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REVIEW

Should we be testing for urogenital *Mycoplasma hominis, Ureaplasma parvum* and *U. urealyticum* in men and women? – a Position Statement from the European STI Guidelines Editorial Board

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**Running head:** Testing for urogenital mycoplasmas in men and women?

**Keywords:** *Mycoplasma hominis, Ureaplasma parvum, Ureaplasma urealyticum,* sexually transmitted infection (STI), urethritis, cervicitis

**Conflicts of interest**

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Abstract

At present, we have no evidence that we are doing more good than harm detecting and subsequently treating *Mycoplasma hominis*, *Ureaplasma parvum* and *Ureaplasma urealyticum* colonisations/infections. Consequently, routine testing and treatment of asymptomatic or symptomatic men and women for *M. hominis*, *U. urealyticum*, and *U. parvum* is not recommended. Asymptomatic carriage of these bacteria is common and the majority of individuals do not develop disease. Although *U. urealyticum* has been associated with urethritis in men, it is probably not causal unless a high load is present (likely carriage in 40-80% of detected cases). The extensive testing, detection and subsequent antimicrobial treatment of these bacteria performed in some settings may result in selection of antimicrobial resistance, in these bacteria, “true” STI agents, as well as in the general microbiota, and substantial economic cost for society and individuals, particularly women. The commercialisation of many particularly multiplex PCR assays detecting traditional non-viral STIs together with *M. hominis*, *U. parvum* and/or *U. urealyticum* have worsened this situation. Thus, routine screening of asymptomatic men and women or routine testing of symptomatic individuals for *M. hominis*, *U. urealyticum*, and *U. parvum* is not recommended. If testing of men with symptomatic urethritis is undertaken, traditional STI urethritis agents such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *M. genitalium* and, in settings where relevant, *Trichomonas vaginalis* should be excluded prior to *U. urealyticum* testing and quantitative species-specific molecular diagnostic tests should be used. Only men with high *U. urealyticum* load should be considered for treatment, however, appropriate evidence for effective treatment regimens is lacking. In symptomatic women, bacterial vaginosis (BV) should always be tested for and treated if detected.
Introduction

Mycoplasmas and ureaplasmas belong to the class Mollicutes. *Mycoplasma genitalium* is a “true” STI causing male urethritis, and is associated with cervicitis and an increased risk of pelvic inflammatory disease (PID), endometritis and infertility.\(^1,2\) However, *Mycoplasma hominis*, *Ureaplasma urealyticum* (previously *U. urealyticum* biovar 2) and *U. parvum* (earlier *U. urealyticum* biovar 1)\(^3\) are frequently found in the human urogenital tract in both healthy individuals and symptomatic patients.\(^4\) Comprehensive testing and subsequent antimicrobial treatment of these three urogenital mycoplasma species in adults is performed in several settings in, e.g., Eastern Europe, Southern Europe, South America and Asia. In many countries, this testing has also increased due the introduction of multiplex PCR assays detecting traditional non-viral “true” STI agents together with *M. hominis*, *U. parvum* and/or *U. urealyticum*.\(^5-7\) These multiplex PCR assays can additionally have suboptimal specificity and/or sensitivity, particularly when home-sampled and self-sampled specimens, e.g. using sampling kit purchased on internet, are analysed. Nevertheless, the evidence base for these three mycoplasmas as aetiological agents of STI syndromes and complications in adult men and women can be questioned. Most older studies used culture and this is still commonly used due to the availability of simple and easy to use culture kits with inappropriate antimicrobial susceptibility testing. However, culture does not distinguish between *U. urealyticum* and *U. parvum*, and results are often reported as *U. urealyticum* instead of *Ureaplasma* spp. leading to further confusion. Qualitative PCR assays are also commonly used without species differentiation and with inappropriate reporting. Furthermore, in most studies, the strong association between bacterial vaginosis (BV) in “patient” and/or BV-associated bacteria in sexual partner of women with BV has not been adjusted for. This is particularly an issue for *M. hominis* but also for ureaplasmas.\(^4,8-10\) These and additional confounding factors make interpretation of many previous studies exceedingly difficult.

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We reviewed the evidence for *M. hominis*, *U. parvum* and *U. urealyticum* as aetiological agents of urethritis, cervicitis and additional STI syndromes and complications in adult men and non-pregnant women. Based on current evidence, we conclude that routine testing and treatment of asymptomatic or symptomatic men and women for *M. hominis*, *U. urealyticum*, and *U. parvum* is not recommended. Furthermore, we suggest further research, and design of appropriate research studies, crucial to provide adequate evidence for any unresolved questions. To avoid some of the confounding factors, we focused on international peer-reviewed papers using molecular diagnostics and appropriate species differentiation. Relevance of these bacteria in pregnancy or in neonates was not addressed, because this has been reviewed recently elsewhere.11-15

**Men**

**Male urethritis**

There is no evidence from case control studies that *M. hominis* causes non-gonococcal urethritis (NGU).16-20 It appears to be a relatively uncommon microorganism in men attending departments of sexual health (2-4%), although colonisation can be as high as 20%.16-18,21

*U. urealyticum* and *U. parvum* can both be detected in men with and without NGU. Earlier studies did not differentiate between *U. urealyticum* and *U. parvum*, which continues to be the case if culture alone is used.9,10 *U. parvum* is detected more often in controls than cases in most studies, which probably explains why earlier studies failed to demonstrate a consistent association of ureaplasmas with NGU.9,10
The population prevalence of *U. parvum* in men is unknown but it is likely more common than *U. urealyticum* as it is detected more frequently in men without urethritis than *U. urealyticum*. A recent meta-analysis of case control studies demonstrated no association of *U. parvum* with NGU. This was also observed by Frølund et al. but not in a few other studies of non-chlamydial NGU where *U. parvum* was associated with microscopy-confirmed non-chlamydial NGU (≥5 polymorphonuclear leukocytes in urethral swab) and/or disease, in particular when present in high loads. Additional large and well-designed studies using quantitative molecular detection of *U. parvum* with appropriate cut-off for high bacterial load and microscopy to evaluate inflammation (polymorphonuclear leukocytes) in men with symptomatic urethritis might be valuable.

The population prevalence of *U. urealyticum* is unknown but is probably 5-15% in men aged 16-44 years old, being more common in younger men and associated with a recent change in sexual partner. *U. urealyticum* is associated with NGU. However, although detected in 5-24% of men with NGU it is probably only causal in 3-11% of NGU cases, i.e. in 40-80% of cases it is probably only carriage. A recent meta-analysis demonstrated a significant association with 18.3% of men with NGU and 13.7% of controls being *U. urealyticum*-positive with a pooled odds ratio (OR) of 1.57 (95% CI: 1.05–2.35), p=0.029. Although, NGU caused by *U. urealyticum* is more likely to develop in younger men, the majority of men carrying *U. urealyticum* will not develop NGU. The development of NGU is associated with a higher bacterial load and fewer lifetime sexual partners. As *U. urealyticum* carriage in men without urethritis is associated with younger age, this
suggests that the adaptive immunity attenuates the clinical manifestation of *U. urealyticum* infection; repeated or prolonged exposure to *U. urealyticum* via multiple sex partners may result in either asymptomatic colonisation without signs of urethral inflammation or shorter duration of symptoms.\(^{22,27,31}\) Using quantitative molecular detection of *U. urealyticum* with appropriate cut-off for high bacterial load in men with symptomatic urethritis can significantly increase the positive predictive value.\(^{22,23,30,32}\) However, additional studies using different quantitative molecular tests and examining symptomatic and asymptomatic male populations in different settings are required before any exact cut-off levels can be recommended.

**Male infertility**

A recent meta-analysis\(^ {33}\) as well as two studies (which did not exclude “true” STIs or BV, and only included *M. hominis* culture positive samples)\(^ {34,35}\) have suggested an association of *M. hominis* with infertility in men. However, *M. hominis* is strongly associated with several “true” STIs that can cause infertility as well as with BV,\(^ {4,36}\) which is common in women, and two recent studies indicated that sexual partners share their genital tract microbiome, suggesting that molecular detection in men is likely to reflect the carriage in their female sexual partner.\(^ {37,38}\) BV is more common in women with infertility and is associated with tubal factor infertility as well as with poor implantation of the embryo as suggested by a study of women undergoing in vitro fertilisation (IVF).\(^ {39,40}\) Thus, considerable caution should be exercised in attributing the detection of *M. hominis* as causal of male infertility before additional studies have been performed. These studies should be appropriately designed and use quantitative PCR, and address “true” STIs and BV as confounders (in infertile men and
their sexual partners) as well as showing that treating the *M. hominis* infection in infertile men will restore fertility.

A recent meta-analysis demonstrated no association with *U. parvum* but suggested an association between *U. urealyticum* and male infertility.\(^3\) Of the five included studies, three were from China where a high prevalence was observed in both cases (19.6%) and controls (8.3%)\(^3\) compared to a study from Jordan 1.1% vs. 2.9%\(^4\) and Iran 9% vs. 1%\(^4\), respectively. Whether *U. urealyticum* actually causes male infertility remains unclear, some studies do not differentiate *U. urealyticum* and *U. parvum*, further complicating interpretation of the data.\(^3\)\(^4\)\(^3\)\(^4\)\(^3\) Possible explanations for an inconsistent association in case control studies of male infertility include, failure to differentiate *U. urealyticum* and *U. parvum*\(^4\)\(^4\)\(^5\)\(^4\)\(^5\) and association by confounding as *U. urealyticum* is associated with younger age, recent change in sexual partner and fewer lifetime sexual partners and the association of ureaplasmas with BV.\(^4\)\(^2\)\(^6\)\(^2\)\(^7\)\(^3\)

**Women**

The prevalence of *M. hominis*, *U. urealyticum* and *U. parvum* in non-pregnant sexually active symptomatic and asymptomatic women, measured by molecular tests including species differentiation, has ranged between 3.1-15%, 5.2-20%, and 20-89%, respectively.\(^5\)\(^6\)\(^5\)\(^4\)\(^6\)\(^5\) The large variation in prevalence probably reflect both methodological and true population differences, in particular in the prevalence of BV, the most important confounder. *M. hominis* and ureaplasmas can be horizontally transmitted and although colonization tends to decrease with age until puberty, detection of these bacteria in prepubertal girls even in the absence of
sexual abuse is not unusual,\textsuperscript{55,56} which illustrates that sexual transmission is not required. Nevertheless, among adults most cases of new colonisation with \textit{M. hominis} and ureaplasmas occurs from sexual contact\textsuperscript{57} and is correlated to the number of sexual partners.\textsuperscript{58}

Overall, in symptomatic women with dysuria, vaginal discharge, painful intercourse, and/or lower abdominal pain the spectrum of symptoms do not differ in ureaplasma-negative women compared to women positive for \textit{U. urealyticum} or \textit{U. parvum}.\textsuperscript{48} However, both of these ureaplasmas are frequently associated with increased positivity for several traditional STIs, e.g. \textit{C. trachomatis} and \textit{M. genitalium}, and/or BV.\textsuperscript{8,46,49,59} The bacterial load of particularly \textit{M. hominis} and to a lesser extent \textit{U. parvum} and \textit{U. urealyticum} can be significantly increased in the dysbiosis of BV.\textsuperscript{4,48,60} However, despite the association between particularly \textit{M. hominis} and BV, \textit{M. hominis} cannot be detected in approximately one third of women with BV and, accordingly, it is neither a sufficiently sensitive nor specific bacterial marker for diagnosis of BV.\textsuperscript{8,61-63} Despite not being susceptible to metronidazole, eradication or a decrease in the \textit{M. hominis} load after BV treatment has also been reported,\textsuperscript{64-66} further indicating that \textit{M. hominis} frequently belongs to the dysbiosis of BV. BV treatment studies using quantitative molecular detection methods for \textit{M. hominis}, \textit{U. urealyticum} and \textit{U. parvum} are required. In many studies, appropriate species differentiation of \textit{U. urealyticum} and \textit{U. parvum} have not been performed and/or traditional STIs and especially BV have not been addressed as confounding factors, making disease association with the urogenital mycoplasmas exceedingly difficult.
**Vulvovaginitis**

There are no case control studies or other appropriate evidence that *M. hominis, U. parvum or U. urealyticum* causes an inflammatory vulvovaginitis. No case control studies or other appropriate evidence that *M. hominis, U. parvum or U. urealyticum* causes an inflammatory vulvovaginitis. The number of leukocytes in vaginal smears are also not increased in women positive for only ureaplasm.48

**Cervicitis**

No case control studies using sensitive and specific molecular diagnostic tests have provided appropriate evidence that *M. hominis, U. parvum or U. urealyticum* causes cervicitis. For example, the unadjusted prevalence ratios of cervicitis have been reported as 1.00, 1.09, and 0.96 for *M. hominis, U. parvum or U. urealyticum*, respectively.67 Also in additional cervicitis studies, none of these three urogenital mycoplasmas was associated with cervicitis68 and the bacterial load of neither *U. parvum* nor *U. urealyticum* has been associated with symptoms or signs of genital infection.49 Nevertheless, in one molecular study of non-gonococcal non-chlamydial cervicitis, despite no difference in *U. parvum* and *U. urealyticum* presence in women with cervicitis and controls69 the bacterial load of *U. parvum* and *U. urealyticum* were significantly higher in women with cervicitis compared to controls.69

**Female urethritis and urethral pain syndrome**

Appropriate studies are mainly lacking, however, no case control or other studies providing evidence that *M. hominis, U. parvum or U. urealyticum* causes urethritis in women are available. One study of the urethral pain syndrome in women showed that 46% of women with urethral pain carried *Ureaplasma* species compared with 64% of the controls. The prevalence of *U. parvum* and *U. urealyticum* were similar in women with the urethral pain...
Using undifferentiated quantitative ureaplasma culture, early work suggested some evidence of a role of high bacterial loads in women with acute urethral syndrome. Studies using up-to-date quantitative techniques for ureaplasma detection are recommended.

**Pelvic Inflammatory Disease (PID), salpingitis and infertility**

Studies are few and no case control studies have yet provided appropriate evidence that *M. hominis, U. parvum* or *U. urealyticum* causes PID, salpingitis or infertility. Although *M. hominis* has been isolated from laparoscopically obtained samples, it was always found also in the vagina, so it may well be present in a background of BV-associated bacteria which were not cultured. In another study, the detection of *M. hominis* in the lower genital tract was not associated with *C. trachomatis*-negative and gonorrhoea-negative salpingitis and was not isolated from the salpinges indicating that it is unlikely to be causal. However, it is occasionally the sole pathogen isolated from the upper genital tract. In infertility, pooled data for non-pregnant women were analysed in a systematic review, and both *M. hominis* (11.5% vs. 14.5%, p=0.03) and *U. urealyticum* (19.5% vs. 25.0%, p=0.004) were more common among asymptomatic women presenting for infertility (n=1205) compared with symptomatic women (n=1131; with vulvovaginitis signs), possibly indicating an association with infertility. In general, *C. trachomatis* infection, gonorrhoea and/or BV as confounding factors have been present or not appropriately excluded in most studies, and BV is strongly associated with infertility. Microbiota studies of invasive samples in women with verified PID, e.g. laparoscopically taken specimens, would be valuable to adequately address this as the BV associated bacteria are often uncultivable.
**Ectopic pregnancy**

There is no clear evidence that any of the urogenital mycoplasmas, including the “true” STI agent *M. genitalium*, result in ectopic pregnancy.\(^7\)

**Discussion and conclusions**

In men, *M. hominis* does not cause disease and is probably mostly a reflection of BV in their sexual partner and the presence of *U. parvum* is not evidently associated with NGU or infertility.\(^9,16-18,22\) *U. urealyticum* is associated with a small proportion of NGU cases, in particular in younger men with fewer lifetime sexual partners and a high *U. urealyticum* load. However, in ~40-80% of cases where it is detected, it is not the aetiological agent.\(^9,21-23,25,28-32\)

It remains unlikely that *U. urealyticum* can cause infertility.

In women, there is no adequate evidence that *M. hominis*, *U. parvum* or *U. urealyticum* causes an inflammatory vulvovaginitis, cervicitis, urethritis, PID or infertility.\(^4,48,51,67-69,71,72,76,77\) In many studies, appropriate species differentiation of *U. urealyticum* and *U. parvum* has not been performed and/or important confounding factors such as recognized STIs and especially BV have not been addressed, making disease associations with the urogenital mycoplasmas mostly undocumented.

There are no international evidence-based management guidelines for *M. hominis*, *U. parvum* and *U. urealyticum*, and appropriate evidence for effective treatment regimens is lacking. Because mycoplasmas lack the rigid cell wall of other bacteria, they are intrinsically
resistant to β-lactam antimicrobials, such as penicillins and cephalosporins, and other antimicrobials targeting the cell wall. *M. hominis* is additionally naturally resistant to 14- and 15-membered macrolides (azithromycin, clarithromycin, erythromycin), but not to 16-membered macrolides such as josamycin and the *in vitro* susceptibility to doxycycline is high for strains lacking the *tetM* gene. *U. urealyticum* is moderately sensitive to 14-membered macrolides. In general, urogenital *M. hominis*, *U. parvum* and *U. urealyticum* can be difficult to eradicate in many individuals because of true antimicrobial resistance but also because of lower activity of the antimicrobials at low pH and lack of bactericidal activity.\(^4,27,78-80\) Additionally, suboptimal antimicrobial susceptibility testing methods, including many commercial kits, are frequently used.\(^80\) The extensive treatment of these commonly colonising commensals with suboptimal antimicrobial regimens selects for antimicrobial resistance in these bacteria and in many of the more severe bacterial “true” STI agents as well as in the general microbiota. Overall, the extensive testing, detection (using microscopy, culture or PCR) and subsequent antimicrobial treatment of urogenital *M. hominis*, *U. parvum* and *U. urealyticum* in some settings result in a substantial burden and economic cost for society (e.g. unnecessary use of diagnostic tests, health care visits, antimicrobial misuse, and emergence of antimicrobial resistance) and individuals (e.g. economical burden, stigmatization, anxiety, and possibly breakdown of relationships including marriages). The commercialisation of many particularly multiplex PCR assays detecting traditional non-viral STIs together with *M. hominis*, *U. parvum* and/or *U. urealyticum* have worsened this situation. At present, we have no evidence that we are doing more good than harm detecting and subsequently treating these bacteria. Increased awareness and education internationally regarding all these issues among laboratory staff, clinicians and other health care professionals as well as among the general population is essential.
Should testing for *M. hominis*, *U. urealyticum*, and *U. parvum* be undertaken in STI syndromes?

- *U. urealyticum* in high bacterial loads might cause a small proportion of male NGU, but the majority of men and women infected/colonised with *U. urealyticum* do not develop disease. Antimicrobial treatment which results in eradication is difficult⁴,²⁷,⁷⁸,⁷⁹ and cure is not associated with eradication.⁷⁸ Treatment may result in development of antimicrobial resistance in urogenital mycoplasmas but also in other bacteria including the traditional, more severe “true” STI agents. Routine testing and/or treatment is therefore not recommended. If testing of men with symptomatic urethritis is undertaken, traditional STI urethritis agents such as *N. gonorrhoeae*, *C. trachomatis*, *M. genitalium* and, in settings where relevant, *Trichomonas vaginalis* should be excluded prior to *U. urealyticum* testing and quantitative molecular diagnostic tests should be used. Only men with high *U. urealyticum* load should be considered for treatment, however, appropriate evidence for effective treatment regimens is lacking.

- Testing for *M. hominis* and *U. parvum* and subsequent antimicrobial treatment of positive men or women is currently not recommended. Instead, “true” STIs and BV in symptomatic women should be diagnosed and treated.

Well-designed, large, randomised controlled studies to investigate unresolved issues regarding *M. hominis*, *U. parvum* and/or *U. urealyticum* and their independent associations with STI syndromes and complications such as possibly infertility,³³-³⁵ PID and prostate cancer⁸¹-⁸⁴ could be valuable. In these studies, it is recommended to control age, sexual behaviour (number and change of sexual partners), use quantitative molecular diagnostic tests investigating bacterial load and microscopy to evaluate inflammation (polymorphonuclear leukocytes), distinguish *U. urealyticum* and *U. parvum*, and exclude traditional STIs such as...
gonorrhoea, chlamydia, *M. genitalium* and trichomoniasis. Furthermore, it is crucial to address aerobic vaginitis and particularly BV and ideally also the specific BV-associated bacteria in controls and symptomatic individuals positive for urogenital mycoplasmas and their sexual partners. It is also important to show that antimicrobial treatment eradicates the mycoplasmas and that lack of eradication is associated with persistent symptoms and signs, documenting that it is not only an effect of treating a general dysbiosis.

**Key messages**

- Routine screening of asymptomatic men and women or routine testing of symptomatic individuals for *Mycoplasma hominis, Ureaplasma urealyticum*, and *Ureaplasma parvum* is not recommended.
- The extensive testing, detection and antimicrobial treatment of urogenital *M. hominis, U. parvum* and *U. urealyticum* performed in some settings result in a substantial burden and economic cost for society and individuals, particularly women. Instead, the diagnostics and treatment of traditional, more severe “true” STIs and BV in symptomatic women need to be improved.
- *U. urealyticum* in high bacterial loads might cause a small proportion of male NGU, but the majority of men and women infected/colonised with *U. urealyticum* do not develop disease. Antimicrobial treatment resulting in eradication is difficult, and eradication is not unequivocally associated with cure. Thus, treatment may select/induce resistance in urogenital mycoplasmas and other bacteria including the more severe “true” STI agents.
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