

## Review Article

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# *Mycoplasma genitalium*: An emerging sexually transmitted pathogen

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*Mycoplasma genitalium* is a member of genital mycoplasmas, which is emerging as an important causative agent of sexually transmitted infections both in males and females. The advent of polymerase chain reaction and other molecular methods have made studies on *M. genitalium* more feasible, which is otherwise a difficult organism to isolate. Besides *Chlamydia trachomatis*, *M. genitalium* is now an important and established cause of non gonococcal urethritis (NGU) in men, more so in persistent and recurrent NGU. Multiple studies have also shown a positive association of *M. genitalium* with mucopurulent cervicitis and vaginal discharge in females as well. The evidences for *M. genitalium* pelvic inflammatory diseases and infertility are quite convincing and indicate that this organism has potential to cause ascending infection. Lack of clear association with *M. genitalium* has been reported for bacterial vaginosis and adverse pregnancy outcomes. Diagnosis of *M. genitalium* infections is performed exclusively using nucleic acid amplification tests (NAATs), owing to poor or slow growth of bacterium in culture. Although there are no guidelines available regarding treatment, macrolide group of antimicrobials appear to be more effective than tetracyclines. The present review provides an overview of the epidemiology, pathogenesis, clinical presentation and management of sexually transmitted infections due to *M. genitalium*.

**Key words** Emerging infection - *Mycoplasma genitalium* - non-gonococcal urethritis - sexually transmitted diseases

### Introduction

According to the WHO there are an estimated 448 million new cases of sexually transmitted infections (STIs) which are acquired worldwide annually<sup>1</sup>. If diagnosed in time, these infections can be treated easily with minimal morbidity as well as decreased economic burden. *Mycoplasma genitalium* is an emerging cause of STIs and has been implicated in urogenital infections of men and women around the world. More than 25 years after its initial isolation from men with non-gonococcal urethritis (NGU)<sup>2</sup>, *M. genitalium* is now recognized as an important aetiologic agent of acute and persistent

male NGU and is responsible for approximately 20-35 per cent of non-chlamydial NGU cases<sup>3,4</sup>. The role of this organism in male urogenital disease was a significant advancement in our knowledge of STIs, but its role in the inflammatory reproductive tract diseases of women is still not very clear<sup>5</sup>.

The risk factors for *M. genitalium* infection are typical as for any STI. Researchers in various study groups of women and men aged 21-23 yr identified behavioural risk factors for *M. genitalium* infection<sup>6</sup>: higher number of partners, younger age during first intercourse, having a partner with infection symptoms,

co-infection with other sexually transmitted pathogens like *Chlamydia trachomatis*<sup>6</sup>. Manhart *et al*<sup>7</sup> showed that the prevalence of *M. genitalium* increased by 10 per cent for every additional sexual partner. Asymptomatic carrier state<sup>7</sup> is also a serious epidemiological problem because of transmission to sexual partners and may be vertical transmission from mother to the newborn. Some investigators have reported infection as common as chlamydial infection and trichomoniasis, and more common than gonorrhoea<sup>8,9</sup>.

In studies conducted in Denmark, the prevalence of infection was 2.3 and 1.1 per cent in women and men, respectively<sup>9</sup>. Takahashi *et al*<sup>10</sup> demonstrated the positivity rate of *M. genitalium* DNA in urine from asymptomatic healthy young Japanese men as 1 per cent; among female students in Japan the prevalence of *M. genitalium* was 2.8 per cent<sup>6</sup>. Tosh *et al* showed high prevalence rate of 13.6 per cent in young (14-17 yr of age) sexually active women who tested positive for *M. genitalium*<sup>11</sup>. Among women and men reporting to an STD clinic in Seattle, *M. genitalium* was detected in 14 and 9 per cent cases, respectively<sup>12</sup>. Table gives a chronological account of important studies reporting geographic prevalence of *M. genitalium*.

### Taxonomy and general characteristics

*Mycoplasma* (Latin: fungus form) belongs to class *Mollicutes* (Latin: soft skin). Despite the lack of a cell wall, many taxonomists have classified *Mycoplasma* and relatives in the phylum *Firmicutes*, consisting of low G+C Gram-positive bacteria such as *Clostridium*, *Lactobacillus*, and *Streptococcus* based on 16S rRNA gene analysis. The order *Mycoplasmatales* contains a single family, *Mycoplasmataceae*, comprising two genera: *Mycoplasma* and *Ureaplasma*. These are amongst the smallest free living microorganisms capable of self-replication. *M. genitalium* is one out of the so far 15 named mycoplasma species of human origin<sup>13</sup>. In 1981, two mycoplasma strains, G-37 and M-30 were first isolated from two men with nongonococcal urethritis (NGU)<sup>2,14</sup>. Later in 1983 these were named as *M. genitalium*, the twelfth mycoplasma species recovered from humans<sup>15</sup>.

The genus *Mycoplasma* contains very small bacteria, with sizes ranging from 0.2 to 0.7 µm. The shape depends on the particular mycoplasma species which may vary from spherical, filamentous or flask/pear-like. *M. genitalium* is a motile flask-shaped mycoplasma with terminal tip-like structure which

**Table.** Chronological account of important representative studies reporting geographic prevalence of *M. genitalium*

Year	Author	Geographic location	Clinical presentation	Prevalence (%)
2002	Casin <i>et al</i> <sup>109</sup>	France	Cervicitis	38.2
2003	Manhart <i>et al</i> <sup>62</sup>	United States	Cervicitis	7.0
2005	Pepin <i>et al</i> <sup>110</sup>	Ghana	Vaginal discharge & Cervicitis	26.3
2005	Anagrius <i>et al</i> <sup>111</sup>	Sweden	Urethritis & Cervicitis	6.3
2005	Falk <i>et al</i> <sup>112</sup>	Sweden	Urethritis & Cervicitis	5.0
2006	Korte <i>et al</i> <sup>113</sup>	Unites States	Vaginal discharge & Cervicitis	42.0
2007	Cohen <i>et al</i> <sup>114</sup>	Kenya	Vaginal discharge & Cervicitis	16.0
2007	Tosh <i>et al</i> <sup>11</sup>	Unites States	Vaginal discharge	13.6
2007	Hogdahl <i>et al</i> <sup>115</sup>	Sweden	Urethritis & Cervicitis	6.5
2008	Huppert <i>et al</i> <sup>116</sup>	Unites States	Vaginal discharge & Cervicitis	22.4
2008	Arraiz <i>et al</i> <sup>117</sup>	Venezuela	Cervicitis	7.6
2008	Manhart <i>et al</i> <sup>118</sup>	Kenya	Cervicitis	17.2
2009	Moi <i>et al</i> <sup>119</sup>	Norway	Urethritis & Cervicitis	4.5
2009	Gaydos <i>et al</i> <sup>73</sup>	Unites States	Cervicitis	19.3
2009	Manhas <i>et al</i> <sup>55</sup>	India	Urethritis	6.0
2010	Thurman <i>et al</i> <sup>120</sup>	Unites States	Vaginal discharge	9.2
2010	Le Roux <i>et al</i> <sup>121</sup>	South Africa	Urethritis	17.3
2011	Shigehara <i>et al</i> <sup>122</sup>	Japan	Urethritis	18
2011	Zimba <i>et al</i> <sup>123</sup>	Mozambique	Urethritis	4.0

assists in attachment to various surfaces and provides gliding motility. It does not have a peptidoglycan cell wall and, therefore, lacks cell surface markers. The absence of a cell wall also means that this bacterium has less osmotic stability in the host environment. Lack of a cell wall is responsible for a negative Gram stain reaction and non-susceptibility to  $\beta$ -lactam antibiotics that inhibit bacterial cell wall synthesis<sup>16</sup>. As *M. genitalium* is too small to be visible singly under a light microscope, the first detailed study of its structure was conducted under a transmission electron microscope (TEM)<sup>16</sup>. These organisms are difficult to culture and it may take from a few weeks to a few months before a culture shows visible growth. The mycoplasmas penetrate the surface of the agar and grow in the underlying medium through deposition of manganese dioxide (MnO<sub>2</sub>). The colonies appear brown and have a typical "fried egg" appearance (size: 200-400  $\mu$ m): a dense, dark, granulated nucleus, bordered by a thin, light zone<sup>2,17</sup>.

## GENOME

In 1995 *M. genitalium* complete genome sequence was published making it the second bacterium after *Haemophilus influenzae* to have its genome fully sequenced<sup>18</sup>. It is the species with the smallest genome of all mycoplasmas studied so far with a genome of only 580 kb. Although most mycoplasma genomes have a low guanine plus cytosine (G+C) content, with the range of 24-33 per cent G+C, the *M. genitalium* genome has a comparatively higher G+C content of 32 per cent<sup>18</sup>. The small genome of *M. genitalium* made it the organism of choice in 'The Minimal Genome Project', a study to find the smallest set of genetic material necessary to sustain life<sup>19</sup>.

## PATHOGENESIS

The pathogenesis of *M. pneumoniae* has been studied extensively and due to the close genetic resemblance, certain features in the pathogenesis of *M. pneumoniae* can be applied to *M. genitalium*. Though *M. pneumoniae* is primarily found in the respiratory tract, and *M. genitalium* in the urogenital tract, both organisms have been shown to cross tissue barriers. *M. genitalium* has been shown to attach to different cell types, including erythrocytes, Vero cells, fallopian tube cells, respiratory cells and spermatozoa<sup>13,20</sup>.

*M. genitalium* has several virulence factors that are responsible for its pathogenicity. These include the ability to adhere to host epithelial cells using the terminal tip organelle with its adhesins, intracellular

localization, the release of enzymes and the ability to evade the host immune response by antigenic variation. *M. genitalium* lipid-associated membrane proteins (LAMs) play an important role in the genito-urinary tract inflammatory reaction<sup>20</sup>. Although mycoplasmas are shown to produce hydrogen peroxide and superoxide metabolites, much of the tissue damage is related to the host cell responses. The stimulation and suppression of cells (including lymphocytes, monocytes, macrophages) through induction of cytokine production (mainly TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 and IL-10) have significant role in pathogenesis. Some of the mycoplasmal cell components can also act as superantigens

**(i) Adhesion to host epithelium** - For any Mollicute to colonize and cause infection, adhesion is the first prerequisite. Mycoplasmas are primarily considered surface parasites of mucous membrane cells as these adhere tenaciously to the epithelial linings of the respiratory or urogenital tract, rarely invading tissues. The urogenital tract appears to be the primary tissue infected by *M. genitalium*, but the adherence does not appear to be restricted to uroepithelial cells *in vitro*<sup>13</sup>. Adhesin molecules, clustered at the tip structure of the flask shaped polar cell have been intensely studied both in *M. genitalium* and *M. pneumoniae*<sup>21</sup>. Both species attach to erythrocytes from a variety of species, eukaryotic cells, such as Vero cells, but more importantly *M. genitalium* binds to the epithelial cells of human fallopian tubes<sup>13</sup>.

Twenty one different putative protein genes have been identified in the *M. genitalium* genome, but only a few have been characterised as adhesions. The major adhesin in the attachment protein complex is the MgPa protein<sup>22,23</sup> and, collectively with the P32 (MG318)<sup>24</sup> protein, makes up the terminal tip organelle. The MgPa encodes the P140 (MG191) and P110 (MG192) cytodherence proteins (cytadhesins) at the tip area<sup>13</sup>. These proteins are immunogenic both in experimental animals as well as in humans. Loss of either of these two proteins results in loss of motility and adherence properties of the entire MgPa attachment organelle<sup>25</sup>, thus showing the importance of these proteins in attachment. It has been shown that the 140 kDa P140 (MG191) is related to the 170 kDa main adhesion protein (P1) of *M. pneumoniae* whereas the 32 kDa *M. genitalium* protein P32 (MG318) closely resembles P30 of *M. pneumoniae*<sup>26</sup>.

The MG218 and MG317 cytoskeletal proteins were shown to have a role in terminal organelle organisation, gliding motility and cytodherence. The

genes encoding the adherence proteins are located in three different regions of the *M. genitalium* genome. The genes coding for the MgPa adhesins are organised in an operon with three genes<sup>22,23</sup>. The P32 (MG318) of *M. genitalium* adherence components found on the tip-like terminal structures is located in operons that are a distance away from the MgPa operon. The accessory proteins and their analogues in *M. genitalium* are important for clustering of the adhesin at the tip and maintaining the tip of the organism and the shape of the cell, thereby acting like a cytoskeleton. MG218 is grouped in an operon with MG217 and MG219<sup>13</sup>.

**(ii) Intracellular localization** - From the very beginning of mycoplasma study<sup>2,15</sup>, the controversy regarding the surface versus intracellular location of mycoplasma remains. Although the intracellular location of some of the *Mycoplasma* spp, e.g. *M. penetrans*, *M. fermentans* and *M. hominis* was successfully proved<sup>27,29</sup>, the same about *M. genitalium* is not clear. Though various studies<sup>30,32</sup> have been conducted regarding location of *M. genitalium*, any conclusion is yet to be arrived. It appears that *M. genitalium* behaves like a facultative intracellular pathogen *i.e.* capable of retaining viability both extracellularly and intracellularly *in vitro*<sup>13</sup>. Whether the same occurs *in vivo* has not been proven. However, from clinical experience with patients receiving multiple courses of highly active antibiotic treatment with temporary improvement but subsequent relapse, it seems likely that such mechanisms may act in at least in a subset of patients<sup>13</sup>.

**(iii) Antigenic variation** - Mycoplasmas, in order to evade host immune response, show antigenic variation of their surface components. Surface lipoproteins of mycoplasmas show antigenic variation and over generations these have evolved to generate higher frequency of phenotypic heterogeneity even in a small population of these organisms. Other mechanisms such as mimicry of host antigens may also assist in their survival within the host cells<sup>33,34</sup>.

There are two basic mechanisms for this. Either the organism controls the expression of the virulence factors molecules in accordance to the environmental changes through a signal transduction pathway or the organism may spontaneously generate mutant phenotypes which survive the changes in their environment *i.e.* host cell responses<sup>33</sup>. In order to evade the host immune system, proteins P140 and P110 of the MgPa have the capacity to undergo antigenic variation, thus changing the genetic sequence of the MgPa with subsequent

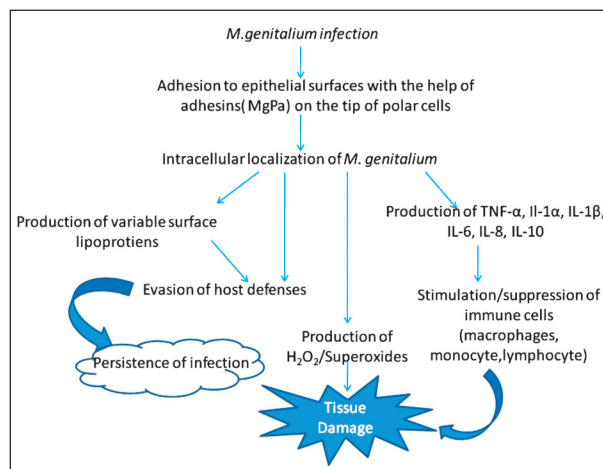
production of variants that are not recognised by the host immune system<sup>13,35</sup>. Other survival mechanisms of this organism may be the ability to mimic host cell antigens and the intracellular location within professional macrophages<sup>13</sup>.

Tissue damage observed in *M. pneumoniae* infections is due to the host cell response and this may indicate what occurs during in *M. genitalium* infections. Mycoplasmas interact with many components of the immune system, which may lead to production of cytokines and macrophage activation. Some cell components may also act as super antigens and induce a heightened autoimmune response<sup>36</sup>.

**(iv) Enzymes** - Alvarez *et al*<sup>37</sup> found that in the glycolytic pathway, the activity of the glycolysis enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) brings about attachment of *M. genitalium* to human vaginal and cervical mucin in female. Thus GAPDH acts as a ligand to receptors mucin and fibronectin, particularly in vaginal and cervical disease. *M. genitalium* translocates its cytoplasmic enzymes to the cell membrane surfaces to enhance host tissue colonization<sup>38</sup>. In addition, methionine sulphoxide reductase (MsrA) can be released to enhance the pathogenicity of its small genome<sup>39</sup>. MsrA is an antioxidant repair enzyme of the bacterium. It restores proteins that have lost their biological activity due to the oxidation of their methionines, thereby protecting the bacterium protein structure from the host oxidative damage<sup>39</sup>. The Figure shows a flow diagram depicting the pathogenesis in *M. genitalium*.

## INFECTIONS IN HUMANS

**(A) Urogenital infection in men** - Urethritis, which is a common sexually transmitted disease (STD) in



**Fig.** An overview of the pathogenic mechanisms of *M. genitalium*.

heterosexual men, is classified as gonococcal or non-gonococcal depending on the presence or absence of *Neisseria gonorrhoeae*<sup>3</sup>. In approximately 30 per cent of the patients with NGU, neither a bacterial nor a mycoplasmal agent is recovered on usual laboratory medium but some in whom a pathogenic microorganism cannot be identified, respond to treatment with antimicrobial agents that are active against *C. trachomatis* or *U. urealyticum*<sup>40,41</sup>. These observations imply that other more fastidious mycoplasmas or other microorganisms susceptible to antimicrobial agents, such as tetracycline, macrolides and fluoroquinolones may be involved in NGU<sup>3</sup>. Due to difficulties in culturing *M. genitalium*, only a few epidemiological studies have been conducted after the discovery of the organism. Since DNA probe<sup>42</sup> and PCR assays<sup>43</sup> were developed for detecting *M. genitalium*, this mycoplasma has now been detected more often in patients with acute NGU than in those without urethritis<sup>44</sup>. It has been shown to cause urethritis in subhuman primates inoculated intraurethrally<sup>45,46</sup>.

The results of many studies tend to indicate that *M. genitalium* can cause NGU. According to these data *M. genitalium* is detectable in 14.1<sup>47</sup> to 33.3 per cent<sup>48</sup> of men with acute NGU recruited at STD clinics<sup>4,44,48-52</sup> and in 13.2<sup>51</sup> to 15.6 per cent<sup>54</sup> of those at urology clinics. An earlier study from our centre showed that *M. genitalium* was detected in 6 per cent (6/100) of NGU cases. The infection rate was 7.1 per cent (5 of 70) among the HIV positive individuals whereas only one HIV negative NGU case was found to be positive (3.3%). The association of *M. genitalium* was independent of the presence of *C. trachomatis* and *U. urealyticum*<sup>55</sup>. The prevalence of this mycoplasma in men without urethritis who attended STD clinics is 0.8 to 9.1 per cent<sup>4,44,48,50-52</sup>, whereas that in asymptomatic men who attended urology clinic is 0 to 2 per cent<sup>53,54</sup>. Thus, most studies document that *M. genitalium* is evident significantly more often in patients with acute NGU than in controls without urethritis. Further, the prevalence of *M. genitalium* is 18.4 to 45.5 per cent in patients with acute nonchlamydial nongonococcal urethritis (NCNGU) and in most studies the prevalence is significantly higher than that in patients with acute chlamydia positive NGU or without urethritis regardless of whether they were recruited at STD or urology clinics<sup>4,44,51,53,54</sup>. The prevalence of *M. genitalium* in men with gonococcal urethritis is likely to be lower than that of *C. trachomatis*<sup>3</sup>. Most patients with NGU associated with *C. trachomatis* or *U. urealyticum* respond to antibiotic therapy. However, when neither organism is detected, NGU sometimes persists or recurs

within 6 wk of treatment<sup>56,57</sup>. Various studies show that *M. genitalium* may have a significant role in persistent or recurrent NGU<sup>5,52,58</sup>. It has been detected in the prostate of about 4 per cent of the patients with chronic abacterial prostatitis<sup>59</sup> but there is currently insufficient evidence to suggest that it could be an important cause of the disease<sup>60</sup>.

**(B) Urogenital infections in women** - Compared to the number of studies in men, only a few studies on the role of *M. genitalium* in women have been conducted<sup>5</sup>.

**(i) Cervicitis** - A few studies have addressed the correlation between *M. genitalium* infection and cervicitis. One of the major problems in interpreting these studies has been the varying definitions of cervicitis. Some studies have considered the presence of 10 polymorphonuclear lymphocytes (PMNLs)/high power field (hpf) in a cervical smear significant, whereas others considered 30 PMNLs/hpf to define cervicitis<sup>13</sup>. One early study by Uno *et al*<sup>59</sup> defined cervicitis by endocervical discharge and > 20 PMNL/hpf and detected *M. genitalium* in 5 of 64 women as compared to none of 80 asymptomatic pregnant women<sup>61</sup>. In a US study using archived cervical secretions from 719 women collected on filter paper, it was found that *M. genitalium* was strongly associated with cervicitis being detected in 7 per cent of all the women examined but in 11 per cent of women with cervicitis (> 30 PMNLs/hpf) and in 5 per cent of women without cervicitis<sup>60</sup>. In a multivariate logistic regression analysis correcting for other factors found to be associated with cervicitis, and excluding the 172 women infected with *N. gonorrhoeae* and/or *C. trachomatis*, *M. genitalium* remained strongly associated with cervicitis (OR 3.1; 95% CI 1.5-6.8), further supporting an independent role for *M. genitalium* as a cause of cervicitis. The attributable risk per cent was 70 per cent, suggesting that among women with cervicitis and *M. genitalium*, 70 per cent of cervicitis can be attributed to *M. genitalium*<sup>62</sup>.

**(ii) Pelvic inflammatory diseases (PID)** - PID is the clinical syndrome caused by the spread of microorganisms from the lower to the upper genital tract. Several bacteria including those found in bacterial vaginosis, *C. trachomatis*, and *N. gonorrhoeae* can cause PID. Establishing a connection between *M. genitalium* and upper genital tract infection is of major importance in determining the significance of the infection<sup>13</sup>. Earlier studies relying on serology have been controversial. In a study of 115 women presenting to an STD clinic in Kenya with acute

pelvic pain, plasma cell endometritis was found in 58 and *M. genitalium* was detected in cervical swabs or endometrial biopsies in 16 per cent compared to only 2 per cent of women without endometritis<sup>63</sup>. As many as 33 per cent of the women studied were HIV positive, but *M. genitalium* was not detected more often in the HIV infected women. In another study on women with clinically suspected PID<sup>64</sup>, *M. genitalium* was detected more often in HIV positive women (19 vs 5%). In a case-control study, Simms and colleagues<sup>65</sup> examined 45 women with clinically diagnosed PID and found *M. genitalium* DNA by PCR in endocervical swabs from nine (16%) as compared to none of 37 control patients.

**(iii) Bacterial vaginosis (BV)** - Although in one of the early reports, *M. genitalium* was found in 4 (16%) of 25 unselected women and in 3 of 10 women with BV<sup>66</sup>, subsequent studies showed lack of association of *M. genitalium* with BV. Keane *et al*<sup>67</sup> studied 15 women with BV, none of whom was *M. genitalium* positive, as were only 2 (12%) of 17 women without BV attending an STD clinic. Thus, unlike *M. hominis*, *M. genitalium* probably is not associated with BV<sup>17</sup>.

**(iv) Adverse pregnancy outcome and infertility** - There is little information available on the role of *M. genitalium* in causing adverse pregnancy outcome, either as preterm labour, abortion or stillbirth. In one study *M. genitalium* was detected in only 4 per cent of mid-trimester vaginal swabs from 124 women delivering preterm<sup>68</sup>. This is probably slightly higher than the prevalence in a normal population of pregnant women as estimated in another study<sup>69</sup> where only 0.7 per cent of women pregnant in the first trimester were *M. genitalium* positive. Regarding infertility, only indirect evidence has been presented. In a Danish study<sup>70</sup>, serum samples from 308 infertile women were investigated for antibodies to *M. genitalium* by immunoblotting against whole cell proteins of *M. genitalium* and *M. pneumoniae* as well as against a recombinant MgPa antigen. Among the women with tubal factor infertility, 22 per cent were seropositive as compared to 7 per cent of women with either male factor infertility or unexplained infertility with normal tubes.

The results of animal experiments offer substantial evidence for the pathogenicity of *M. genitalium* for the urogenital tract of subhuman primates and the agent is sexually transmitted between partners<sup>13</sup>. It seems reasonable to conclude, that *M. genitalium* can cause mucopurulent cervicitis although the demonstration

of an antibody response is lacking. *M. genitalium* is sexually transmissible with transmission rates similar to those of *C. trachomatis*<sup>13</sup>. The role of *M. genitalium* in women is less well established than in men, and further studies are needed to address its relation to PID and late sequelae such as infertility. Only indirect evidence exists for infertility, and BV and adverse outcome of pregnancy does not appear to be associated with *M. genitalium* infection. As in men, randomised controlled clinical trials aiming at determining the optimal treatment of the infection are needed.

### Association of *M. genitalium* with other STDs

As the mode of transmission is common amongst all sexually transmitted infections, co-infection of *M. genitalium* with other pathogens has been observed by many authors. In a study by Yokoi *et al*<sup>71</sup>, rates of co-infection with *M. genitalium* among men with gonococcal urethritis were shown to be low (4.1%), compared with the *C. trachomatis* co-infection rate (21.2%). In another study, when 45 males with gonococcal urethritis were simultaneously screened for *M. genitalium* using PCR, 4.4 per cent showed positivity<sup>72</sup>. Gaydos *et al*<sup>73</sup> while conducting studies on STIs in male attending STD clinics showed 5.9 per cent of them to be co-infected with *C. trachomatis*. In West Africa, Pepin *et al*<sup>74</sup> showed that almost half of the infections due to *M. genitalium* occurred as co-infections. The prevalence of co-infection with gonococcal urethritis, *C. trachomatis* and *Trichomonas vaginalis* was 37.9, 10.6 and 7.6 per cent, respectively<sup>74</sup>. Co-infections amongst genital mycoplasmas have also been reported. In a study by Amirmozafari *et al*<sup>75</sup> simultaneous occurrence of *M. genitalium* and *U. urealyticum* was shown in 1.4 per cent of women with genital infections, while triple infection of *M. genitalium*, *U. urealyticum* and *M. hominis* was seen in 0.5 per cent of these patients. Other authors have also demonstrated co-existence of *M. genitalium* with other pathogens<sup>76,77</sup>.

### ANIMAL MODELS

Animal studies are useful to establish the transmissibility of *M. genitalium* in human. Although there are severe limitations of urogenital studies in animals, till now chimpanzees are considered the best animal models for studying male urethritis caused by *M. genitalium*<sup>13</sup>. Intraurethral inoculation of *M. genitalium* in male chimpanzees has been shown to lead to infection in them and the organisms were isolated from blood in some cases<sup>45,46</sup>. Animal studies

of female *M. genitalium* infections have been more rewarding than those performed in male animals. Lower primates like rhesus and cynomolgus monkeys have decreased susceptibility to infection<sup>13</sup>. Studies performed in men with NGU and their female partners showed that *M. genitalium* was transmitted sexually as efficiently as *C. trachomatis*, which has been studied in more details<sup>48,78,79</sup>. Based on both the concordance rates among partners and on DNA typing showing the same sequence type among partners, it is beyond doubt that *M. genitalium* is a sexually transmitted pathogen<sup>80</sup>, but further studies are required to confirm.

## DIAGNOSIS

Mycoplasma identification and laboratory diagnosis of mycoplasmal infections has been based on classical bacteriological tests including morphology, cultural characteristics, physiological and serological properties. New tests based on molecular analysis of genomic DNA, ribosomal RNAs, cell proteins and lipids appear to push aside the classical tests and one may expect that the molecular tests will shortly become the prevailing tests in mycoplasma identification<sup>17</sup>.

### (i) Sample collection

Specimens appropriate for the detection for *M. genitalium* by culture or non-cultural methods include urethral swab, urine, endocervical swab and endometrial biopsy. Calcium alginate, Dacron or polyester swabs with aluminium or plastic shafts are preferred. Wooden-shaft cotton swabs should be avoided because of potential inhibitory effect. Swabs should always be removed from specimens before transportation to the laboratory<sup>81</sup>. Specimens should be inoculated at the bedside whenever possible by using appropriate transport and/or culture media. SP4 is considered to be a good transport as well as culture medium for *M. genitalium*. If immediate transportation to the laboratory is not possible, specimens should be refrigerated, but not beyond 24 h. Mollicutes can be stored for long periods in appropriate growth and transport medium at -80°C or in liquid nitrogen. Storage at -20°C is deleterious to detection, even by non-culture methods. Mycoplasmas of human origin can be undertaken on the laboratory bench and/or in a class 2 safety cabinet<sup>81</sup>.

### (ii) Staining and Culture techniques

Direct detection of *M. genitalium* in the samples with the help of different staining techniques is nearly impossible due to their small size and lack of a cell wall.

However, DNA fluorochromes like Hoechst 33258 or acridine orange stain may be applied to body fluids after centrifugation. *M. genitalium* grows slowly with the prototype strain G37 producing colonies in 6 wk or more<sup>82</sup>. After initial isolation of two *M. genitalium* strains in 1981, no subsequent isolate was recovered from the genital tract until 1996, when Jensen *et al*<sup>83</sup> reported isolation of the organism on agar using tissue culture as an enrichment culture for eventual growth on Friis medium.

Sucrose phosphate based culture medium (SP4) played a major role in the discovery of *M. genitalium*. This medium designed to isolate mycoplasmas and spiroplasmas was developed by Tully *et al*<sup>2</sup> at the National Institutes of Health (NIH), Maryland, USA, and consisted of mycoplasma broth base supplemented with glutamine, yeast extract, bovine serum, penicillin and phenol red as the indicator. Evidence of mycoplasmal growth was an increase in turbidity as well as the acidic pH change from red to yellow of the phenol red indicator, due to glucose fermentation by the organism<sup>84</sup>. The layer of cells adhering to the container surface was scraped off to inoculate a solid agar medium (0.6% agarose or 0.8% Noble agar in broth base) before anaerobic incubation at 37°C. Signs of growth in the medium occurred very slowly; with a colour change only occurring after 50 days.

Another medium is the (Pleuro Pneumonia like Organisms) PPLO broth which has been extensively used for the culture of *M. genitalium*. It can also be used for transport of urethral swabs. The procedure can be made selective for mycoplasmas by filtering the sample through 0.45 µm syringe filter. The tubes are incubated in 5 per cent CO<sub>2</sub> and observed thrice daily for any colour change till 15 days. As soon as the colour change to red-pink is seen in the absence of any turbidity of the medium, the growth is subcultured to corresponding PPLO agar plates. PPLO agar plates are incubated at 37°C with 5 per cent CO<sub>2</sub> for 15 days. The plates are observed daily under the microscope for any growth till 15 days. Once the colonies appeared, the organism is identified according to standard biochemical methods and staining by Dienes and Giemsa stains as per the standard methods<sup>81</sup>. The shape of the colonies is like other mycoplasmas but not typically 'fried egg'.

Considering the susceptibility of cultured cells to mycoplasma infection with a variety of reported species, and also with the knowledge of propagation of fastidious strains of *M. hyorhinae* in cell cultures<sup>85</sup>, attempts were made to grow *M. genitalium* in cell

cultures (*e.g.* Vero cell lines)<sup>83</sup>. This approach proved to be very efficient but extremely time-consuming<sup>13</sup>. As there is no reliable medium for the direct isolation of *M. genitalium*, detection is carried out by PCR. Nevertheless, culture is important for obtaining organisms for susceptibility testing and is a topic of continuing research.

### (iii) Serodiagnosis

The classical recommended tests include growth and metabolic inhibition by specific antiserum, as well as direct and indirect immunofluorescence tests applied to mycoplasma colonies. In some cases more sensitive tests based on principles of ELISA, immunobinding, immunoblotting, immunofluorescence and immunoperoxidase test have been applied to mycoplasma diagnosis. The cross-reactions between *M. pneumoniae* and *M. genitalium* have significantly hampered the use of specific serology for diagnosis and epidemiological studies<sup>17</sup>. Till date, no serologic test for genital mycoplasmas have been standardized and made commercially available for diagnostic use.

### (iv) DNA probes and PCR

Before the development of PCR methods, a few attempts were made using DNA probes. The design of radiolabelled oligonucleotide probes targeting the 16S rRNA was reported to have a detection limit of approximately 1000 organisms<sup>86</sup> and could be tailored to detect only *M. genitalium*. Clinical studies using such probes have not been reported, but actually, the sequence information from this study was used in one of the first publications on PCR for *M. pneumoniae*<sup>87</sup>. Risi *et al*<sup>88</sup> used a cloned 256 bp fragment of unknown function to probe simulated female genital tract specimen and found a detection limit of approximately 10,000 genome copies. A whole-genomic, nick-translated probe with a reported detection limit of 10<sup>4</sup> to 10<sup>5</sup> genome copies was also used to study urethral specimens from 203 men<sup>89</sup>. No evidence suggesting an important role of *M. genitalium* in acute NGU was found, but men with recurrent NGU were more often positive. Considering that a high proportion of the patients have a very low DNA load in urogenital specimens<sup>90</sup>, it is surprising that Hooton *et al*<sup>89</sup> found an overall rate of 15 per cent *M. genitalium* positive in their study. Highly sensitive assays are needed for detection of *M. genitalium* with a high clinical sensitivity. At present, only nucleic acid amplification tests (NAATs) offer the sensitivity needed and only PCR based assays have been reported so far<sup>5,82</sup>.

The first PCR based test was published in 1991<sup>43</sup> and shortly after, Palmer and colleagues<sup>64</sup> published their method. Both were based on the MgPa DNA sequence. At that time, it was believed that an adhesin gene would be rather conserved, since it has an important role in the pathogenesis of the infection. It was, therefore, surprising that the region of MgPa flanked by the MgPa-1 and MgPa-3 primers<sup>43</sup> appeared to be variable. Different other sequence variants were found by restriction enzyme analysis, and later by sequencing<sup>90</sup>. This observation led to further studies into the genetic variability of *M. genitalium*<sup>91</sup> and to the need for alternative PCR assays<sup>92,93</sup>. The assay developed by Palmer *et al*<sup>94</sup> was a hemi-nested PCR. The experience with the MgPa-476/MgPa-903 primer-set provided further impetus to develop an assay based on the 16S rRNA gene<sup>93</sup>. One of the more recent developments in diagnostic PCR is the homogeneous real-time PCR. The first real-time PCR assay for *M. genitalium* was developed by Yoshida *et al* in 2002<sup>95</sup>. The assay was based on detection of the 16S rRNA gene. Subsequently, a LightCycler assay detecting the P115 gene (MG299) with LNA (locked nucleic acid) probes was applied for detection of *M. genitalium* and for monitoring the response to treatment in nine infected men<sup>96</sup>.

Research workers have noted the unreliability of immunological assays and some PCR tests used for detection of *M. genitalium*<sup>97</sup>, probably related to the extensive antigenic recombination characterized in genomic studies<sup>98</sup>. Several assays testing first void urine (FVU) and self-collected vaginal swabs have some evidence-based utility (sensitivity or specificity >95%) for detection of *M. genitalium* and these include multi-target real-time PCR, Gen-Probe (Gen-Probe, San Diego, CA, USA) transcription-mediated amplification research assay (GPTMARA), real-time PCR (RTPCR), MPCR Reverse Line Blot (MPCRRLB), LightCycler real-time PCR (LCRTPCR) (Roche Applied Science, Indianapolis, IN, USA) and TaqMan® real-time PCR<sup>99</sup>. In the absence of a 'gold standard' assay, local availability may govern the choice of assay used. Vaginal swabs reportedly have high sensitivity for detecting *M. genitalium*<sup>12</sup>. Presently, there is a reasonable amount of information that supports the utility of MgPa, regardless of its potential variability, as a NAAT target.

## TREATMENT

Mycoplasmas including *M. genitalium* are normally susceptible to tetracyclines and fluoroquinolones<sup>100</sup>



but resistant to those that act on bacterial cell wall components, e.g.  $\beta$ -lactams due to lack of cell wall. Due to difficulty in isolating *M. genitalium* from clinical samples, only a few reports exist of the minimum inhibitory concentration (MIC) of various antibiotics. These MICs were determined only for a few isolates of *M. genitalium* that were established and maintained in the laboratory. The antimicrobial susceptibility profile of such isolates may not precisely confirm that of current clinical isolates of *M. genitalium*. However, these studies provide some useful information, when antimicrobial agents were chosen to treat *M. genitalium* infection. Data from these studies indicate that the *in vitro* antimicrobial susceptibility profile of *M. genitalium* is similar to that of *M. pneumoniae*<sup>101</sup>. Tetracycline, erythromycin and some newer macrolides, such as clarithromycin and azithromycin, are highly active against *M. genitalium*<sup>101-105</sup>. The MIC of tetracycline, including doxycycline and minocycline, for *M. genitalium* is 0.01 to 0.05  $\mu\text{g/ml}$ <sup>101</sup>. Information on tetracycline resistance in *M. genitalium* does not exist, but *M. genitalium*-positive men with NGU and women with cervicitis may respond better to azithromycin than they to a tetracycline, possibly because of lower MICs. The MIC of erythromycin and the newer macrolides is 0.01  $\mu\text{g/ml}$  or less<sup>101</sup>. Telithromycin, which is a member of the newest macrolide derivatives called ketolides, is also highly active against *M. genitalium* with a MIC of 0.015  $\mu\text{g/ml}$  or less<sup>102</sup>. Some newer quinolones are highly active. For example, the reported MIC is 0.06 to 0.12  $\mu\text{g/ml}$  for sparflaxacin, 0.06 to 0.12  $\mu\text{g/ml}$  for grepafloxacin, 0.05  $\mu\text{g/ml}$  for gemifloxacin and 0.03 to 0.06  $\mu\text{g/ml}$  for trovafloxacin<sup>103-105</sup>. However, ofloxacin and ciprofloxacin show only moderate activity at 0.5 to 1 and 2  $\mu\text{g/ml}$ , respectively<sup>101,102</sup>. Till now no universally accepted standards are established for pH, medium, incubation conditions, or duration of incubation for performing mycoplasmal susceptibility test. No MIC breakpoints specific for these organisms are endorsed by any regulatory agency.

Till date, there are no published guidelines or recommendations for treating *M. genitalium* positive urethritis. Only a few results of antimicrobial chemotherapy in men with *M. genitalium* positive urethritis have been reported<sup>44,50,52,58</sup>. In addition, these studies have some limitations, such as a small number of patients and no detection of other potentially important genital mycoplasmas or other types of organisms. Therefore, it is impossible to draw conclusions on the best strategy for managing *M. genitalium* positive nongonococcal urethritis<sup>3</sup>. As

a specific microbiological diagnosis of mycoplasmal infection is seldom made, appropriate treatment provides antimicrobial coverage for the organisms that cause the particular syndrome. Accordingly NGU is treated with doxycycline (100 mg orally twice a day for 7 days) or azithromycin (1.0 gm as a single oral dose) to provide activity against *C. trachomatis*, *U. urealyticum* and *M. genitalium*<sup>106</sup>. Gambini *et al*<sup>50</sup> observed that doxycycline treatment resulted in symptom alleviation and microbiological cure of infection in 94.3 per cent patients with *M. genitalium* positive nongonococcal urethritis. In NGU cases persistent *M. genitalium* in the urethra is associated with recurrent NGU. Thus, in patients with *M. genitalium* induced NGU a longer course of antibiotic treatment may be recommended as recommended a 2 or 3 wk course of antibiotic treatment in case of *M. pneumoniae* pneumonia<sup>3</sup>. The slow growth *M. genitalium*, and its ability to invade and multiply in the epithelial cells might account for its persistence in urethra or other sites, specially following treatment<sup>60</sup>. *M. genitalium* was found in some patients with chronic NGU who had been treated originally with doxycycline (100 mg/day for 10 days), and a 6 wk course of erythromycin (500 mg QDS)<sup>107</sup>. Others are found *M. genitalium* persisting in some patients with acute NGU after treatment with doxycycline (200 mg followed by 100 mg for 13 days) or erythromycin<sup>108</sup>, or after treatment with tetracycline (500 mg BD for 10 days)<sup>58</sup> or after treatment with levofloxacin (100 mg TDS for 14 days)<sup>58</sup>. In one study an azithromycin regimen comprising 500 mg in a single dose on day 1 followed by 250 mg OD for 4 days provided excellent cure rates<sup>60</sup>. Further studies are needed to determine the most appropriate treatment for *M. genitalium* positive acute and chronic NGU and other *M. genitalium*-associated infections.

## Conclusion

*M. genitalium* is one of the smallest prokaryote capable of replication, lacks a cell wall and has a characteristic pear/flask shape with a terminal tip organelle. *M. genitalium* has several virulence factors that are responsible for its pathogenicity. The latter includes its ability to adhere to host epithelial cells using the terminal tip organelle with its adhesins, the release of enzymes and the ability to evade the host immune response by antigenic variation. *M. genitalium* has emerged as an important cause of sexually transmitted infections in recent times and it is now known as established cause of non gonococcal urethritis (NGU) in men. It is reasonable to conclude

that it is strongly associated with mucopurulent cervicitis in women. The evidence for *M. genitalium* PID and infertility are quite convincing and indicate that this organism has potential to cause ascending infection but more studies are needed to understand the relationship between *M. genitalium* and reproductive tract disease in women. Further research is required to understand the dynamics of HIV and *M. genitalium* co-infections. Though there are variety of in-house PCRs which have been developed for diagnosing the *M. genitalium* infections, there is a need of highly accurate internationally validated and approved commercial NAAT. All the present evidences support the use of azithromycin as first drug of choice for *M. genitalium* infections. In conclusion, *M. genitalium* is an important cause of sexually transmitted infections in both men and women and should be treated with azithromycin when required.

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