Mycoplasma genitalium: A new superbug

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Abstract

Mycoplasma genitalium (MG) is an emerging sexually transmitted pathogen. It is an important cause of nongonococcal urethritis in men and is associated with cervicitis and pelvic inflammatory disease in women, putting them at risk of infertility. Multiple factors that aid pathogenesis of MG include its ability of adhesion, gliding motility, and intracellular invasion by means of the tip organelle. Through intracellular localization and antigenic variation, MG could result in treatment-resistant chronic infection. There are limited data on the prevalence of MG in Indian patients with urogenital syndromes. Recently, a high prevalence of extra genital infection with MG has been reported. Molecular assays are the major diagnostic techniques of MG infection. Antimicrobial agents such as macrolides, along with fluoroquinolones, are the treatment of choice for MG infections. The issue of drug resistance to azithromycin and fluoroquinolones in MG is rising globally. As molecular tests are becoming available for MG, both for the diagnosis and the detection of antimicrobial resistance, any patient with MG infection should then be tested for antimicrobial resistance. Consideration of MG as a cause of sexually transmitted disease in the Indian population is crucial in diagnostic algorithms and treatment strategies. The purpose of this review is to understand the prevalence of MG in different clinical scenarios, molecular mechanisms of pathogenesis, current status of antimicrobial resistance, and its impact on MG treatment.

Key words: Antimicrobial resistance, extragenital infections, *Mycoplasma genitalium,* pathogenesis

Introduction

Mycoplasma genitalium (MG) is an emerging cause of sexually transmitted infection (STI) in both men and women. It is a common cause of nonchlamydial nongonococcal urethritis (NCNGU) in men and cervicitis, pelvic inflammatory diseases (PIDs), and tubal infertility in women.[1] The organism may also play a role in increasing the risk of human immunodeficiency virus (HIV) infection.

MG was first isolated in 1980 from the urethral swabs of two homosexual men. G37 and M30 were the first MG strains to be isolated.[2] MG is the smallest free living, self-replicating microbe with minimal genome (580 kb). It was the first bacteria to be fully sequenced and the first genome to be chemically synthesized.[3]

MG belongs to the class *Mollicutes* and family *Mycoplasmataceae* that colonizes the male and female reproductive tract. Multiple species of *Mycoplasmataceae* family are present as both commensals and pathogens of human genital tract. The pathogenic genital mycoplasmas include MG, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Mycoplasma fermentans,* whereas *Mycoplasma penetrans* is of doubtful pathogenicity.

Mycoplasma primatum and *Mycoplasma spermatophilum* are the nonpathogenic genital mycoplasmas.[4]

Remarkably, MG has a disturbing capacity to develop resistance to the major antimicrobials available against it: macrolides and fluoroquinolones. The lack of peptidoglycan in MG precludes the use of antibiotics acting on the cell wall. Although resistance in other STI pathogens such as gonococcus has increased insidiously, resistance in MG has emerged at a relatively greater speed belying its small size.[2] This review on MG focuses on the recent advances in the developments of its molecular pathogenesis and antimicrobial resistance. We surveyed PubMed literature and Google Search engine using the terms "*Mycoplasma,*" "MG," and "Genital *Mycoplasma.*" The relevant literatures were selected to provide current perspectives of MG.

Genome of *Mycoplasma Genitalium*

MG has a minimal genome size of 580 kbp. The GC content is approximately 31%. MG evolved from clostridium-like Gram-positive bacteria by genomic reduction process during which it lost most of the genes

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including the genes for enzymes involved in amino acid synthesis, *de novo* nucleic acid synthesis, and synthesis of fatty acid. Hence, MG relies on the host for several metabolic growth factors. MG type strain G37 was the second bacterial genome to be fully sequenced.

Pathogenesis

Multiple factors that aid pathogenesis of MG include the ability for adhesion, gliding motility, and cell invasion. All these functions are performed by a specialized tip structure. However, the mechanisms by which MG is able to maintain infection within the stratified squamous epithelia of the vagina and ectocervix despite normal sloughing of the apical most layers remains unknown.[5]

Tip organelle

The tip organelle is a multisubunit dynamic motor[6] made of the following three parts: terminal button, segmented pair plates, and wheel complex. Each part consists of different proteins. Table 1 shows genes encoding for tip organelle proteins and their subcellular localization.[7]

Terminal button

The terminal button is the distal end of the tip organelle. It comprises P110, P140, P65, P32, and HMW1 proteins. Among these proteins, P110 and P140 are the major adhesions encoded by *MG192* and *MG191,* respectively.[8] These two proteins are immunological determinants and important for the organism to adhere to the host epithelial cells. They are also required for the proper development of the terminal organelle. Burgos *et al.* showed that *MG191* and *MG192* mutant cells showed a loss of terminal organelle, suggesting the absolute requirement of both the P140 and P110 proteins for the proper development of such a structure.[9]

Adhesion proteins of MG and P1 protein of *Mycoplasma pneumoniae* exhibit homology at both DNA and protein level. At DNA level, 48% of coding sequence of adhesion genes of MG was 60%–70% homologous to the sequence of P1 adhesion gene. Figure 1 is showing clinical samples positive for MG using MgPa1 and MgPa3 primers for amplification. At protein level, 85% of the deduced amino acid sequence of MG adhesin exhibited 42%–74% identity with *M. pneumoniae* P1 protein.[10]

The functions of P65 and HMW1 proteins include the determination of the curvature of terminal button and for assembly of the tip organelle, respectively.[9]
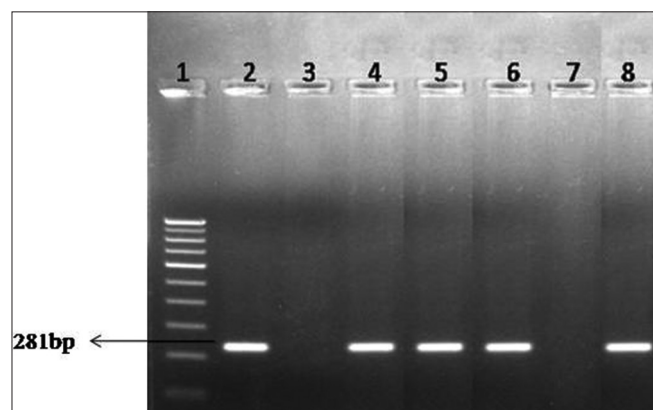

Figure 1: Agarose gel electrophoresis for ***Mycoplasma genitalium.*** Lane 1: 100 bp DNA ladder, Lane 2: Positive control, Lane 3: Negative control, Lane 4–6: Clinical samples-positive, Lane 7: Clinical sample-negative, Lane 8: Clinical sample-positive

Segmented pair plates

They form an electron dense core that is a part of the mycoplasma cytoskeleton. HMW3 protein is involved in the formation of terminal button and contributes to anchoring the electron dense core to the cell membrane.[7]

Wheel complex

The proximal end of the electron dense core is in contact with the wheel complex. It is connected to the cell periphery by fibrils and it is thought to be the connecting link between terminal organelle and cell body. The wheel complex contains MG219, MG200, MG386, and MG491 proteins and which have been implicated in gliding motility.[7]

Virulence Factors

Adhesion

It is an essential process for MG pathogenicity. Adhesion is the primary step in the initiation of an infection/colonization. MG adheres to the plastic and glass surfaces, epithelial cells,[11] spermatozoa,[12] and erythrocytes. Adhesion process is achieved by the major adhesion proteins P140 and P110.

Gliding motility

MG does not contain flagellum for its motility. Gliding motility of this organism is achieved with the help of the specialized tip organelle, mainly by the proteins that are forming the wheel complex (MG200, MG386, MG219, and MG491). Of these proteins, MG 200 and MG386 are specific for gliding motility.[13]

Luca observed that MG491-deficient MG showed alteration in gliding motility.[7] Burgos *et al.* showed that *MG191* and *MG192* negative mutants showed decreased MG386 protein. This observation reinforces the close connection between gliding motility and adherence machineries.[9]

Gliding motility is essential for the penetration of MG into the mucus layer covering the epithelial cells followed by adhesion to the epithelial cells and invasion.[2]

Intracellular localization

MG is a facultative intracellular pathogen. After attaching to the epithelial cells, cell entry is mediated by the tip organelle. Exact mechanism of entry of the organism is still not known. In a study by Mernaugh *et al.,* attachment of MG into human lung fibroblasts was followed by the formation of cup or depression in the plasma membrane. The membrane pockets resembled clathrin-coated pits,

Table 1: Genes encoding for tip organelle proteins and their subcellular localization[7]

Gene	Protein	Sub cellular localization
MG191	P140	Surface of TO
MG192	P110	Surface of TO
MG200	DNAj like protein	Wheel complex
MG217	P65 homolog	Terminal button
MG218	HMW2	Rod
MG491	MG491	Wheel complex
MG219	MG219	Wheel complex
MG312	HMW1	Rod
MG317	HMW3	Terminal button
MG318	P32	Terminal button
MG386	P200	Wheel complex

TO=Tip organelle; MG=*Mycoplasma genitalium*; HMW=High molecular weight

suggesting that the mycoplasma might adhere to and enter the cells by a site-directed, receptor-mediated event resembling cell entry by Chlamydiae.[14] Ueno *et al.* observed intranuclear localization of MG proteins.[15] Intracellular localization protects the organism from the both host immune system and antibiotics. It also promotes chronic and latent infection.[16,17]

Enzymes

MG lacks toxins and secreted virulence factors. However, MG186 acts as a calcium-dependent membrane-associated nuclease which degrades the host nucleic acid and provides nucleotide precursors for growth and pathogenic processes.[18]

Genetic variation

MG is able to generate a high frequency of intragenomic variation in nucleotide sequence or DNA arrangement at selected chromosomal loci (*Mgpar* islands and *Mgpa* operon) promoting random phenotypic variation as a result of constantly changing host environment.

Mgpar islands are found in the genome of MG. *Mgpar* islands are a family of repetitive DNA elements with homology to the *MgPa* adhesin gene (*MgpB*/*MgpC*).[19] As explained earlier, *MG191* (*MgpB*) and *MG192* (*MgpC*) encode for P140 and P110 major adhesins, respectively. Both these genes are present in the *Mgpa* operon. These *Mgpar* islands do not express protein coding sequences but are involved in genetic variation through recombination between *Mgpar* islands and *Mgpa* operon.[20]

There are two types of genetic variation; antigenic variation and phase variation.

Antigenic variation

In order to escape host immune response, MG alters its entire genetic sequence of the *Mgpa* adhesion gene with subsequent generation of variants that are not recognized by the host immune system on subsequent encounters.

Mechanism for antigenic variation

Antigenic variation arises when the variable region of the expression site (*MgpB*/*MgpC*) exchanges sequences through segmental recombination with ≥1 of *Mgpar* sites.[20] This recombination process is reciprocal. Furthermore, antigenic diversity achieved by initial recombination within *Mgpar* islands followed by recombination with *MgpB*/*MgpC* genes.[21]

Phase variation

In phase variation, the organisms will lose their ability to adhere to the epithelial cells due to loss of their major adhesion proteins.

Mechanism of phase variation

It arises by multiple recombination processes involving both the variable and conserved regions of expression sites (*MgpB*/*MgpC*) with *Mgpar* islands. This recombination results in translocation of the conserved *MgpB*/*MgpC* sequences to participating *Mgpar* sites, thereby leaving an incomplete *Mgpa* operon.[20]

Regulation of recombination

Recombination is a highly regulated process. In MG, MG428 is a positive regulator of this recombination process.[22] It co-ordinates the expression of the key genes of recombination such as *recA, ruvA, ruvB,* and other proteins required for recombination.[23] MG428 is considered as an alternative sigma factor since, it binds to the RNA polymerase and unique promotor sequence present upstream of the MG428-activated gene in response to external stimuli.

Clinical Scenarios

MG has potential to cause clinical disease, in both men and women but more so than women. Despite its identification nearly four decades ago, much remains unclear. While there is a clear association with NGU in men, the clinical evidence that it causes epididymo-orchitis, proctitis, reactive arthritis, and facilitates HIV transmission in men is weak, although biologically plausible. It is not known how long asymptomatic infection persists in untreated men, nor the risk of developing disease if left untreated. Although there is evidence of sexual transmission from male to female, it is unclear how often this occurs and of the risk of developing reproductive tract disease. Summary of studies showing the prevalence of MG in different population groups is mentioned in Table 2.

Relationship Between *Mycoplasma Genitalium* and Disease in Men

Acute *nongonococcal urethritis*

MG was isolated initially from men with acute nongonococcal urethritis (NGU). In numerous studies, MG has been strongly and almost uniformly associated with acute NGU, in which diagnosis was done by microscopy. In one study,[24] in which the diagnosis was based on clinical symptoms and signs only, the association with MG was weaker, as those subjects were not recorded as having NGU but had microscopic evidence of disease. Overall, MG has been detected in the urethras of 15%–25% of men with symptomatic NGU, compared to about 5%–10% of those without disease. Among sexually transmitted disease (STD) clinic populations, 90% of MG-infected men have microscopic evidence of urethritis, with a complaint of discharge being more common than in NGU of other etiologies.[25] Indeed, there is evidence that MG is more closely associated with symptomatic than with asymptomatic NGU.[26,27] Furthermore, the development and the use of quantitative polymerase chain reaction (PCR) assays for MG have shown greater MG DNA loads in urine from men with NGU than in urine from those without the disease.[28,29] It is noteworthy that the association between mycoplasma and disease is even stronger for acute NCNGU,[30,31] with the mycoplasma being found in more than one-third of men with such disease, indicating that MG and *Chlamydia trachomatis* act as separate causes of the condition.

Chronic nongonococcal urethritis

Persistent or recurrent NGU following an acute attack was noted by Hooton *et al.*[32] to be associated with MG. Since then, MG has been found in up to 40% of men presenting with chronic disease after treatment with doxycycline.[33] Indeed, in several clinical studies,[27,34,35] a strong correlation was found between MG infection and persistent or recurrent NGU, probably due to tetracyclines and more recently, azithromycin[36] eradicating MG from only a subset of the patients.[37]

Balanoposthitis

Inflammation of the glans penis (balanitis) and inflammation of the prepuce (posthitis) frequently occur together (balanoposthitis). In one study,[38] MG was associated significantly ($P = 0.01$) with balanitis and/or posthitis in 114 men with acute symptomatic NGU.

Chronic *prostatitis*

Association of MG with chronic prostatitis is sparsely evidenced. In a study by Doble *et al*., MG could not be detected by PCR on ultrasound guided transperineally derived prostatic biopsy samples in fifty patients with chronic abacterial prostatitis.[39] In another study,[40] MG was detected by a PCR assay in prostatic biopsy specimens from 5 (4%) of 135 men, and in yet another study,[41] MG was detected in semen from 2 (11%) of 18 men with chronic abacterial inflammatory prostatitis, compared to 20 controls, showing insufficient evidence to suggest any significant association.

Acute epididymitis

Detection of MG in few patients during an antibiotic trial[42] indicated that MG may be a cause of acute epididymitis in some patients. An undoubted causal involvement is certainly true in the case of *C. trachomatis* and by analogy might well be so for MG. To firmly establish this, epididymal fluid should be examined whenever possible in addition to urine and/or urethral swabs.[43]

Diseases in Women

Nongonococcal urethritis

There is evidence for an association between MG and urethritis in women attending STD clinics.[44,45] The observations have been made mainly in Scandinavia, where examination of urethral smears from women is a part of routine STD examinations, and the numbers of these infections are few compared with those in men. It is clear that further studies are warranted, as it is not yet fully clear to what extent MG is involved in symptomatic or asymptomatic pyuria or in the so-called "urethral syndrome" (dysuria and frequency in women with apparently sterile urine).

Bacterial vaginosis and vaginitis

MG was first detected in the lower genital tract of about one-fifth of women attending an STD clinic at St. Mary's Hospital, London, United Kingdom,[46] and in cervical samples from 5 of 74 women in Copenhagen, Denmark.[47] However, unlike *M. hominis*, which is very strongly associated with bacterial vaginosis (BV),[48] the association of MG with BV is controversial. In some studies,[49,50] there was no evidence that MG played any part in BV, while in a one of the study,[51] the presence of MG in women was independently associated with BV, being more common in women with BV than in those without the condition. Gonococcal and chlamydial infections are not known for causing inflammation of the vagina in sexually mature women. However, aerobic vaginitis with aerobic bacteria has been described,[52] and infection of vaginal cells *in vitro*[17] and skin cells in balanoposthitis[38] by MG raises the intriguing question of whether it might cause vaginitis *in vivo*.

Cervicitis

The first evidence of an association of MG with cervicitis came from a Japanese study, reported in 1997,[53] in which MG was detected in the cervices of 5 (9%) of 57 women with cervicitis but in none of 79 women without the condition. Subsequently, the results of other studies,[30,54] to a large extent attest to MG having a significant role in causing cervicitis. In another study,[55] MG was the only genital mycoplasma/ureaplasma regarded as causing cervicitis.

Pelvic inflammatory disease

Further evidence for MG causing PID is (i) the ability of the organisms to adhere to Fallopian tube mucosal epithelial cells in organ culture[56] and to affect the cells and cause ciliary damage,[57] (ii) the production of endometritis and salpingitis experimentally in several subhuman primate species[58,59] and hydrosalpinx formation in mice,[60] (iii) the association of tubal factor infertility with a previous infection with MG,[61] and (iv) the demonstration of MG antibody responses in one-third of women with acute PID, a finding disputed by some investigators.[62,63] In summary, the overall supportive aspects have led to the conclusion that MG is one of the causes of PID.[64] PID comprises endometritis and/or salpingitis.

Endometritis

In an early study[65] on endometritis, MG was reported to have been detected in endometrial biopsy specimens from women with clinically suspected PID. In another study,[66] MG was found to be strongly associated with acute endometritis, being detected in 9 (16%) of 58 women with histologically diagnosed endometritis, but in only one (2%) of 57 women without endometritis.

Salpingitis

There have been few studies, in which the fallopian tubes have been examined at laparoscopy. In one study,[67] MG was detected in the cervix/endometrium of 9 (7%) of 123 women with acute salpingitis but in only a single tube.

Reproductive Disease in Women

In relation to pregnancy outcome, there is evidence that MG alone or in combination with other microorganisms causes some cases of PID. As this disease damages Fallopian tubes,[57] there is a small chance that such a prior mycoplasmal infection could be responsible for an ectopic pregnancy. However, a serological study provided no support for this.[62] In consideration of the poor pregnancy outcomes of spontaneous preterm labor (SPTL) and preterm birth (PTB), which have been shown to occur for women with BV,[68] few studies[69-71] suggested that MG was unlikely to be responsible for such outcomes, whereas in two other studies,[72,73] it was reported to be a significant independent risk factor for SPTL and PTB. *M. hominis* is considered to be responsible for some cases of maternal fever after a normal delivery or abortion,[74] but the role, if any, of MG has not been assessed.

Complications
Infertility

MG affects the motility of human spermatozoa.[12] Whether this could reduce male fertility *in vivo* is unknown. MG is known to cause PID, this could result in tubal damage and occlusion and subsequent infertility. Two seroepidemiological studies[61,75] have shown an association with tubal factor infertility, with 17%–22% of women having MG antibodies, compared to 4%–6% of women with normal tubes. There is a chance that women who have had a prior infection with MG could also be at a higher risk for infection by sexually transmitted organisms other than *C. trachomatis*, which might cause infertility.

Arthritis

Sexually acquired reactive arthritis or the less common Reiter's disease, in which conjunctivitis also develops, occurs in men who have or have recently had NGU and less often in women. MG was detected from adult with conjunctivitis which was not a part of Reiter's disease.

Table 2: Studies from India showing prevalence of *Mycoplasma genitalium* in different population groups using polymerase chain reaction

Year of study	Study population	Sample tested	Test method	Prevalence of MG (total number of sample tested), *n* (%)
Dhawan *et al.*, 2020[80]	MSMs	FVU Rectal swab Oropharyngeal swab	PCR	46 (30.4)
Rekha *et al.*, 2019[115]	Infertility	Peritonial fluid	PCR	Infertile 162 (6.1) Fertile 162 (0.6)
Saigal *et al.*, 2016[116]	STIC	Endocervical/urethral swabs, FVU	PCR	164 (1.2)
Rajkumari *et al.*, 2015[117]	Infertility	FVU Endocervical swab Endometrium biopsy	PCR	100 (16)
Kokkayil *et al.*, 2013[89]	Infertility	Endocervical swab	PCR	Co-infection of MG and CT in one patient
Ghosh *et al.*, 2011[118]	HIV population	FVU	PCR	100 (0)
Manhas *et al.*, 2009[119]	HIV infected men with NGU	Urine	PCR	HIV positive: 70 (7.1) HIV negative: 30 (3.3)

STIC=Sexually transmitted infection clinic; FVU=First void urine; PCR=Polymerase chain reaction; MSMs=Men who have sex with mens; MG=*Mycoplasma genitalium*; CT=*Chlamydia trachomatis*; NGU=Nongonococcal urethritis; HIV=Human immunodeficiency virus

MG was detected in the knees of 2 of 13 patients with arthritis, one of whom had Reiter's disease and another one had seronegative arthritis.[76] In addition, clinical experience indicates that reactive arthritis occurs occasionally in patients with MG genital tract infections. MG has also been reported with or without *M. fermentans* and *C. trachomatis* in 9 (35%) of 26 "deranged" temporomandibular joints considered possibly of a reactive nature.[77]

Extragenital Manifestations

Proctitis
Trends in oral and anal sex have increased over the past decades; anal intercourse has doubled over a 10-year period. Sexually transmitted proctitis is commonly caused by *Neisseria gonorrhea* and *C. trachomatis*. Proctitis is commonly observed in MSM. MG is also a cause of sexually transmitted proctitis. It is mostly an asymptomatic infection. In symptomatic patients, it manifests as rectal pain and anal discharge. However, severity of symptoms of proctitis due to MG is less as compared to *C. trachomatis* and *Neisseria gonorrhea*. Prevalence of proctitis associated with MG showed in studies done by different countries. In a study by Francis *et al.* from the USA, 5.4% of the rectal swabs collected from 500 MSM positive for MG.[78] Bissessor *et al.* from Australia observed that the prevalence of MG in MSM with HIV infection was more as compared to those without HIV (21% vs. 8%) also the load of the organism was higher in symptomatic patients as compared to asymptomatic patients.[79]

In a study from India, the prevalence of MG in MSM was 41.3% of the infected patients, anorectal infection was observed in 68.4% of cases.[80] Furthermore, there is a co-infection of MG with *C. trachomatis* and *N. gonorrhea*. Latimer *et al.* reported co-infection of MG with *C. trachomatis* and *N. gonorrhea* at the anorectal site ranging from 13% to 14%.[81] MG has been found in the anorectal region, but its pathogenicity in causing clinical proctitis has not been elucidated and more research is required.

Oropharyngeal infection
Oropharyngeal infection is usually asymptomatic. Prevalence of pharyngeal infection due to MG is less as compared to anorectum. Couldwell *et al.*[82] and Dhawan *et al.*[80] in their studies did not detect any MG in oropharyngeal infection. However, Jiang *et al.* showed a prevalence of 13.5% oropharyngeal infection.[83]

Co-infections
Co-infection of MG with other pathogens has been observed by many authors. In a study by Getman *et al.*,[84] a lower prevalence of co-infections of MG with other sexually transmitted organisms was seen. However, in a study by Gaydos *et al.*,[85] the percentage of co-infections of MG with another organism for those infected with at least one organism ranged from 30.6% for TV-infected women to 73.3% for NG-infected women. In a study by Yokoi *et al.*,[86] rates of co-infection with MG among men with gonococcal urethritis were shown to be low (4.1%), compared with the *C. trachomatis* co-infection rate (21.2%). Another study evaluated the prevalence of MG co-infection in 302 chlamydia-infected women at a STD clinic in Birmingham. Co-infection of MG was detected in 22 (7.3%).[87] In West Africa, Pépin *et al.*[88] showed that almost half of the infections due to MG occurred as co-infections. The prevalence of co-infection with gonococcal urethritis, *C. trachomatis,* and TV was 37.9%, 10.6%, and 7.6%, respectively. In a study from India, co-infection of both MG and *C. trachomatis* was found in 5 (10.8%) in MSMs diagnosed with urethritis[80] co-infection in patient with MG and *C. trachomatis* in an infertile female patient with genital tuberculosis has also been reported. Co-infections among genital mycoplasmas have also been reported.[89] In a study by Darkahi,[90] simultaneous occurrence of MG and *U. urealyticum* was shown in 1.4% of women with genital infections, while triple infection of MG, *U. urealyticum,* and *M. hominis* was seen in 0.5% of patients.

Infection in Immunodeficient or Immunosuppressed Patients

About a decade ago, it was reported that more than 50% of men who had AIDS but no urethritis were MG positive. A study by Loubinoux *et al.*[91] failed to detect MG in urine from 54 HIV-positive patients. More recently, it was reported[92] that MG was found much more frequently at both urethral and rectal sites of HIV-positive MSM than HIV-negative MSM. MG-induced cervicitis[55] has been shown to occur more often in HIV positive than in HIV-negative women, and the mycoplasma has been found more frequently in endometrial biopsy specimens of women who were HIV positive[65] and can persist longer in HIV-positive women.[93]

Diagnostic Tests for *Mycoplasma Genitalium*
Availability of nucleic acid amplification testing for MG is limited in India. Testing is currently available at some

tertiary care hospitals. A summary of studies from India showing prevalence of MG in different population groups using PCR is shown in Table 2. Isolation and culturing of MG is slow, time consuming, and not feasible when there is a need to institute immediate antimicrobial therapy. Therefore, nucleic acid amplification test (NAAT) is the preferred diagnostic method where feasible.

Although research companies have quantitative PCR detection kits in the market, the United States Food and Drug Administration has not approved any of these methods for the clinical screening or detection of MG Vandepitte *et al.*[94] compared two commercially available kits (TIB MOLBIOL LightMix kit) and the Diagenode MG (real-time PCR kit) as well as an in-house PCR method using the Roche Diagnostics cobas z 480 analyzer[95] TIB MOLBIOL LightMix kit targeted the MG219 gene, Diagenode MG real-time PCR kit targeted the *gap* gene, and the in-house kit targeted the *MgPa1* adhesion protein gene. The commercial kits had a sensitivity of 92.6% and 87%, respectively, and a specificity of 100% which was concordant with the in-house kit that was >95%.

In an effort to establish a simpler and streamlined protocol for MG detection, Takanashi *et al.* developed a PCR test using Invader Plus technology, carrying out both the endonuclease and PCR in the same simple step.[96] This approach would require less genetic material and would be of less labor and would be time consuming. The approach was tested with first-void urine samples. The Invader Plus assay was comparable to typical hybridization microtiter PCR and was able to detect as few as 10 DNA copies per reaction.

Genotype Assays for Predicting Resistance Phenotype

Another opportunity for the detection of MG is establishing genetic markers of resistance to first-line therapy. It is currently recommended that detection of MG is followed by testing for mutations associated with macrolides and fluoroquinolone resistance in order to guide antibiotic treatment.[97]

There has been considerable recent progress in developing molecular tests to evaluate resistance mechanisms for MG that is difficult (or slow) to culture and for which resistance to frontline antibiotics is a serious concern. MG exhibits considerable resistance to fluoroquinolones and macrolides. A previously published report demonstrated excellent results for a multiplex PCR assay designed to detect MG, as well as mutations in MG 23S rRNA associated with macrolides resistance.[98] Fernández-Huerta *et al.* described an assay for simultaneous detection of MG and mutations to subunit A of topoisomerase IV (Par C) that lead to fluoroquinolone resistance.[99] In both studies, multiplex PCR assays were compared to Sanger sequencing. Macrolide resistance was predicted in 63% of MG clinical isolates;[98,99] fluoroquinolone resistance was predicted in 8.8% (Spanish cohort) to 23.4% (Australian cohort) of MG clinical isolates.[99] A similar approach was taken to detect macrolide resistance in *M. pneumoniae* in a Pennsylvania cohort,[100] wherein investigators found 7.5% of isolates were predicted to be resistant to macrolides. Summary of the laboratory studies of MG antimicrobial susceptibility and genotypic resistance testing in the literature subsequent to the report by Couldwell *et al.*, 2015, is mentioned in Table 3.

Resistance Issues

M. genitalium is intrinsically resistant to cell wall-acting agents because of lack of cell wall and is sensitive to limited group of antibiotics. In addition to this, it developed resistance to most of the available antibiotics.

Azithromycin

Mechanism of resistance

Azithromycin inhibits the protein synthesis by binding to the A2058 and A2059 residues of region V of 23S rRNA in 50S ribosomal subunit, thereby inhibits the translation of mRNA and thus interfere with protein synthesis and also binds to the L4 and L22 proteins which are important for the assembly of ribosome.[101]

MG develops resistance to azithromycin by single nucleotide polymorphism at A2058G, A2058C, A2059G, and A2059C of V region. Thereby, it prevents the binding of drug. MG develops resistance also by mutation in L4 and L22 proteins.[101]

Reason for high Azithromycin resistance

Differentiation between *C. trachomatis* and MG cannot made clinically, because both are the cause of NGU. Infection with *C. trachomatis* is treated with single dose azithromycin 1 g, but this is a suboptimal dose for treating MG. Instead of eradication, it will select the resistance.

Resistance can also be explained by intranuclear localization of MG. Azithromycin is capable of entering eukaryotic cells, it primarily accumulates in the cytoplasm and only low concentrations were observed in the nucleus. This reduces the efficacy of azithromycin and selects the resistance.[101]

Cure rates with single dose azithromycin regimen decreased over the period of time which is shown in Table 4.[37,102-104] Azithromycin resistance rate in different countries from 2011 to 2018 varied from 5.3% to 75%,[101] Mulligan *et al.* in Ireland showed the highest resistant rate of 75%.[105]

Moxifloxacin

Moxifloxacin is a fourth-generation fluoroquinolone and is used as the second-line treatment against MG in most of the countries.

Mechanism of resistance

It acts by inhibiting DNA replication process by inhibiting two enzymes involved in DNA replication process. First enzyme is DNA gyrase which is encoded by *gyrA* and *gyrB* genes. Function of this enzyme is to introduce the negative supercoils, thereby unwind the DNA and initiates the replication process. Second enzyme is topoisomerase IV which is involved in the release of daughter DNA from the parent DNA and this enzyme is encoded by *parC* and *parE* gene. Mutation in Quinolone Resistance Determining Region of *gyrA* and *parC* gene is the most common mechanism for moxifloxacin resistance.

Moxifloxacin resistance was first reported from Sydney with resistance rate of 16.1%.[106] The resistance rate for moxifloxacin ranged from 5% to 47.1%, as was observed from various studies done in the different parts of the world from 2008 to 2018.[101] Maximum resistance (47.1%) was reported from Japan.[107]

Josamycin

It is a 16 membered lactone ring macrolide antibiotic. In Russia, josamycin is a first-line drug for treatment of MG

Table 3: Summary of the laboratory studies on *Mycoplasma genitalium* antimicrobial susceptibility and genotypic resistance testing in the literature subsequent to the report by Deborah L Couldwell and David A Lewis, 2015

Reference	Study type	Population	MG DNA extracts or isolates examined	Macrolide resistance (MIC data/resistance mutations)	Fluoroquinolone resistance (MIC data/resistance mutations)	Comments
Huerta *et al.*, 2020[120]	Prospective study	95 positive specimens from 89 individuals included 8 vaginal swabs, 20 endocervical swabs, 8 urethral swabs, 25 first-void urine, and 34 rectal swabs	90 DNA extracts	The rate of MRMM in MG among the study population was 41.8%	Not done	The ResistancePlus® MG FleXible a rapid, simple, and accurate cartridge-based assay for simultaneous detection of MG and MRMM in clinical settings
Pitt *et al.*, 2020[121]	Laboratory analysis	Sexually active British general population	66 DNA extracts	Mutations in 23 S rRNA gene were detected in 9/56 (16%) specimens, with the A2058G mutation being most common (*n*=7), followed by A2059G (*n*=1) and A2059C (*n*=1)	*parC* gene mutations associated with fluoroquinolone resistance were detected in 2/61 (4%)	Specimens with macrolide resistance were more likely to come from participants reporting a history of diagnosed bacterial STIs or recent sexual health clinic attendance
Martens *et al.*, 2019[122]	Retrospe-ctive study	Tested 28,408 samples from 20,537 patients for the presence of STD organisms. Most (*n*=25,132) samples were provided by general practitioners, 3087 (10.9%) by hospitals, and 189 (0.7%) from other and unknown locations	894 DNA extracts	Single-nucleotide polymorphisms A2058C, A2058G, A2058T, and A2059G in the 23S ribosomal RNA-encoding region of MG, which together account for >95% of the cases of azithromycin resistance	Not done	The rate of MRMM positivity rose from 22.7% in 2014 and 22.3% in 2015 to 44.4% in 2016 but decreased to 39.7% in 2017
Sweeney *et al.*, 2019[123]	Retrospective study	Patients with genital symptoms urine (*n*=280), cervicovaginal swabs (*n*=90), urethral swabs (*n*=10), anal/rectal swabs (*n*=60), throat swabs (*n*=1), and samples from unknown sites (*n*=6)	447 DNA extracts	277/447 (61%) carried strains which harbored MRDR 35/447 (8%) patient samples harbored both MRDR and QRDR mutations	47/447 (11%) samples harbored MG strains with *parC* or *gyrA* mutations in QRDR	The levels of antibiotic resistance may differ between populations within the same state, which has implications for clinical management and treatment guidelines
Hokynar *et al.*, 2018[124]	Laboratory analysis	Specimens from heterosexual population included swabs from vagina (*n*=30), urethra (*n*=8), rectum (*n*=1), cervix (*n*=31) and FVU, (*n*=233)	17 DNA extracts	4 mutation associated with macrolide resistance A2058/9G and 9 were wild type by sequence	Only one specimen contained a mutation at the QRDR area *parC* gene leading to fluoroquinolone resistance	Recommend testing for the MG positive samples for mutations leading to macrolide resistance but not for fluoroquinolones to guide in selecting treatment
Mondeja *et al.*, 2018[125]	Retrospe-ctive study	280 MG positive DNA extracts conserved at the Cuban National Reference Laboratory of Mycoplasma Research between 2009 and 2016 from Cuban patients with urogenital syndromes, spontaneous abortion and infertility	280 DNA extracts	52/64 (82%) samples were identified as A2058G/A2059G and 12/64 (19%) as A2058C/T Three new MG isolates confirmed phenotypic resistance to macrolides in a cell-culture assisted susceptibility test	Not done	Rapid emergence and high prevalence of MRMM in MG-infected patients and confirmed the phenotypic resistance in isolates carrying MRMM
Le Roux *et al.*, 2018[126]	Reterospe-ctive study	Vaginal swab samples from 100 and 104 termination of pregnancy attendees at a tertiary hospital in Pretoria, South Africa during 2012 and 2016 respectively	13 clinical isolsates	2 isolates had A2059G mutation in region V of the 23S rRNA gene	One a fluoroquin-olone resistance-associated mutation in the *parC* gene	Increase in macrolide and fluoroquinolone resistance among local MG strains
Braam *et al.*, 2017[127]	Laboratory analysis	147 women and 73 men (general population)	220 DNA extracts	Mutation at position A2058G (n=18/46), (39%) followed by A2059G (n=16/46), (34%) A2058T (n=10/46) (21%) and A2058C (n=2/46) (5%)	Not done	Molecular methods designed to detect all macrolide resistance-associated mutations, patients infected with proven macrolide-resistant strains can be empirically treated with moxifloxacin
Forslund *et al.*, 2017[128]	Retrospe-ctive study	3167 males and 5636 women who were seeking care at diverse clinics were routinely tested for MG during 2015	271 clinical isolates	Macrolide associated resistance mutations in the 23S rRNA gene 8.8% and 4.2% of the isolates had point mutations of the 23S-gene at position 2072 and 2071, respectively	Not done	Relatively low rate of macrolide-resistant MG

Contd...

Table 3: Contd...

Reference	Study type	Population	MG DNA extracts or isolates examined	Macrolide resistance (MIC data/resistance mutations)	Fluoroquinolone resistance (MIC data/resistance mutations)	Comments
Mondeja et al., 2016[129]	Laboratory analysis	7 strains isolated from endocervical and urethral swab specimens from cuban patients	7 DNA extracts	A2059G transition was detected in the phenotypically macrolide resistant B19 strain	No mutations detected in the QRDR of the parC gene	None
Kristiansen et al., 2016[130]	Laboratory analysis	113 samples were obtained from females (92 cervical swabs, 17 urethral swabs, and 4 urine samples), and 146 were obtained from males (94 urethral swabs and 52 urine samples)	253 DNA extracts	109=Wild type 75=A2058G mutation 65=A2059G mutation 2=A2058T mutation 1=A2058C mutation	Not done	5'nuclease genotyping assay is easily interpretable and allows timely reporting of macrolide resistance in MG The assay can genotype a large proportion of samples and displays a high concordance with sequencing

MRMM=Macrolide-resistance mediating mutations; MG=Mycoplasma genitalium; QRDR=Quinolone resistance-determining regions; MRDR=Macrolide resistance-determining region; STIs=Sexually transmitted infections; STD=Sexually transmitted disease; FVU=First void urine

Table 4: Change in the azithromycin cure rates over a period of 10 years

Study	Year	Cure rate (%)
Twin et al. Australia[102]	2005-2007	84
Björnelius et al. Scandinavia[37]	2009	85
Bradshaw et al. Australia[103]	2007-2009	69
Manhart et al. Washington[104]	2013	40

infections. In other parts of the world, it is not commonly used. Not much data about josamycin are available. Guschin et al. showed the eradication rate with josamycin was 93.5% and faster and higher eradication was seen in patient with lower pretreatment load. In contrast, 50% of patients with higher load were resistant to josamycin and mutations were detected at A2059G and A2062G residues of 23S rRNA.[108]

Management Issues in the Treatment of Mycoplasma Genitalium Infections

Syndromic treatment of NGU has focused on the eradication of C. trachomatis, a well-established cause of reproductive morbidity in women, and is usually instituted at initial presentation before results of investigations to detect specific bacterial causes are made available. In most cases of sexually acquired urethritis and cervicitis, tests are only performed for N. gonorrhoeae and C. trachomatis. Few countries offer routine screening for MG and where this is performed, it typically relies on the use of in-house NAATs performed on specimens collected at either the initial visit or after failure of first-line therapy. Importantly, there are still no validated and commercially available assays for routine diagnostic testing although these may be available in the near future.

Treatment Options for Mycoplasma Genitalium Infection are Limited by Antimicrobial Resistance

Treatment of MG urogenital infection is important from the view point of transmission and complications. Due to the lack of cell wall, limited antibiotic options are available.[13] Tetracyclines, macrolides, and fluoroquinolones have activity against mycoplasmas. Therapy for MG is indicated if detected in any genitourinary sample in symptomatic patients or as part of an epidemiological survey. Macrolides remain the mainstay of therapy in susceptible infections and have been covered under the syndromic approach for genito-urinary discharge. It achieves a good cure rate of 85%–95% in susceptible infections as single dose therapy [Table 4]. However, increasing macrolide resistance has been reported with the widespread use of azithromycin 1 g single dose without test of cure.

Azithromycin is recommended as the first-line agent for the treatment of uncomplicated MG infections (including in pregnancy). Individuals who have not received previous empirical treatment for urethritis or cervicitis with single-dose azithromycin should receive an extended oral macrolide regimen with azithromycin 500 mg on day 1, then 250 mg on days 2–5. In treatment failure or with confirmed macrolide-resistant infection, moxifloxacin is recommended. Treatment failure with moxifloxacin is uncommon. Test of cure is recommended only in those with persistent symptoms after treatment.[109]

An extended oral macrolide regimen with azithromycin or Josamycin 500 mg three times daily for 10 days drastically improves the cure rate. Macrolide resistance rates vary significantly geographically, but where azithromycin 1 g single dose is used for the treatment of NGU, it is usually found in 30%–45% of samples.[110-112]

Josamycin is widely used in Russia with 500 mg three times a day for 10 days but will not eradicate macrolide-resistant strains. Moxifloxacin can be used as second line therapy or for complicated cases for 7–14 days.[111] Moxifloxacin is the most commonly used second line antimicrobial. It is bactericidal and has a cure rate approaching 100% in infections with susceptible strains. However, resistance has developed with treatment failures ranged from 5% to 47.1% primarily in patients from the Asia-Pacific region.

Doxycycline in a dose of 100 mg two times daily for 14 days has a low cure rate of 30%–40% but does not increase resistance.

Emerging Treatment Options

Pristinamycin

Pristinamycin is a bactericidal streptogramin group of drug. It is a third-line treatment option for MDR strains and is effective against macrolide-susceptible MG. In a study by Bissessor et al. showed that pristinamycin was highly effective in treating macrolide- and quinolone-resistant strains.[113] The maximal recommended dose is 1 g four times a day for 10 days. Due to the high price, lack of clinical registration of drug, and

patient compliance for the drug issues, this drug has not been established as a second-line drug.

Other drugs used were solithromycin, lifamulin, sitafloxacin, and spectinomycin. However, the clinical efficacy of these drugs is still under evaluation.

Newer Drug Targets

Because of rising of resistance rate to all the available antibiotics, it is essential to identify the newer drug targets. Butt *et al.* identified 67 nonhomologous essential proteins using comparative genomic and metabolic pathway analysis. Enzymes from Thiamine, protein, and folate biosynthetic pathways were identified. These proteins could serve as novel drug targets for MG.[114]

Conclusion

MG has emerged as a superbug and the rising resistance in this bacterium with only a few treatment options in hand is an imminent problem. Future research should look toward to developing newer antimicrobials and proper management algorithms. Monotherapy should no longer be used. Etiology-based treatment will be a definitive solution to this emerging antimicrobial resistance due to the misuse of antibiotics as a part of syndromic management.

Conflicts of interest
There are no conflicts of interest.

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