1.1Introduction

*Mycoplasma genitalium*was first isolated in 1981 by Tully,*et al.* from two men with non-gonococcal urethritis(Tully *et al*., 1981).

Two isolates were grown on SP4, a transport medium that they had developed two years earlier(Tully *et al*., 1977).

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 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 inthe years following its discovery(Ueno *et al*., 2008).

However, after the introduction of molecular diagnostic assays, many clinical studies were performed, mainly in developed countries.Themajorityof these studies have shown an association of *M. genitalium*with NGU(Yu *et al*., 2008).

Improvement in laboratory detection methods, particularly with theintroduction 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(Clarivet *et al.*, 2014).

1.2. Rationale

*Mycoplasma genitalium*is a serious public health problem in young women in the true burden of infection and related sequelae are unknown. Considering *Mycoplasma* is often asymptomatic and testing rates for *Mycoplasma* remain low, the notification data for *Mycoplasma* are likely to underestimate the extent of *Mycoplasma* in the population. Further, there are few population based prevalence estimates and no incidence data for women. Internationally re-infection rates for Mycoplasmas are very high which is concerning considering repeated infections are more likely to cause serious upper genital tract infections, including PID and tubal factor infertility (Haggerty, 2008). However higher rate was reported byGaydos that had found in the STD clinic USA, the prevalence of *M. genitalium*was found to be 19.2% (Gaydos *et al.*, 2009), 4.5% was found in study done in UK the positivity *M .genitalium* infection in asymptomatic sexual-health clinic attendees (Mirnejad et al., 2011). Reported in Mozambique, but lower (4.3%) than that reported in United Kingdom(Zimba *et al*., 2011, Oakeshott *et al*., 2010).Moreover higher incidence was reported in other areas such as West Africa (26%), Kenya (16%), and Uganda (14%).

The infection rates for *Mycoplasma* in Sudan are unknown, it will be essential for developing clinical guidelines, in particular retesting guidelines and partner management for women who test positive. Re-infection rates and incidence data will be important data for the development of *Mycoplasma* control strategies.

1.3. Objectives

1.3.1. General Objective:

To determine the molecular characterization of *Mycoplasma genitalium*detected among Sudanese women with genitourinary infections.

1.3.2. Specific Objectives:

1. To perform molecular detection of *M. genitalium* in urine and high vaginal swabs specimens using Tag Man real-time PCR.
2. To determine the relative sensitivity of the urine and high vaginal swabs specimensinvestigated in the study.
3. To estimate the frequency rate and diseases attributable to *M. genitalium*among Sudanese women.
4. To determine the risk factors associated with genitourinaryinfections caused by *M. genitalium.*
5. To evaluate the leucocytes esterase test of urine and high vaginalswab specimens as a screen tool for *M .genitalium*genitourinaryinfections.
6. To perform molecular genotyping of partialmgpB gene of *M.genitalium*
7. To perform molecularscreening for macrolide antibiotics resistance at domain V 23SrRNA gene among positive *M genitalium*specimens.

2.Literature Review

2.1.Taxonomy:

Mycoplasmas are prokaryotes belonging to the family *Mycoplasmataceae*within the order *Mycoplasmatales*(Prescott LM, 2005).

The genera *Mycoplasma* and*Ureaplasma*are of the class *Mollicutesmollis*(soft); *cutis* (skin)] whichencompasses bacteria without a cell wall and are popularly termed thenaked bacteria(Prescott LM, 2005, Yu *et al*., 2008)**.**

The genus *Mycoplasma* contains more than 100 species, of which 13 are present as human flora. *Mycoplasmas* were initially thought to be viruses, since they couldpass 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**.**

In 1995, the International Committee onSystematic Bacteriology Subcommittee on the Taxonomy of *Mollicutes*defined new *Mycoplasmas* based on their ability to be filtered at verylow pore size and absence of a wall(BACTERIOLOGY, 1996).

In 2007, these standards were revised to include the deposition of the 16S rRNA gene sequenceinto a public database, and a phylogenetic analysis of the relationships among the 16S rRNA gene sequences of the novel species and itsneighbours(Brown *et al*., 2007).

The phylogenetic tree of evolution shows that *Mycoplasmas* may be descendants of Gram-positive bacteria, presumably of *clostridial*origin(BACTERIOLOGY, 1996, Yu *et al*., 2008)**.**

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(Himmelreich *et al*., 1997).

2.2. 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.*M. genitalium*and*M.pneumoniae*have the characteristic morphology with a terminal/apical tip organelle(Taylor-Robinson, 1995)

The electron micrograph of G-37 and M-30 *M. genitalium*strains (Fig.1) 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(JOSEPH G. T U L L Y, 1983).

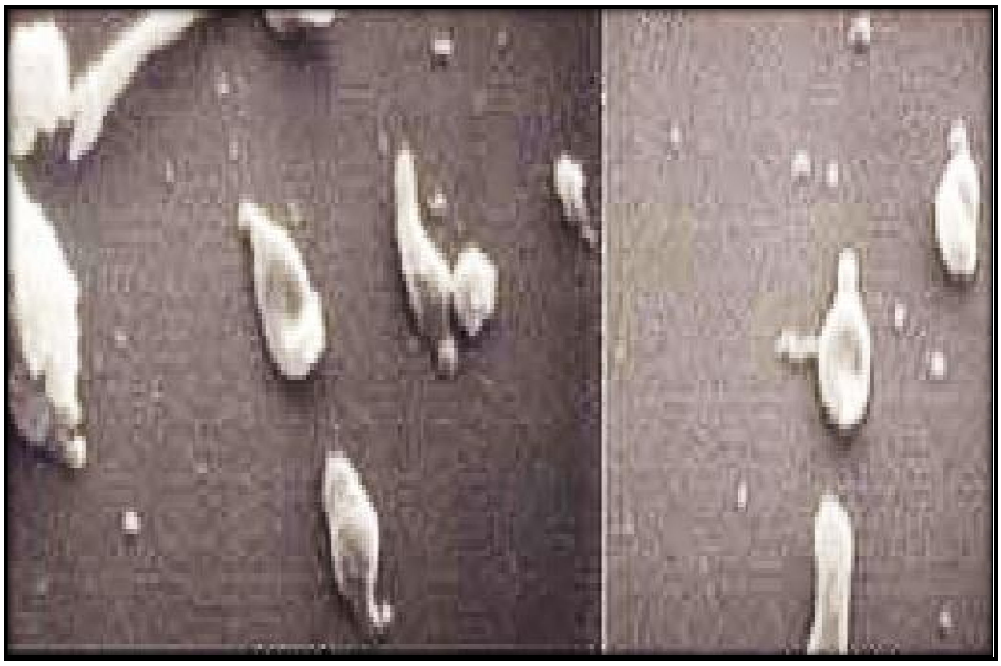


Fig. 2.1Electron microscope of *M. genitalium*showing pear-like appearance(Google, 2014b).

The electron micrograph demonstrates G-37 and M-30 *M. genitalium*strains, 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 terminal tip organelle is specialized 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(Tully *et al.*, 1981, Gasparich, 1995)**.**

*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 change in its flask-like shape (Fig. 1.2). 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(Taylor-Robinson, 1995).

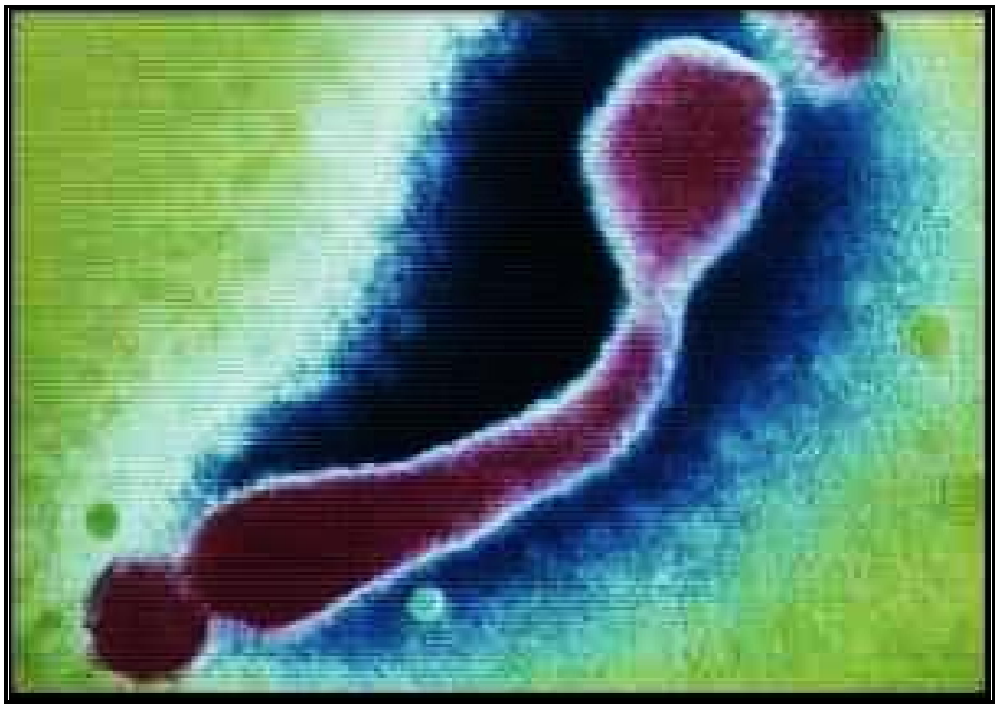


Fig. 2.2 Flask-like shape of *M. genitalium*in the host environment(Google, 2014a).

*M. genitalium*does not have a peptidoglycan cell wall. Its absence means that this microorganism has less osmotic stability in the host environment and therefore is prone to modify in its flask-likeshape.

2.3. Metabolism

In spite of the small genome possessed by the *Mycoplasmas*, they have the ability to self-reproduce. *M. genitlium*metabolizes glucose, resulting in the production of acid(Gasparich,1995, Taylor-Robinson,1995)

The metabolism of substrate (glucose) phosphorylation 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(Pollack *et al.*, 2002)

2.4.Genomicstructure

The fastidious nature of *M .genitalium* can be explained by its reduced genome, which at 580kb, makes thus smallest genome of any known self replicating cellular organism(Iverson, 2008)

In 2006, Glass *et al.* identified 382 of the 482 *M. genitalium*protein coding genes as essential(Glass *et al.*, 2006)

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. influenzae*.Ueno *et al.* (2008) found 484 coding regions(Ueno *et al.*, 2008)

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 synthesize truncated proteins(Seto *et al.*, 2001)

*M. genitalium*falls under the so-calledlow 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(Mombach *et al.*, 2006, Bizarro CV, 2007)

The G+C content in the DNA of most *Mycoplasmas*ranges from 24% to 33%, with *M. genitalium*at 32%.The significance of the low G+C content is that *M. genitalium*would have a lower melting temperature (Tm) during the doublestranded 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 gene (coding for the adhesin proteins), the rRNA genes, and the housekeeping gene, gap coding for GAPDH(Jensen *et al*., 1991).

Annotation of*M.genitalium* genome suggests that this organismsar deficient for many cellular pathways deemed essential in layer, more complex bacterial organisms. To overcome reduced genomic capabilities *M. genitalium*parasitizes the mucosa of the human urogenital tract and through thus intimate association is provided with necessary supply of the compounds these bacteria are unable to synthesize(Ueno *et al*., 2008).

2.5. Pathogenesis *M. genitalium*

*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 adhesions, the release of enzymesand the ability to invade the host immune response by antigenic variation(Razin *et al*., 1998).

Attachment and entry:*Mycoplasma* lack cell walls and cell wall-associated structure 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(Pich *et al*., 2009)**.**

The majoradhesion in the attachment protein complex is the MgPa protein and, together with the P32 (MG318) protein, makes up the terminal tip organelle(Inamine *et al*., 1989, Reddy *et al*., 1995).

The MgPa encodes the P140 (MG191) and P110 (MG192) cytoadherence proteins (cytoadhesions) at the tip area(Yu *et al*., 2008, Burgos *et al*., 2006)**.**

These proteins are immunogenic both in immunizedanimals and in humans. Loss of either P140 or P110 results in loss of motility and adherence properties of the entire MgPa attachment organelle(Pich *etal.*, 2009, Gasparich, 1995)**.** Thus showing the importance of these proteins in attachment. The MG218 and MG317 cytoskeletal proteins were shown to play a role in terminal organelle organization, gliding motility and cytoadherence(Pich *et al*., 2008)**.**

The genes encoding the adherence proteins are located in three different regions of the *M. genitalium*genome. The genes coding for the MgPaadhesions are organized in an operon with three genes**,** consisting of ORF-1 (MG190), ORF-2 (MG191), and ORF-3 (MG192)(Inamine *et al*., 1989, Inamine *et al*., 1988).

The target cell membrane then invaginates in a manner similar to the clathrin-coated pits mechanism of endocytosis observed in *C. trachomatis*(Yu *et al*., 2008)**.**

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. This internuclear localization process may take place within 30 minutes after infection(Yu *et al*., 2008, Ueno *et al*., 2008)**.**

2.6. Enzymes secreted by *M. genitalium:*

Besides the role played by the adhesions, Alvarez *et al.* (2003) 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(Alvarez *et al*., 2003).

Thus GAPDH, among other binding proteins, acts as a ligand to receptors mucin and fibronectin, particularly in vaginal and cervical disease. In addition to GADPH, another enzyme, methionine sulfoxidereductase (MsrA), can be released to enhance the pathogenicity of its small genome(Alvarez *et al*., 2003, Dhandayuthapani *et al*., 2001)**.**

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(Dhandayuthapani *et al*., 2001)**.**

2.7. Invasion 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 invade the host immune response. The major antigenic determinants of the Mollicutes are their membrane proteins that are expressed on the surface. They are able togenerate a highfrequency 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(Moxon *et al*., 1994, Arber, 2000)**.**

Multiple copies of partial gene sequences have been found in most pathogenic bacteria, but as mycoplasmas have very small genomes, the number of *Mycoplasma* genes involved in diversifying the surface antigens is markedly high(Yu *et al.*, 2008)**.**

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(Razin *et al.*, 1998).

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 recognized by the host immune system on subsequent encounters(Yu *et al.*, 2008, Ueno *et al.*, 2008).

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(Yu *et al.*, 2008).

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(Svenstrup *et al.*, 2006).

2.8. Transmission:

Evidence suggests that *M. genitalium*is sexually transmitted. First, detection of the *M. genitalium*has been associated with a history of sexual intercourse and with increasing number of sexual partners(Burgos *et al.*, 2006, Inamine *et al.*, 1989).

Second, sequence-based typing of *M. genitalium*has revealed sexual transmission. In a study of 19 couples conducted by Hjorth *et al*, sequence-based typing of *M. genitalium*revealed sexual transmission, for the sequence type found in specimens from the female partner was identical to that found in the male partner in all the couples studied(Hjorth *et al.*, 2006).

On the contrary, *M. genitalium*has been detected in women who denied ever having any sexual contact. In the Manhart*et al* study, 2 of the 34 (0.05%) women who tested positive for *M. genitalium*denied sexual activity(Manhart LE, 2007)**.**

Tosh reported that only one of the 65 (1.5%) adolescent women who tested positive for *M. genitalium*denied sexual activity(Tosh AK1, 2007)**.**

Very little is known of vertical transmission and subsequent colonisation of newborn infants by *M. genitalium*(Yu *et al*., 2008)**.**

However, Waites*et al*. (2005) 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 fetus 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 colonization of the skin, mucosal membranes and respiratory tract of the neonate(Waites *et al*., 2005)**.**

2.9. Clinical presentations of *M. genitalium*among women:

*M. genitalium*is commonly asymptomatic in infected women. This is supported by a number of studies which have found no association with infection and any genital symptoms(Tosh AK, 2007, Andersen *et al*., 2007, Huppert *et al*., 2008).

In some cases, women infected with *M. genitalium*can present with genito-urinary symptoms including vaginal discharge and dysuria(Korte *et al*., 2006, Moi *et al*., 2009)**.**

Overall, the research suggests that*M.genitalium*presents similarly to *Chlamydia* but appears to cause milder symptoms than gonococcal infections in women(Moi *et al.*, 2009, Short VL, 2007)**.**

2.10. Clinical signs and symptoms:

Lower genital tract infection:In women, *M. genitalium*has been established as a cause of urethritis inwomen(Falk et al., 2005, Anagrius *et al.*, 2005) and cervicitis(Gaydos *et al.*, 2009, Moi *et al.*, 2009).

Cervicitis is a particularly important diagnosis as it increases the risk of developing an upper genital tract infection. The extent of *M. genitalium*related cervicitis.In two studies of women recruited from STD clinics inSweden, onestudy found 10.2% of women with cervicitis tested positive for *M. genitalium*(Falk *et al.*, 2005) and in the other study population showed 13.3% tested positive for *M. genitalium*(Anagrius *et al.*, 2005)**.**

Pepin *et al* reported a weak association with symptoms of cervicitis in West African female sex workers based on symptoms of cervical discharge, cervical pus, easily induced bleeding and inflammatory cervix ($p \leq 0.05$ for each of the four signs) which was similar to findings for *Chlamydia*(Pepin *et al*., 2005).

All these studies demonstrated a significantly higher proportion of women with cervicitis had *M. genitalium*than women without *M. genitalium*, however one study of a consecutive cohort of women with vaginal discharge at an STD clinic in Paris found a high prevalence of *M. genitalium*(38% ,95% CI, 31–46%) but found no association with *M. genitalium*infection and cervicitis. The limitations to these studies include the variability in the diagnostic criteria for cervicitis which is

based on clinical symptoms and/or the presence of inflammatory.Gaydos had found that the prevalence of women with cervicitis was 28.6% which means *M. genitalium* was significantly associated with cervicitis in multiple logistic regression models(Gaydos *et al.*, 2009).

It was found that*M. genitalium* has been positively associated with urethritis, vaginal discharge, and microscopic signs of cervicitis and/or mucopurulent cervical discharge in seven of 14 studies(McGowin and Anderson-Smits, 2011)**.**

Another supported this that *M. genitalium* cause severe urethritis and more often lead to symptomatic urethritis/ cervicitis than non-chlamydia-non gonococcal(Jernberg and Moi, 2007)**.**

Upper genital tract infections and *M. genitalium*:

A) Pelvic inflammatory disease (PID): The aetiology and natural history of pelvic inflammatory disease (PID) is not well understood, and is difficult to definitively diagnose as it can be asymptomatic and requires a clinical diagnosis. *N. gonorrhoeae*and*Chlamydia*are known causes of PID, however there is a large proportion non-gonococcal, non-chlamydial PID where the primary pathogen is unknown(Haggerty, 2008).

There is more recent evidence to support the association of *M. genitalium*with PID using more accurate diagnostic techniques such as PCR. One study reported 7 of 50 women (14%) diagnosed with PID tested positive for*M. genitalium*and negative for *Chlamydia*and *N. gonorrhoeae*(Haggerty CL, 2006).

A recent review of the literature also stated there is a strong evidence that *M. genitalium*is associated with PID considering all the evidence

associating *M. genitalium*with acute endometritis and adnexitis, independent of gonococcal and chlamydial infection(Haggerty, 2008)**.**

The proportion of PID attributable to *M. genitalium*is unclear, particularly as there are limited and inconsistent PID data and few *M. genitalium*studies. Predictors for *M. genitalium*associated PID have been reported as younger age (under 25 years) [AOR: 2.7 (95% CI: 1.5, 4.7)], douching two or more times per month [AOR: 2.0 (95% CI 1.2, 3.4)], and smoking [AOR:2.0 (95% CI 1.3, 3.3)](Short VL, 2009).

A recent study specifically designed to determine the attributable risk of *M. genitalium*with PID found that *M. genitalium*was unlikely to be a major contributor to PID infection in young women in the UK [risk ratio: 2.35 (95% CI: 0.74, 7.46)(p=0.14)]. This study involved a large population of women followed up over at least one year, and the clinical records of any of the patients diagnosed with PID were reviewed by two physicians who did not know the bacteriological results(Oakeshott *et al*., 2010).

B) Tubal factor infertility (TFI): There has been some evidence Tubal factor infertility can follow an episode of PID if the fallopian tubes undergo cellular and sub-cellular damage and ciliary motion of damaged epithelial cells is reduced(Wiesenfeld H, 2008)to support *M. genitalium*associated salpingitis and *M. genitalium*has been detected in the fallopian tube tissue of a woman with acute salpingitis(Cohen *et al*., 2005).

Svenstrup*et al* followed this up in 2007 and determined *M. genitalium*was detected in 17% of women with tubal factor infertility compared with 4% of women with normal tubes [OR: 4.5 (95% CI 1.2, 15.6)](Svenstrup *et al*., 2008).

As with *Chlamydia*, fallopian tube damage is hypothesized to be caused by *M. genitalium*damaging ciliated human fallopian tubes causing scarring and occlusion(Baczynska *et al*., 2007).

Jaanna*et al* had found that *M. genitalium*is a species having an impact on impaired fertility in women(Grzesko *et al*., 2009).

*M. genitalium*was found in the cervical canal of 19.6% of all infertile patients and in 4.4% of fertile patients. In addition, the pathogen was discovered in the cervical canal of 29% patients with unexplained (idiopathic) infertility, whichin comparison with the fertile group was a statistically significant difference(Grzesko *et al*., 2009).

C) Endometritis: There is also evidence to support *M. genitalium*as a cause of endometritis, in a study of 115 women who presented to an STD clinic in Nairobi with persistent acute pelvic pain for no more than 14 days. Of the 58 women who had histologically confirmed endometritis, 9 (16%) tested positive for*M. genitalium*in either their cervix, endometrium or both, compared with only one women (2%) who tested positive for *M. genitalium*out of the 57 women without endometritis(p=0.02).In another study, 9 (16%) of women with histological confirmed endometritis tested positive for *M. genitalium*compared with one (2%) of women without *M. genitalium*[p=0.02](Cohen *et al.*, 2002)**.**

2.11. Adverse pregnancy outcomes:

Because *M. genitalium*may be associated with PID and TFI, it is plausiblethat *M. genitalium*can infect the upper genital tract during pregnancy, resulting in adverse pregnancy outcomes. The prevalence

of *M. genitalium*in pregnant women has ranged from less than 1% to over 20%(Kataoka *et al*., 2006)**.**

However, the consequences of prenatal *M. genitalium*are unknown.Results have been conflicting about the potential role *M. genitalium*has played in causing adverse pregnancy outcome. Edwards *et al* found an association between*M. genitalium*infection and preterm labour [OR 3.5 (95% CI: 1.4, 8.6)](Edwards RK, 2006)**.**

Other studies have found no relationship between pregnancy complications and *M. genitalium*infection. In Japan, women were tested for mycoplasmae, ureaplasmae, chlamydiaeand gonorrhea, and although adverse pregnancy outcomes were associated with infection with*Ureaplasmaparvum*there was no association with *M. genitalium*and adverse pregnancy outcome(Kataoka *et al*., 2006)**.**

In a more recent study done by Short *et al* in pregnant women presenting at the emergency department of a hospital in the United States of America, no association was found with spontaneous abortion during pregnancy [AOR:0.9 (95% CI 0.2, 3.8)](Short VL, 2007)**.**

2.12. Risk factors of *M. genitalium*:

Young age is a risk factor for STDs, especially for females. Biologically, younger females are more susceptible to bacterial infections than older females due to cervical ectopy. During adolescence, endocervical columnar epithelial cells extend to the vaginal surface, increasing the surface areaand increasingthe number of receptive cells which may favor the growth of some mucosal pathogens(Sharpe *et al*., 2010)**.**

Younger women may also be at a greater risk for STDs because they may be more likely to engage in risky sexual behaviors, such as unprotected intercourse and multiple sexual partners. Non-white race is also associated with STDs(Delcher *et al*., 2008)**.**

Douching can alter the vaginal microflora, remove protective components from the vagina or cervix, and/or promote the ascension of microorganisms from the lower to the upper genital tract, all increasing a woman's susceptibility to infection(Hillier S, 2008).

Manhart*et al* used data from a sub sample of participants in Wave III of the National Longitudinal Study of Adolescent Health (Add Health) to examine the potential risk factors of *M. genitalium*.PCR was used to test the urine of 1714 women and 1218 men aged 18 to 27 years. *M genitalium*infection was strongly associated with ever having engaged in vaginal intercourse (Prevalence Ratio (PR) 22.5, 95% CI 4.3-116.6), and in multivariate analyses the prevalence of *M. genitalium*increased by 10% with each additional vaginal intercourse partner in the past year (PR 1.1 per partner in the past year, 95% CI 1.0-1.2).Further, *M. genitalium*was more prevalent in individuals that ever lived with a sexual partner (PR 11.2, 95% CI 3.2-39.5), and in individuals who reported Black race (PR 7.2, 95% CI 2.9-17.9) and condom use during last sexual intercourse (PR 3.9, 95% CI 1.3-11.5).*M. genitalium* was not associated with age, age at sexual debut, or correct and consistent condom use over the past year(Inamine *etal*., 1989).

In another study conducted in the U.S., Huppert *et al* tested vaginal swabs from 331 sexually active female adolescents aged 14 to 21 years recruited from inner-city medicalcentrefor *M. genitalium*using PCR.Sexual intercourse within the last 7 days was associated with a

2-fold increase in the odds of*M. genitalium*infection (OR 2.0, 95% CI 1.1-3.2), after adjusting for *C. trachomatis.M. genitalium*infection was not independently associated with demographic variables including age, race and sexual behaviors such as inconsistent condom use, new sexual partner, or multiple sexual partners(Huppert *et al.*, 2008)**.**

Tosh *et al* used PCR to test vaginal samples from 383 female adolescents aged 14-17 years enrolled in urban primary health care clinics in the U.S.With the exception of one individual, *M. genitalium*was identified exclusively amongindividuals reporting history of vaginal intercourse. Having a recent sexual partner (OR 1.4, 95% CI 1.2-1.7) was the only behavioural characteristic independently associated with *M. genitalium*(Glass *et al.*, 2006).

2.13. Laboratory diagnostics methods:

Clinical isolation and *in vitro* growth:The most widely researched *on M.genitalium* strain G-37. This typeof strain was isolated by Tully in the decades since its initial identification, fewer than twenty clinical strains been isolated, but the ability to isolate axenically growing *M.genitalium* directly from patient specimens is time consuming and laborious process that involves blindly passaging samples in tissue culture and, then slowly, adapting growth to cell free media condition. Thus still unclear why many clinical strains require the initial cell assisted growth. Once*M.genitalium* has been adapted to in vitro broth condition, growth is detected by a red to yellow colorchanges as medium pH shift by production of acid through glucose fermentation. Growth of*M.genitalium* on solid medium required a soft 1% agar and relatively long incubation period. Colonies with the characteristic fried -egg (Fig.2. 3) morphology

generally appears after 2-3 weeks and can be visualized using a dissecting microscope. When isolating a single–colony- clone, it is recommended that cultures be subjected to filter cloning at least three times due to the adherent nature of *M. genitalium*cells(Ma *et al*., 2010).

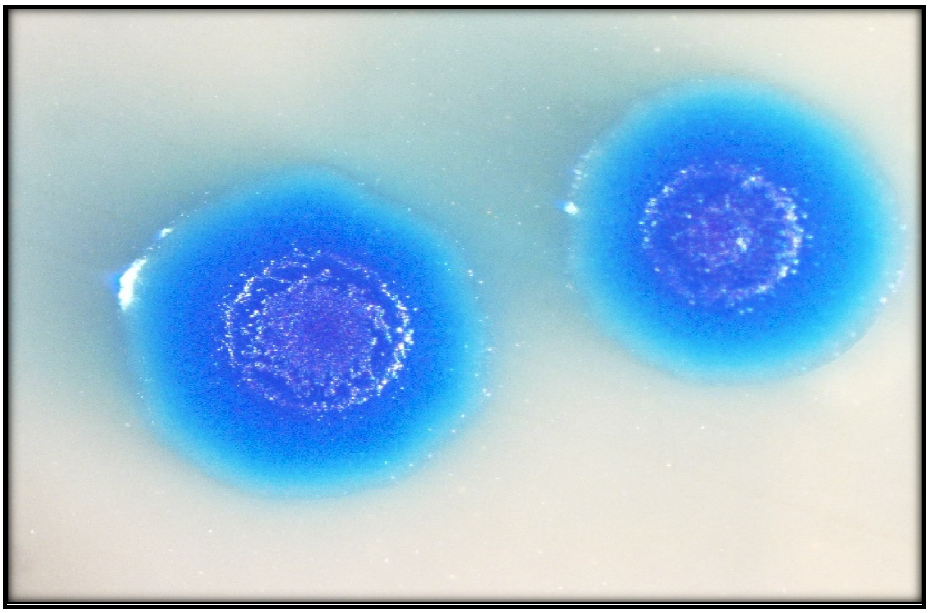


Fig. 2.3Fried-egg colony appearance of *M. genitalium* after2-3 weeks incubation(google, 2014c).

2.14. Serlogical tests for *M. genitalium:*

*M. genitalium*shares several structural properties with another human pathogen, *M. pneumoniae*, and cross-reactivity between these two *Mycoplasma* species can result in lack of adequate specificity when using serology for diagnosis(Huppert *et al*., 2008)**.**

Since traditional diagnostic methods, such as culture and serology, are not suitable for routine diagnosis of *M. genitalium*, identification of infected individuals has been entirely dependent on nucleic acid amplification tests (NAATs).

2.15. Molecular diagnosis:

A) Real-time PCR: Jensen *et al* in 1991 had developed a PCR test based on the MgPa-1/MgPa-3 primer set located in the conserved regions of the mgpB gene which can be detected at very low levels, making testing for diagnostic purposes in clinical samples possible(Jensen *et al*., 2003, Jensen *et al*., 1991).

*M. genitalium*strains isolated from clinical samples showed a degree of diversity in the main gene of the MgPa gene sequence which led to the development of a PCR amplification test based on therRNA gene sequences(Jensen *et al*., 2003), specifically relying on the detection of the 16S rRNA sequence specific to *M. genitalium*(Jensen *et al*., 1996).

In a comparative study by Edberg *et al* to determine the differences between MgPa gene PCR and 16S rRNA gene PCR, the results reported real-time MgPa gene PCR detected 97.4% of *M. genitalium*in true-positive samples, conventional 16S rRNA gene PCR detected 80.3% and real-time 16S rRNA gene PCR detected 68.4%(Edberg *etal*., 2008).

Contrary to this, in 2009, a sample of 830 stored vaginal swab samples were tested for *M. genitalium*with both the 16S rRNA gene PCR and the real-time MgPa gene PCR. The 16S rRNA gene PCR was found to have a concordance of 98.9% (sensitivity 95.0 % and specificity 99.1%) when compared with real-time MgPa gene PCR, suggesting it is as valuable an assay to use for *M. genitalium*diagnosis in clinical samples as the MgPa gene PCR assay(Wallander *et al*., 2012)**.**

Although true sensitivity and specificity of any of the PCR assays in women are unknown, one study demonstrated a high specificity of

PCR tests 99.6% relative to 68 infected patients who tested positive with two different assays There is no 'gold standard' test as yet for *M. genitalium* detection(Wroblewski *et al.*, 2006)**.**

B) Transcription-Mediated Amplification (TMA): Alternative to PCR, a transcription-mediated amplification (TMA) assay targeting the 16S rRNA, a molecule present in up to 100–1000 copies per bacterial cell, thereby increasing the sensitivity of detection compared with the PCR assays that target single-copy genes, and has been offered recently. This method was shown to be a sensitive, specific and high-through test for *M. genitalium*detection(Wroblewski *et al.*, 2006)**.**

2.16. *M. genitalium*sampling methods:

*M. genitalium*can be detected from a vaginal swab, endocervical swab or a first catch urine sample (FVU). Wroblewski*et al* estimated the relative sensitivities for the detection of *M. genitalium* is 91% for vaginal specimens, 53% for cervical specimens and 65% for FVU specimens using PCR compared with transcription mediated amplification test (TMA)(Wroblewski *et al.*, 2006)**.**

Although Moi*et al*(2009) found a higher sensitivity in cervical swabs than FVU (86% versus 62% respectively..

The high sensitivity of self administered vaginal swabs makes home based testing for *M. genitalium*possible and increases accessibility and population based research options. Other study by (Rebecca*et al.*,2011) hadassessed the utility of urine, vaginal, cervical, and rectal specimens for the detection of*Mycoplasma genitalium* in women by using laboratory-developed PCR assay. The relative sensitivity was 85.7% for the vaginal swab specimen, 74.3% forthe endocervical

swab specimen, 61.4% for the urine specimen,and 24.3% for the rectal swab specimen.

2.17. Molecular typing:

Molecular typing is an important tool that can be used for show transmission of infection and the geographic distribution of strains providing valuable insights regarding *M.genitalium* virulence. The most reliable and best characterization method was developed by (Jensen*et al*.,1991) which distinguished *M. genitalium* strains based on nucleotide polymorphisms within the conserved ′5 region of the mgpB gene. The typing system has a high discriminatory index (0.95) for unrelated strains and has used to show strain stability over time in infected individuals concordance among sexual partners.The mgpB strain typing system has been the most extensively validated, but alternative molecular typing system based polymorphism with the rRNA operon and variation in the numbertandem repeats in the MG 309 gene have been developed. Additional, a recent report by Musatovova, *etal* proposed that the repeated proximal region of the mgpC gene might be useful for strain discrimination. Their evaluation of various *M .genitalium* strains showed a high degree of sequence diversitythat they interpreted to be indicative of strain variation(Ma *et al*., 2010)

2.18. Prevalence of *M. genitalium*in women:

Information of the prevalence of *M. genitalium*in general populations not seeking health care because of symptoms are limited. In one study reported from Denmark 731 men and 931 women, 21–24 years old,

who were participating in a population based *M.genitalium*screening program, were tested. The prevalence was 2.3 % in women and 1.1 % in men(Andersen *et al*., 2007).

In another large population based adolescent health study from USA (Manhart*etal*.,2007). used urine specimens *M. genitalium* was detected in 0.8% of women and in 1.1 of the men.

In Recently study among young women seeking legal abortion in New Zeelandshowed *M. genitalium*8.7%[(66)] while another study from Denmark reported a low prevalence in a similar population 0.98 %(Baczynska *et al*., 2008).

Japanese study of female students in vocational schools. *M .genitalium*was detected in 2.8% and *Chlamydia trachomatis* in 8.8% in firstvoid urine specimens(Manhart LE, 2007).

Another study has been conducted in Sweden by Bjartling, among 7.598 women the prevalence of *M .genitalium*was 2.1%(Bjartling, 2009) .

However higher rate 4.5% was found in study done in UK the positivity *enitalium* infection in asymptomatic sexual-health clinic attendees is comparable with that of gonorrhea or *chlamydia*, no significant association were found between *M. genitalium* (RFLP)(Mirnejad et al., 2011).

Moi*et al* used PCR to test urine and cervical from 7646 women attending Norway sexual transmitted disease (STD) clinic, they found that the prevalence of *M.genitalium* was 4.5%(Moi *et al*., 2009).

The result was less in compared with female sex workers in STD grouped tested positive in Kenya 16 %(Cohen *et al*., 2007).

(Rahman,2008) has determined the prevalence of *M. genitalium*among health clinic attendees complaining of vaginal discharge in Bangladesh, 399 vaginal and cervical swabs was randomly collected and tested by PCR. the prevalence was found low in the population tested 0.8%(.

(Gaydos,2009) had done cross-section study of 324 women attending Baltimore city STD clinic USA, the prevalence of *M. genitalium*was found to be 19.2%.

In the same manner (Shipitsyna, 2009) has assessed the performance of five PCRs developed and currently used in Russia for diagnosis of M .*genitalium* by collecting vaginal swabs and first voided urine samples (FVU) from 281 female, the prevalence of *M. genitalium* was 2.5% among females.

2.19. Treatment of *M. genitalium*infection:

There are no available guidelines regarding the most effective *M. genitalium* treatment due to the lack of data from controlled clinical trials and in vitro susceptibility assays, which are problematic due to the low number of clinical *M. genitalium* isolates available for testing, in additional to lack of cell wall.*M .genitalium* cells are intrinsically resistant to penicillin and other beta-lactam antibiotics, but are likely to be susceptible to antibiotics in other classes including azithromycin and doxycycline.Of these, azithromycin appears to be the most effective with five-days treatment clinically curing *M. genitalium* infection in men with urethritis and women with cervicitis(Iverson, 2008). Now epidemiological studies have established a conclusive link between *M. genitalium* and reproductive tract infections in men and women, research examine the most effective antibiotic treatment and dosage, as well as investigations of treatment failure and possible

antibiotic resistance, are clearly needed for successful treatment and cure, however in US currently recommended by physician for women with pelvic inflammatory disease, to take ofloxacin, levofloxacin, doxycycline, cefoxitin, and/or metrionidazole for outpatient treatment(Iverson, 2008).

2.20. Current treatment recommendations:

The recommended treatment for *M. genitalium*is 1g azithromycin stat[(75)]. An important part of *M. genitalium*treatment is treatment of the patient's current and recent sexual partners with azithromycin, and having no unprotected sexwith an untreated partner for 7 days. A 'test of cure' is recommended one monthafter treatment to determine the infection has not persisted; particularly considering *M. genitalium*can be azithromycin resistant. If *M. genitalium*persists, it is importantto determine if the treatment has not been adhered to or not absorbed, or if it is are-infection from an untreated partner or from a new partner. If the infection is determined to be an azithromycin resistant infection, the recommended treatment is 400 mg/day of moxifloxacin for 10 days(Bradshaw *et al*., 2006, Bradshaw *et al*., 2008).

2.21. Antibiotic resistance:

*M. genitalium*has been found to be resistant to many antibiotics including quinolones and tetracyclines, except for moxifloxacin which appears to be effective against all the strains examined thus far(Bradshaw *et al*., 2008, Jensen *et al*., 2008, Bradshaw *et al*., 2006).

The current recommended treatment of azithromycin (1 g) has only an 85% efficacy at best for uncomplicated *M. genitalium*infections in both men and women(Bradshaw *et al*., 2008, Jensen *et al*., 2008, Bjornelius *et al*., 2008).

Other macrolide resistant strains of *M. genitalium*have been identified which are resistant to erythromycin and clarithromycin(Jensen *et al.*, 2008).

There is also concern that azithromycin resistance is increasing and moxifloxacin resistance might be developing. Resistance appears to be dependent on background use of azithromycin. In countries where azithromycin is used as a first line treatment for non-gonococcal urethritis (NGU) macrolide resistance is very high. Greenland for example uses azithromycin for NGU and one study had shown 100% (95% CI: 71.7, 100) of *M. genitalium*cases were resistant to azithromycin, whereas, in Sweden doxycycline is used for NGU and the same study was found 1.6% (5% CI: 0.4, 4.4%) *M. genitalium*macrolide resistance(JS., 2009).

Some clinicians are now suggesting that treatment for *M. genitalium*should include an extended course of azithromycin of 500 mg stat followed by 250 mg daily for 4 days. Future resistance to azithromycin appears to beinevitable and less expensive medication other than moxifloxacin will have to be explored to treat *M. genitalium(Hay P, 2009).*

3. Materials and Methods

3.1. Study approach:

This was a qualitative, prospectivestudy.

3.2. Study design:

The study design was a hospital-based, analytical study.

3.3. Study type:

The study type was a descriptive, cross-sectional study.

3.4. Study setting:

It was a hospital study setting. Specimens were collected from participantswith genitourinary infections.

3.5. Study duration:

This study was carried out during the period from June 2011 to July 2014,including the pilot study, literature review, specimens collection, laboratory investigations, data analysis, and thesis writing.

3.6. Study area:

This study was conducted at Khartoum North Teaching Hospital, and Al Hayat Charity Health Center (Khartoum).

3.7. Study population:

Non-pregnant, married women with symptoms of genitourinary infections were recruited for this study covering different age groups.

3.8. Inclusion criteria:

Non-pregnant, married women with symptoms of genitourinary infections.

3.9. Exclusion criteria:

Pregnant women, non-married women, menstruating women, women on antibiotic therapy, and women without symptoms of genitourinary infections were excluded from this study.

3.10. Study variables:

Demographic data: age, occupation, residence, education.

Molecular sensitivity pattern.

Molecular genotypes and sequencing

3.11. Sampling:

Non- probability purposive sampling.

Sample strategy: convenience where participants were chosen on the basis of accessibility.

Sample frame: participants with genitourinary infections at Khartoum North Teaching Hospital, and Al Hayat Charity Health Center (Khartoum).

Sample size: 200 urine and high vaginal swabs were collected from100 participants, by applying the below sample size formula:

$$N = Z^2P / q^2$$

Key: **z** = critical standard score(95%CI 1.96);

p= population incidence(0.04);**q**=1-P; $\mathbf{E^2}$= margin error (0.04).

3.12. Validity and pre-testing:

All reagents and primers were pre-tested using control strains; and equipment wascalibrated.

3.13. Ethical considerations:

Maintaining confidentiality of information obtained from participantsinvestigated.

Written consent of the participants was obtained before being enrolled in the study (Appendix II).

Laboratory results of specimens collected were handed to all participantsincluded in the study or dispatched to physicians treating those participantsfor prescription.

Permission to collect the specimens was obtained from the Federal Ministry ofHealth (Khartoum), Khartoum North Teaching Hospital&Al Hayat Charity Health Center (Appendix II).

Complete information regarding risk factors, if any, was handed to all participants under the study and no concealment what so ever.

3.14. Data analysis:

The software used for the analysis of data was the Statistical Package for Social Sciences (SPSS) program (version 14). For categorical variables, proportions were compared by the Chi-square test as appropriate. The means and medians of the continuous variables were compared by Student's *t*test program depending on the sample distribution. Frequencies, percentages, tables and graphs were used for presentation of the data.

3.15. Plan of dissemination of results:

The study findings may be shared with others by the following means:

Presentations in conferences, symposia, workshops, and scientific meetings.

Publishing results in scientific journals, memoranda, textbooks, and websites.

Lectures, seminars, and tutorials addressed to undergraduate and postgraduatestudents as well as university graduates.

3.16. Methodology:

Demographic and clinical data were collected using a structuredquestionnaire with a written informed consent (appendix (I).

Thundered high vaginal swabs were collected by insertion of a non-lubricated sterile disposable plastic speculum (Welch AllayKlein speculum) into vaginaand high vaginal swab was collected from the posterior vaginal fornix. The collected swab was inserted in a tube containing 5ml Tris-HClbuffer. The mixture was centrifuged at 2000 rpm for 10 minutes and the pellet was transferred to cryogenic tube and kept at -70°C until DNA extraction.

The Tris-HCl buffer was prepared by adding A 2.24 g Tris to 100 ml of distilled water,B 1.7 ml hydrochloric acid in 100 ml distilled water, and 25 ml of A+13.4 of B (pH 8.0) made of up to 100 cm^3 with distilled water.

20 ml first voided urine was collected in sterile leak-proof urine containers. Then 10 ml of the urine specimen were transferred to clean Falcon centrifuge tubes, and centrifuged at 2000 rpm for 10 minutes. The pellet was suspendedin one ml phosphate buffer saline (PBS), and kept at -70° C for later PCR investigation.

The phosphate buffer salinewas prepared by dissolving 800g NaCl, 20g KCl, 144g $Na_2PO_4.2H_2O$, 24g KH_2PO_4,and 8 litersofdistilled water. After complete mixing, final solution was topped up to 10 liters. The pH of the 10x stock was approximately 6.8, but when diluted to 1x PBS itwas 7.4. The solution was autoclaved for 15 min.

at 121° C and dispensed in one ml cryogenic tubes and stored at room temperature.

3.17. Urine leucocytes esterase detection test:

It is a test used for screening of urinary infection. It detects white blood cellsassociated with infection. However the test may be positive even if leucocytes have lysed.

Principle: Leucocytes esteraseis an enzyme present in granulocytes and hydrolyzesindoxyl carbonic acid esterase to produce indoxyl, which reacts with adiazonium salt to form a purple colorwithin two minutes.

Procedure: A dip strip was immersed in fresh, well mixed urine specimen. The strip was removed immediately to avoid the dissolving out of reagent area. The strip was blotted on an absorbent filter paper and the result was read by comparing each reagent area to the color blocks, shown on thecolor chart. Reagent area was read within 1-2 minutes to confirm a positivespecimen.

Quality control: For best results, two reagent strips were used**:**

(1) Uristikstrip (United Kingdom).

(2) Combostikstrip (Korea).

3.18. DNA extraction:

The DNA was extracted from specimens according to manufacturer's instructions (Aidlab Biotechnologies, China). This was done as follows:

Harvests of 5-50 ml urine and high vaginal swab (HVS) were placed incentrifuge tubes, centrifuged at 3000 rpm, cell pellet was harvested, and supernatant was discarded. Then200 µl of buffer UB were added,

pellet suspended, 20 µlofproteaseK(20mg/ml) were added,and the solution was mixed thoroughly. 200 µlCB binding solution were added to the mixtureimmediate vortex was done, andmixed thoroughly.

The mixture was incubated at 70°C for 10 minutes. Then the solution was cooled, 100 µlisopropanol were added, immediate vortex, andmixed thoroughly. The resulting flocculent precipitate was immediately vortexed, mixture was mixedthoroughly. Specimen was vortexed for 15 seconds, mixed, and mixture was transferred (including the pellet) to the adsorption column (AC).

This mixture was centrifuged at 13,000 rpm for 30-60 seconds, and flow-through was discarded. Then 500 µlinhibitor removal solution (IR) wereadded, centrifuged at 12,000 rpm for 30 seconds, flow-through wasdiscarded,500 µlwashsolution (WS) were added, centrifuged at 12,000 rpm for 30 seconds, flow-through was discarded, again 450 µl wash solution were added, centrifuged at 12,000 rpm for 30 seconds, and flow-through was discarded. Then the solution was transferred to the adsorption column (AC) into an emptyEppendrof tube,centrifuged at 13,000 rpm for 2 minutes.

The solution was then took out of the adsorption column, and placed into another cleanEppendroftube. In the middle partof theadsorption film, 30 µl of warmed (70°C)elution buffer (EB) were added, incubated at room temperature for 3-5 minutes, and centrifuged at 12,000 rpm for 1 minute. The flow-through was placed into the adsorption column, incubated at room temperature for 2 minutes, and then centrifuged at 12,000 rpm for 1 minute. The DNA so extracted was stored at -20°C until PCR test was conducted.

3.19. Detection of *Mycoplasmagenitalium* by Real Time PCR:

The extracted DNA from both HVS and urine specimens was subjected to Real Time PCR detection using the primers:

MgPa-355F (5'-GAGAAATACCTTGATGGTCAGCAA-3')

MgPa-432R(5'-GTTAATATCATATAAAGCTCTACCGTTGTTATC-3')

MgPa-380 FAM (5'-ACTTTGCAATCAGAAGGT-3) MGB Probe

These primers to detect a 78pb fragment of the MgPa operon sequence (Accession No.M31431)were designed by Jensenfor TaqMan assay(Jensen *et al.*, 2004).

The components of PCR reaction mixture were:

1X PCR buffer (20 mMTris-HCl-pH 8.4), 50 mMKCl, Platinum, Invitrogen, Carlsbad, and California).Together with 5 mM MgCl2; 1 uM each primer MgPa-355F; MgPa-432R; TaqMan probe; 75 nM FAM-labeled MgPaTaqMan MGB probe; 62.5 uMof each dATP, dGTP, and dCTP;125 uMdUTP; 10% glycerol (Sigma-Aldrich Denmark A/S, Copenhagen, Denmark); 1 ul of 6-carboxy-x'-rhodamine reference dye (Invitrogen); and 2 U of *Taq*DNA polymerase (Platinum *Taq*, Invitrogen).

An ABI 7500 Real-Time PCR instrument (Applied Biosystems) was used witha 96-well block and MicroAmp Optical 96-well reaction plates covered with ABI prism Optical Adhesive Covers (Applied Biosystems).

All tests were performed in with 5 µl of template DNA at 50°C for1sec (stage 1), 95°C for 10 min. (stage 2), and 50 cycles of denaturation at 95°C for 15 sec,annealing and extension at 60°C for 1

min.Standard curves were produced by analyzing 10-fold dilutions of *M. genitalium*DNA containing 5 to 500,000 genome equivalents (geq). The *M. genitalium*DNA was diluted in TE buffer (pH 8.0) containing 1 µg of calf thymus DNA per ml (from D-8661; Sigma-Aldrich).

3.20. Detection of the 23S-rRNA gene of *M. genitalium:*

Detection of *M.genitalium* in both HVS and urine specimens was confirmed by the amplification of the 23S-rRNA gene in the MgPaTaqMan real time assay positive samples using theprimers:

Mg23S-1992F (5-CCATCTCTTGACTGTCTCGGCTAT-3)

Mg23S-2138R (5-CCTACCTATTCTCTACATGGTGGTGTT-3)

Flanks mutations found in the V region of the 23S-rRNA gene and producing a 147-bp amplicon.

3.21. Primerspreparation:

The primers used in thestudy were in the form of lyophilized powderpurchased from Macrogen (Korea).

The primers sequences were first checked for quality assurance .Primer was spun for few seconds, the required volume of sterile water was added according to manufacturer's instructions in a bio-safety hoodusingautomatic pipette withsterile filter tips, labeled with date of preparation. Then the primers solutionwas mixed well and kept in a refrigerator at 4°C overnight. The solution was vortexed, and 2-3 primers aliquots were made.

Primers dilution was made by taking 10 µl of primers stock (100 pmol/µl) and added to 90 µlsterile distilled water in a sterile Eppendrof tubeto get a final concentration of 10 pmol/µl. After

dilution, the primer was labeled andstored at -20°C until later used in PCR reactions.

Specimens were denatured at 95°C for 1 min. A total of 35 cycles were performed. In the 35thcycle, the extension time was increased to 6 min. andprimers were annealed at 65°C for 1 min. and extended at 72°C for 1 min.

The 4ul specimen to be analyzed was adjusted to a total volume of 20 µlMaxime PCR PreMix(iNtRON Technology, Korea). Ready composed i-TagDNApolymerase (5U/ µl) equal to 2.5 U, dNTPs 2.5 mM each, reaction buffer (10X) 1X and gel loading buffer 1X.

One µlof each primer was added to 14µlnuclease- free water to get a final volume of 20µl which was entered an automated DNA thermal cycler (Convergys® TD Peltier thermal cycler-Germany). This cycler was programmedto run at 95°C for 2 min. followed by 40 cycles each consisting of incubation at 95°C for 15 seconds. Combined with the 60 seconds,annealing and extensionsteps were performed at 60 °C(Jensen *et al.*, 2008).

20 ul of the amplified PCR product were analyzed on a 2% agarose gel, stained with ethidium bromide, and photographedby UVlight. Specimens showing a band of 147-bp were considered positive.

3.22. Preparation of agarose gel for electrophoresis:

Agarosegel was prepared at a concentration of 1.5% as follows:

1.5gof agarose powder (Ambion, USA) was dissolved by heating in a microwave after adding 50 ml 1X TBE buffer (AppliChem). Then it was cooled to 55°C in a water bath. Then2µl of 10mg/ml ethidium bromide were added, mixed and poured on to the casting tray that has been taped up appropriately and was equipped with suitable comb to

form well in place. Any bubbles were removed and the gel was allowed to set at room temperature. After solidification, the comb was gently removed and the spacer from the opened sides was removed.

To visualize PCR product, the gel casting tray was flooded with 1xTBE bufferto cover the gel surface. 5µl of the PCR product from each specimen wereloaded directly, and then the gel was electrophoresed at 100V for 25min. (Serva Blue Power 500, Germany). The gel was visualized by UV trans-illuminator(Genius, UK).

3.23. Amplification of *M.genitalium*mgpa B genes by conventional PCR:

All Real Time PCR positive specimens were selected and subjected to the conventional PCR testing using primers of:

- MgPa-1: 179 to 206

Forward primer(5'AGTTGATGAAACCTTAACCCCTTGG3')

Reverse primer: (5'CCGTTGAGGGGTTTTCCATTTTTGC3')

- **MgPa-3: 435 to 460**

Primers correspondto the sequence base of coding strand of the same coding for the 140-kDa adhesion protein gene of *M .genitalium*.mgpB gene**:**

Amplification of this gene was developed by Jensen(Jensen *et al*., 1991).

3.24. Interpretation of PCR results:

A blotting chart was drawn to compare the size of each amplicon against the DNA marker 100 bp (Vivantis, Malaysia). Positive results of *M. genitalium* will produce mgpBband of 281 bp**.**

3.25QiaQuick PCR purification kit protocol:

The PCR products of both 23S-rRNA and mgpBgenes were subjected topurification using the QiaQuick PCR purification kit (QIAGEN, Hidlden,Germany).500 µl of PB buffer were added to100 µl PCR specimen.The QiaQuick spin column was placed in the provided 2 ml collection tube to bind DNA. Then the specimen was applied to theQiaQuickcolumn and centrifuged for 30-60 seconds. Flow-through was discarded, and the QiaQuickcolumn wasreturned back to the same tube. The QiaQuickcolumn was washed by adding 0.75 ml PE buffer and centrifuged for 30-60 seconds. Flow-through was discarded;the QiaQuickcolumn was returned again to the same tube, and centrifuged for an additional one min. at a maximum speed. Then the QiaQuickcolumn was placed in a clean 1.5 mlmicro-centrifuge tube. The DNA was eluted by adding 50 µlEB buffer (10mM Tris-Cl, pH 8.5) or H_2O to the center of the QiaQuickmembrane, and centrifuged for one min**.**

3.26. Sequencing of 23S-rRNA gene and mgpBgene:

Sequencing was performed by ABI Big Dye Terminator kit v. 2.0 (Applied Biosystems, Foster City, USA), and read by ABI 3100 genetic analyzer(Applied Biosystems,Foster City, USA). Both strands of amplified fragmentswere sequenced using the same PCR primers(SSI, Denmark).

3.26(a)Analysis of sequence data for MgPa1-3:

The amino acid sequencesof (SDN19,SDN51and SDN151) werealigned and comparedwith reference strainof *M.genitalium* G-37 (GenBank, Accession number NC000908)using CLUSTAL W 2.1 andMEGA5.2.2software. This typing method was developed by Hjorth(Hjorth *et al*., 2006).

3.26.(b)Analysis of sequence data for 23S-rRNA:

Sequences of the 23SrRNA gene amplified in this study were assembled,consensus files were aligned with *Mycoplasmagenitalium* wild-type positions 2,058/ 2,059. and analyzed by software packages DNA Baser Sequence Assembler for detection of mutation. Mutations further, amplified sequence and looked for (Fig.3. 4).

CCATCTCTTGACTGTCTCGGCTATAGACTCGGTGAAATC CAGGTACGGGTGAAGACACCCGTTAGGCGCAACGGGAC GG*AA*AGACCCCGTGAAGCTTTACTGTAGCTTAATATTGA TCAA

AACACCACCATGTAGAGAATAGGTAGG

Fig3.1 Sequence of the amplified 23S rRNA gene. The primers binding sites are underlined and the 2,058 and2,059 positions are marked in Italic

3.27.Translation ofM.genitalium codons to protein sequence

Codons sequences were translated to correspondence putative protein using online Genemark software Genemark S http://exon.gatech.edu/GeneMark/genemarks.cgi

3.28.Alignment of protein sequences

The obtained protein sequences were alignment using MEGA and CLUSTAL W software, to detect the presence of mutation

3.29.Protein modeling

The protein sequences were first blast into NCBI to find 3D structure model with ID number .If the homologies models were not found ,then another online CHP software was used, by coping the sequences and submitted. Furthermore, the obtained homologies protein structure in Query pbd format was open by Chimera software to locate the positionof mutation in 3D structure protein. http://www.cbs.dtu.dk/services/CPHmodels/

4. Results

Hundred high vaginal swabs and 100 first void urine were investigated from 100 women with genitourinary infection in this study by real-time PCR.Four urine specimens were positivefor *M. genitalium*mgpBout of 100 (4%), high vaginal swabs 2 (2%). seeAmplification plot Fig 3.1

M.genitalium DNA load in each specimen were quantified byReal-time PCR standard curve.high vaginal swabs revealed higher mean (640.92 geg/ml+SD±10.4) in comparison with urine specimens mean (314.86geg/ml+SD±5.4) (Fig. 3.2 and Table1).

The meanage of infected womenwere found 31years(age,range18-44). The most infected women against age fell in first group range (18-25 with 7.4%.)(Table 2).

With respect to residence study showed that all of infected women were from Gezira andOmdurman 16.7% and 4.4% respectively (Table 3).

Infected women with *M.genitalium*with respect to educational level were found among women with university and higher secondary school 6.3% and 5.3% (Table 4).

Infectivity of *M. genitalium* with respect to occupationwere higher among teachers and housekeeping wife's 8.3% and 3.9% respectively(Table 5).

Among the studied women positive *M.genitalum*vaginal discharge, Itching burningmicturition, and lower abdominal pain symptoms were found 3.3%3.6%, 1.5%and 2.7% respectively (Tables6,7,8 and 9).

No association was found with history of abortion and *M.genitalium* positivity among women under study 2.7%(Table 10).

leucocyte esterase test as primary screening test for detection *M.genitalium*,was detected only 6.5 % of positive cases were showed in (Table11).

Confirmatory assay targeting23S rRNA genesof *M.genitalium*amplicon fragment length 147-bp was electrophoresed(Fig 3.3)

Detection of *M.genitalium* macrolides resistance at domain Vregion 23S rRNA geneswas Showed in*AA* Italic at positions 20,58 and 20,59(*Escherichia coli* numbering). Fig2.1.

The *M.genitalium*mgpBgeneamplicon fragments length281-bpwereelectrophorese in 1.5% agarose(Fig3.4)

The purified PCR products of mgpB*M.genitalium* were sequenced by ABI sequencer in chromatograms format Fig:(3.5,3.6,3.7,3.8,3.9 and 3.10).

The characterizedsequencesobtained in thisstudy(SDN19,SDN51andSDN151)were submitted to the GenBank with the accession numbers: KF612736 to KF612738.(Tables 12,13,14).

The isolate codon sequences in this study were translated toamino acid sequencesin order todetect the presence of mutation(Table 15).

Sequence alignment and phylogenic analysis

Amino acids alignmentof *M.genitalium*mgpBgene (position 79-136) of Sudanese sequences (SDN19,SDN51 &SDN151) together with *M.genitalium*reference strain G37**.**Revealed fouramino acidmutation in SDN19 isolatesequenceposition(D^{96}/E,S^{101} /A, S^{107}/V A^{117} /S)and two mutation in isolatesequences SDN51 and SDN151 position (S^{107}/V, G^{124}/D)Fig 3.11.

Phylogenetic analysis of amino acid sequences of isolated sequences in this study with reference G37 sequences obtained from gene bank showed that the Sudanese isolates sequences SDN51 and SDN151 were clustering with genotype 25 (RYH217 accession number GU226228)(boot strap value 94)whereas isolate SDN19 was assigned to separate close to TX84.Fig.3.12

The 3DProtein structure of modeled *M.genitalium* was constructed to show protein structure and to localize the mutation positionFig 3.13.

Quantification of *M. genitalium* in unknown samples

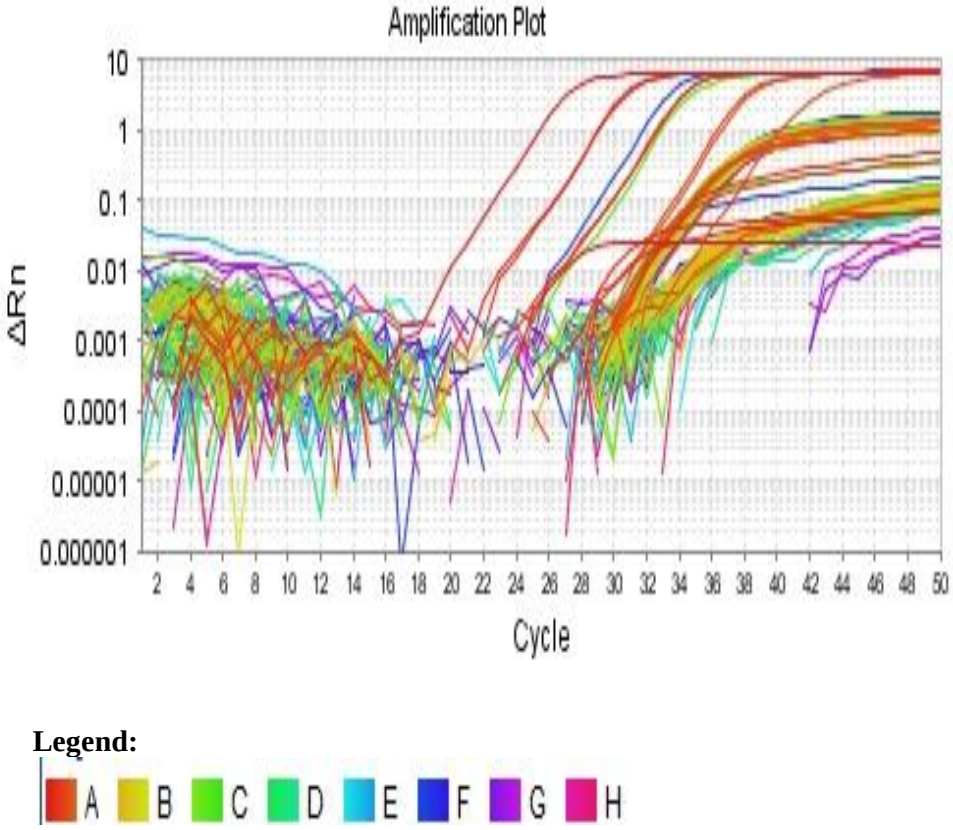
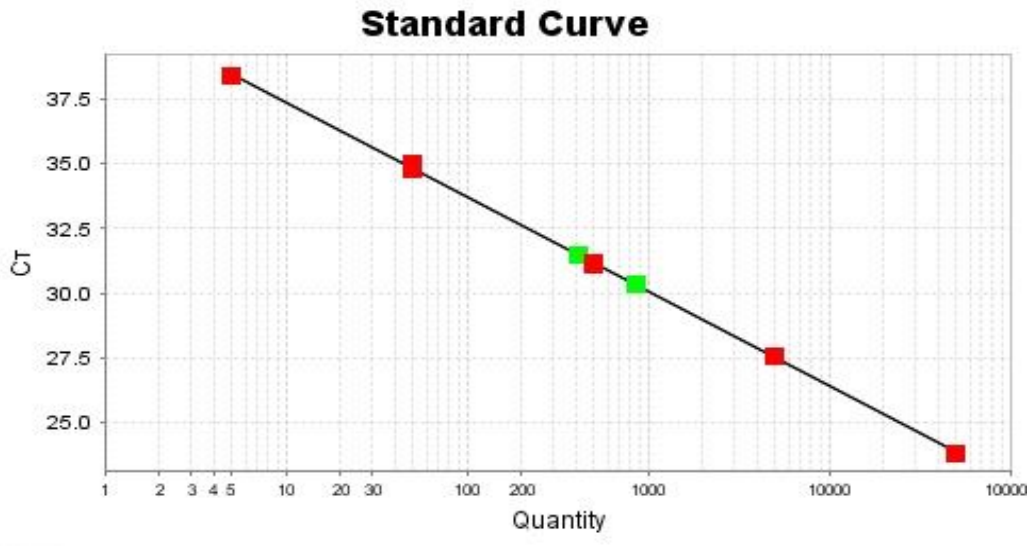


Fig. 4.1 Amplification Plots of *M .genitalium* fromHVS and FVUspecimensusing Real-Time PCR

This plot is used to calculate the baseline and threshold cycle for the standard curve, as demonstrated in the below fig.

Quantificationof *M.genitalium*



Key: Slope: -3.642; Y. inter: 41.01; r^2 =1; Eff %: 88.187 Ct = Threshold cycle.

andard; nown; own (flagged)

Fig. 4.2Shows the standard curve of *M.genitalium* quantification by real-time PCR TagMANassay.

Ten folds serial dilutions (from 5 – 500x10^3) were prepared from the genomic *M.genitalium*, from which the number of cycles were computed. Therefore, the unknown samples were calculated, using the below equation:

Y= m x +b

Quantity log =Ct –b/m

Key: m= slope; x =unknown specimen quantity;

b= intersection point; Ct = threshold cycle

Table 4.1: Comparison between loaded DNA inHVS against FVU

	HVS(±SD)	**FVU (±SD)**
Ct	30.9 (0.8)	36.1**(1.1)
Quantity	640.9 (10.4)	314.9* (5.4)

Key: Significance level at P<0.05 *; Significance level at P<0.01**

Studied samples were classified into three categories, (18- 27), (28 - 37) and (38 - 47) based on their age ranges. Of these categories, the positive cases were 6%, 2% and 5.5 %, whereas the negative individuals were 34%, 48% and 18% respectively. Most positive cases were detected among participants, who were less than 28 Yrs.

Table 4.2: *M. genitalium* positivity according to age

Age group	**Result**	
	Negative % (n)	**Positive %(n)**
(18-27)	94 (34)	6 (2)
(28-37)	98 (48)	2 (1)
(38-47)	94.5(17)	5.5 (1)

Key: (X^2, 1.22, P>0.05)

Table 4.3: Positivityof *M. genitalium*based ongeographical distribution of participants.

Area	(-ve)*M. genitalium*	(+ve)*M. genitalium*
Khartoum North	100 %	0%
Gezira	83.3 %	16.7%
Khartoum	100 %	0 %
Kordofan	100 %	0 %
Omdurman	95.6 %	4.4 %
Port Sudan	100 %	0 %
River Nile	100 %	0 %
West Darfur	100%	0 %

Table 4.4: Results of *M. genitalium* according to education level

Educational level	(-ve)*M. genitalium*	(+ve) *M. genitalium*
Secondary school	94.7 %	5.3 %
Illiterate	8%	0 %
Intermediate school	100 %	0%
Khalwa	100 %	0 %
Primary school	100%	0 %
University	93.8%	6.3 %

Key: (X^2, 1.83, P>0.05)

Table 4.5: Findings of *M. genitalium*based on occupational status.

Diagnosis	White colalar	Cleaning worker	House wife	Lawyer	Nurse	Student	Teacher
-Ve*M. genitalium*	100 %	100 %	96.1%	100%	100%	100%	91.7 %
+Ve*M. genitalium*	0%	0 %	3.9%	0 %	0%	0%	8.3%
Total	100 %	100%	100 %	100%	100%	100%	100 %

Key:(X^2, 1.04, P>0.05)

Table 4.6: *M.genitalium* positivity with discharge symptom.

Diagnosis	Negative	Positive	Total
-Ve*M. Genitalium*	90 %	10 %	100
+Ve*M. genitalium*	96.7 %	3.3 %	100

Key:(X^2, 1.04, P>0.05)

Table 4.7: *M.genitalium* positivity with an itching symptom.

Diagnosis	Negative	Positive	Total
-Ve*M. genitalium*	95.3%	4.7 %	100
+Ve*M.genitalium**	96.4%	3.6%	100

Key:* severity of itching (X^2,0.15, P>0.05)

Table 4.8: *M. genitalium* positivity with buringmicturation symptom

Diagnosis	Negative	Positive	Total
-Ve*M. genitalium*	91.2%	8.8%	100
+Ve*M. genitalium*	98.5 %	1.5 %	100

Key:(X^2, 3.12, P>0.05)

Table 4.9: *M.genitalium* positivity with lower abdominal pain symptom

Diagnosis	Negative	Positive	Total
-Ve*M. Genitalium*	92.6 %	7.4 %	100
+Ve*M. genitalium*	97.3 %	2.7 %	100

Key:(X^2,1.11, P>0.05)

Table 4.10: *M. genitalium* positivity with abortion

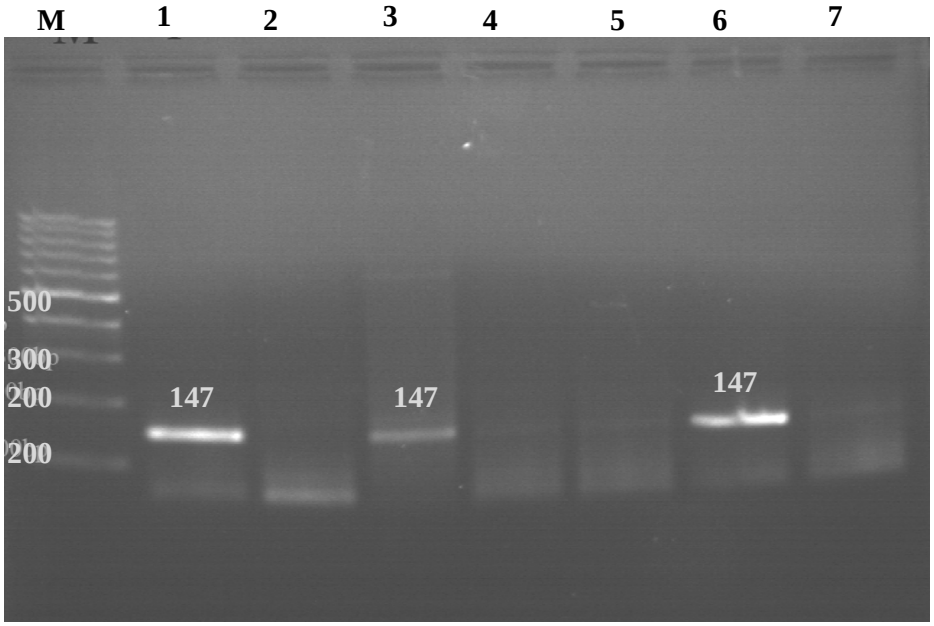
Diagnosis	**Negative**	**Positive**	**Total**
-Ve*M. genitalium*	95.5%	4.8 %	100
+Ve*M. genitalium*	97.3 %	2.7 %	100

Key: (X^2,0.25, P>0.05)

Table 4.11: *M.genitalium* positivity with Leucocyte esterase test

Diagnosis	Negative	Positive	Total
-Ve*M.genitalim*	97.1 %	2.9%	100
+Ve*M.genitalim*	93.5%	6.5 %	100

(X^2,0.70, P>0.05)



Key: Lane M= DNA marker; 1 = Positive control; 2 = Negative control; 3 and 6 were positive sample, 23s RNA gene of M. genitalium, (147 bp).

Fig. 4.3Detection of the 23S rRNA Gene of *M. genitalium*

The 23s RNA gene after PCR on 2% agarose gel:

The DNA product of positive samples, 3 and 6 were analyzed for DNA sequencing. After that the sequences were assembledto detect substitution mutations at position 2,058 and 20, 59 marked in italic, usingDNA Baser Sequence Assemblersoftware 3.3.5 (HeracleBioSoft S.R.L.)**.**No mutation was detected in the studied positive samples at nucleotides position 2,058 and 20, 59 (*Escherichia coli*. Numbering).

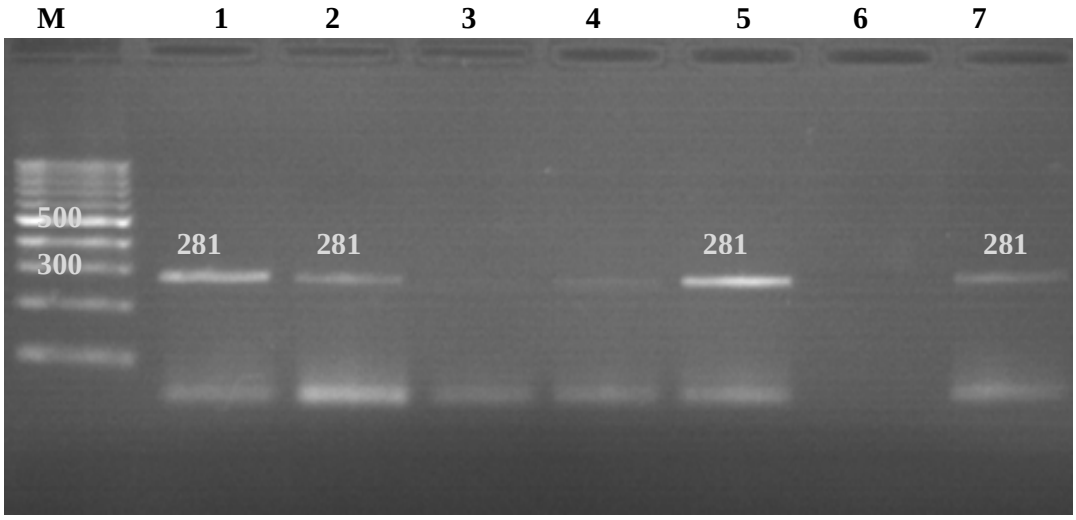


Fig. 4.4Detetion and chacterization of mgpBgene

themgpBgene after PCR on 1.5% agarose gel.

Lane M= DNA marker; 1= positive control; 3= negative control; 2,4,5 and 7 were positive samples. mgpBgene of *M. genitalium* (281-bp).

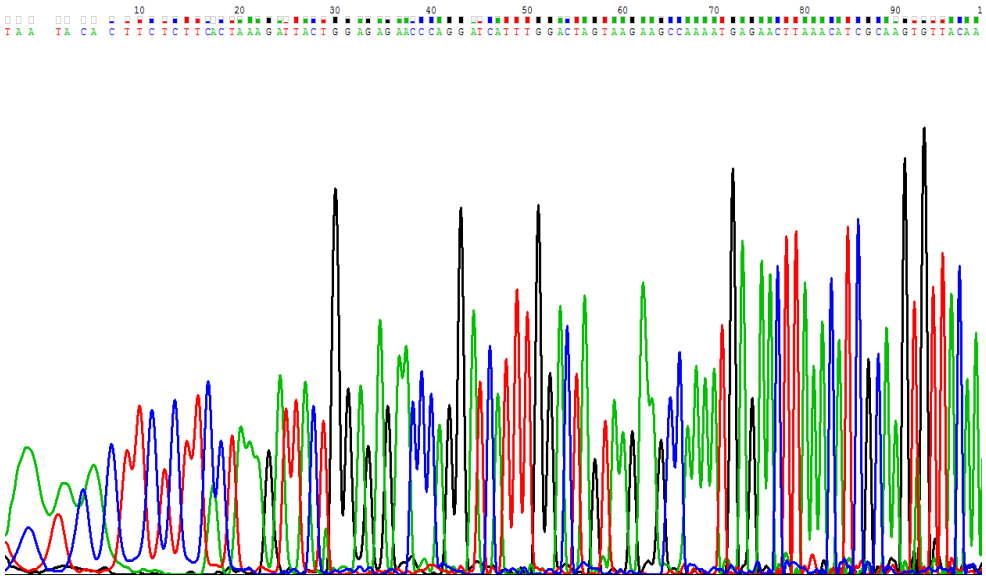


Fig. 4.5Sudan19-MgPa-1(forward) sequence chromatogram

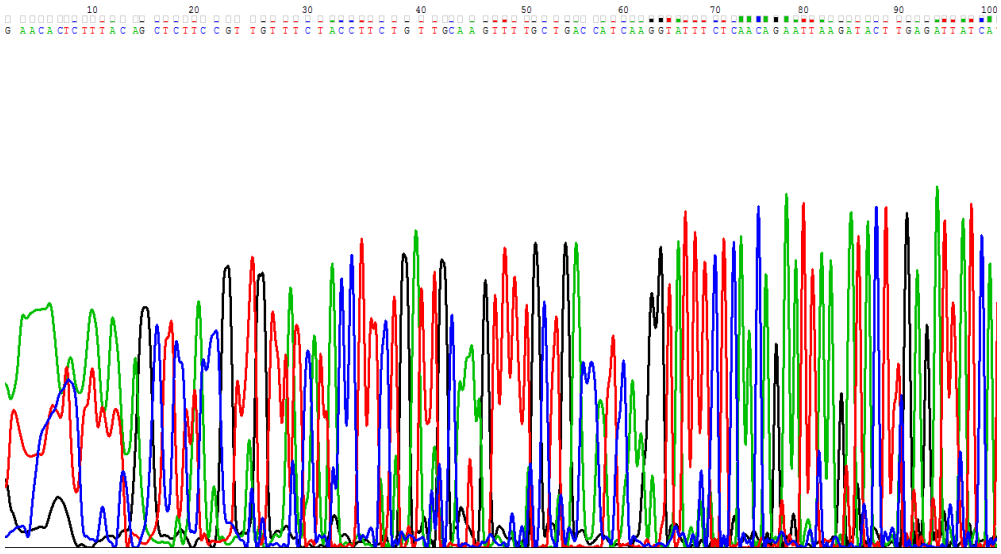


Fig. 4.6Sudan19-MgPa-3(Reverse) sequence chromatogram

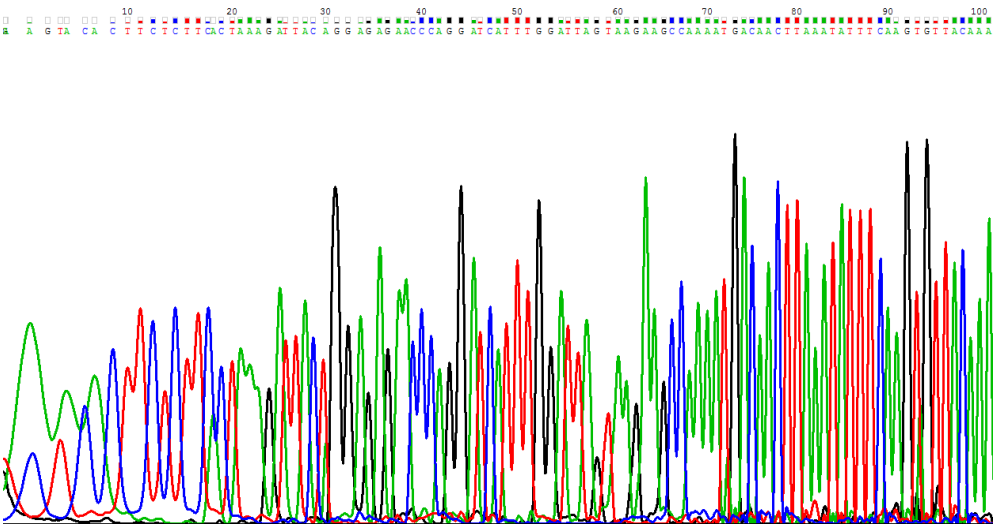


Fig. 4.7Sudan51-MgPa-1(forward) sequence chromatogram

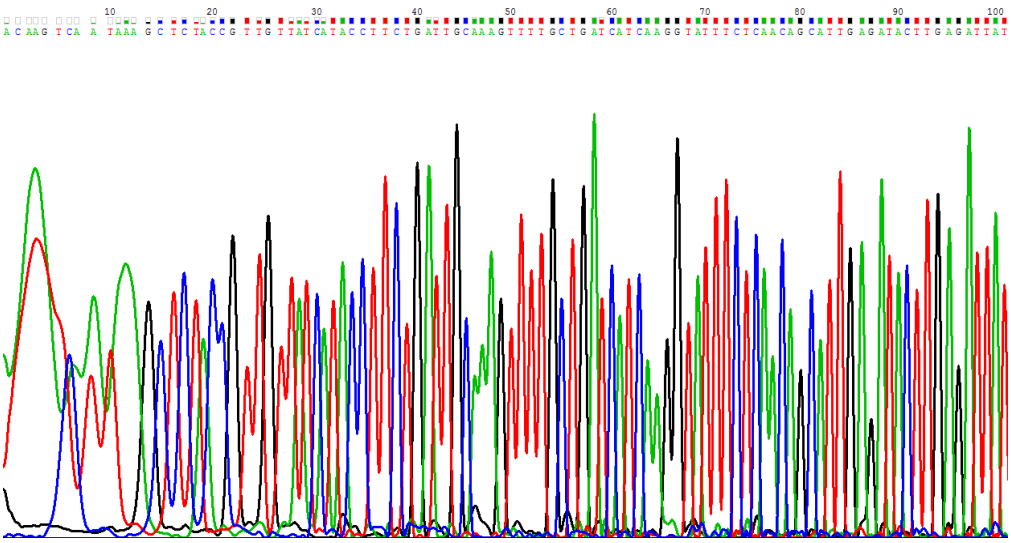


Fig. 4.8Sudan51-MgPa-3(Reverse) sequence chromatogram

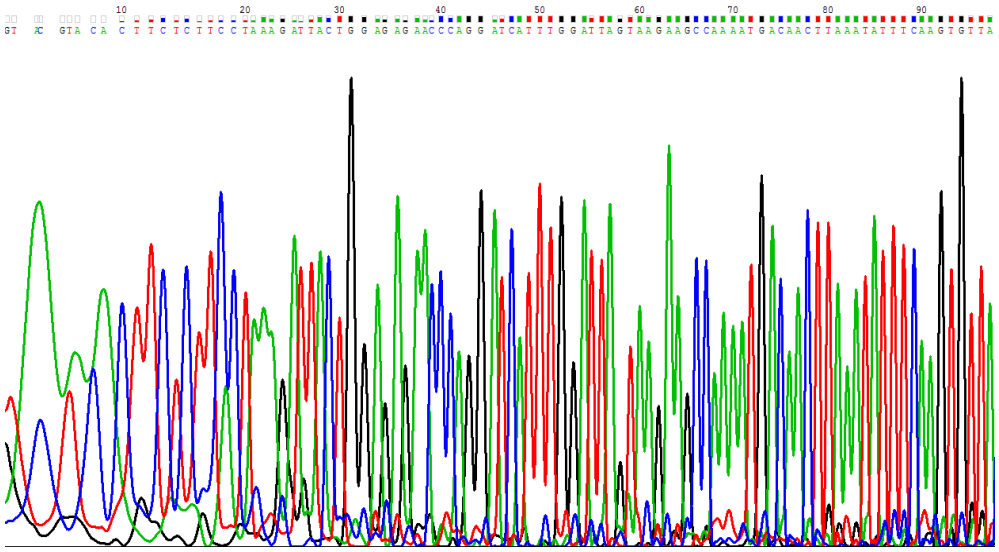


Fig. 4.9Sudan151-MgPa-1 (forward) sequence chromatogram

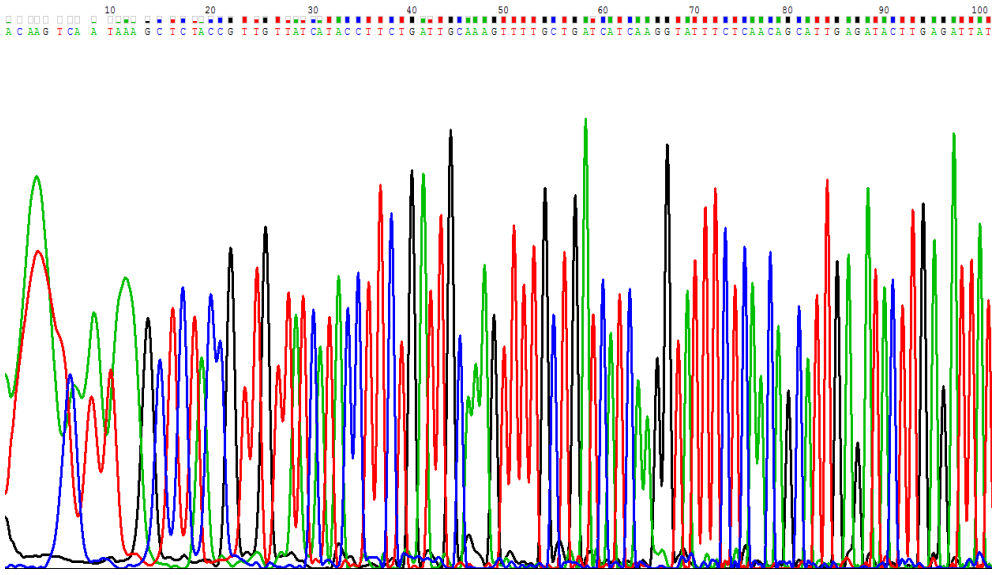


Fig. 4.10Sudan151-MgPa-3(Reverse) sequence chromatogram

Table (4.12): Shows sequence submitted to GenBank Accession N: KF612736

Sudan19-MgPa B gene partial codons sequence
>SDN19 ACTTGAAACAATAACAACTTCTCTTCACTAAAGATTACTGGAGAGAACCCAGGATCATTTGGACT AGTAAGAAGCCAAAATGAGAACTTAAACATCGCAAGTGTTACAAAGAATGTTAGTGATGATAATC TCAAGTATCTTAATTCTGTTGAGAAATACCTTGATGGTCAGCAAAACTTTGCAATCAGAAGGTAT GATAACAACGGTAGAGCTTTATATGATATTAACTT

Table (4.13): Shows sequence submitted to GenBank Accession N: KF612737

Sudan51-MgPa-b gene partial codons sequence
>SDN51 ACTTGAAACAATAACAACTTCTCTTCACTAAAGATTACTGGAGAAAAACCCAGGATCATTTGGAT TAGTAAGAAGCCAAAATGACAACTTAAATATTTCAAGTGTTACAAAGAATGTTAGTGATGATAAT CTCAAGTATCTCAATGCTGTTGAGAAATACCTTGATGATCAGCAAAACTTTGCAATCAGAAGGTA TGATAACAACGGTAGAGCTTTATATGATATTAACTTA

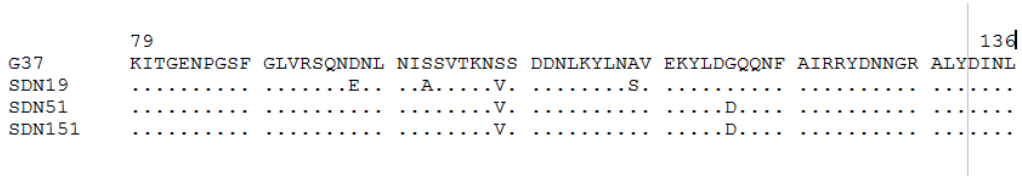
Table (4.14): Shows sequence submitted to GenBank Accession N: KF612738

Sudan151-MgPa-b gene partial codons sequence
>SDN151 CCAAAATGACAACTTAAATATTTCAAGTGTTACAAAGAATGTTAGTGATGATAATACTTGAAACA ATAACAACTTCTCTTCACTAAAGATTACTGGAGAGAACCCAGGATCATTTGGATTAGTAAGAAGC TCAAGTATCTCAATGCTGTTGAGAAATACCTTGATGATCAGCAAAACTTTGCAATCAGAAGGTAT GATAACAACGGTAGAGCTTTATATGATATTAACTTA

Table (4.15): Translation of DNA sequences to amino acid sequenes by Genemark S online software.

Sequence name	Protein sequence
G-37	>G37 TWNNNNFSSLKITGENPGSFGLVRSQNDNLNISSVTKNSSDDNLKYLNAVE KYLDGQQNFAIRRYDNNGRALYDINL
SDN19	>SDN19 TWNNNNFSSLKITGENPGSFGLVRSQNENLNIASVTKNVSDDNLKY LNSVEKYLDGQQNFAIRRYDNNGRALYDINL
SDN51	>SDN51 TWNNNNFSSLKITGENPGSFGLVRSQNDNLNISSVTKNVSDDNLKY LNAVEKYLDDQQNFAIRRYDNNGRALYDINL
SDN151	>SDN151 TWNNNNFSSLKITGENPGSFGLVRSQNDNLNISSVTKNVSDDNLKY LNAVEKYLDDQQNFAIRRYDNNGRALYDINL

Fig. 4.11Amino acids alignmentof *M.genitalium*mgpBgene (position 79 - 136) of Sudanese sequences (SDN19,SDN51 &SDN151) together with *M.genitalium*reference strain G37.



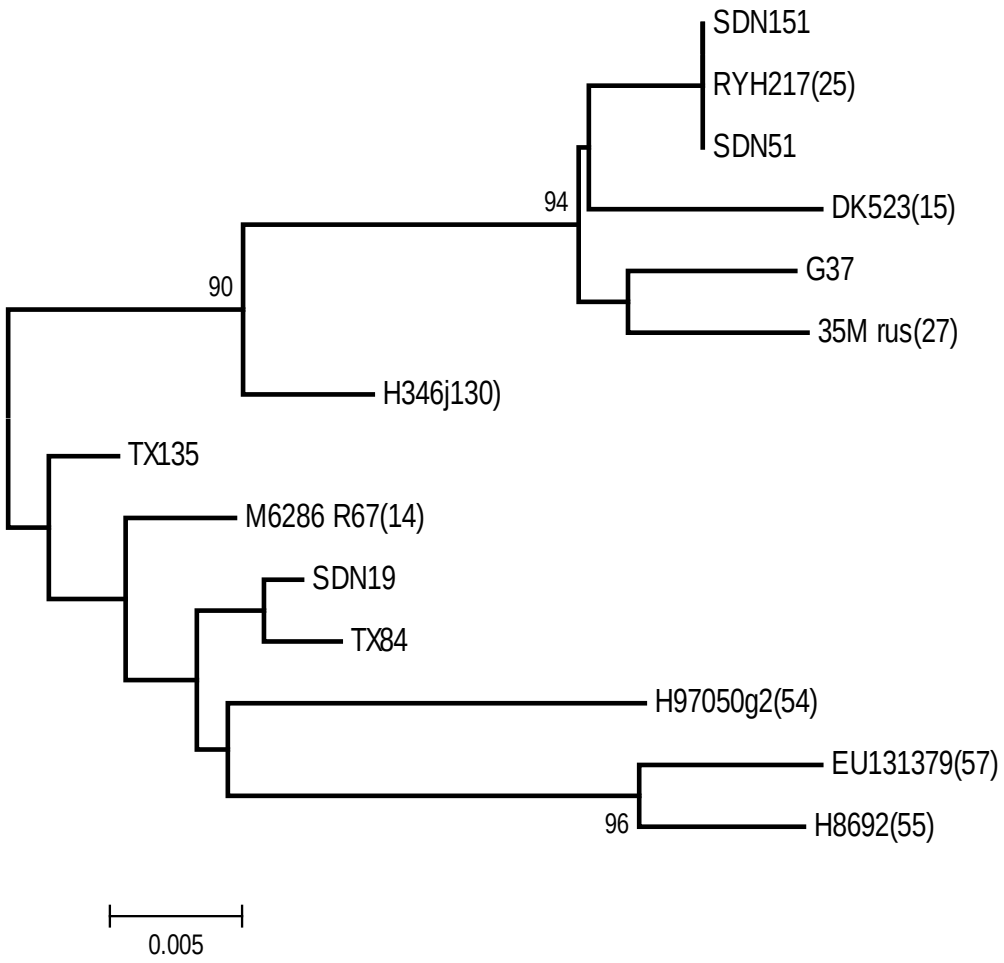
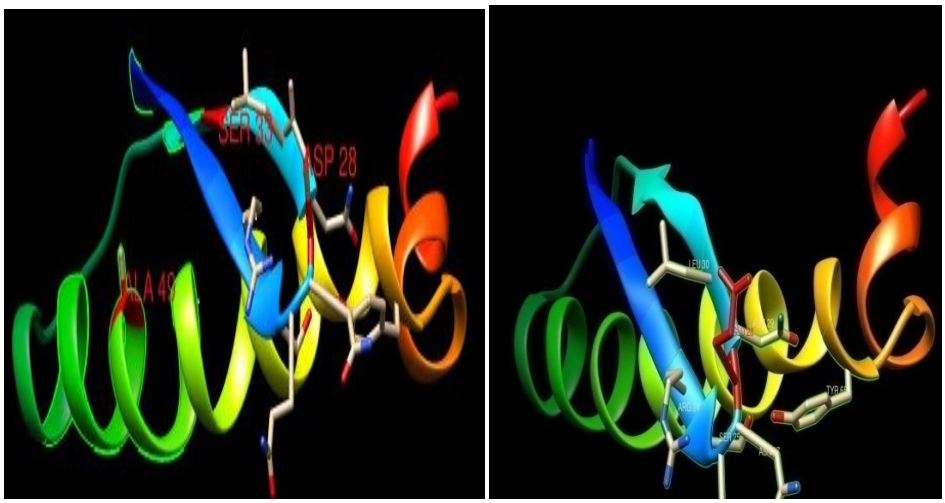


Fig. 4.12Shows phylogenetic tree analysis of amino acids with reference strain G37 and clinical sequences obtained in his studywith previously published sequences on GenBank were aligned by CLUSTAL W.(2007)



(A) **(B)**

Fig. 4.13Modeled 3D protein structureof*Mycoplasmagenitalium*Mgpa

(a) Templatenative site conserved with aspartic acid -28/ *G*lutamate

(b) mutated template at S33/ Al and A49/ S.

4. Discussion

4.1. Discussion

M .genitalium is an emerging cause of sexually transmitted diseases and has been implicated in urogenital infections of men and women around the world more than 25 years after its isolation(Tully *et al.*, 1981).

In Sudan the prevalence of *M .genitalium*or the partial characterization of its genome have never been reported as far as our knowledge. Thefrequency rate of *M.genitalium*was 4% among the Sudanese women enrolled in this study. This finding is in agreement (4%) with results reported in Mozambique, but lower (4.3%) than that reported in United Kingdom(Zimba *et al.*, 2011, Oakeshott *et al.*, 2010).

Moreover higher incidence was reported in other areas such as West Africa (26%), Kenya (16%), and Uganda (14%).In addition, *M. genitalium*burden is associated with the shedding of HIV-1 DNA from the cervix(Manhart *et al.*, 2008). This suggests *M. genitalium* infection may facilitate HIVtransmission**.**

Also high frequency rates of *M. genitalium* infections were reported in USA (9.5%) and India (6.5%)(Thurman et al., 2010, Manhas *et al.*, 2009). The molecular technique applied in this study to amplify mgpB gene that encoded for MgPa protein which mediated attachment to ciliated epithelium of human fallopian tubes(Collier A M, 1990).

Significant difference ($p<0.05 = 0.001$)in the DNA load were found in vaginal swab in comparison to the first void urine, this may either to due to patient swab specimen was taken before the first void urine , or colonization of *M. genitalium* to vagina, these finding results were supported by other study done by Rebeeca and *et al* whom found the vaginal sensitive for detection *M. genitalium* as highest 85.7 % in

comparison to other clinical specimens(Lillis *et al*., 2011). Moreover the vagina pH may be also a useful indicator to suspect the presence of *M. genitalium*, especially in the absence of BV and TV(Huppert *et al*., 2013).

The mean age of infected women was found to be 31years(age range18-44years).The younger age women infected in this study was in good agreement with a recent study performed in United States of America, result hasshown that there was association with *M. genitalium* infection and younger age, decrease by10% for each year(Lisa E. Manhart, 2007).

However the low level of education foundashigher secondaryobtained in study($p>0.05$) was in disagreement with reported in west African that had found a significant association($p<0.050$)between *M. genitalium* infection with low-level education(Pepin *et al*., 2005). This may be due to poor health education and inadequate health services(Wang *et al*., 2010).

The lack of association ($p<0.05$)between*M. genitalium* infection and the presence of sexually transmitted infections signs and symptoms may be due to the following reasons. Firstpresence of symptoms in the past year were self reported and recall bias might exist with *M. genitalium* infections, Second, if symptoms were very slight, they could be ignored. Third reason, younger women may have more biological susceptibility to some sexually transmitted infections due to cervical ectopy and less likelihood of acquired immunity from previous sexually transmitted infections exposure(Pettifor *et al*., 2007, Rekart and Brunham, 2008).Clinical presentation of *M. genitalium* is commonly asymptomatic in infectedwomen thus is supportedby numbers of studies which have found no association with infection and any genital symptoms such as vaginal itching, vaginal burning and dyspareunia(Tosh AK1, 2007,

Andersen *et al*., 2007).More over all, the research suggests that *M.genitalium* present similarly to *Chlamydia* but appears to cause milder symptoms than Gonococcal infections in women. The finding of our study suggests that *M. genitalum* infection in women is more likely to be asymptomatic or have few slightly symptoms, which may easily be ignored and undiagnosed.

In this study the leucocytes esterase screening test identified 50 % frequency rate of *M .genitalium* infection among the women investigated. This result was inconsistent (76%) with other studies(Hogdahl and Kihlstrom, 2007). These observable four substitution mutations were observed in SDN19 (D^{96}/E, S^{101}/A, S^{107}/V,A^{117} /S)and two in SDN51and SDN151 were(S^{107}/V, G^{124}/D).These mutations may indicate local predominant clinical variant of *M. genitalium*among Sudanese patients need to be confirmed by further studies.However, this substitution mutation were also detected in strains prevalent in other geographic regions and proved to be having an identical protein variants. This finding was confirmed by the presence of 65 different MgPa-13 genotype variantsall recognized in 267 gene sequences found in nine countries(Hjorth *et al*., 2006)

Also other 8 different strains were identified among female prostitutesin Kenya(Hogdahl and Kihlstrom, 2007). These identical MgPa-13 variants showed clearly the existence ofseveral commongenotype sequences world-wide. This may be due to extensive sequence variability resulting from recombination between repetitive elements of mgpBand MgPars, which was recently found associated with clinical strain and *M.genitalium* strain G37(Ma *et al*., 2007, Hogdahl and Kihlstrom, 2007, Iverson-Cabral SL, 2007). These variants can facilitate survival under host-immune pressure. However it was found that the first gene MG190

of MgPa operon can encode an enzyme controlling the homologous recombination of the MG191 and MG192 repeatedregions with MgPars(Hogdahl and Kihlstrom, 2007, Ma *et al*., 2007)**.**This facility had allowed the organism to evade the host immune system and leads to genetic sequence changes of MgPa with subsequent production of genotypic variants that were not recognized by the host immune system(Alvarez *et al*., 2003, Ueno *et al*., 2008)**.**

Other supportive survival mechanisms may be found to make *M. genitalium*to have the power to mimic host cell antigens and to exist inside the professional macrophage(Jensen, 2006)**.**

Furthermore study was done by(Alvarez *et al*., 2003) found that enzyme glyceraldehyde 3-phospate dehydrogenase (GAPDH) bring attachment of *M. genitalium* to human vaginal and cervical mucin in female.

In 2011, the frequency ratesof macrolide resistance were 21%, 40% and 100% in Sweden, Denmark, and Greenland respectively(Anagrius *et a*l., 2013, Gesink et al., 2012)**.** In this study, all DNAsequencestrains of*M. genitalium* werefound susceptible to macrolide antibiotics. This may be due to proper prescribed treatment of *M.genitalium* infections in Sudan.

The obtained Id number 3D protein structure 3H49 pbd was found 30 % identical but not matching the *M. genitalium* model. It was belonging to other bacterial species such as *Escherichriacoli*.This may be due to similar protein domains. The obtained Id number wastaken from the CHP server (Fig. 4.13); using Chimera1.8 software; and the mutation was further located in a 3D model.

4.2. Conclusion:

The 23S-rRNA mutant strains of *M.genitalium*are susceptible to the macrolide antibiotic.

Several amino acids substitutions mutations of *M.genitalium* were revealed by alignments analysis techniques.

Leucocyte esterase test as screen test for detection of *M.genitalium* was detected only 50% of positive case in comparison to molecular technique was 4%.

Urine specimens were showed high sensitive in detection of *M genitalium*($p<0.05$) in comparison with high vaginal swabs.

4.3. Recommendations:

- Recent molecular diagnostic techniques of *M. genitalium* should be available to cliniciansto facilitate proper management of *M. genitalium*infections.
- Screening programs of asymptotic pregnant women for *M. genitalium.*

  This procedure may help in the control of *M.genitalium*infections, improve the reproductive health, and limit the complications *M. genitalium*infections among the population.
- Further studies are recommended to study the characterization mgpBgene of *M. genitalium* to:
  1. Reveal more new *M. genitalium*variants among the Sudanese population.
  2. Justify the role of protective immunity against *M .genitalium* infections.

3. Clarify the role played by mgpBgene variant of *M. genitalium*in the severity of infections.
4. Study the sequence variability of the whole MgPa operon of *M. genitalium*and its role in pathogenesis of *M. genitalium*infections, and the possibility of development of new molecular diagnostic techniques.
5. Study *M. genitalium*MgPa gene recombination and variation existence in the human reproductive tract.
6. Determine the potential effect of *M. genitalium*load on the treatment efficiency of its infections.
7. Determine the contribution of macrolide antibiotic resistance in treatment failure of *M. genitalium* infections.

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Appendix (i)

Study Questionnaire

Lab. No.....Name: …….....

Age: .....Occupation: .....

Residence:
.....Education:.....

Urine leucocyte esterase testing urine:Pos. ☐ **Neg.** ☐

Type of specimen: Urine ☐ **High vaginal swab** ☐

Clinical features:

Burning micturition: Yes ☐ **No** ☐

Lower abdominal pain: No ☐ **Yes** ☐

Vaginal discharge: Yes ☐ **No** ☐

Itching Yes ☐ **No** ☐

Abortion: Yes ☐ **No** ☐

If yes , How many? Once ☐ **Two** ☐ **Three or more** ☐

PCR Result :Positive …… Negative……

Molecular GenotypingCharacterization:
……

Appendix (ii)

Federal Ministry of Health
Khartoum North Teaching Hospital

In the Name of Allah the Gracious the Merciful

Date 27/04/2011

ETHICAL CLEARENCE CERTIFICATE

This is to certify that, the proposal entitled:

(Molecular characterization and frequency of Mycoplasma genitalium among Sudanese women presenting with genitourinary infections In Khartoum north teaching hospital)

Submitted by: Elfatih Yousif Eldowma

the approved by the Khartoum north teaching hospital Health Research Ethics Committee to be carried out in the Sudan.

Dr. ABD ALLAH KANAN AHMED

Head of the

Khartoum north teaching hospital Health Research Ethics Committee

نموذج طلب الموافقه من المريض

انا الدارس لنيل درجه الدكتوراه: الفاتح يوسف الدومه

تخصص احياء دقيقه سريريه – قسم المختبرات الطبيه- جامعة السودان للعلوم والتكنولوجيا

ابحث عن بكتريا المياكوبازما جنتليم التي عرفت بانها تتسبب في كثير من امراض النساء مثل التهابات المهبل , عنق الرحم , انابيب المبيض وبالتالي تؤدي الي الاجهاض والعقم.

تهدف الدراسه الى الكشف عن هذه البكتريا وعلاجها وتحديد نسبتها بين النساء المرضي.

العينات:

1. **عينه من البول**
2. **عينه مسحه المهبل: تؤخذ بواسطه أخصائياو طبيب أمراض النساء والتوليد.**

هذه العينه سهله وبسيطه وغير مؤلمه ولا تسبب أي ضرر او مضاعفات جانبيه للمريضه.

- **اذا كان نتيجه المريضه موجبه يتم الاتصال بالمريضه لعلاجها بواسطه الأخصائي او الطبيب المعالج.**

اسم الدارس: الفاتح يوسف الدومه

ت: 0912204177

التوقيع.....

موافقةالمريضة:

المريضه.....

التوقيع .....

تلفون.....