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REVIEW ARTICLE

Potential therapeutic targets for combating *Mycoplasma genitalium*

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Abstract

Mycoplasma genitalium (M. genitalium) has emerged as a sexually transmitted infection (STI) all over the world in the last three decades. It has been identified as a cause of male urethritis, and there is now evidence that it also causes cervicitis and pelvic inflammatory disease in women. However, the precise role of *M. genitalium* in diseases such as pelvic inflammatory disease, and infertility is unknown, and more research is required. It is a slow-growing organism, and with the advent of the nucleic acid amplification test (NAAT), more studies are being conducted and knowledge about the pathogenicity of this organism is being elucidated. The accumulation of data has improved our understanding of the pathogen and its role in disease transmission. Despite the widespread use of single-dose azithromycin in the sexual health field, *M. genitalium* is known to rapidly develop antibiotic resistance. As a result, the media frequently refer to this pathogen as the "new STI superbug." Despite their rarity, antibiotics available today have serious side effects. As the cure rates for first-line antimicrobials have decreased, it is now a challenge to determine the effective antimicrobial therapy. In this review, we summarise recent *M. genitalium* research and investigate potential therapeutic targets for combating this pathogen.

Keywords *Mycoplasma genitalium* · Urethritis · Cervicitis · Pelvic inflammatory disease · Sexually transmitted infections

Introduction

Mycoplasma genitalium (M. genitalium) is known to be a growing source of sexually transmitted infections (STIs) over the world, and it has been linked to both male and female urogenital infections, that include urethritis, cervicitis, pelvic inflammatory disease and preterm birth (Smolarczyk et al. 2021; Sethi et al. 2012; Jensen 2004; Lis et al. 2015; Lewis et al. 2020; Averbach et al. 2013). Non-gonococcal urethritis (NGU) cases were recorded continuously in about 30% of total STI cases (Manhart et al. 2013; Greydanus et al. 2022). *M. genitalium* co-infections with other human STIs such as the human immunodeficiency virus (HIV), chlamydia, and gonorrhoeae have also been reported in various studies (Napierala Mavedzenge et al. 2015; Fernández-Huerta and Espasa 2019; Harrison et al. 2019; Mahlangu et al. 2019). The Centers for Disease Control and Prevention (CDC) includes a guideline for *M. genitalium* infection diagnostics, management, and therapy in the Sexually Transmitted Diseases Treatment Guidelines, 2021, due to its clinical significance (Workowski et al. 2021). Despite these recommendations, treating *M. genitalium* infection remains difficult (Horner and Martin 2017; Sweeney et al. 2022b; Ross and Jensen 2006). It soon became evident that treatment was being restricted by antibiotic resistance (Raj et al. 2022). The degrees of resistance vary by location and amongst populations within a given area (Jensen et al. 2008; Workowski and Bolan 2015; Deguchi et al. 2017; Valentine-King et al. 2019). Another problem while treating *M. genitalium* is the high persistence of this bacterium in humans (Cina et al. 2019; Romano et al. 2019). This emphasizes the significance of conducting antibiotic susceptibility test prior to starting treatment. As this pathogen lacks a cell wall, it is immune to antibiotics that target cell wall production, such as beta-lactams (penicillins and cephalosporins) (Jensen and Bradshaw 2015; Iwuji et al. 2022). Antibiotics such as macrolides, tetracycline, and quinolones have been shown to be useful in treating this infection in various studies (Horner et al. 2016; Bradshaw et al. 2017; Sweeney et al. 2022a). The rapid rise in antimicrobial resistance to these drugs, on the other hand, is concerning. The recommended 1 g single dose of azithromycin for NGU treatment, for example, has not only been ineffective against *M. genitalium* but has

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also resulted in macrolide-resistant *M. genitalium* (Taylor-Robinson 2014; Jensen et al. 2016; Gnanadurai and Fifer 2020). Second-line antibiotics (such as moxifloxacin) are also effective against *M. genitalium*, however, resistance to these antibiotics has been reported in several countries (Manhart et al. 2015; Murray et al. 2017; Muller et al. 2019; Ke et al. 2020; Pitt and Fifer 2022). Researchers are exploring novel therapeutic targets in *M. genitalium* to develop new lead/drug molecules to cure this devastating infection because of the growing antibiotic resistance.

In this review, we have focused on the therapeutic targets of *M. genitalium*. A summary of all therapeutic targets is shown in Table 1. We hope that the reader will have a better understanding of these therapeutic targets that can aid in the current treatment of *M. genitalium* resistance development and the development of novel lead/drug molecules against these targets.

Table 1 Therapeutic targets of *M. genitalium* identified through comparative genomics approaches

Targets	Uniport ID	Genes/ORF	Mechanism of action	References
Chromosomal replication initiator protein DnaA	P35888	dnaA/MG469	Binds to Origin of Replication	(Kitchen et al. 1999; Fatoba et al. 2021)
Phosphomannomutase	P47299	manB/MG053	Conversion of mannose-1-phosphate to Mannose-6-phosphate	(Yung-Hun et al. 2010; Fatoba et al. 2021)
Protein translocase subunit SecA	P47318	secA/MG072	Coupling the hydrolysis of ATP to the transfer of proteins	(Veenendaal et al. 2004; Fatoba et al. 2021)
Acyl carrier protein homolog	P47529	MG287	Carrier of the growing fatty acid chain in fatty acid biosynthesis	(Byers and Gong 2007; Fatoba et al. 2021)
Phosphate acyltransferase	Q49427	plsX/MG368	Reversible formation of acyl-phosphate from acyl-acyl-carrier-protein	(Zhang and Rock 2008; Fatoba et al. 2021)
Processive diacylglycerol beta-glycosyltransferase	Q9ZB73	MG335.2	Biosynthesis of beta-diglycosyldiacylglycerol	(Cantarel et al. 2009; Butt et al. 2012)
Thymidylate kinase	P47252	Tmk/ MG006	Phosphorylation of dTMP to form dTDP	(Reichard 1988; Huang et al. 1994; Lavie et al. 1997; Butt et al. 2012; Kazakiewicz et al. 2015)
type I restriction modification protein	Q49434	MG438	Determines the specificity of DNA sequence	(Vasu and Nagaraja 2013; Nogueira et al. 2021)
Hydroperoxide reductase	P47666	MG427	Reduction of hydrogen peroxide and organic hydroperoxides to water and alcohols	(Seaver and Imlay 2001; Nogueira et al. 2021)
ribosome-binding factor A	P47389	rbfA/MG143	Interact with the 5'-terminal helix region of 16S rRNA	(Bylund et al. 1998; Huang et al. 2003; Xia et al. 2003; Nogueira et al. 2021)
Hypothetical protein	P47538	MG296	Not Available	(Nogueira et al. 2021)
DUF3217 domain-containing protein	P47616	MG376	Not Available	(Nogueira et al. 2021)
50S ribosomal protein L32	P47603	rpmF/ MG363	The structural component of the ribosome	(Brauer and Röming 1979; Walleczek et al. 1989; Nogueira et al. 2021)
class Ib ribonucleoside diphosphate reductase assembly flavoprotein NrdI	P47472	nrdI/MG230	Involved in ribonucleotide reductase function	(Nordlund and Reichard 2006; Nogueira et al. 2021)
Nucleotide adenylyltransferase	P47482	MG240	Reversible adenylation of nicotinate mononucleotide (NaMN) to nicotinic acid adenine dinucleotide	(Rodionova et al. 2014; Yang et al. 2020)
Phenylalanine-tRNA ligase	P47436	PheS/ MG194	Phenylalanyl-tRNA aminoacylation	(Mermershtain et al. 2011; Yang et al. 2020)
ribosomal protein 30S ribosomal protein S17	P47406	rpsQ/MG160	Binds specifically to the 5′-end of 16S ribosomal RNA	(Held et al. 1974; Yang et al. 2020)
Phosphoglycerate mutase	P47669	gpmI/MG430	Interconversion of 2-phosphoglycerate and 3-phosphoglycerate	(Jedrzejas 2000; Yang et al. 2020)
Phosphate acetyltransferase	P47541	Pta/MG299	Synthesises acetyl-CoA from acetate in acetyl-CoA biosynthesis	(Shin et al. 1999; Yang et al. 2020)

Drug target identification

Target identification in a biological pathway is one of the important steps in drug development process. In theory, when a pathogen's target is identified, it is critical that the putative target is either absent in the host or significantly different from the host homolog so that it may be used as a therapeutic target (Zhang et al. 2022). The evolution of antimicrobial resistance in Gram-positive bacteria has prompted researchers to look for new therapeutic targets that could help in the development of new antibiotics. Their cell organization differs greatly from that of mammalian cells, making it possible to identify pathogen-specific targets. The chosen target must be extremely important for the pathogen's survival (Naorem et al. 2022). The biochemical properties of the target should also be considered. It must have a small molecule binding pocket so that specific inhibitors could be designed, and if the target protein is an enzyme, its inhibition should result in a loss of cell viability. It is critical that the target chosen be assayable (Uddin et al. 2021; Chandela et al. 2022). A general workflow for drug target identification is depicted in Fig. 1. The application of bioinformatics tools in comparative genomics is extremely beneficial in predicting novel therapeutic targets against a pathogen. The goal is to identify essential and non-homologous proteins in the pathogen with distinct metabolic pathways that could be used as novel drug targets. Based on this, a manual comparison of *M. genitalium* metabolic pathways with human host is performed in order to identify pathogen-specific metabolic pathways. Furthermore, the entire proteome of *M. genitalium* is analysed using various bioinformatics databases and tools to identify essential, non-homologous, and cytoplasmic proteins involved in pathogen-specific metabolic pathways. These proteins are then tested for druggability in the DrugBank database. Following further investigation, druggable proteins are chosen as novel putative therapeutic targets (Nogueira et al. 2021; Fatoba et al. 2021). For high-throughput screening of compounds against these targets, an affordable and specific assay system should be available (Fatoba et al. 2021).

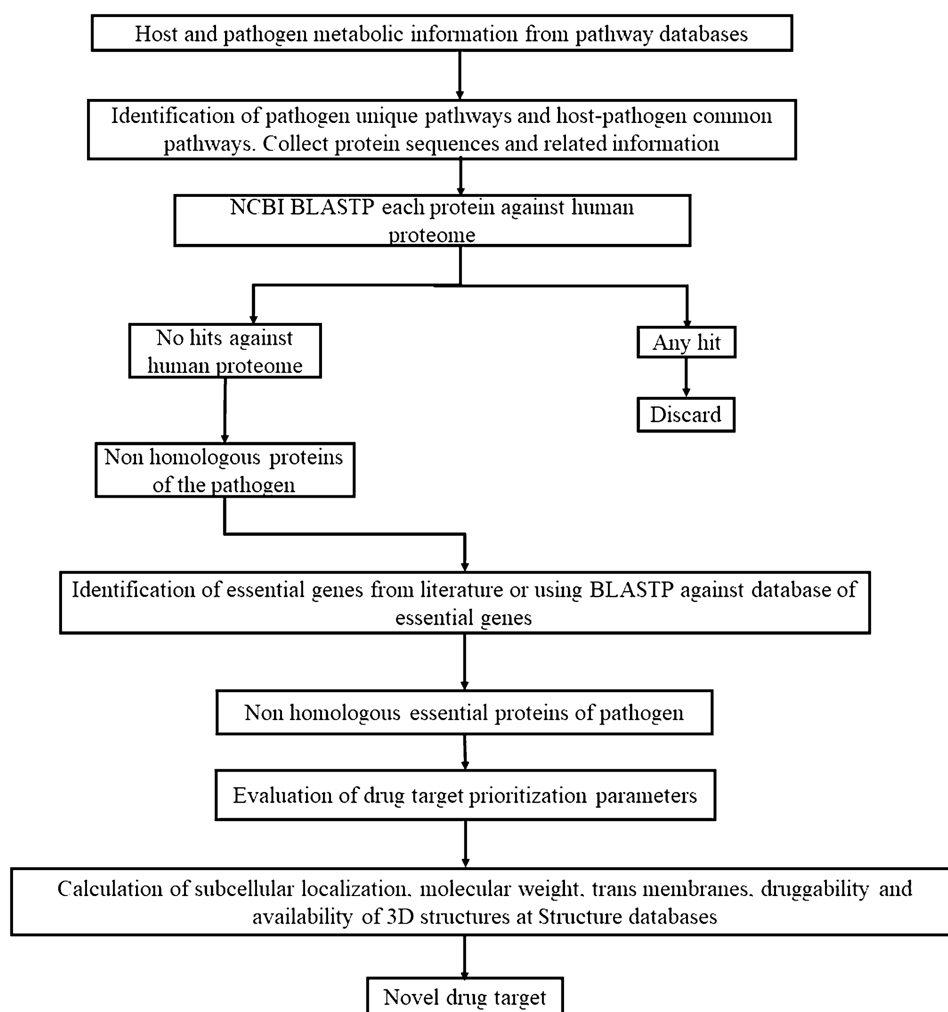

Fig. 1 General workflow of Drug target identification through comparative genomics approaches

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Drug targets in *Mycoplasma genitalium*

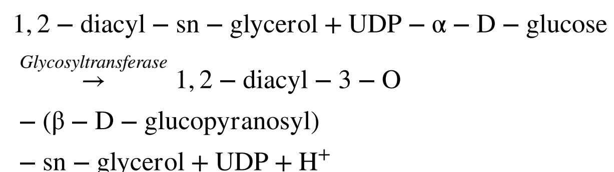
Prior knowledge

M. genitalium is a common sexually transmitted bacterium that causes up to 25% of cases of nongonococcal urethritis and has been linked to cervicitis and pelvic inflammatory disease. The macrolide antibiotic azithromycin is the standard first-line treatment, however, an increase in treatment failure over the last five years shows the development of antibiotic resistance. In many *M. genitalium* populations, mutations causing macrolide resistance have been discovered in the 23S rRNA gene. When azithromycin became ineffective, the fluoroquinolone, moxifloxacin was deemed to be a viable option. However, several investigations confer the fluoroquinolone (second-line antibiotic) resistance due to mutations in the *parC* and *gyrA* genes. However, various quinolones, like moxifloxacin, show substantial antimicrobial activity against *M. genitalium*, and it is recommended as a second-line treatment for persistent or recurrent NGU (Jernberg et al. 2008). Quinolones shows their antimicrobial properties by inhibiting topoisomerases II, DNA gyrase (composed of two GyrA and two GyrB subunits), and topoisomerase IV (composed of two ParC and two ParE subunits). Quinolone resistance has been linked to alterations in the genes that determine quinolone resistance of the genes encoding DNA gyrase and/or topoisomerase IV in different species of bacteria, including mycoplasmas (Yoshida et al. 1990; Pan et al. 1996; Bebear et al. 1999; Gruson et al. 2005).

Current advances

Glycosyltransferase

Glycoglycerolipids (galactolipids) are membrane components linked with photosynthetic tissues found in plant chloroplasts as well as in cyanobacteria (Hölzl and Dörmann 2007; Benning 2008). Gram-positive bacteria create glycolipids with a wider structural diversity, in which the diacylglycerol headgroups contain glucosyl, galactosyl, or mannosyl units with a range of glycosidic linkages (Hölzl and Dörmann 2007). Their functions range from serving as membrane anchors for other biomolecules (such as lipoteichoic acids) to act as free components in the bilayer stability of membranes (Kiriukhin et al. 2001; Gründling and Schneewind 2007; Hölzl and Dörmann 2007). As part of the subsequent role, glycosyl residues are incorporated into monoglycosylidiacylglycerol to produce diglycosylidiacylglycerol that forms bilayers (Wieslander et al. 1980; Lindblom et al. 1986; Dahlqvist et al. 1995). Glycosyltransferases (GTs) function on a diacylglycerol substrate to start the synthesis of glycoglycerolipids. Glycolipid synthases are divided into GT families based on their sequence (carbohydrate-active enzyme classification). Despite the fact that only a few have been studied enzymatically, most have mono- or di synthase ability, with one enzyme for diacylglycerol glycosylation and another for further glycosylation to produce the diglycosyldiacylglycerol product (Cantarel et al. 2009). Monoglucosyldiacylglycerol (MGlcDAG) synthases belong to the GT2 family of enzymes in cyanobacteria (Awai et al. 2006), while digalactosyldiacylglycerol (DGalDAG) synthases are tentatively referred to the GT4 family (Sakurai et al. 2007). It has been shown that *Mycoplasma pneumoniae* contains a processive GT2 that produces mostly galacto-glycerolipids (Klement et al. 2007). It is believed that GTs implicated in the biosynthesis of glycoglycerolipids could be viable therapeutic targets due to the critical significance of these components in membrane stability in *Mycoplasma* and the fact that these lipid structures are lacking in the animal host cells. A high-throughput screening test for GT based on fluorescence polarisation (FP-tag) was utilised to discover marine natural compounds as lead inhibitors (Gao et al. 2019). GT was also considered a suitable target in various other studies on *Staphylococcus aureus, Citrobacter rodentium* and *Escherichia coli* (*E. coli*) (El Qaidi et al. 2018; Kattke et al. 2019; Zhu et al. 2021). GT2 enzymes are found in the majority of *Mycoplasma* genomes (over 18 species) (Klement et al. 2007). They could be used to develop novel antibiotics.

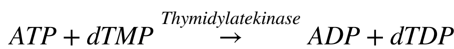


$$1,2-\mathrm{diacyl}-\mathrm{sn}-\mathrm{glycerol}+\mathrm{UDP}-\alpha-\mathrm{D}-\mathrm{glucose}\xrightarrow{Glycosyltransferase}1,2-\mathrm{diacyl}-3-\mathrm{O}-(\beta-\mathrm{D}-\mathrm{glucopyranosyl})-\mathrm{sn}-\mathrm{glycerol}+\mathrm{UDP}+\mathrm{H}^{+}$$

Thymidylate kinase

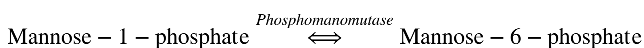
Thymidylate kinase (TMK) has been proposed as a potential therapeutic targets by various researchers in *M. genitalium* (Kazakiewicz et al. 2015; Butt et al. 2012). It catalyses the phosphorylation of thymidine 5′-monophosphate (dTMP) in the presence of adenosine triphosphate (ATP) and Mg2 + to generate thymidine 5'-diphosphate (dTDP), which is then transformed to thymidine 5′-triphosphate (dTTP) by nucleoside-diphosphate kinase (NDK) (Reichard 1988; Huang et al. 1994; Lavie et al. 1997). dTTP is a crucial component of DNA synthesis, and its activity is carefully regulated throughout the cell cycle and during cell proliferation (Reichard 1988; Ke et al. 2005). TMK is also the last specific enzyme in the routes for the synthesis of dTTP, a critical component of DNA synthesis. To prevent the onset of such diseases interrupting dTTP metabolism could play as key role (Cui et al. 2013). TMKs

are intensively investigated as prospective therapeutic targets in various other infectious diseases such as infections caused by *Staphylococcus aureus and Mycobacterium tuberculosis* (Martínez-Botella et al. 2013; Gul et al. 2020). In addition, Keating et al. reported piperidinylthymines (a group of synthetic molecules) as potential inhibitors of TMK in *Staphylococcus aureus* infection in mouse model (Keating et al. 2012).

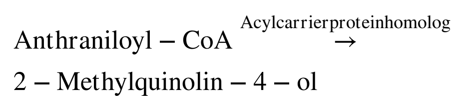

$$ATP + dTMP \xrightarrow{Thymidylatekinase} ADP + dTDP$$

Biosynthesis of secondary metabolites

Small bioactive compounds produced by bacteria in the stationary phase of growth are known as secondary metabolites (Ruiz et al. 2010). They are not needed for bacteria's growth and survival, but they do give them a competitive advantage (Craney et al. 2013). Several studies have demonstrated the modulation of bacterial secondary metabolite pathways and their use in developing antibiotics, antiviral medications, and other pharmaceuticals (Weber et al. 2003; Ozcengiz and Demain 2013; Onaka 2017). Phosphomannomutase, Acyl carrier protein homolog, and Phosphate acyltransferase are all discovered in *M. genitalium* and are found to be involved in secondary metabolite biosynthesis (Fatoba et al. 2021). The conversion of mannose-6-phosphate to mannose-1-phosphate, the main function of phosphomannomutase (ManB) is involved in the biosynthesis of guanosine diphosphate (GDP)-mannose for a variety of processes in prokaryotes and eukaryotes, including the synthesis of structural carbohydrates, the production of alginates and ascorbic acid, and the post-translational modification of proteins (Yang et al. 2010).


$$\text{Mannose} - 1 - \text{phosphate} \overset{Phosphomanomutase}{\Longleftrightarrow} \text{Mannose} - 6 - \text{phosphate}$$

Universal and highly conserved Acyl carrier protein (ACP) plays a major role in fatty acid biosynthesis as a carrier of acyl intermediate. The fatty acid synthase polyprotein (type 1 FAS) found in yeast and mammals contains ACP as a separate domain, whereas the type II FAS found in bacteria and plastids contains ACP as a small monomeric protein. A bacterial acyl carrier protein is involved in the synthesis of different products like endotoxin and acylated homoserine lactones as acyl donors. These products have an essential role in quorum sensing (Byers and Gong 2007). ACP-dependent enzymes are attractive antimicrobial drug targets because of their distinct and essential roles in growth and pathogenesis (Fatoba et al. 2021; Byers and Gong 2007). Furthermore, ACP homologs have a role in the production of secondary metabolites such as polyketides and nonribosomal peptides (Byers and Gong 2007).


$$\text{Anthraniloyl} - \text{CoA} \xrightarrow{\text{Acylcarrierproteinhomolog}} 2 - \text{Methylquinolin} - 4 - \text{ol}$$

The role of Phosphate acyltransferase is the reversible synthesis of acyl-phosphate (acyl-PO4) from acyl-[acyl-carrier-protein], which is catalysed by this enzyme. It uses acyl-ACP instead of acyl-CoA as a fatty acyl donor (Zhang and Rock 2008).

$$\text{Fattyacyl} - [\text{ACP}] + \text{Phosphate} \overset{Phosphateacyltransferase}{\Longleftrightarrow} \text{Acylphosphate} + \text{Acylcarrierprotein}$$

These three proteins involvement is invariable with observations from earlier studies showing the proteins implicated in this pathway are essential, non-homologous, and specific to various bacterial pathogens such as *Staphylococcus saprophyticus* and *Vibrio parahaemolyticus* (Hasan et al. 2020; Shahid et al. 2020).

Quorum sensing

Communication between microbial cells is known as quorum sensing (QS) and it relies on the detection and creation of signals called autoinducers (AIs) to access cell density and species complexity in populations. QS supports the coordination of collective behaviour among bacteria. AI ligands are quite specific to some QS receptors, while others are more promiscuous. QS is required for bacterial species to regulate crucial cellular processes that are required for surveillance, survival, and adaptation to changing environments. Bacteria can correlate with population density and species complexity changes in the vicinity and respond as a group when they monitor the accumulation of specific AIs (Hawver et al. 2016). The discovery of microbial quorum-sensing systems in recent years has sparked renewed interest in investigating drug resistance mechanisms as well as combating drug resistance. The findings reveal that the quorum-sensing system regulates a variety of cellular functions in microbes, including pathogenic gene expression, toxin production, and extracellular polysaccharide synthesis. It also plays a major regulatory role in drug efflux pumps and microbial biofilm development (Turan et al. 2017; Bäuerle et al. 2018; Wei and Zhao 2018). Antibiotic synthesis and sporulation are two further activities governed by quorum sensing (Abisado et al. 2018). In *M. genitalium*, the protein translocase subunit, which has been identified as a potential therapeutic target, is implicated in the quorum-sensing metabolic pathway (Fatoba et al. 2021). The SecYEG pre-protein conducting channel interacts with a part of the Sec

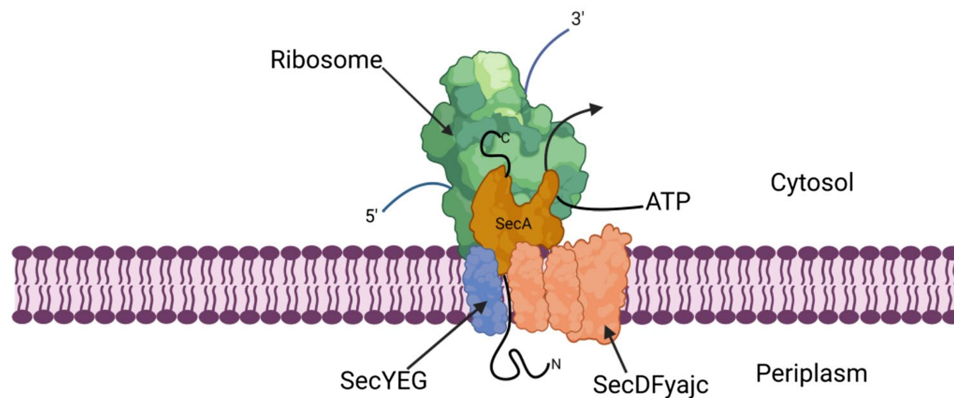


Fig. 2 Schematic representation of the preprotein translocase of *E. coli*. The cartoon shows the different units of the preprotein translocase including the motor protein SecA which drives preproteins across the membrane via a channel made up of an oligomeric assembly of SecYEG complexes. The SecDFyajC is another heterotrimeric membrane protein complex that stimulates the preprotein translocation. The ribosome contacts the SecYEG complex and SecA protein to facilitates the co-translational insertion of membrane proteins (Veenendaal et al. 2004)

protein translocase complex, as shown in Fig. 2, serves as an ATP-driven molecular motor that drives the progressive translocation of polypeptide chains across the cell membrane, connecting ATP hydrolysis to protein transfer into and across the cell membrane (Veenendaal et al. 2004). Biofilm production contributes to antibiotic resistance in *M. genitalium*, according to a recent study by Daubenspeck and co-workers (Daubenspeck et al. 2020). Proteins from this metabolic pathway have been identified as possible therapeutic targets due to the substantial involvement of quorum sensing in biofilm development in most bacteria (Haque et al. 2018; Jiang et al. 2019; Daubenspeck et al. 2020). Furthermore, the protein translocase component was found to be involved in the metabolic process of the bacterial secretion system (Butt et al. 2012). In the development of an effective antibacterial treatment against *M. genitalium*, the bacterial secretion system was also proposed as a novel therapeutic target to consider. These systems have been considered as therapeutic targets in many bacterial species such as *Staphylococcus aureus, Listeria monocytogenes, Vibrio cholerae, Klebsiella pneumoniae, and Yersinia enterocolitica* (Cegelski et al. 2008; McShan and De Guzman 2015; Green and Mecsas 2016).

Bacterial secretion system

Typically bacterial secretion system allows proteins to be transported from their cytoplasm to their host environment or straight into their host cells. This results in serious damage to the host cell due to increased virulence and pathogenicity of the bacteria (Waksman 2012). Proteins involved in bacterial secretion system such as protein translocase subunit SecA could be a suitable therapeutic target in *M. genitalium* (Fatoba et al. 2021). SecA is required for bacterial pathogenicity and survival as it aids in the release of important proteins, toxins, and virulent factors. Furthermore, since SecA is a membrane protein in its translocation functional form, SecA inhibitors can directly access SecA without entering the cytoplasmic space (Chaudhary et al. 2015). Sodium azide, an inorganic compound, was the first described inhibitor of SecA in *E. coli* (Oliver et al. 1990). Later, various classes of small molecule SecA inhibitors were discovered which include Rose bengal, Thiouracil-pyrimidine and Triazole-pyrimidine analogues (Jin et al. 2016).

Two-component system

In response to environmental signals, bacteria commonly use a two-component system (TCS). Histidine kinase and a sensor regulator make up TCS. It is related to the expression of virulence and antibiotic resistance responses in pathogenic bacteria (Lingzhi et al. 2018). VicRK (a TCS), for example, has been shown to influence biofilm formation, lipid metabolism, and pathogenicity (Ng et al. 2005; Ahn and Burne 2007). Similarly, in enterococci, the VanS/VanR two-component system regulates vancomycin resistance (Gagnon et al. 2011). Chromosomal replication initiator protein (DnaA) is predicted as a novel drug target involved in the TCS metabolic pathway (Fatoba et al. 2021). It is essential for the beginning and control of chromosomal replication. It binds to the replication origin at a 9-bp consensus (DnaA box): 5′-TTATC [CA]A[CA]A-3′ (Kitchen et al. 1999). In the literature, no TCS has been found in *M. genitalium* (Martinez et al. 2013). Signal transduction systems such as serine/threonine phosphatases (STP) and serine/threonine kinases (STK) have been shown to fulfill similar activities to TCS (Hoch 2000). Martinez and co-workers

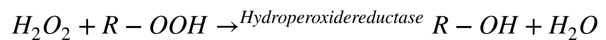
also discovered that STP plays a role in *M. genitalium* pathogenicity (Martinez et al. 2013). Other TCSs involved in bacterial pathogenicity and biofilm formation include DegS-DegU, Pho regulon (such as PhoR-PhoP, PhoR–PhoS, and PhoP-PhoQ), and QseC-QseB, which are found in *Bacillus subtilis*, *Corynebacterium glutamicum*, *Shigella* species, and enterohaemorrhagic *Escherichia coli.* As a result, a TCS can be used as an antibiotic target (Hirakawa et al. 2020).

Type I restriction-modification protein

Comparative genomics approaches have suggested that Type I restriction-modification protein could be a suitable therapeutic target against *M. genitalium* as well as in various other bacteria (Nogueira et al. 2021; Nammi et al. 2016). The restriction-modification system (RM system) is made up of big pentameric proteins known as type I restriction enzymes (REases) (Calisto et al. 2005). Restriction (R), methylation (M), and DNA sequence recognition (s) are all distinct subunits. Bacteria and other prokaryotic species have RM systems that defend them from foreign DNA such as bacteriophage DNA (Luria 1953). A restriction endonuclease and a methylase are the main components of this defence system. In foreign DNA such as viruses (phases) the restriction endonuclease cuts at a specific recognition site. The methylase enzyme adds a methyl group to a specific nucleotide shortly after replication, in the same target position as the restriction enzyme, to protect the bacterial DNA as illustrated in Fig. 3 (Vasu and Nagaraja 2013). Although Type I REases were the first to be found and purified, they have proven difficult to define. As genome analysis reveals their genes and methylome research reveals their recognition sequences, more information is getting available on these proteins for the researchers (Loenen et al. 2014; Nogueira et al. 2021).

Hydroperoxide reductase

Hydroperoxide reductase has been found a potential therapeutic target against *Mycobacterium tuberculosis* and *Candida albicans* in various studies (Seneviratne et al. 2008; Dhiman and Singh 2018). It has also been identified as a suitable therapeutic target against *M. genitalium* in various comparative genomics studies (Nogueira et al. 2021). Hydroperoxide reductase catalyses the conversion of hydrogen peroxide and organic hydroperoxides to water and alcohols, respectively (Seaver and Imlay 2001). It is an essential enzyme for cell defence against oxidative stress by detoxifying peroxides and preventing bacterial damage as hydrogen peroxide is produced during aerobic metabolism and can cause significant damage to biomolecules (Jacobson et al. 1989).



$$H_2O_2 + R - OOH \rightarrow^{Hydroperoxidereductase} R - OH + H_2O$$

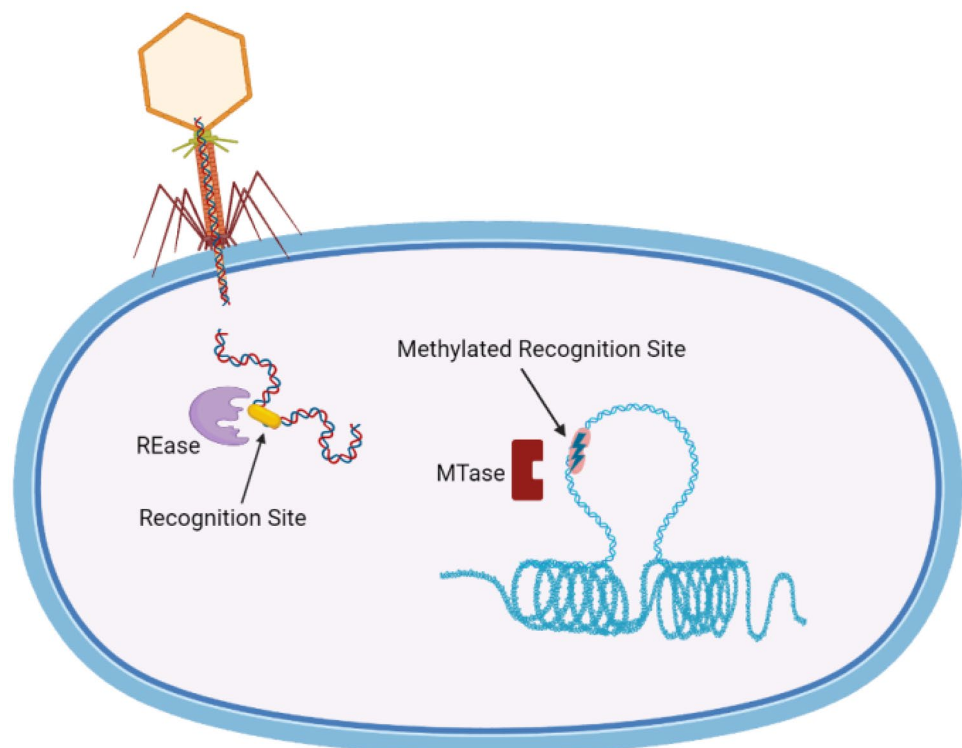


Fig. 3 Restriction-modification (R-M) system as defence mechanism. Incoming foreign DNA, such as phage genomes, is recognised by R-M system for its methylation status. Methylated sequences are recognised as self, but nonmethylated sequences on incoming DNA are recognised as nonself and cleaved by restriction endonuclease (REase). The cognate methyltransferase (MTase) of the R-M system maintains the methylation state at the genomic recognition sites (Vasu and Nagaraja 2013)

Ribosome-binding factor A

Ribosome-binding factor A (RbfA) was discovered to interact with free 30S subunits but not with 70S or polysomes. RbfA binds to the 5′ terminal helix region of 16S rRNA. It is an assembly cofactor that is hypothesised to enhance assembly more quickly in-vivo in comparison to in-vitro. RbfA mutations impair 17 s rRNA maturation as well as the capacity of the 30S subunits to bind with the 50S (Maguire 2009; Shajani et al. 2011). It is one of the primary proteins involved in the late maturation of the functional core of the 30S subunit, and it also functions as a cold-shock adaptation protein in *Escherichia coli*. RbfA-deficient cells are unable to adapt to low temperatures and have slower growth rates as well as altered ribosome profiles (Bylund et al. 1998; Xia et al. 2003). It is required for successful pre-16S rRNA processing and belongs to a broad family of small proteins found in many bacterial species, making it a prime target for structural proteomics (Huang et al. 2003). This protein is identified as a potential therapeutic target for therapeutic applications against *M. genitalium* (Nogueira et al. 2021).

Hypothetical protein

Gene identification techniques anticipate hypothetical proteins during genome analysis, but there is no experimental proof that they are expressed in vivo (Prava et al. 2018). Bioinformatics tools are currently used to identify new genes during the annotation stages of genome assembly, and when it finds open reading frames (ORFs) in the genome that show less identity than required to a known, identified protein in the database, the sequence is labelled as "putative" or "hypothetical protein." Structural and functional characterisation of such hypothetical proteins can be done by various in-silico and in-vitro approaches. The hypothetical proteins which have been found to play a vital role in the growth and pathogenesis of the organism can be considered potential therapeutic targets. These approaches are very crucial for developing therapeutic agents against drug-resistant infections caused by various organisms such as *Plasmodium falciparum*, *Chlamydia trachomatis* and *Klebsiella pneumonia* (Singh and Gupta 2022; Turab Naqvi et al. 2017; Pranavathiyani et al. 2020). Structural and functional characterisation of such hypothetical proteins present in *M. genitalium* provides novel therapeutic targets for screening to find new lead molecules against *M. genitalium* infection (Araújo et al. 2019; Nogueira et al. 2021).

DUF3217 domaincontaining protein

DUFs (domains of unknown function) are a huge collection of uncharacterized protein families that are assembled in the Pfam database, accounting for 20% of all known protein families (Bateman et al. 2010). The Pfam database is a database of protein families and domains that is frequently used to annotate sequenced genomes and assemblies (Finn et al. 2014). The DUF3217 (PF11506) domain is found in *Mycoplasma* proteins and appears to be confined to them. All proteins having this domain appear to belong to *Mycoplasma*, according to the Pfam database. While certain members of this protein family may be classified as MG376, this has yet to be proven. These proteins are suitable therapeutic targets for screening for a variety of disorders including *M. genitalium* infection (Nogueira et al. 2021).

Ribosomal proteins

Ribosomal proteins (L32 and S17) have been considered as therapeutic targets in *M. genitalium* by various researchers (Yang et al. 2020; Nogueira et al. 2021). The 50S ribosomal subunit contains the L32 protein that, along with L17 and L22, forms a cluster that holds all of the 23S rRNA domains together (Walleczek et al. 1989). The 50S ribosomal protein L32 is present on the solvent side of the large subunit and belongs to the bacterial ribosomal protein bL32 family (Brauer and Röming 1979). The ribosomal protein S17 binds directly to 16S rRNA and assists in the nucleation of assembly of the platform and body of the 30S subunit by bringing together and stabilising connections between numerous distinct RNA helices. The shoulder and platform of the 30S subunit appear to be held together by a cluster of proteins derived from S8, S12, and S17 (Held et al. 1974; Yang et al. 2020).

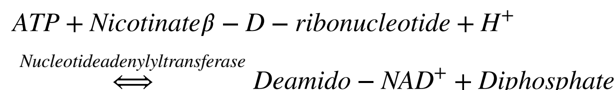
Class Ib ribonucleosidediphosphate reductase assembly flavoprotein (NrdI)

Ribonucleotide reductases (RNRs) are enzymes that convert ribonucleoside diphosphates to deoxyribonucleoside diphosphates, which are required for DNA synthesis and repair (Reichard 1988). Till date, three classes of RNRs (I, II, and III) have been identified based on their diverse processes, quaternary structural differences, and cofactor differences. Class I RNRs are further subdivided into classes Ia and Ib (Jordan et al. 1997). A dimanganese-tyrosyl radical is found in Class Ib (NrdI) enzymes on a cysteine residue and it is involved in the formation of a transient thiyl (sulfanyl) radical. It was found in Salmonella that in presence

of NrdI, NrdH and NrdEF have a stimulatory effect on their ribonucleotide reductase activities. In *M. genitalium*, protein NrdI has been discovered as a novel therapeutic drug target (Jordan et al. 1996; Nogueira et al. 2021).

Nucleotide adenylyltransferase

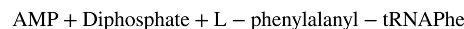
The Nucleotide adenylyltransferase is a crucial enzyme for nicotinamide adenine dinucleotide (NAD) production and plays an important role in a variety of redox processes. This protein is engaged in the first step of the deamido-NAD (+) synthesis pathway from nicotinate D-ribonucleotide. This subpathway is a part of NAD (+) biosynthesis route, which is part of the cofactor biosynthesis pathway (Rodionova et al. 2014). This protein has previously been identified as a therapeutic target in *M. tuberculosis* and has been linked to the development of new anti-mycobacterial drugs (Petrelli et al. 2011). Later, it was also suggested as a potential therapeutic target against *M. genitalium* by Yang et al. (Yang et al. 2020).



$$ATP + Nicotinate\beta - D - ribonucleotide + H^{+} \overset{Nucleotideadenylyltransferase}{\Longleftrightarrow} Deamido - NAD^{+} + Diphosphate$$

Phenylalanine–tRNA synthetase

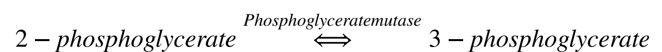
The phenylalanine tRNA synthetase (PheRS), a member of the class II aminoacyl-tRNA synthetase family, catalyses the synthesis of phenylalanyl-tRNAPhe (Phe-tRNAPhe) (Mermershtain et al. 2011). As a result, PheRS, like other aminoacyl-tRNA synthetases, plays an important part in the cellular translation process. Its structural characterisation reveals remarkable architectural diversity ranging from monomer to hetero-oligomer. An editing domain in heterotetrameric PheRS prevents the inclusion of non-cognate amino acids (Banerjee and Chakraborty 2016). Monomeric PheRS, on the other hand, lacks editing activity. PheRS has also exhibited certain noncanonical capabilities including DNA binding and its involvement in complicated regulatory circuits. Engineered mutants of PheRS with relaxed substrate specificities could be a useful tool in chemical and synthetic biology (Banerjee and Chakraborty 2016). PheRS was considered a possible therapeutic target in drug development for the treatment of *S. aureus* multidrug resistance, as it has a natural substrate binding site (Uddin and Saeed 2014). Subsequently, PheRS was suggested as a potential therapeutic target against *M. genitalium* through comparative genomics approaches (Yang et al. 2020).



$$\mathrm{ATP + L - phenylalanine + tRNAPhe} \rightarrow^{Phenylalanine-tRNAsynthetase} \mathrm{AMP + Diphosphate + L - phenylalanyl - tRNAPhe}$$

Phosphoglycerate mutase

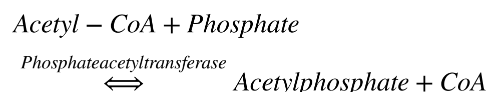
Phosphoglycerate mutase (PGM) is an enzyme that involves in the interconversion of 3–phosphoglyceric acid (3PGA) and 2–phosphoglyceric acid (2PGA) in the glycolysis and gluconeogenesis pathways. There are two forms of PGMs: cofactor-dependent PGMs (dPGMs), which require 2, 3–diphosphoglyceric acid (DPG) for catalysis and use this molecule as a phosphate donor; and cofactor-independent PGMs (iPGMs), which do not use DPG. In vertebrates (including humans), certain invertebrates, fungi, and some bacteria, notably Gram-negative bacteria like *Escherichia coli*, the dPGMs are the most abundant and often the only PGMs present. The phosphoglycerate mutase enzyme in *Geobacillus stearothermophilus* is a well-studied drug target (Jedrzejas 2000). As a novel drug target phosphoglycerate mutase was considered to be a potential drug target in drug development in *Mycoplasma genitalium* (Yang et al. 2020).



$$2 - phosphoglycerate \overset{Phosphoglyceratemutase}{\Longleftrightarrow} 3 - phosphoglycerate$$

Phosphate acetyltransferase

Phosphate acetyltransferase has been explored as a potential drug target against various organisms such as *Bacillus subtilis* and *Staphylococcus aureus* (Yang et al. 2020; Morya et al. 2012). It has been identified as a suitable target against *M. genitalium* in comparative genomics (Yang et al. 2020). This enzyme is engaged in step 2 of the acetate-to-acetyl-CoA conversion process from acetate. This process is part of the acetyl-CoA biosynthesis pathway, which is part of the metabolic intermediate biosynthesis pathway (Shin et al. 1999). In a computer simulation of *M. genitalium*, phosphate acetyltransferase, which is involved in acetyl-CoA production, was considered as a potential therapeutic target. Acetylphosphate inhibits phosphate acetyltransferase, a therapeutic target in *Bacillus subtilis* (Yang et al. 2020).



$$Acetyl - CoA + Phosphate \overset{Phosphateacetyltransferase}{\Longleftrightarrow} Acetylphosphate + CoA$$

Conclusion

M. genitalium is a sexually transmitted pathogen that causes various human diseases such as acute and chronic urethritis, cervicitis, pelvic inflammatory disease, and possibly female infertility. It has emerged as a superbug, with rising resistance in this bacterium and only a few treatment options available. Future research should focus on finding new potential therapeutic targets and the development of novel antimicrobials. As monotherapy is no more effective, combination therapy along with antimicrobial resistance testing is essential. Because of the misuse of antibiotics as part of syndromic management, etiology-based treatment could help against this emerging antimicrobial resistance. In this article potential therapeutic targets of *M. genitalium* have been discussed. Inhibiting or modulating these targets could be a rational strategy for developing novel antimicrobial drug molecules against various *M. genitalium* infections which require extensive further investigations.

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Declarations

Conflict of interest The authors declare that there is no competing conflict of interest.

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Consent to participate Not applicable.

Consent for publication Not applicable.

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