasuna R, Osada Y, Jensen JS. Isolation of *Mycoplasma genitalium* from first-void urine specimens by coculture with Vero cells. *J Clin Microbiol* 2007; **45**: 847–850.
42 Jensen JS, Hansen HT, Lind K. Isolation of *Mycoplasma genitalium* strains from the male urethra. *J Clin Microbiol* 1996; **34**: 286–291.
43 Shipitsyna E, Zolotoverkhaya E, Dohn B *et al.* First evaluation of polymerase chain reaction assays used for diagnosis of *Mycoplasma genitalium* in Russia. *J Eur Acad Dermatol Venereol* 2009; **23**: 1164–1172.
44 Jensen JS, Bradshaw CS, Tabrizi SN, Fairley CK, Hamasuna R. Azithromycin treatment failure in *Mycoplasma genitalium*-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. *Clin Infect Dis* 2008; **47**: 1546–1553.
45 Twin J, Jensen JS, Bradshaw CS *et al.* Transmission and selection of macrolide resistant *Mycoplasma genitalium* infections detected by rapid high resolution melt analysis. *PLoS ONE* 2012; **7**: e35593.
46 Jensen JS. Protocol for the detection of *Mycoplasma genitalium* by PCR from clinical specimens and subsequent detection of macrolide resistance-mediating mutations in region V of the 23S rRNA gene. In MacKenzie CR, Henrich B, eds. Diagnosis of Sexually Transmitted Diseases; Methods and Protocols, 903 edn. Humana Press, Springer, New York, 2012: 129–139.
47 Touati A, Peuchant O, Jensen JS, Bebear C, Pereyre S. Direct detection of macrolide resistance in *Mycoplasma genitalium* isolates from clinical specimens from France by use of real-time PCR and melting curve analysis. *J Clin Microbiol* 2014; **52**: 1549–1555.
48 Wold C, Sorthe J, Hartgill U *et al.* Identification of macrolide-resistant *Mycoplasma genitalium* using real-time PCR. *J Eur Acad Dermatol Venereol* 2015; **29**: 1616–1620.
49 Deguchi T, Maeda S, Tamaki M *et al.* Analysis of the *gyrA* and *parC* genes of *Mycoplasma genitalium* detected in first-pass urine of men with nongonococcal urethritis before and after fluoroquinolone treatment. *J Antimicrob Chemother* 2001; **48**: 742–744.
50 Pond MJ, Nori AV, Witney AA *et al.* High prevalence of antibiotic-resistant *Mycoplasma genitalium* in nongonococcal urethritis: the need for routine testing and the inadequacy of current treatment options. *Clin Infect Dis* 2014; **58**: 631–637.
51 Shimada Y, Deguchi T, Nakane K *et al.* Emergence of clinical strains of *Mycoplasma genitalium* harbouring alterations in *ParC* associated with fluoroquinolone resistance. *Int J Antimicrob Agents* 2010; **36**: 255–258.
52 Couldwell DL, Tagg KA, Jeoffreys NJ, Gilbert GL. Failure of moxifloxacin treatment in *Mycoplasma genitalium* infections due to macrolide and fluoroquinolone resistance. *Int J STD AIDS* 2013; **24**: 822–828.
53 Kikuchi M, Ito S, Yasuda M *et al.* Remarkable increase in fluoroquinolone-resistant *Mycoplasma genitalium* in Japan. *J Antimicrob Chemother* 2014; **69**: 2376–2382.
54 Hardick J, Giles J, Hardick A *et al.* Performance of the Gen-Probe transcription-mediated amplification research assay compared to that of a multitarget real-time PCR for *Mycoplasma genitalium* detection. *J Clin Microbiol* 2006; **44**: 1236–1240.
55 Wroblewski JK, Manhart LE, Dickey KA, Hudspeth MK, Totten PA. Comparison of transcription-mediated amplification and PCR assay results for various genital specimen types for detection of *Mycoplasma genitalium*. *J Clin Microbiol* 2006; **44**: 3306–3312.
56 Carlsen KH, Jensen JS. *Mycoplasma genitalium* PCR: does freezing of specimens affect sensitivity? *J Clin Microbiol* 2010; **48**: 3624–3627.
57 Reinton N, Moi H, Olsen AO *et al.* Anatomic distribution of *Neisseria gonorrhoeae, Chlamydia trachomatis* and *Mycoplasma genitalium* infections in men who have sex with men. *Sex Health* 2013; **10**: 199–203.
58 Björnelius E, Anagrius C, Bojs G *et al.* Antibiotic treatment of symptomatic *Mycoplasma genitalium* infection in Scandinavia: a controlled clinical trial. *Sex Transm Infect* 2008; **84**: 72–76.
59 Mena LA, Mroczkowski TF, Nsuami M, Martin DH. A randomized comparison of azithromycin and doxycycline for the treatment of *Mycoplasma genitalium*-positive urethritis in men. *Clin Infect Dis* 2009; **48**: 1649–1654.
60 Schwebke JR, Rompalo A, Taylor S *et al.* Re-evaluating the treatment of nongonococcal urethritis: emphasizing emerging pathogens - A Randomized Clinical Trial. *Clin Infect Dis* 2011; **52**: 163–170.
61 Manhart LE, Gillespie CW, Lowens MS *et al.* Standard treatment regimens for nongonococcal urethritis have similar but declining cure rates: a randomized controlled trial. *Clin Infect Dis* 2013; **56**: 934–942.
62 Schönwald S, Gunjaca M, Kolacny Babic L, Car V, Gosev M. Comparison of azithromycin and erythromycin in the treatment of atypical pneumonias. *J Antimicrob Chemother* 1990; **25** (Suppl A): 123–126.
63 Horner P, Blee K, Adams E. Time to manage *Mycoplasma genitalium* as an STI: but not with azithromycin 1 g!. *Curr Opin Infect Dis* 2014; **27**: 68–74.
64 Anagrius C, Lore B, Jensen JS. Treatment of *Mycoplasma genitalium*. Observations from a Swedish STD Clinic. *PLoS ONE* 2013; **8**: e61481.
65 Falk L, Enger M, Jensen JS. Time to eradication of *Mycoplasma genitalium* after antibiotic treatment in men and women. *J Antimicrob Chemother* 2015; **70**: 3134–3140.
66 Nijhuis RH, Severs TT, Van der Vegt DS, Van Zwet AA, Kusters JG. High levels of macrolide resistance-associated mutations in *Mycoplasma genitalium* warrant antibiotic susceptibility-guided treatment. *J Antimicrob Chemother* 2015; **70**: 2515–2518.
67 Gesink DC, Mulvad G, Montgomery-Andersen R *et al. Mycoplasma genitalium* presence, resistance and epidemiology in Greenland. *Int J Circumpolar Health* 2012; **71**: 1–8.
68 Guschin A, Ryzhikh P, Rumyantseva T, Gomberg M, Unemo M. Treatment efficacy, treatment failures and selection of macrolide resistance in patients with high load of *Mycoplasma genitalium* during treatment of male urethritis with josamycin. *BMC Infect Dis* 2015; **15**: 40.
69 Pereyre S, Guyot C, Renaudin H *et al.* In vitro selection and characterization of resistance to macrolides and related antibiotics in *Mycoplasma pneumoniae*. *Antimicrob Agents Chemother* 2004; **48**: 460–465.
70 Bradshaw CS, Jensen JS, Tabrizi SN *et al.* Azithromycin failure in *Mycoplasma genitalium* urethritis. *Emerg Infect Dis* 2006; **12**: 1149–1152.
71 Jernberg E, Moghaddam A, Moi H. Azithromycin and moxifloxacin for microbiological cure of *Mycoplasma genitalium* infection: an open study. *Int J STD AIDS* 2008; **19**: 676–679.
72 Terada M, Izumi K, Ohki E, Yamagishi Y, Mikamo H. Antimicrobial efficacies of several antibiotics against uterine cervicitis caused by *Mycoplasma genitalium*. *J Infect Chemother* 2012; **18**: 313–317.
73 Gundevia Z, Foster R, Jamil MS, McNulty A. Positivity at test of cure following first-line treatment for genital *Mycoplasma genitalium*: follow-up of a clinical cohort. *Sex Transm Infect* 2015; **91**: 11–13.
74 Bissessor M, Tabrizi SN, Twin J *et al.* Macrolide resistance and azithromycin failure in a *Mycoplasma genitalium*-infected cohort and response of azithromycin failures to alternative antibiotic regimens. *Clin Infect Dis* 2015; **60**: 1228–1236.

75 Judlin P, Liao Q, Liu Z *et al.* Efficacy and safety of moxifloxacin in uncomplicated pelvic inflammatory disease: the MONALISA study. *BJOG* 2010; **117**: 1475–1484.
76 Workowski KA, Bolan GA. Sexually transmitted diseases treatment guidelines, 2015. *MMWR Recomm Rep* 2015; **64** : 1–137.
77 Horner P, Blee K, O'Mahony C *et al.* 2015 UK National Guideline on the management of non-gonococcal urethritis. *Int J STD AIDS* 2016; **27**: 85–96.

Appendix 1: Search strategy

A Medline search was conducted in May 2015 using PubMed. The search heading was kept broad (Mycoplasma genitalium) to include epidemiology, diagnosis, antimicrobial resistance, drug therapy, clinical trials and prevention and control. Only publications and abstracts in the English language were considered. The Cochrane library was searched for all entries related to mycoplasma. Sexually transmitted diseases' guidelines produced by the US Centers for Disease Control (www.cdc.gov/std/) and the British Association for Sexual Health and HIV (www.bashh.org) were also reviewed.

Appendix 2: Levels of evidence and Grading of recommendations

http://iusti.org/regions/Europe/pdf/2013/Levels_of_Evidence.pdf.

Appendix 3: Declarations of interest

Jørgen Skov Jensen, Marco Cusini, Mikhail Gomberg, Harald Moi: None.