

Review Article

Genital infections with *Mycoplasma genitalium*

生殖支原體所致的生殖道感染

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Mycoplasma genitalium has an unequivocal and significant association with Non-gonococcal urethritis (NGU), especially chlamydia negative NGU. *M. genitalium* is sexually transmitted with transmission rates comparable to chlamydia. Testing for *M. genitalium* in the western world is limited to a few centers only, due to the non-availability of standardized commercial test kit. A recent study from Hong Kong failed to show significant association between *M. genitalium* infection and NGU. There may be a type II error due to the low prevalence of *M. genitalium* detected in this study. Larger studies will be necessary to determine the prevalence of *M. genitalium* infection in the local population, especially for high risk groups.

生殖支原體病菌與非淋病尿道炎（尤其是衣原體陰性的）有著明確及重要的關聯。它同樣經由性接觸傳染，傳染機率與衣原體相約。在西方國家，生殖支原體的測試僅限於少數醫療中心，皆因市面沒有標準化的測試商品。一個香港近來的研究未能展示出生殖支原體與非淋病尿道炎這一重要關聯，可能由於那研究中的生殖支原體低盛行率引致第二型誤差，故需要更大型的研究來測定本地人口尤其是高風險族羣的生殖支原體感染盛行率。

Keywords: *Mycoplasma genitalium*, Non-gonococcal urethritis

關鍵詞：生殖支原體，非淋病尿道炎

Introduction

Non-gonococcal urethritis (NGU) is the most prevalent reproductive tract infection recorded in men in local Social Hygiene Clinics. It is defined

as the inflammation of the anterior urethra in male not caused by *Neisseria gonococchoea*. The most frequent cause of NGU is *Chlamydia trachomatis* (Group D to K), whilst other possible associations identified less frequently include *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Trichomonas vaginalis*, *Herpes simplex virus*, *Candida* species etc. There is increasing evidence in the literature to show *M. genitalium* as a cause for non-Chlamydia associated NGU in men. Meanwhile there is accumulating evidence that *M. genitalium* causes cervicitis, but further studies are needed to establish its causative role for upper genital tract infection in women.

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The first-line treatment for NGU is tetracycline which is effective against Chlamydia, the most common cause of NGU. *M. genitalium* NGU is however more responsive to azithromycin than tetracycline. Therefore there are clinical and public health relevance in better knowing and defining the role of *M. genitalium* in NGU.

1) Mycoplasma genitalium as a cause of disease in men

The traditional Koch's postulates are not well suited to investigate the aetiology of syndromes such as urethritis that may have several different causes.¹ Therefore a modification of Koch's postulates has been suggested (Table 1).² This has formed the basis for evaluating evidence on the causative role of mycoplasmas in genital tract infection.³

a) Epidemiology

Mycoplasma genitalium was first isolated in 1980 from 2 of 13 men with NGU.⁴ Epidemiological studies have been few for more than a decade since the discovery of *M. genitalium*, because of difficulty in culturing the organism. The development of sensitive and specific polymerase chain reaction (PCR) assays in the early 1990's has made clinical studies possible. A significant number of publications from around the world have shown a strong association between *M. genitalium* and NGU, independent of *C. trachomatis* (Table 2).

In a review of 19 studies retrieved from Medline search, *M. genitalium* was positive in 21% (436/2069) of patients with NGU as compared to 6.7% (121/1810) without NGU. This resulted in a pooled OR of 3.8 (95%CI 3.0-4.9, $p < 0.0001$).³ In all studies, *M. genitalium* has been detected more often in men with NGU than in those without.

In 15 studies, the prevalence of *M. genitalium* was 19.2% in the NGU group compared to a *C. trachomatis* prevalence of 28%. *M. genitalium* was found less often in the chlamydial NGU

(12.9%, 62/479) than in the non-chlamydial NGU (21.7%, 267/1233) ($p < 0.0001$, Fischer's exact test). Thus *M. genitalium* behaves largely independent of *C. trachomatis* as an aetiological agent causing NGU.³

b) Antibody response

Cross reactions between *M. pneumoniae* and *M. genitalium* have hindered the usage of serology for diagnosis and epidemiological studies. Taylor-Robinson observed >4 fold rise in *M. genitalium* antibodies in 29% of patients with NGU and 12% of those without, but the correlation between antibody response and urethral detection of *M. genitalium* was not very convincing.¹²

Wang studied the seroprevalence of *M. genitalium* antibodies in different populations with ELISA to lipid associated membrane proteins (LAMP).¹³ Serum and urine specimens were collected from 104 patients and 38% (40/104) were found to have antibodies to *M. genitalium*. Of these 38% (15/40) were urine PCR positive for *M. genitalium*, indicating that antibodies from previous exposure were persisting. However none of the 64 who were negative for mycoplasma antibodies was urine PCR positive, indicating that LAMP-ELISA is a sensitive test.

Although further studies are required, the second of the modified Koch's postulates appears partially fulfilled (Table 3).

c) Effect of treatment

Tetracyclines have been the drug of choice for treatment of NGU in most countries because of low cost and good clinical efficacy towards *C. trachomatis* infections. However in vitro antibiotic susceptibility studies indicate that *M. genitalium* are more susceptible to macrolides than tetracyclines.¹⁴ Clinical studies showed that tetracycline treatment for chlamydial NGU did not always eradicate *M. genitalium*.^{15,16}

One study found that all men (7/7) who had *M. genitalium* detected after doxycycline treatment

Table 1. Modified Henle-Koch postulates²

| | |
|------------------------------|--|
| Epidemiology | The organism should be detected more frequently and in larger numbers from patients with disease than from those without. |
| Antibody response | This should be demonstrated in infected host. |
| Response to treatment | Clinical and microbiological cure after treatment with an antimicrobial agent which the organism is susceptible in vitro. |
| Transmissibility | The organism should infect an animal host from which they can be recovered, and produce disease similar to that seen in man. |

Table 2. Recent publications on association of *M. genitalium* with NGU

| | Symptomatic NGU cases (≥5 PML on smear) | Asymptomatic controls | Symptomatic cases versus controls |
|--|---|-----------------------|--------------------------------------|
| Leung et al⁵ 2006 UK | 302 | 378 | 11 vs 2% (p<0.001) |
| Mena et al⁶ 2002 New Orleans, US | 97 | 184 | 21 vs 8% (p<0.002) |
| Morency et al⁷ 2001 Central African Republic | 136 | 100 | 42 vs 15% (p<0.001) |
| Totten et al⁸ 2001 Seattle, US | 121 | 117 | 22 vs 4% (p<0.01) |
| Johannisson et al⁹ 2000 Sweden | 115 | 118 | 15 vs 1% (p<0.001) |
| Janier et al¹⁰ 1995 Paris | 100 | 96 | 29 vs 9% (p<0.001) |
| Horner et al¹¹ 1993 UK | 103 | 53 | 23 vs 6% (p=0.006) |

Table 3. Summary on strength of evidence that *M. genitalium* has a causative role in genital tract disease

| | Non-gonoccal Urethritis | Cervicitis | Pelvic Inflammatory Disease |
|----------------------------|-------------------------|------------|-----------------------------|
| Epidemiology | +++ | + | + |
| Antibody response | + | ? | ? |
| Effect of treatment | + | + | ? |
| Transmissibility | ++ | + | + |

+++Strong evidence with meta-analysis and many controlled studies; ++Moderate evidence with few controlled studies
+Weak evidence with isolated studies; ?No significant evidence found yet

had urethritis at follow up.^{15,16} A second study found that 63% (10/16) of men treated with tetracyclines were *M. genitalium* positive at follow up.¹⁷ These patients' symptoms responded to treatment, but urethritis remained. Another 6 men treated with azithromycin were all PCR negative at follow up ($p=0.015$).¹⁷

Due to diversity in dosages of tetracyclines and azithromycin used in published studies, randomised controlled trials are necessary to compare efficacy of doxycycline (100 mg BD for 7 days) and azithromycin (1 g as single dose) as recommended in clinical effectiveness guidelines.¹⁸

Although published studies were small, there appeared to be a strong correlation between eradication of *M. genitalium* and clinical cure. Patients with microbiological treatment failure appeared to have recurrent or persistent disease (Table 3).

d) Transmissibility

Animal studies of male urethritis caused by *M. genitalium* were performed in chimpanzees. In one study, 4 chimpanzees were inoculated intra-urethrally with G37 stain of *M. genitalium*.¹⁹ Two of the chimpanzees became persistently infected for 13 weeks. They developed an antibody response and a urethral inflammatory response. In a second study, 6 chimpanzees were inoculated with strains re-isolated from the two chimpanzees in the first study.²⁰ All of the 6 chimpanzees became infected.

In a study of 39 men with NGU (index patients) and their female partners, *C. trachomatis* was detected in 43% (6/14) female partners of *C. trachomatis* positive men, and 11% (4/37) female partners of *C. trachomatis* negative men. *M. genitalium* was detected in 58% (7/12) female partners of *M. genitalium* positive men, and 10% (2/22) female partners of *M. genitalium* negative men. The concordance rate for *M. genitalium* is as at least as high as that for *C. trachomatis*.²¹ Thus *M. genitalium* appears at least as efficiently

transmitted as *C. trachomatis* by human sexual transmission. Consistent condom use reduces the risk of *M. genitalium* infection compared with those who never used condoms.²²

In summary, *M. genitalium* satisfies most of the modified Koch's criteria for its causative role in NGU in men (Table 3). *M. genitalium* DNA has been found in the urethra of men with epididymitis, and in prostatic tissue of men with prostatitis. Further studies are necessary to see if *M. genitalium* is associated, perhaps causally with acute or chronic prostatitis, epididymo-orchitis, neonatal disease and reactive arthritis.

2) M. genitalium as a cause of disease in women

a) Epidemiology

One US study found that after excluding women infected with *N. gonorrhoeae* and *C. trachomatis*, women with mucopurulent cervicitis were 3.1 times more likely to be infected with *M. genitalium* than those without cervicitis.²³

In a Swedish study, urethritis and/or cervicitis was defined by >5 PMN/hpf and >30 PMN/hpf respectively. Among 445 women attending STD clinic, *M. genitalium* PCR was positive in 9.7% (21/217) women with cervicitis/urethritis as compared with 2.6% (6/228) without ($p=0.003$).²⁴ The overall prevalence of *M. genitalium* was 6.3%.²⁵

Pelvic inflammatory disease (PID) is a more severe disease, with loss of working days and long term complications such as tubal factor infertility, ectopic pregnancy and pelvic pain. In the PID Evaluation and Clinical Health Study (PEACH) study, 8% (50/611) of women with clinically suspected PID were tested positive for *M. genitalium* in the endometrium by PCR.²⁶ These women were 3 times more likely to have histologically confirmed endometritis compared with women without *M. genitalium* identified at this site (OR 3.0, 95% CI 1.5-6.1).

b) Antibody response

In a study of 308 infertile women, 22% of 132 women with tubal factor infertility had antibodies to *M. genitalium* as compared to 7% of 176 women with other causes of infertility.²⁷ After the correction of *C. trachomatis* antibodies, *M. genitalium* is still strongly associated with tubal factor infertility with an OR of 5.6.

There were no studies where antibody response has been evaluated in women with acute infections documented by PCR. The simultaneous detection of *M. genitalium* in lower genital tract and development of antibody response would be invaluable, particularly for PID studies. Such studies are necessary to fulfill the second criteria of modified Koch's postulates.

c) Effect of treatment

In a prospective study on prevalence of *M. genitalium* in Swedish STD patients, 26 *M. genitalium* PCR +ve women were identified. Among 14 women treated with tetracycline, 71% (10/14) were still PCR +ve at follow up >3 weeks after treatment.¹⁷

Whereas in 12 women treated with azithromycin, all 10 who returned for follow up were found to be *M. genitalium* PCR-ve.¹⁷ The 10 patients who had persistent *M. genitalium* +ve specimens after tetracycline were subsequently also treated with azithromycin, and all had become PCR-ve on follow up.¹⁷

Like male patients, no controlled trials on treatment efficacy in women have been published. Randomised controlled trials are urgently needed.

d) Transmissibility

Four female chimpanzees inoculated intravaginally with *M. genitalium* shed organisms for 15 weeks. A vaginal PMNL response was noted but without overt discharge. All inoculated chimpanzees developed a clear cut antibody response.²⁰ When *M. genitalium* was inoculated into oviducts of marmosets and grivet monkeys they developed endosalpingitis, with inflammatory

cell infiltration of tubal epithelium, luminal exudates and adhesions similar to that seen after *C. trachomatis* inoculation.²⁸

In a study where index patients infected with *M. genitalium* underwent partner tracing, *M. genitalium* was found positive in 38% (10/26) partners of infected female index patients, and 45% (10/27) partners of infected male index patients.²⁴ In comparison with the transmission of *C. trachomatis*, *M. genitalium* is at least as efficiently transmitted.²¹ In conjunction with concordance rates among partners, DNA typing showing the same sequence typing among partners in contrast to unrelated *M. genitalium* positive patients may provide further proof of transmission in future.

In summary, the sexual transmissibility of *M. genitalium* has been documented in animal studies and in the concordance rates of the infection between patients and their partners.

3) Update and local epidemiology

A survey of male patients attending a government STI clinic in Hong Kong has been published recently.²⁹ While *C. trachomatis* was significantly associated with symptomatic NGU, neither *U. ureaplasma* nor *M. genitalium* demonstrated similar significant association with symptomatic NGU (Table 4).²⁹ The reasons for this will be discussed later. In contrast, clinical studies performed in other parts of the world had shown a significant association between *M. genitalium* and NGU, independent of *C. trachomatis* (Table 2).

A recent survey aims to determine the prevalence and risk factors associated with *M. genitalium* infection in young adults from the United States.³⁰ Among adolescents who participated in a nationwide health study, 4.2% were infected with chlamydia, 2.3% with trichomoniasis, 1% with *M. genitalium* and 0.4% with gonorrhoea. Thus *M. genitalium* appears to have surpassed gonorrhoea in prevalence to become the third

Table 4. Patients with organisms detected by PCR in NGU and asymptomatic controls²⁹

| Organisms | Symptomatic NGU (n=98) | Asymptomatic controls (n=236) | P value (with logistic regression) |
|-----------------------|---------------------------|----------------------------------|---------------------------------------|
| <i>C. trachomatis</i> | 49 (50%) | 14 (6%) | <0.001 |
| <i>U. urealyticum</i> | 22 (22%) | 47 (20%) | 0.535 |
| <i>M. genitalium</i> | 10 (10%) | 5 (2%) | 0.799 |

most common STD among young people in United States.

The study also showed that *M. genitalium* was strongly associated with sexual activity. The prevalence of *M. genitalium* among those who reported to have vaginal intercourse was 1.1%, compared with 0.05% among those who did not. In multivariate analysis, The prevalence of *M. genitalium* was 11 times higher among the respondents who reported living with a sexual partner, and the prevalence of *M. genitalium* increased by 10% for each additional sexual partner.

A molecular epidemiological study of *M. genitalium* infection in high risk populations of sexually transmitted diseases in Mainland China had been performed.³¹ It found that the positive *M. genitalium*-DNA detection rate among high risk populations of STDs (16% for STD clinic attendees, 14% for promiscuous group) was significantly higher than that of the control group (1.8% for healthy subjects) (Chi Square=7.82, $p<0.01$). The positive detection rates in Guandong STD clinics were higher than those from Shanghai, Nanjing and Changzhou areas (Chi Square=8.54, $p<0.01$).

There was also significant difference in *M. genitalium* detection rate in patients with or without urethritis symptoms ($p<0.05$).³¹ In Nanjing, 20% (10/50) were positive for *M. genitalium* in symptomatic group compared with 3% (1/34) in asymptomatic controls. In Jiangmen, the detection rate of *M. genitalium* was 27% (18/66) in symptomatic individuals compared with 6.7% (2/33) in asymptomatic controls.

4) Utility of molecular diagnostics for detecting genital mycoplasmas in STD clinic settings of western world

In contrast to chlamydia PCR, the routine usage of molecular diagnostics in detecting genital mycoplasmas in STD clinics in western world has not been widespread. Most of the diagnostic work has been performed in a few centers only. Good diagnostic tools are only available in the form of 'in-house' PCR assays and commercial nucleic acid amplification tests (NAATs) are not yet available. The access to commercially available NAATs would facilitate further studies, and provide external reference standard.

While treatment failure in *M. genitalium* positive NGU appears common with doxycycline, early reports suggested cure rates of 85% with azithromycin 1 g single dose, and prolonged azithromycin treatment (500 mg on day 1, and 250 mg daily on days 2 to 5) eradicates *M. genitalium* in 95% of cases.³² Recently one Australian study reported a significant failure rate of 28% (95%CI 15-45%, 9/32) with azithromycin in *M. genitalium* positive NGU, which is supported by in vitro evidence of reduced susceptibility to macrolides.³³ Recurrent urethral symptoms following azithromycin therapy only occurred in person with persistent *M. genitalium* infection and resolved with moxifloxacin. Reports indicated differential activity of fluoroquinolones against *M. genitalium*, with moxifloxacin being more active than levofloxacin and ciprofloxacin in vitro.³⁴

A cross sectional survey in Australia showed that 73% of sexual health physicians believed that

female partners of men who present with chlamydia negative NGU were at risk of adverse reproductive health outcomes, and 62% initiate some kind of partner notification for female partners of men with chlamydia negative NGU.³⁵ However only 19% (21/111) of sexual health physicians routinely test for, and 65% sometimes test for, pathogens other than *N. gonorrhoeae* and *C. trachomatis* in men presenting with NGU. These included *M. genitalium*, herpes virus, ureaplasma species, *T. vaginalis* and adenoviruses.

The Melbourne Sexual Health Centre in Australia has recently developed treatment guidelines for *M. genitalium* genito-urinary infections (personal communication). A PCR test has been developed by Department of Molecular Microbiology at Royal Women's Hospital to detect *M. genitalium* in the first pass urine specimens and in urethral, endocervical, throat and anal specimens. A single dose of 1 g azithromycin is used as first line for treatment of uncomplicated *M. genitalium* urinary infection. Contacts should be screened and treated empirically with azithromycin also. As around 16% will be resistant to azithromycin, a test of cure 2-4 weeks following treatment is arranged. If treatment failure occurs and re-infection has been excluded, then moxifloxacin 400 mg daily for 10 days is considered after discussion with patient. They should be warned that this is an 'off-label' use as there are no formal evidence based guidelines published yet, and there is a quinolone risk of Achilles tendon rupture. Because of the risk of development of resistance this treatment should be considered second line.

There have been concerns with development of macrolide resistance and subsequent azithromycin treatment failure, which may be associated with widespread single dose azithromycin treatment for NGU of unknown aetiology.³⁶ The genetic basis for the drug resistance of *M. genitalium* was shown to be mutations in region V of the 23S rRNA gene. Consequently there have been advocates for an extended 5-day course of azithromycin instead of single dose as first line therapy.³⁷

Meanwhile British and European Guideline recommendations for persistent or recurrent NGU include azithromycin for 5 days plus metronidazole for *M. genitalium* and trichomonal infection respectively, notwithstanding the non-availability of molecular diagnostics. If the above fails then moxifloxacin may be used as second line.^{38,39}

5) Further study/evidence before introducing tests into local STD services

In the recent local study, there were 10% (10/98) symptomatic patients with NGU tested positive for *M. genitalium*, compared with 2% (5/236) asymptomatic patients without NGU.²⁹ This difference was not statistically significant when compared by logistic regression analysis ($p=0.799$). However clinical studies performed in other parts of the world have shown a strong association between *M. genitalium* and NGU, independent of *C. trachomatis* (Table 2). The low prevalence of *M. genitalium* in this study means that a type II error may have been inadvertently introduced. A larger study, appropriately designed and well performed, will be needed to clarify any possible association of *M. genitalium* with NGU in Hong Kong.

The probability that a test will accurately determine true infection status of a person being tested depends on the prevalence of the infection in population tested. The positive predictive value is very low when one tests a population with a low prevalence of the illness. Thus positive non-culture results in low prevalence settings should be considered presumptive until confirmed.

Conclusions and way forward

Molecular diagnosis of genital *M. genitalium* infection is only performed in few centers as part of their clinical management protocol for those *C. trachomatis* -ve persistent non-gonococcal urethritis patients in the developed countries. Available evidence showed that the local prevalence of *M. genitalium* was low, but this finding may be limited by the study size and hence

power. Currently there is no conclusive evidence to support the introduction of molecular diagnosis for *M. genitalium* genital infection in local STD clinics. Further studies to better define the local prevalence are however of value. Information in this article has been presented at the 15th Meeting of the Scientific Committee on AIDS and STI in June 2009.

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