Mycoplasma genitalium: a brief review

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Mycoplasma genitalium *belongs to the class Mollicutes and is the smallest prokaryote capable of independent replication. It was originally isolated from the urethras of two men with non-gonococcal urethritis (NGU). It has a number of characteristics which are similar to its genetically close relative,* Mycoplasma pneumoniae, *which is an established pathogen of the respiratory tract.* M. genitalium *lacks a cell wall and has a characteristic pear/flask shape with a terminal tip organelle. This organelle enables* M. genitalium *to glide along and adhere to moist/mucous surfaces, including host cells.* M. genitalium *has minimal metabolism, and when compared to the other genital mycoplasmas, has the ability to metabolise glucose. The organism is the smallest self-replicating prokaryote with a genome of only 580 kb pairs and was the second bacterium to have its genome fully sequenced. Its DNA falls under the low G+C category and thus has a lower melting temperature during denaturation in polymerase chain reaction (PCR) assays. The target genes for PCR assays include MgPa, rRNA and gap.* 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, the release of enzymes and the ability to evade the host immune response by antigenic variation.*

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Introduction

Mycoplasma genitalium was first isolated in 1981 by Tully *et al.*[1] from two men with non-gonococcal urethritis (NGU). Two isolates were grown on SP4, a transport medium that they had developed two years earlier.[2] The strains were designated G-37 and M-30, and shown to be distinct from all other mycoplasma species. These unique isolates were subsequently named *Mycoplasma genitalium*. The G-37 isolate has become an American Type Culture Collection (ATCC 33530) strain with its genome being fully sequenced in 1995.[3]

Due to its slow cell replication and fastidious growth requirements, culture is not usually used for laboratory diagnosis of *M. genitalium*, hence few epidemiological studies were done in the years following its discovery. However, after the introduction of molecular diagnostic assays, many clinical studies were performed, mainly in developed countries. If one reviews the studies that have been performed to date, *M. genitalium* is found in 21% of men with NGU (range 9.7% - 43.2%) and in 6% of asymptomatic men (range 0% - 16%).[4] The majority of these studies have shown an association of *M. genitalium* with NGU, whilst in one study no association could be shown.[5]

Improvement in laboratory detection methods, particularly with the introduction of the newer nucleic acid amplification tests (NAATs), is playing an important role in elucidating the place of *M. genitalium* among sexually transmitted pathogens, and especially its role in NGU and cervicitis.

Characteristics of *M. genitalium*

Most of the characteristics of *M. genitalium* are known through the findings from its thoroughly studied, genetically close relative, *Mycoplasma pneumoniae*.[4,6] While *M. genitalium* can cause genitourinary tract disease, *M. pneumoniae* is an established pathogen of the respiratory tract and is an important cause of atypical pneumonia.[7]

Taxonomy

Mycoplasmas are prokaryotes belonging to the family *Mycoplasmataceae* within the order *Mycoplasmatales*.[8] The genera *Mycoplasma* and *Ureaplasma* are of the class *Mollicutes* [*mollis* (soft); *cutis* (skin)] which encompasses bacteria without a cell wall and are popularly termed the naked bacteria.[4,8] The terms Mycoplasma and Mollicute are occasionally used interchangeably to refer to the species that belong to this class. The genus *Mycoplasma* contains more than 100 species, of which 13 are present as human flora.[9]

Mycoplasmas were initially thought to be viruses, since they could pass through filters that were meant to trap bacteria. However, they became accepted as bacteria when the concept of viruses was much better defined in the 1930s.[10] In 1995, the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Mollicutes* defined new mycoplasmas based on their ability to be filtered at very low pore size and absence of a wall - the latter being the case even after incubation in a medium without antibiotics.[11] In 2007, these standards were revised to include the deposition of the 16S rRNA gene sequence into a public database, and a phylogenetic analysis of the relationships among the 16S rRNA gene sequences of the novel species and its neighbours.[12]

The phylogenetic tree of evolution shows that mycoplasmas may be descendants of Gram-positive bacteria, presumably of clostridial origin.[4,7] This transformation is thought to have occurred through a genome reduction process leading to *M. genitalium* having the smallest genome of all self-replicating prokaryotes.[13] The level of the phylogenetic tree following the 16S ribosomal ribonucleic acid (rRNA) gene sequence

has revealed that *M. genitalium* and *M. pneumoniae* belong to the same cluster within the *Mycoplasma* genus, thus making the two organisms closely related.[14] The close relationship was confirmed by the similarity in many morphological and antigenic characteristics, including lipid components that the two bacteria share.[15]

Morphology

The genus *Mycoplasma* contains very small bacteria, with sizes ranging from 0.2 to 0.7 µm depending on the shape of the various species.[16] The shape depends on the particular mycoplasma species, which may be spherical, filamentous or flask/pear-like.[7] *M. genitalium* and *M. pneumoniae* have the characteristic morphology with a terminal/apical tip organelle.[16]

As *M. genitalium* is too small to be visible under a light microscope, it was first viewed under a transmission electron microscope (TEM).[17] The electron micrograph of G-37 and M-30 *M. genitalium* strains shows an organism of 0.6-0.7 µm in length, 0.3-0.4 µm wide near the base and 0.06-0.08 µm wide at the terminal tip. The core of the tip has dense parallel tracts called a nap (N) at the neck-like structure that protrudes from the main cell, giving it a pear-like appearance.[17] This differentiated tip structure is commonly known as the terminal organelle**.**

The neck-like region of *M. genitalium* is shorter than that of *M. pneumoniae*.[18] The terminal tip organelle is specialised to enable *M. genitalium* to glide along moist/mucous surfaces, as well as to adhere to surfaces such as plastic, red blood cells, Vero monkey kidney cells, and epithelia of eukaryotic host cells.[1,7] This attachment organelle is a membrane-bound extension of the cell and is further characterised by an electron-dense core that is part of the mycoplasma cytoskeleton.[19]

M. genitalium 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 and is prone to changes in its flask-like shape. This lack of a cell wall is a feature that is largely responsible for the two biological properties of *M. genitalium,* namely no Gram stain reaction and non-susceptibility to common antimicrobials of the β-lactam class that inhibit bacterial cell wall synthesis.[16]

Metabolism

In spite of the small genome possessed by the mycoplasmas, they have the ability to self-reproduce. Whilst other mycoplasmas may utilise arginine (*M. hominis*) or urea (*U. urealyticum*), *M. genitalium* metabolises glucose, resulting in the production of acid.[7,16] In keeping with the Mollicutes, the metabolism of *M. genitalium* makes use of substrate (glucose) phosphorylation that is associated with glycolytic kinase enzymes, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), pyruvate kinase or phosphoglycerate kinase for the synthesis of essential nucleotriphosphates (NTPs) for its genome.[20] *M. genitalium* exclusively makes use of GAPDH, retained in its small genome, during the process of glycolysis, generating energy for the organism.[7,21]

Genetic make-up

M. genitalium is the smallest existing self-replicating prokaryote with a genome consisting of only 580 kb. In 1995, *Haemophilus influenzae* was the first pathogen with a fully sequenced genome.[22] This was followed shortly thereafter with the publication of the complete genomic sequence of *M. genitalium.*[13] When the genome of *M. genitalium* is compared to the slightly bigger genome of *M. pneumoniae* (816 kb), it has been shown that *M. genitalium* contains some subsets of *M. pneumoniae*'s genomic complement and that the coding genes in the *M. genitalium* genome correspond to certain sequences of *M. pneumoniae*'s genome.[14]

The small genome of *M. genitalium* gives a good indication of the minimal set of genes needed to sustain bacterial life. The minimum set of genes, also called essential genes, in both prokaryotes and eukaryotes, are those described as indispensable for the survival of an organism and are therefore the basis of life for a particular organism. In 2006, Glass *et al.* identified 382 of the 482 *M. genitalium* protein-coding genes as essential.[23] A more recent study by Zhang and Lin (2009) showed that *M. genitalium* needed only 381 essential genes compared to the 642 required by *H. influenza.*[24] This highlights how the very small *M. genitalium* is capable of surviving on its own. When studying the open reading frames (ORFs) of the *M. genitalium* genome, Taylor-Robinson (1995)[16] and Su *et al.* (2007)[25] identified only 480 protein-coding regions, while a later study by Ueno *et al.* (2008)[3] found 484 coding regions. These identified coding regions include genes for DNA replication, transcription, translation, DNA repair, cellular transport and energy metabolism. It has also been found that *M. genitalium*, unlike other bacteria, uses the UGA codon to code for tryptophan instead of a stop codon, suggesting that expression of its genes is complicated since it would synthesise truncated proteins.[26]

To characterise its genome, *M. genitalium* falls under the so-called 'low G+C' mycoplasmas because its DNA genome typically has fewer guanine (G) and cytosine (C) DNA bases than adenine (A) and thymine (T), as compared to other bacteria.[27,28] The G+C content in the DNA of most mycoplasmas ranges from 24% to 33%, with *M. genitalium* at 32%.[4] The significance of the low G+C content is that *M. genitalium* would have a lower melting temperature (T_m) during the double-stranded DNA denaturation stage of polymerase chain reaction (PCR) assays. However, *M. genitalium* has a significantly higher G+C content (44%) in its ribosomal rRNA gene.[4]

A few genes have been used as target for PCR assays, with the most popular the *MgPa* DNA gene (coding for the adhesin proteins), the rRNA genes and the housekeeping gene, *gap* (coding for GAPDH).[21,29]

Pathogenesis of *M. genitalium*

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.*[4] Although *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.[30,31] *M. genitalium* has been shown to attach to different cell types, including erythrocytes,[32] Vero cells,[33] Fallopian tube cells,[34] respiratory cells[35] and spermatozoa.[36]

Virulence

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, the release of enzymes[3,4] and the ability to evade the host immune response by antigenic variation.[37]

Attachment and entry

For all intracellular pathogenic microorganisms, adhesion is a pre-requisite for colonisation and infection. Since mycoplasmas lack cell

walls and cell wall-associated structures such as fimbriae that are normally associated with adhesion, the process of adhesion is mediated by cell membrane-bound components that are collectively called adhesions.[38] *M. genitalium* utilises the terminal tip organelle that is a complex protein structure, to mediate adhesion. This cell membrane-bound protein complex is required for intimate adherence to host target cells. Although surface-exposed, these adhesins are linked to the internal cytoskeleton of the tip organelle.[3]

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) of mutant strains that are incapable of haemadsorption has been widely used for the characterisation of proteins involved in adhesion.[39] A total of 21 putative protein genes have been identified in the *M. genitalium* genome, but only a few have been characterised as adhesions.[13,14]

The major adhesin in the attachment protein complex is the MgPa protein and, together with the P32 (MG318) protein, makes up the terminal tip organelle.[40,41] The MgPa encodes the P140 (MG191) and P110 (MG192) cytadherence proteins (cytadhesins) at the tip area.[4,38] These proteins are immunogenic both in immunised animals and in humans. Loss of either P140 or P110 results in loss of motility and adherence properties of the entire MgPa attachment organelle,[7,38] thus showing the importance of these proteins in attachment. It was shown that the 140 kDa P140 (MG191) closely resembles the 170 kDa main adhesion protein (P1) of *M. pneumoniae* whereas the 32 kDa *M. genitalium* protein P32 (MG318) resembles P30 of *M. pneumonia.*[7,30]

The MG218 and MG317 cytoskeletal proteins were shown to play a role in terminal organelle organisation, gliding motility and cytadherence.[42] The MG317 protein contributes to anchoring the electron-dense core of the tip to the cell membrane.

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,[40,43] consisting of ORF-1 (MG190), ORF-2 (MG191), and ORF-3 (MG192).[44] 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. They are expressed together with adherence accessory proteins (MG312, MG317 and MG320). 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.[45,46] MG218 is grouped in an operon with MG217 and MG219.[47]

M. genitalium penetrates host epithelial cells after attachment in a similar way as *M. penetrans* and *M. fermentans.*[4,49] The target cell membrane then invaginates in a manner similar to the clathrin-coated pits mechanism of endocytosis observed in *C. trachomatis.*[4] Clathrin is a large protein that helps in the formation of a coated pit on the inner surface of the plasma membrane of a cell. The pit later buds into the cell to form a coated vacuole in the cytoplasm of the cell through which the infecting organism is delivered into the cell. Following entry into the target cell, the organism appears to reside in the membrane-bound vacuoles closer to the target cell nucleus.[3,4] This internuclear localisation process may take place within 30 minutes after infection.

Enzymes

Besides the role played by the adhesins, Alvarez *et al.* (2003)[48] found that during the enzyme-mediated glycolytic pathway, it is the activity of the glycolysis enzyme GAPDH that brings about attachment of *M. genitalium* to human vaginal and cervical mucin in female disease. Thus GAPDH, among other binding proteins, acts as a ligand to receptors mucin and fibronectin, particularly in vaginal and cervical disease.

M. genitalium has the ability to translocate its cytoplasmic enzymes to the cell membrane surfaces to enhance host tissue colonisation[3,50] (Blaylock et al., 2004; Ueno et al., 2008). In addition to GADPH, another enzyme, methionine sulfoxide reductase (MsrA), can be released to enhance the pathogenicity of its small genome.[48,51] 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.[51]

Evasion of the host immune response

Pathogenesis in mycoplasmas is dependent on an intimate contact with the host cell and therefore they have to be able to evade the host immune response, adapt to the environment and change phenotypically.[37] The major antigenic determinants of the Mollicutes are their membrane proteins that are expressed on the surface. They are able to generate a high frequency of intragenomic variation in nucleotide sequence or DNA arrangement at selected chromosomal loci, promoting random phenotypic variation as a result of constantly changing host environments.[52,53] The adaptive potential has been maximised without compromising household functions. Multiple copies of partial gene sequences have been found in most pathogenic bacteria, but as mycoplasmas have very small genomes, the number of mycoplasmal genes involved in diversifying the surface antigens is markedly high.[4]

The basic mechanisms observed in antigenic variation are regulation of the expression of virulent factors by signal transduction pathways, or natural generation of new phenotypes that are able to survive the host immune response.[37] When there are many regulatory genes available that could serve as sensors to environmental stimuli, or that can encode transcriptional factors, *M. genitalium* can employ antigenic variation caused by molecular switching of events.[54]

In order to escape the host immune attack, proteins P140 and P110 of the MgPa have the ability to undergo antigenic variation, thus altering the entire genetic sequence of the MgPa with subsequent generation of variants that are not recognised by the host immune system on subsequent encounters.[3,4] This is a limitation when using this gene as target in PCR. Other survival mechanisms of this organism may be the ability to mimic host cell antigens and the intracellular location within professional macrophages.[4]

Tissue damage seen in *M. pneumoniae* is due to the host cell response and this may reflect what happens in *M. genitalium* infection. Mycoplasmas have been found to interact with many components of the immune system. This may lead to production of cytokines and macrophage activation. Some cell components may act as super antigens and induce an autoimmune response.[55]

Transmission

M. genitalium is commonly detected in urogenital specimens of sexually active people and their partners attending sexually transmitted infections clinics, indicating sexual transmission.[56,57] Like other sexually transmitted pathogens, *M. genitalium* is transmitted between heterosexual partners during unprotected coital activity.[58,59] Information on prevalence of this organism in non-sexually active persons is lacking. *M. genitalium* has been shown to adhere to spermatozoa.[36]

Bradshaw *et al.* (2009) showed that oral sex is not a significant mode of transmission of this organism,[60] as *M. genitalium* was not detected in the pharyngeal swabs of 521 men who have sex with men (MSM) who met at male-only saunas. The organism was present in the urethral and rectal swabs of these men. Further studies are required to determine whether the presence of this organism in the rectum plays a role in the subsequent development of proctitis.

Very little is known of vertical transmission and subsequent colonisation of newborn infants by *M. genitalium*.[4] However, Waites *et al.* (2005)[49] have mentioned that *M. hominis* and *Ureaplasma* species, both belonging to the same family (*Mycoplasmataceae*) as *M. genitalium*, can be transmitted from an infected female to the foetus or neonate by gaining access to the amniotic sac through ascending intrauterine infection, the haematogenous route through placental infection where umbilical vessels are involved, or the perinatal route during passage of the neonate through the infected maternal birth canal with the resultant colonisation of the skin, mucosal membranes and respiratory tract of the neonate.

Conclusion

M. genitalium is the smallest prokaryote capable of independent replication, lacks a cell wall and has a characteristic pear/flask shape with a terminal tip organelle. This organelle enables *M. genitalium* to glide along and adhere to moist/mucous surfaces, including host cells.

M. genitalium is transmitted sexually and 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 male urethritis.

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