An update on prevalence, diagnosis, treatment and emerging issues of genital mycoplasma infection in Indian women: A narrative review

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Abstract
Despite adequate treatment of reproductive tract infection, there is persistence of symptoms in some patients. This raises the possibility of existence of other silent microbes with pathogenic potential. Apart from the common sexually transmitted organisms such as Chlamydia trachomatis and Neisseria gonorrhoeae, there are other silent and emerging pathogens, like genital mycoplasma, which have been associated with cervicitis, pelvic inflammatory disease, infertility, and pregnancy-related complications in women. Although these organisms were identified decades ago, they are still overlooked or ignored. There is a need to understand the role played by these organisms in Asian populations and their susceptibility to the standard line of treatment. Data on genital mycoplasma infections in Indian women is heterogeneous, with limited evidence of pathogenicity. Although known for their wide spectrum of reproductive morbidities in western counterparts, these microorganisms are yet to gain the attention of Indian clinicians and microbiologists. There is paucity of adequate information in India regarding these infections, so Indian literature was compiled to get an overview of these pathogens, their association with reproductive morbidities, and their response to treatment. Thus, there is a need to explore genital mycoplasma infections in Indian women, especially in the arena of antimicrobial resistance among genital mycoplasma, which has the potential to become a major problem. A literature search with keywords focusing on "genital mycoplasma", "sexually transmitted infections India", "sexually transmitted mycoplasma", and "characteristic of mycoplasma" was carried out through computerized databases like PubMed, MEDLINE, Embase, and Google Scholar.

Key words: Characteristics of mycoplasma, genital mycoplasma, sexually transmitted infections India, sexually transmitted mycoplasma

Introduction
Reproductive tract infections (RTIs) have a profound impact on sexual and reproductive health worldwide. More than 30 different bacteria, viruses, and parasites are known to be transmitted through sexual contact.1 World Health Organization (WHO) report on global sexually transmitted infection surveillance 2015 has estimated that the global estimate of new curable sexually transmitted infection (STI)/RTI cases attributed to Chlamydia trachomatis, Neisseria
gonorrhoea, and Trichomonas vaginalis was about 130.9, 78.3, and 142.6 million, respectively. This roughly contributes to 1 million RTI/STI per day. Prolonged lower genital tract infections from different pathogens may result in inflammation of the upper genital tract leading to morbidities such as infertility, ectopic pregnancy, and chronic pelvic pain. Therefore, it is essential to evaluate various microbial pathogens causing RTIs including the silent pathogens. Genital mycoplasmas are amongst these silent pathogens whose prevalence, pathogenic potential, and response to treatment in Indian women have been explored very minimally. Due to this paucity of adequate information about genital mycoplasma in Indian women, this review was undertaken. The objectives of the review were:

1. To summarize the existing literature on prevalence of genital mycoplasma and the reproductive morbidities caused by them in Indian women.
2. To provide clinical guidance regarding the detection and treatment of genital mycoplasma in susceptible women.

Materials and Methods
An English language literature search was performed using electronic database of PubMed, MEDLINE, Embase, and Google Scholar. Grey literature search was done by going through government policies, information manuals and WHO Fact sheet which are available online. The following keywords were used for the search: genital mycoplasma OR sexually transmitted infections India OR sexually transmitted mycoplasma OR characteristic of mycoplasma. The personal knowledge and experience of authors in the field helped in archiving the relevant articles. Studies which met the following criteria were included in the current review:

1. English language publications.
2. Those focusing only on genital mycoplasma.
3. Studies pertaining to the association of genital mycoplasma only in Indian women.

All the studies meeting the inclusion criteria have been described in brief. Data from these has been compiled and interpreted to give a comprehensive overview of the role of mycoplasma infections in Indian women.

Results
The early diagnosis and treatment of STI/RTI can prevent serious complications and long-term sequelae affecting reproductive health. Other than the common organisms causing STI/RTIs, there are other silent microorganisms such as Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum, and Ureaplasma parvum which are associated with cervicitis, pelvic inflammatory diseases, infertility, and lower genital tract infections in women.

Most of these organisms are found very frequently in the urogenital tract, and according to circumstances they can be commensals or pathogens. They have been associated with various morbidities individually, among which Mycoplasma hominis is well associated with bacterial vaginosis, Ureaplasma is known for adverse pregnancy outcomes, and more recently Mycoplasma genitalium is being associated with cervicitis and infertility.9-14

Mycoplasma – characteristics and pathogenesis
Genital mycoplasma belongs to family Mycoplasmataceae and order Mycoplasmatales.15 This family consists of genera Mycoplasma and Ureaplasma. Ureaplasma can be distinguished from Mycoplasma by the presence of a urease enzyme, which hydrolyses urea to produce 95% of its energy requirements.16 Although known as a separate group of organisms since 1898, it was only in 1937 that the first isolation of genital mycoplasma in humans was done. This discovery further led to extensive investigations of the interactions of mycoplasmas with the human host.17 Further studies showed that mycoplasmas were frequent inhabitants of the mucous membranes of the urogenital and upper respiratory tracts. Initially, these genital mycoplasmas were considered just as commensals, but subsequently some of the mycoplasma species were attributed to have a definitive or possible pathogenic role in reproductive morbidities.12,14,18-20 The lack of knowledge regarding the commensal versus pathogenic potential of these organisms in developing countries is due to their fastidious growth and poor laboratory infrastructure for their diagnosis.

Out of the six commonly found genital mycoplasmas, four species viz. Mycoplasma hominis, Ureaplasma, Mycoplasma genitalium, and Mycoplasma penetrans have a definitive or possible pathogenic role as discussed in Table 1. The other two species Mycoplasma primatum and Mycoplasma spermaphilum are considered to be non-pathogenic in humans.21 The majority of human Ureaplasma isolates belong to the proposed new species U. parvum (biovar 1, parvo), which includes serovars 1, 3, 6, and 14. Ureaplasma urealyticum (biovar 2, T960) includes all other serovars and is isolated less frequently.22

| Number | Organism                  | Colonization            | Commonly associated diseases                        |
|--------|---------------------------|-------------------------|-----------------------------------------------------|
| 1      | M. hominis                | Genital tract, urinary tract | Bacterial vaginosis13 Decreased gestational age14 PPROM19 |
| 2      | Ureaplasma species        | Genital tract, urinary tract | Adverse pregnancy outcome12 Infertility17 PPROM19 |
| 3      | M. genitalium             | Genital tract, urinary tract | Cervical inflammation14 Infertility7 |
| 4      | M. penetrans              | Genital tract, urinary tract | Antiphospholipid syndrome20 |

PPROM: preterm premature rupture of membranes; M. genitalium: Mycoplasma genitalium; M. hominis: Mycoplasma hominis; M. penetrans: Mycoplasma penetrans
This review focuses on prevalence, diagnosis, treatment, and emerging issues.

**Prevalence of genital mycoplasma among women with genital tract infections in India**

There is paucity of adequate information from India regarding the prevalence of Mycoplasma in women with genital infections. A literature search retrieved eight studies which have been done to estimate the prevalence of genital mycoplasma in Indian women (tabulated in Tables 2–4). This section has been subclassified into prevalence of various genital mycoplasmas as estimated in different studies.

**Prevalence of Ureaplasma species and Mycoplasma hominis in genital infections**

*Ureaplasma* species and *Mycoplasma hominis* are the more commonly investigated genital mycoplasma. Studies as summarized in Tables 2 and 3, carried out in sexually active Indian women with genital infections showed a prevalence of *Ureaplasma* species ranging from 31% to 44.5%, and of *Mycoplasma hominis* 14 per cent. All these studies showed a higher prevalence of *Ureaplasma* species when compared with *Mycoplasma hominis* in patients with genital infections. The first study was done in India by Kapur et al. in 1975 at All India Institute of Medical Sciences on 70 married females and 50 controls suffering from leucorrhoea to estimate the prevalence of “T” *Mycoplasma*. Twenty-two (31.5%) and five (10%) patients were found to be positive for “T” *Mycoplasmas* among leucorrhoea and non-leucorrhoea cases, respectively. This difference was highly significant. These “T” *Mycoplasma* were named so because of their tiny colony size when compared with *Mycoplasma hominis* which are large colony forming bacteria, and were later termed as *Ureaplasma urealyticum*. This study attributed promiscuity as an important risk factor for mycoplasma infections. Bhatt et al. in 1985 reported the isolation of *Mycoplasma hominis* or *Ureaplasma urealyticum* in 27% in women with genital tract infection. *Ureaplasma* was considered to be the predominant organism by both Bhatt et al. in 1989 and Bhandari et al. in 2000 with a prevalence of 38 percent. Dhawan et al. in 2006 and 2012 did more extensive studies determining not only prevalence of *Ureaplasma* species but also comparing their pathogenicity and their antimicrobial susceptibility patterns. They reported that *Ureaplasma parvum* (biovar 1) was predominant in Indian women with genital tract infection. Of these, *Ureaplasma parvum* serovar 3/14 was the most frequent isolate in women with genital infections followed by either serovar 6 or serovar 1. But a recent study done by Arif et al. in 2017 shows a shift toward biovar 2.

| Author and year | Location  | Test used | Sample size | Study population | Prevalence (%) |
|-----------------|-----------|-----------|-------------|------------------|----------------|
| Kapur et al., 1975 | New Delhi | Culture   | 70          | Vaginitis         | 31.5           |
| Bhatt et al., 1989 | Bombay    | Culture   | 225         | Genital tract infection | 38.6          |
| Bhandari et al., 2000 | Chandigarh | -         | 50          | Cervicitis       | 38             |
| Dhawan et al., 2006 | New Delhi | PCR       | 83          | Symptomatic sexually active | 44.5         |
| Dhawan et al., 2012 | New Delhi | Multiplex PCR | 88 | LGTI                  | 31             |

LGTI: lower genital tract infection; PCR: polymerase chain reaction

| Author and year | Location  | Test used | Sample size | Case population | Organisms tested | Prevalence (%) |
|-----------------|-----------|-----------|-------------|-----------------|------------------|----------------|
| Sethi et al., 2013 | Chandigarh | PCR       | 200         | Women with mucopurulent cervicitis | *M. genitalium* | 11             |
| Saigal et al., 2016 | New Delhi | PCR       | 164         | Male with urethral discharge and female with vaginal discharge | *U. urealyticum* | 15.2          |
| Arif et al., 2017 | New Delhi | PCR       | 221         | Male with urethral discharge and female with vaginal discharge | *U. urealyticum* | 22.6          |
| Arif et al., 2017 | New Delhi | PCR       | 221         | Male with urethral discharge and female with vaginal discharge | *M. hominis* | 29             |
|                |           |           |             |                  | *M. genitalium* | 1.2            |
|                |           |           |             |                  | *M. hominis* | 5              |

PCR: polymerase chain reaction; *M. genitalium*: *Mycoplasma genitalium*; *U. urealyticum*: *Ureaplasma urealyticum*; *M. hominis*: *Mycoplasma hominis*
Prevalence of Mycoplasma genitalium in genital infections

*Mycoplasma genitalium* discovered in 1981 has been a well-established cause of nongonococcal urethritis in males.\(^{30}\) Recent literatures from western population have attributed it to be a causative agent for cervicitis, pelvic inflammatory diseases, and lower genital tract infection.\(^{6,31,32}\) Indian studies to determine the prevalence of *Mycoplasma genitalium* in Indian women are few in number. We found three studies (tabulated in Table 4), that have shown the prevalence of *Mycoplasma genitalium*. The studies done by Saigal *et al.* and Arif *et al.* have shown much lower prevalence between 0.5% and 1.2% when compared with Sethi *et al.* which estimated the prevalence to be 11% among women diagnosed to have clinical cervicitis.\(^{4,27,28}\) This difference is probably due to diversity in the recruitment of susceptible population. Although the studies done by Saigal *et al.* and Arif *et al.* are among the recent ones to study the prevalence of *Mycoplasma genitalium* in Indian population, the exact burden of infection in female population could not be estimated from these studies. Due to difficulty in diagnosis, potential for damage to reproductive health of women, and lack of data in the Indian population, future research on *Mycoplasma genitalium* is warranted. Negligible literature is available with respect to *Mycoplasma penetrans*.

**Prevalence and association of genital Mycoplasma with other reproductive morbidities**

Data about prevalence of genital mycoplasma in women with other reproductive morbidities such as infertility, preterm birth, low birth weight infants, and bad obstetric history are scanty and varied.

**Infertility**

It is essential to evaluate various microbial pathogens causing RTIs, including the silent pathogens, as they all increase the infectious sequelae and have an ultimate impact on human reproduction. In India, few studies have been carried out to evaluate the role of genital mycoplasma in infertile Indian women. As ascertained by Dhawan *et al.* and Gupta *et al.*, the prevalence of *Ureaplasma* species is about 20.8%–32% which was much more when compared to an average 5% prevalence of *Mycoplasma hominis*.\(^{5,33}\) Only one study by Rajkumari *et al.* has researched the association of *Mycoplasma genitalium* with infertility in women. This case–control study on 100 women found a much higher prevalence of *Mycoplasma genitalium* to be present in cases (16%) when compared with controls which was highly significant.\(^{7}\)

**Birth outcomes**

Vaginal swabs of pregnant women were tested by Choudhury *et al.* in 1994 and Paul *et al.* in 1998 for colonization of genital mycoplasma and the results were correlated with birth outcomes.\(^{34,35}\) These studies were undertaken with the aim to explore the infective etiology in women with adverse pregnancy outcome and thereby initiate prevention by treatment with antimicrobial drugs. However, these two studies found no correlation between low birth weight and infection with genital mycoplasma. Contrary to this, Tellapragada *et al.* in 2016 found positive association between colonization of *Mycoplasma hominis* with preterm birth and low birth weight. The same study found *Ureaplasma urealyticum* to be a significant risk factor for premature rupture of membranes.\(^{36}\)

**Bad obstetric history**

Gogate *et al.* detected IgG antibodies against *Mycoplasma hominis* in 365 women with bad obstetric history. *Mycoplasma hominis*–specific antibodies were found in 27% of women, when compared with controls, which was highly significant.\(^{37}\)

However, considering each subgroup, these studies are inadequate to draw meaningful conclusion from them. More analytical studies are required to determine the association between genital mycoplasma and various reproductive morbidities.

**Diagnosis of genital mycoplasma infection**

Samples used for detection of genital mycoplasma in women are varied. Commonly used samples are endocervical swabs,\(^{2,28}\) first void urine,\(^{38}\) vaginal swabs,\(^{31}\) and endometrial biopsy.\(^{7}\) For swab specimens, the use of only Dacron or polyester swabs with aluminium and plastic shafts is recommended as cotton and wooden swabs can have potential inhibitory effect.\(^{39,40}\) It is important to use appropriate transport medium after collection of samples as they can have a deleterious effect on the performance of the real-time polymerase chain reaction (PCR) assay. Swabs are transported in 2 mL of pleuropneumonia-like organisms’ medium broth containing urea for *Ureaplasma urealyticum*, and arginine for *Mycoplasma hominis*. SP4 is considered to be a good transport and culture medium for *Mycoplasma genitalium*.\(^{39}\) Nowadays, molecular methods are commonly used for detection of genital mycoplasma. Dhawan *et al.* in 2005 was the first to evaluate the diagnostic efficacy of culture versus PCR in Indian adults with symptom of genital infections. The detection rate of *Ureaplasma* was 13% more by PCR when compared with culture.\(^{26}\) A multiplex PCR targeting the urease gene of *Ureaplasma* spp. and 16S rDNA of *Mycoplasma hominis* is the commonly used molecular method for their detection. For slow-growing organisms, such as *Mycoplasma genitalium*, nucleic acid amplification detection is the only practical means for rapid microbiological diagnosis. Therefore, PCR is also being performed to detect *Mycoplasma genitalium* by targeting the 140-kDa adhesion gene using primers MgPa-1 and MgPa-3.\(^{28}\)

**Treatment of genital Mycoplasma – Indian scenario**

National Guidelines on Prevention, Management and Control of RTIs and STIs 2014 released by the Ministry of Health and Family Welfare, Government of India, has identified *Mycoplasma* and *Ureaplasma* as a potential causative agent
for vaginitis, along with the more common organisms such as Trichomonas vaginalis and Candida albicans. Mycoplasma genitalium has been recognized as a potential causative agent for persistent or recurrent cervicitis in these guidelines. Treatment of lower genital tract infection in Indian women is based on the WHO-guided syndromic approach. Although syndromic approach is an excellent management scheme in patients with STI in low-resource settings, it can be revised depending on the epidemiological pattern of various microbes prevalent in the population. Under the National AIDS Control Programme (previously known as National STI/RTI Control Programme), 10 regional STI training, research, and reference laboratories (RSTRRL) have been established, under an overarching apex STD center, across the country through high-quality etiological testing of STI/RTI. But before any steps to revise the treatment of genital infection are undertaken, more extensive studies in India are required for understanding the epidemiology and pathogenesis of genital mycoplasma infections. This will facilitate the development of better strategies for their treatment and prevention.

**Emerging issue of antimicrobial resistance**

Although syndromic management used in the treatment of lower genital tract infections is the most cost-effective approach, periodic surveillance of the susceptibilities of mycoplasma to these antibiotics in use is necessary to ensure treatment efficacy and provide updated information regarding development of antimicrobial resistance. Commonly used drugs in India for treatment of genital tract infections are from the macrolide group, such as azithromycin, quinolones, such as fluorquinolones which include ofloxacin, and doxycycline which is a tetracycline. Emerging antimicrobial resistance especially against Mycoplasma genitalium is a major concern in western countries. The 7-day treatment of doxycycline is highly ineffective against Mycoplasma genitalium with a median cure rate between 21% and 45%. Due to the emerging resistance in western countries, the cure rate of single-dose azithromycin is only 72%–87%. European guidelines have suggested that widespread use of 1 g azithromycin is contributing toward the resistance of Mycoplasma genitalium to this agent. These guidelines recommend a longer course of azithromycin 500 mg stat, followed by 250 mg for 4 days. This is superior to the single dose of azithromycin. A fourth-generation fluorquinolone, moxifloxacin in the dose of 400 mg daily for 7–10 days was recommended as a second-line drug for treatment of uncomplicated macrolide-resistant Mycoplasma genitalium infection. However, there are recent reports of treatment failure with moxifloxacin probably due to resistant mutations. Therefore, newer drugs like pristinamycin, solithromycin, sitafloxacin, and lefamulin are being explored in western countries for their therapeutic potential.

From the Indian perspective, only three studies have been done to evaluate antimicrobial resistance in women with genital mycoplasma infection, none of which includes Mycoplasma genitalium. Saigal et al. in 2014 did a recent study to evaluate the susceptibility of genital mycoplasma to commonly used antibiotics by microbroth dilution method. Although all isolates of Ureaplasma were susceptible to ofloxacin and josamycin, an intermediate level resistance towards doxycycline and azithromycin was noted in 4% and 8% of strains, respectively. All strains of Mycoplasma hominis were uniformly susceptible to doxycycline, ofloxacin, and josamycin. Dhawan et al. in 2012 observed resistance to ofloxacin and azithromycin of intermediate nature against all the ureaplasma isolates. These studies suggest that though previously these organisms were susceptible to all antibiotics as suggested by Ghosh et al. in 2011 an antimicrobial resistance of variable pattern is emerging toward doxycycline and azithromycin in Indian women. An increasing minimum inhibitory concentration against these two drugs was observed by Saigal et al. thereby warranting continuous surveillance of antimicrobial susceptibility patterns. There is a need for updated information on the susceptibility pattern of these microbes which will help in modifying the treatment guidelines if required.

**Discussion**

It is premature to start screening the entire population for genital mycoplasma. Despite the availability of molecular methods, genital mycoplasma infections have often been left undiagnosed when compared with the more common STI/RTIs. This is probably due to financial constraints in developing countries for the use of molecular methods required for detection. Prevalence studies from India are mainly focused on women with lower genital tract infection. Fewer attempts have been made to estimate community-based prevalence and prevalence in women with other reproductive morbidities. The prevalence of Ureaplasma urealyticum is more than Mycoplasma hominis, and least of Mycoplasma genitalium, in Indian women with lower genital tract infection. The result of these studies corresponds to studies conducted in populations of different ethnicity by Zhu et al. and Kechagia et al. Active sexual behaviour and multiple sexual partners are important risk factors for genital mycoplasma infection, along with contributory factors such as smoking, low socioeconomic status, and lack of education. Lack of knowledge about STI/RTI prevention and poor healthcare seeking behaviour, could be other factors contributing.

While association of genital mycoplasma with various morbidities in the western population has been extensively studied, the data from Indian population is sparse and heterogeneous. Mycoplasma infections have been associated with upper genital tract infections and urinary morbidities such as urethritis and pyelonephritis in women. Because of the close anatomical relationship between the female urethra and genital tract, it is assumed that ascending infections can affect the upper genital tract and the urinary tract. The pathogenic potential of genital mycoplasma with respect to these morbidities in Indian women remains unassessed.
There are serious lacunae with respect to the studies evaluating pathogenic potential and response to treatment of genital mycoplasma infections in Indian women. Studies on association of these genital mycoplasmas with disease are expensive and require sophisticated infrastructure to do highly specific serologic, nucleic acid, and epidemiologic tests. It will be worthwhile to generate evidence with test of cure done using molecular methods after treatment with 1-g azithromycin, routinely given under syndromic management. Later, the focus can be shifted to explore other treatment options or mutation studies in treatment-resistant cases.

More analytical studies are required in India for better understanding of not only the prevalence but also the epidemiology and pathogenesis of genital mycoplasma. In future, more randomized control trials are needed to identify the best treatment modality of genital mycoplasma infections. This would facilitate the development of better strategies for their treatment and prevention based on the antimicrobial susceptibility patterns of these microbes.

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