UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

The Prevalence and Risk Factors for Genital Mycoplasmas in Human immunodeficiency virus infected pregnant women from King Edward Vlll hospital

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Dissertation submitted in fulfillment of the requirements for the degree:

Master of Medical Science in the

School of Clinical Medicine, College of Health Sciences

University of KwaZulu-Natal

South Africa

Durban

DECLARATION

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ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude and appreciation to the following persons and organizations:

My supervisor, Prof Nathlee Abbai for all your selfless assistance, time, knowledge, guidance and motivation during this project.

My co-supervisor, Dr Ravesh Singh, the School of Clinical Medicine Laboratory, Members of the Clinical Medicine Laboratory and Nelson Mandela School of Medicine for project supervision, assistance and motivation.

All the participants attending the King Edward VIII antenatal clinic.

Partson Tinarwo, our biostatistician, for performing the statistical analysis for this project.

My laboratory colleagues, Bongekile Ngobese (PhD student), for allowing us to use samples from her previous study, assistance and motivation for this project, Deshanta Naicker (PhD student) and Rowen Govender (PhD student) for all your support, motivation, guidance and friendship throughout this project. To everyone who contributed and played a role towards this study.

God, my family and friends; especially my mum for all the unconditional motivation, love and support throughout this journey.

CONTENTS

ABSTRACT

Background: Genital mycoplasmas can be found amongst the normal human flora mostly in the respiratory, reproductive and urinary tracts as commensal or pathogenic organisms. These bacteria are sexually transmitted and can be linked to sexually transmitted diseases and other conditions. There are a limited number of studies conducted in South African pregnant women especially from KwaZulu-Natal which have assessed the prevalence, co-infection rates and risk factors for genital mycoplasmas. In this study, the prevalence, co-infection rates and risk factors for *Mycoplasma genitalium*, *M. hominis*, *Ureaplasma urealyticum* and *U. parvum* were investigated in a cohort of Human immunodeficiency virus (HIV) infected pregnant women. The data generated in this study, therefore adds to the growing body of knowledge on these pathogens.

Methods: This study included 264 HIV infected pregnant women attending the King Edward VIII antenatal clinic in eThekwini, South Africa. The women were recruited between October 2020 and April 2021. Each enrolled women provided self-collected vaginal swabs (dry swabs) for detection of the vaginal infections. The consenting women had also completed a questionnaire on socio-demographic, behavioural and clinical factors. DNA was extracted from the vaginal samples using the PureLink Microbiome kit. The individual pathogens were detected using the TaqMan Real-time PCR assays using commercially available primers and probes on a QuantStudio 5 Real-time polymerase chain reaction (PCR) platform. The statistical data analysis was conducted in a freely available Statistical Computing Environment, R software, version 3.6.3 using the RStudio platform.

Results: The most prevalent infection in the study population was *U. urealyticum*, 236/264 (89.4%), followed by *M. hominis*, 215/264 (81.4%), *U. parvum*, 203/264 (76.9%) and lastly *M. genitalium*, 7/264 (2.70%). A total of five women (1.90%) were coinfected with all four microorganisms.

Within the group of women who tested positive for *Mycoplasma genitalium* (*M. genitalium*), partner having other partners was the only significant behavioral factor in relation with being positive, p=0.031. However, a smaller proportion of positive women reported that their partner had other partners (28.6%) when compared to 57.1% who reported that their partner did not have other partners and 14.3% who did not know if their partner had other partners. Within the group

of women who tested positive for *Mycoplasma hominis* (*M. hominis*), partner having STI symptoms was a significant clinical factor in relation with being positive, p=0.027. Women that experienced current symptoms of STIs was significantly associated with being positive, p<0.001. In addition, of the *M. hominis* positive women, a higher proportion, 80.5% tested positive for *U. parvum* infection compared to 19.5% who tested negative and this was significant, p=0.004. Partner being circumcised was a significant clinical factor in relation with being positive for *Ureaplasma urealyticum* (*U. urealyticum*), p=0.028. In addition, partner having symptoms of STIs was a significant clinical factor in relation with being positive, p=0.027. The majority of the positive women were in the third trimester of pregnancy and trimester of pregnancy was significantly associated with being positive for infection, p=0.040. Of the women who tested positive for *U. urealyticum*, a higher proportion of women also tested positive for *M. hominis* and this association was significant, p=0.051. Within the group of women who tested positive for *Ureaplasma parvum* (*U. parvum*), partners HIV status was significant in relation with being positive, p=0.049. Lifetime number of sex partners was significantly associated with being positive, p=0.012. Partner having other partners was also a significant factor in relation with being positive, p=0.023. Of the *U. parvum* positive women, a higher proportion of women (85.2%) tested positive for *M. hominis*. This association was significant, p=0.004. In the adjusted analysis, being employed increased the risk of getting infected with *M. hominis* p=0.012. In the adjusted analysis, current STI symptoms increased the risk for *M. hominis* by 95.27 fold, p<0.001. Being *U. parvum* positive increased the risk for *M. hominis* by 8.19 fold, p=0.001. Being *U. urealyticum* positive also increased the risk for *M. hominis*, p=0.039. In the adjusted analysis, having >4 lifetime sex partners increased the risk of infection with *U. parvum* by 88.02 fold. This factor was significant, p<0.001. Partner having other partners increased the risk of infection with *U. parvum*, p=0.008. In the adjusted analysis, being *M. hominis* positive increased the risk for *U. parvum* by 4.33 fold, p=0.008.

Conclusion: The present study provides information on the risk factors associated with genital mycoplasma infections. The identification of risk factors provides the foundation for the development of prevention interventions. In this study, clinical and behavioral factors were shown to be significantly associated with the risk for infection. Based on this finding, it is evident that a single prevention strategy will not be sufficient, what will be needed is a combination prevention strategy for this vulnerable population.

Key words: *Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum,* prevalence, risk factors, HIV infected pregnant women, KwaZulu Natal

Outputs (*Manuscript in progress*)

Significant associations between *Mycoplasma hominis* and *Ureaplasma parvum* infection in Human immunodeficiency virus infected pregnant women

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SYMBOLS AND ABBREVIATIONS

%	Percent
>	Greater-than
<	Less-than
=	Equal to
°C	Degree Celsius
µL	Microlitre
AOR	Adjusted odds ratio
BV	Bacterial vaginosis
BLAST	Basic Local Alignment Search Tool
BREC	Biomedical Research Ethics Committee
CI	Confidence interval
e.g.	Example
FAM	Fluorescein amidite
HIV	Human Immunodeficiency virus
IL	Interleukin
Q1-Q3	Interquartile range
n	Number
Neg	Negative
NGU	Non-gonococcal urethritis
p	Probability value
PCR	Polymerase Chain Reaction
PID	Pelvic inflammatory disease
Pos	Positive
STD	Sexually transmitted disease
STI	Sexually transmitted infection
spp.	Species
UOR	Unadjusted odds ratio
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Mycoplasma species are the smallest bacterial cells to be discovered and have the smallest genomes with the least amount of highly essential organelles (1) (2). There are over 200 different species that fall under this genus and some of them have been known to cause diseases in humans (3). The species that can potentially lead to significant clinical infections in humans are: *Mycoplasma pneumonia, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma parvum* and *Ureaplasma urealyticum* (3). There is an uncertainty of the consequences in women that are infected with genital mycoplasmas due to the fact that only a few studies have been conducted. However, infected women are at high risk for reproductive tract consequences, affecting fertility and pregnancy outcomes. These pathogens can also increase the risk of cervicitis, cystitis, bacterial vaginosis (BV), pelvic inflammatory disease (PID), chorioamnionitis, postpartum fever, infertility, preterm delivery, intrauterine growth retardation, systemic neonatal infections, endometritis and Human immunodeficiency virus (HIV-1) acquisition (4) (5) (6) (7) (8).

In the last few years, researchers have gathered evidence showing that *M. genitalium* is an emerging fastidious pathogen in sexually transmitted diseases (9). Studies have shown that *M. genitalium* prevalence rates were approximately 1% in a screening population (10) and 9% to 50% in populations at high risk for sexually transmitted infections (STIs) (11) (12). In South Africa, a prevalence of 10.8% was reported for *M. genitalium* in women (Hay *et al,* 2015). In addition, the prevalence of this pathogen in HIV infected women was found to be 7.4% (13).

M. hominis has been isolated from the endometrium and fallopian tubes of about 10% of women with salpingitis (14). It has also been isolated significantly more often and in larger numbers from the lower genital tracts of women who delivered a preterm infant when compared to women who had a normal delivery (15). Since *Ureaplasma* species were only first identified around the beginning of the new millennium there are not many studies published on these pathogens. *U. urealyticum* and *U. parvum*, *M. hominis*, *Gardnerella vaginalis* and *Streptococcus agalactiae* have been associated with a broad range of adverse pregnancy and neonatal outcomes such as clinical chorioamnionitis, funisitis, bacteremia and preterm labor and birth (16, 17) (8). Studies have

shown that microbes in the amniotic cavity can also invade the surrounding tissues such as the chorioamniotic membranes (also known as fetal membranes) and placenta (4). Kenichiro *et al*. (2020) showed that *U. parvum* induces adverse pregnancy and neonatal outcomes by causing severe inflammatory responses in the amniotic cavity, the fetus, the gestational and reproductive tissues and the maternal-fetal interface (18). Studies have also shown that *M. hominis* and *U. urealyticum* have also been demonstrated in patients with pyelonephritis (19).

Currently, there are a limited number of studies conducted in South African pregnant women especially from KwaZulu-Natal which have assessed the prevalence and risk factors for genital mycoplasmas. In this study, the prevalence and risk factors for *M. genitalium*, *M. hominis*, *U. urealyticum* and *U. parvum* was investigated in a cohort of HIV infected pregnant women. The data generated in this study, therefore adds to the growing body of knowledge on these pathogens.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of genital mycoplasmas

There are more than one million sexually transmitted infections (STIs) that are spread worldwide each day (20) and every year there are approximately 367 million new infections with either chlamydia, gonorrhoea, syphilis and trichomoniasis (20). STIs are spread via sexual contact such as vaginal, oral and anal sex, non-sexual contact such as via blood or blood products or mother to child transmission during pregnancy and childbirth (20).

The genus *Mycoplasma* are the smallest bacteria to be discovered and have the smallest genomes and the least amount of highly essential organelles (1) (2). They are gram-negative, aerobic or facultative aerobic bacteria. They can be classified as either parasitic or saprotrophic. There are over 200 different species that fall under this genus and some of them have been known to cause diseases to humans (3). The species that can potentially lead to significant clinical infections in humans are: *M. pneumonia, M. hominis, M. genitalium U. parvum* and *U. urealyticum* (3).

2.2 Prevalence estimates of genital mycoplasmas

M. hominis was first identified and isolated in 1937 as the first mycoplasma of human origin (21). The role of this bacteria to cause disease has been researched over the years and is still not yet fully understood (4). A study conducted by Christofolini *et al*. (2012) in a cohort of non-pregnant women observed a prevalence of 11.3 % (12/106) for *M. hominis* infection (22). That same study, also reported on coinfections between *M. hominis* and *Chlamydia trachomatis* (22). A meta-analysis conducted on studies published between 2000 and 2019, reported a prevalence of 9.68% for *M. hominis* for non-pregnant Iranian women (23). A recent study conducted by Naicker *et al*. (2021) reported a prevalence of 48% for *M. hominis* for a population of South African pregnant women (24). A previous study conducted in South Africa by Redelinghuys *et al*. (2013) also reported high prevalence data for *M. hominis* (50.7%) in pregnant women from Gauteng, South Africa (25).

M. genitalium was first reported to be isolated in 1981 using urethral swabs of 13 men with nongonococcal urethritis (NGU) that were attending the sexually transmitted disease (STD) clinic at St Mary's Hospital, Paddington, London, United Kingdom (26). A study conducted by Seña *et al*. (2018), found an overall prevalence of 20.5% for *M. genitalium* in non-pregnant women (27). The prevalence of *M. genitalium* was higher among women aged 15–21 years than among those aged 22–25 years (22.6% vs 17.7%, respectively) (27). The prevalence of *M. genitalium* coinfection was 29.9% among women with chlamydial infection and 23.6% among those with gonococcal infection (27). A recent study conducted by Nye *et al*. (2020), reported a prevalence of 4% for *M. genitalium* in a population of women from the United States (28). A more recent study conducted in South Africa, reported a prevalence of 5.9% for *M. genitalium* in pregnant women (24).

Ureaplasma species were only first identified in the last 20 years (29) (30). In a study conducted by Lee *et al*. (2020) that analyzed 4035 endocervical swab specimens using a *Mycoplasma* IST2 kit, 1589 (39.4%) cases were positive for genital mycoplasmas, which included 49 (3.1%) cases of *M. hominis*, 1243 (78.2%) cases of *Ureaplasma* species and 297 (18.7%) cases of both *M. hominis* and *Ureaplasma* species (31). The prevalence of *Ureaplasma* species (30.8%) was higher than that of *M. hominis* (1.2%). According to several studies conducted in South Korea, the prevalence of *Ureaplasma* species in symptomatic patients was higher than that of *M. hominis*. The prevalence of *Ureaplasma* species and *M. hominis* was 21.3% and 2.9%, as reported by Moon *et al*. (2013), 65.6% and 11.8% by Kweon *et al*. (2016), and 48.8% and 25.3% by Jang *et al*. (2019), respectively (32), (33), (34). Similar rates were reported in Poland (35) and China (36).

In a study conducted by Peretz *et al*. (2020), 214 gravidas women were sampled and their prevalence rates were found as follows: overall, 19 (9.3%) tested positive for any genital mycoplasmas, with five (2.3%) participants testing positive for *M. genitalium*, nine (4.2%) testing positive for *U. parvum*, and five (2.3%) testing positive for *U. urealyticum* (37). It was found that mothers had passed on these bacteria to their newborns who tested positive after birth (37). Previous studies have also shown prevalence rates of 0.7–3.3% for *M. genitalium* (38), and a higher prevalence of *U. parvum* than *U. urealyticum* (2) (30).

2.3 The effect of genital mycoplasmas on women's health

Women infected with genital mycoplasmas are at high risk for severe health consequences. Women are mainly affected in the reproductive tract ultimately affecting fertility and pregnancy outcomes. Infection with mycoplasma can also increase the risk of cervicitis, cystitis, BV, PID, chorioamnionitis, postpartum fever, infertility, preterm delivery, intrauterine growth retardation, systemic neonatal infections, endometritis and HIV-1 acquisition (4), (5), (6-8). Bacterial transmission can occur from mother to child via intrauterine infection, where the bacterium multiplies in the amniotic fluid and is then transmitted to the fetal lungs (4). This kind of infection could occur at the beginning of the pregnancy and when membranes are still intact. Alternatively, infection can be caused via the hematogenous route which involves the navel blood vessels from an infected placenta. A third means of transmission can occur via the respiratory and cutaneous membranes, while the baby passes through the birth canal (4).

A recent meta-analysis showed that infection with *M. genitalium* in women had a two-fold increased risk of cervicitis, PID, preterm delivery, spontaneous abortion, and infertility (39). Previous studies have shown that genital mycoplasmas can cause infections in the lower genital tract. Infection with *M. genitalium* has been associated with high levels of leukocytes causing easily induced cervical bleeding leading to cervicitis (40) (41), (42). PID is a polymicrobial syndrome of the female upper genital tract, including endometritis, salpingitis, pelvic peritonitis, and tubo-ovarian abscess which is an important cause of infertility in women (43). Studies have shown that *M. genitalium* can be detected in the upper genital tract of women with acute PID. Women from a Kenyan STD clinic with acute pelvic pain, were identified using polymerase chain reaction (PCR) to have *M. genitalium* in the endometrium in 12% of women with histologic endometritis. Whereas, it was not present in the endometrium of women without endometritis (44). Moreover, the prevalence of *M. genitalium* in the endometrium in women with endometritis was similar to that of *N. gonorrhoeae* (10%) and *C. trachomatis* (7%) (44). Simms *et al*. (2003) identified *M. genitalium* by PCR on cervical swabs in 13% of women clinically diagnosed with acute PID, whereas none of the 37 control women tested positive (45). Subclinical PID (histologic endometritis in the absence of signs or symptoms of acute PID is often present in women with uncomplicated chlamydial or gonococcal cervicitis or BV (46). Similar to acute PID, a prospective study has demonstrated that women with subclinical PID are at risk for infertility (46). There have

been studies that have suggested that there may be a link between *M. genitalium*, BV and female urethritis (47) (48), (49) and this could lead to an increase in susceptibility to HIV-1.

In previous studies, the highest infection rate of *Ureaplasma* species (40–80%) was found in the vagina and cervix of asymptomatic women. In addition, these bacteria were the most prevalent among all bacteria found in the amniotic fluid and placenta (4). Later studies that distinguished between the two species found a higher prevalence of *U. parvum* than *U. urealyticum* among pregnant versus non-pregnant women (29) (30). In regards to newborns, prospective cohort studies demonstrated that infection with *U. urealyticum* slows fetal growth and is associated with low birth weight, a risk which is high especially in cases of BV (50) (38).

2.4. Mechanisms of pathogenesis

Previous studies have suggested that the biological mechanism by which *M. genitalium* attaches to the host epithelial cells is by the binding of the toll like receptors 2 and 6 to the bacterium which stimulates the host response-chemokines and leukocytes to be produced (51), (52) (53). *M. genitalium* causes proinflammatory responses from monocytes and macrophages that are present in higher numbers to the female reproductive tract tissues (54). In women that are often infected, neutrophils are a prominent component of the proinflammatory response to *M. genitalium* (52).

In vitro and animal models assist in explaining the mechanisms for pathogenesis in the lower reproductive tract. *M. genitalium* has the ability to infect mucosal cells in the female genital tract and induce a prolonged inflammatory response after inoculation (55). Acute *M. genitalium* infection of endocervical cells lead to the destruction of microvilli and an increase in secretory vesicle formation (56). *M. genitalium* infection of endocervical cells *in vitro* also causes a proinflammatory cytokine and chemokine response with secretion of interleukin 6, interleukin 7, interleukin 8, monocyte chemotactic protein 1, and granulocyte-macrophage colony stimulating factor (57). Mycoplasmas can also inhibit the transcriptional activity of p53, resulting in reduced apoptosis of damaged cells as shown in Figure 1. In addition, some Mycoplasmas may have oncogenic potential since they cause phenotypic changes of the cell (58). High proinflammatory cytokine levels with chronic *M. genitalium* infection, may be deleterious to the female reproductive tract (52).

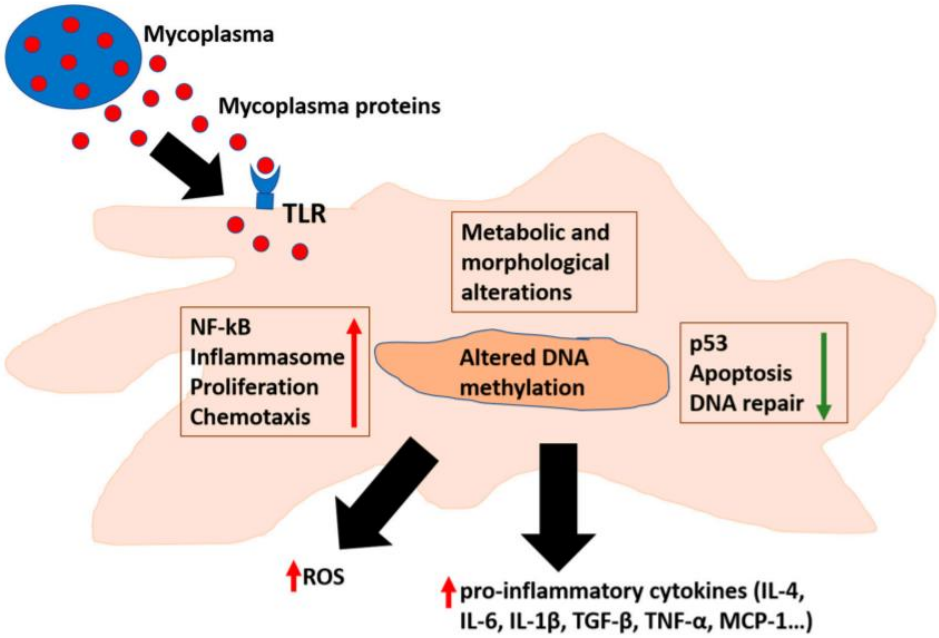

Figure 1: Mycoplasmas affect cellular pathways involved in inflammation and cellular transformation. Mycoplasmas' proteins interact with TLR or enter the cells, where they can alter several pathways responsible for inflammation and DNA repair. In addition, affecting methylation of cellular DNA results in alteration of cellular epigenetic landscape. TLR: Toll Like Receptor; ROS: Reactive Oxygen Species. TGF: Transforming Growth Factor; TNF: Tumor Necrosis Factor; and MCP-Monocyte Chemoattractant Protein (58).

2.5 The effect of genital mycoplasmas on pregnancy

In vitro and animal studies have investigated the effect of mycoplasma infection on the fallopian tube mucosa. There has been evidence of microscopic ciliary damage in human fallopian tube explants infected with *M. genitalium* (59).

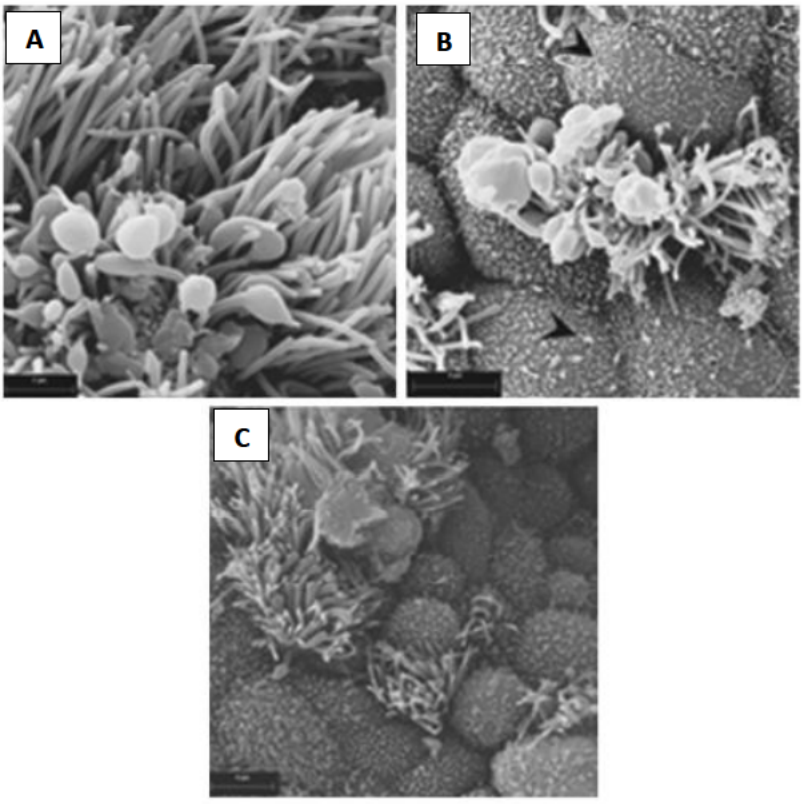

Figure 2: The effect of infection with *Mycoplasma genitalium* on the human fallopian tube epithelium (59).

Baczynska *et al*. (2007) demonstrated that infection with *M. genitalium* damaged the cilia found on the human fallopian tube. The swelled cilia can have many shapes and swelling can appear at different places of the cilia (Figure 2A). Following this, cilia fall apart, are shortened and the number of cilia per cell then decreases (Figure 2B). Affected ciliated cells with two dead cells attach to the human fallopian tube tissue (Figure 2C) (59).

Epidemiological studies have shown mixed results of infection with genital mycoplasmas causing infertility in pregnant women (60) (61) (62) (63). However, the risk of infertility has been shown to be elevated in women who have experienced *M. genitalium* infections (62) (63). Ectopic pregnancy can result from damaged cilia and has been strongly linked to upper tract infection with *N. gonorrhoeae* and *C. trachomatis* (64). The ciliary damage observed in fallopian tube tissue indicates that this can also occur with *M. genitalium* infection as seen in Figure 2 (59). Motomura *et al*. (2020) showed that *U. parvum* induces adverse pregnancy and neonatal outcomes, and

infections caused by *M. hominis* and *U. urealyticum* have also been demonstrated in patients with pyelonephritis (18) (19).

2.6 Laboratory diagnosis of genital mycoplasmas

Several methods have been developed for the detection of *Mycoplasma* species such as microbiological cultivation, biochemical assays, direct or indirect fluorescent staining, immunofluorescence and nucleic acid amplification techniques (65).

Traditional culture-based detection and enumeration methods for genital mycoplasmas are hindered by their strict growth requirements and inability to grow to visual turbidity (66). Real-time polymerase chain reaction (q-PCR), a development and modification of the traditional (conventional) PCR, combines amplification and detection of amplified PCR products (amplicons). It has several advantages over the earlier DNA hybridization probes or PCR based assays (67). It has improved the rapid detection of DNA and RNA from many organisms since it is more specific (99.5%) and sensitive (91.8%) than a conventional PCR assay (68). The simultaneous detection eliminates the labor intensive post amplification agarose gel method of detection as in the conventional PCR assay, or probe hybridization with reduced risk of carryover contamination (67).

Real-time PCR has been shown to be a useful tool for detecting bacterial loads in a range of samples, including vaginal and cervical swabs, urethral swabs, and first-void urine (69). Several qPCR assays are able to detect and quantify Mycoplasmas as low as 2 copies/reaction (70). A study conducted by Keskin *et al*. (2018), showed that *M. hominis* detected by culture and real-time PCR assays was 72% (47/65) and 69% (45/65), respectively (71). The sensitivity and specificity of real-time PCR when compared to culture was 91.5% and 88.9%. According to Keskin *et al*. (2018), real-time PCR can play an important role for the rapid detection of Mycoplasmas in clinical samples (71). The findings of Keskin *et al*. (2018) were confirmed by Leli *et al*. (2018). According to Leli *et al*. (2018) the use of real-time PCR for the detection of mycoplasmas in cervical samples showed a higher rate of recovery when compared to culture-based methods (72).

Yoshida *et al*. (2002) were the first to publish a real-time PCR assay for the detection of *M. genitalium* (73). They used TaqMan probe chemistry to target the *16S rRNA M. genitalium* gene in urine specimens of men with nongonococcal urethritis and found the assay to be highly sensitive

(98.8%) when compared to the sensitivity (87.0%) found by Mena *et al*. (2002) in the same specimen type using conventional PCR (74). In Sweden, Edberg *et al*. (2008) compared real-time PCR to conventional PCR assays and found real-time PCR to be highly sensitive (97.4%) compared to 80.3% of the conventional PCR assay (75). A recent study by Mikami *et al*. (2021), investigated the efficacy of the loop-mediated isothermal amplification (LAMP) assay to detect *Ureaplasma* species in respiratory tract samples of preterm infants (76). In that study, the sensitivity and specificity of LAMP was 73.9% (17/23) and 97.2% (140/144), when compared to real-time PCR which was 73.9% (17/23) and 95.8% when both assays were compared to culture (76).

2.7 Treatment for genital mycoplasmas

During pregnancy the list of antibiotics effective against urogenital mycoplasmas is much shorter (77). Azithromycin has been an ideal treatment for several years in many countries, including South Africa because of its long half-life, excellent tissue penetration, and the fact that it can be administered as a single-dose treatment. Antimicrobial resistance poses a threat to effective treatment (78). Studies have shown that antibiotics such as ciprofloxacin and levofloxacin are less effective against Mycoplasmas (79). In South Africa, the recommended management of urogenital infections is based on symptoms alone (syndromic management). The recommended seven-day course of doxycycline for genital discharge syndromes (i.e. vaginal discharge) was changed in 2015 to include a single-dose of azithromycin only. Doxycycline is no longer in the guidelines for use (78).

Resistance to macrolides has already been reported in South Africa. The first study to report on macrolide resistance in *M. genitalium* in South Africa was conducted in the Limpopo province. In that study, macrolide resistance-associated mutations were detected in four out of 41 (9.8%) *M. genitalium* positive isolates obtained from women attending a primary health care clinic (80). A later study conducted by Le Roux *et al*. (2018) reported a macrolide resistance-associated mutation (A2059G) amongst *M. genitalium* positive isolates obtained in 2016. The mutation was present in 25% of isolates tested (78).

2.8 Rationale for the study

Studies have shown that genital mycoplasmas are associated with causing adverse outcomes in non-pregnant and pregnant women. There are a limited number of studies conducted in South African pregnant women especially from KwaZulu-Natal which have assessed the prevalence and risk factors for genital mycoplasmas. Hence the need for the current study. Through this study, the coinfections between *U. parvum*, *U. urealyticum*, *M. hominis* and *M. genitalium* will also be determined. This information is also lacking in our current setting. The research question/s for this study are, what is the prevalence of genital mycoplasmas in HIV infected pregnant women and what are the risk factors associated with genital mycoplasmas in HIV infected pregnant women?

The objectives are:

1. To determine the prevalence of *M. genitalium*, *M. hominis*, *U. urealyticum* and *U. parvum* in a cohort of HIV infected women
2. To identify the rate of coinfections among these microorganisms
3. To identify risk factors associated with the individual microorganisms

CHAPTER 3

METHODS AND MATERIALS

3.1 Ethics Statement

The current study was approved by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (UKZN), (BREC/00003166/2021) (Appendix I).

3.2 Study design and population

The study was a retrospective laboratory based study using stored vaginal swab samples collected from pregnant women attending the King Edward VIII antenatal clinic. The swabs had been collected through another study (BREC0000/1382/2020) which had obtained ethics permission to store the swabs and use them for future studies. For the larger study, recruitment of the study population took place between October 2020 and April 2021. The larger study included, HIV infected pregnant women, willing to provide written informed consent, willing to provide vaginal swab samples and willing to provide socio-demographic, behavioral and clinical data. The swab samples were self-collected and the women were provided with instructions on proper sample collection. After collection, dry swabs were placed in 2ml of phosphate buffered saline. The solution was vortexed to dislodge the cells from the swabs and the swab was discarded. The solution was stored at -80°C for future use. All sample processing, testing and analysis was performed at the Clinical Medicine Laboratory of UKZN. A total of n=264 swab samples were tested in this study.

3.3 Isolation of DNA from the vaginal swabs

For the DNA extraction, the vaginal fluid solution was removed from the freezer and allowed to thaw prior to the extraction. DNA was extracted from the vaginal fluid using the PureLink Microbiome kit (ThermoFisher Scientific, United States) according to the manufacturer's instructions. Briefly, 2ml of the vaginal fluid samples were centrifuged for 30 minutes at 14 000xg. The supernatant was discarded and 800µl of S1 lysis buffer was added to the pellet and pipetted up and down to mix the sample. The sample was then transferred to the bead tube and 100µl of S2 lysis enhancer was added to the bead tube, capped and vortexed briefly. This was incubated at 95^{o}C for 10 minutes, followed by vortexing at a maximum speed for 7 minutes and further centrifuged at 14 000x g for 1 minute. Thereafter, 500µl of the supernatant was transferred to a clean microcentrifuge tube, avoiding the bead pellet and any cell debris. To bind DNA to the

column, 900µl of binding buffer was added and vortexed briefly. Following this, 700µl of the sample mixture was loaded onto a spin column-tube and centrifuged at 14 000x g for 1 minute. The flow through was discarded and the spin column was centrifuged at 14 000x g for 30 seconds. The spin column was placed in a clean tube and 50µl of S6 elution buffer was added, the tube was incubated at room temperature for 1 minute. After 1 minute, the spin column was centrifuged at 14 000x g for 1 minute, and the column was discarded and the purified DNA was stored at -20^{o}C. The concentration and purity of the DNA was assessed using a Nanodrop spectrophotometer (ThermoFisher Scientific, United States).

3.4 Detection of genital mycoplasmas

M. hominis was detected using the TaqMan Real-time PCR (sensitivity) assay (ThermoFisher Scientific, United States) using commercially available primers and probes specific for *M. hominis* (Ba04646255_s1). The assay targets a Hypothetical protein from this pathogen.

M. genitalium was detected using the TaqMan Real-time PCR (sensitivity) assay (ThermoFisher Scientific, United States) using commercially available primers and probes specific for *M. genitalium* (Ba04646251_s1). The assay targets a Hypothetical protein from this pathogen.

U. urealyticum was detected using the TaqMan Real-time PCR (sensitivity) assay (ThermoFisher Scientific, United States) using commercially available primers and probes specific for *U. urealyticum* (Ba04646254_s1). The assay targets *ureB* gene from this pathogen. *U. parvum* was detected using in-house designed primers and probes specific for this pathogen.

The assays were run on the Quant Studio 5 Real-time PCR detection system (ThermoFisher Scientific, United States). Each PCR reaction was performed in a final volume of 20µl comprising: 2µl FAM-labelled probe/primer mix, 5µl Fast Start 4x probe master mix, (Thermofisher, Part No. 4444434), 2µl template DNA and 11µl nuclease-free water. Non-template and positive controls (TaqMan™ Vaginal Microbiota Extraction Control; cat no. A32039) were also included. Amplification was performed at 95^{o}C for 30 seconds followed by 45 cycles comprising of denaturation at 95^{o}C for 3 seconds and annealing at 60^{o}C for 30 seconds. Detection of amplified fluorescent products was carried out at the end of the annealing phase. The raw fluorescent data that included the C_T mean values were automatically generated by the Quant Studio 5 Real-time PCR system software.

3.5 Statistical Data Analyses

The statistical data analysis was conducted in a freely available Statistical Computing Environment, R software, version 3.6.3 using the RStudio platform. Initially, the population characteristics were described using frequencies stratified by infection status of the pathogens. In addition to the frequencies, univariate analysis was used to assess the relationship between each risk factor and the pathogen infection status. The categorical risk factors were univariately assessed using the Chi-Square test or the Fisher's exact test in the case of smaller expected frequencies. The significant risk factors were used to fit univariate logistic regressions in order to quantify their relationships with the outcome in terms of odds ratios. The analysis further considered multiple logistic regression to assess the influence of these univariately significant risk factors in the presence of the other factors. All the tests were conducted at 5% level of significance. A p-value ≤ 0.05 was considered significant.

CHAPTER 4

RESULTS

4.1 Characteristics of the study population

Table 1 describes the overall characteristics of the study population. Overall, the median age (interquartile range: Q1-Q3) of the study women was 31 years of age (Q1-Q3: 26.0-37.0). The majority of the women had attended high school, 209/264 (79.2%), were unemployed, 188/264 (71.2%) and were unmarried, 234/264 (88.6%). With respect to behavioral factors, 244/264 (92.4%) of the women reported having a regular sex partner, 136/264 (51.5%) had a partner who was HIV positive, 157/264 (59.5%) were not living with their partner, 189/264 (71.6%) had experienced first sex between the ages of 15-20 years, and 134/264 (50.8%) had reported having between 2-4 lifetime sex partners. In addition, 138/264 (52.3%) of the women reported "not knowing" if their partner had other partners, and 167/264 (63.3%) did not use a condom during their last sex act. With respect to clinical factors, 172/264 (65.2%) of the women reported that their partner was circumcised, 60/264 (22.7%) reported that their partner had symptoms of STIs, 161/264 (61.0%) were in their third trimester of pregnancy, 92/264 (34.8%) had been previously treated for STIs, and 16/264 (6.1%) had engaged in intravaginal practices. The majority of the women, 152/264 (57.6%), were asymptomatic for STIs (no current abnormal vaginal discharge) (Table 1).

Table 1: Overall characteristics of the study population

Variable	**Overall (N=264)**
Age	
Mean ±SD (CV%)	30.8±6.62(21.5)
Median(Q1-Q3)	31.0(26.0-37.0)
Min-Max	18.0-44.0
Educational level	
College, University	48 (18.2%)
Did not attend school	1 (0.4%)
High school	209 (79.2%)
Primary school	6 (2.3%)
Employed	
No	188 (71.2%)
Yes	76 (28.8%)
Married	
No	234 (88.6%)
Yes	30 (11.4%)
Regular sex partner	

No	20 (7.6%)
Yes	244 (92.4%)
Partners HIV status	
Don't know	40 (15.2%)
Negative	88 (33.3%)
Positive	136 (51.5%)
Cohabiting	
No	157 (59.5%)
Yes	105 (39.8%)
Missing	2 (0.8%)
Age of 1st sex	
<15	9 (3.4%)
>25	6 (2.3%)
15-20	189 (71.6%)
21-25	60 (22.7%)
Lifetime sex partners	
>4	54 (20.5%)
1	76 (28.8%)
2-4	134 (50.8%)
Partner has other partners	
Don't know	138 (52.3%)
No	61 (23.1%)
Yes	65 (24.6%)
Condom used during last sex	
No	167 (63.3%)
Yes	97 (36.7%)
Partner circumcised	
No	92 (34.8%)
Yes	172 (65.2%)
Trimester	
1st	20 (7.6%)
2nd	82 (31.1%)
3rd	161 (61.0%)
Missing	1 (0.4%)
Previously treated for STIs	
No	172 (65.2%)
Yes	92 (34.8%)
Intravaginal practices	
No	248 (93.9%)
Yes	16 (6.1%)
M. hominis	
Neg	49 (18.6%)
Pos	215 (81.4%)
U. urealyticum	
Neg	28 (10.6%)
Pos	236 (89.4%)
U. parvum	
Neg	61 (23.1%)
Pos	203 (76.9%)
M. genitalium	
Neg	257 (97.3%)
Pos	7 (2.70%)
Partner STI symptom	

No	204 (77.3%)
Yes	60 (22.7%)
Current STIs symptoms	
No	152 (57.6%)
Yes	112 (42.4%)

4.2. Prevalence of infections

The most prevalent infection in the study population was *U. urealyticum*, 236/264 (89.4%), followed by *M. hominis*, 215/264 (81.4%), *U. parvum*, 203/264 (76.9%) and lastly *M. genitalium*, 7/264 (2.70%) (Table 1). A total of 156/264 (59.1%) women were coinfected with *U. urealyticum*, *M. hominis* and *U. parvum*, 35/264 (13.3%) were coinfected with *U. urealyticum* and *M. hominis*, and 24/264 (9.10%) were coinfected with *U. urealyticum* and *U. parvum*. A total of five women (1.90%) were coinfected with all four microorganisms, 12/264 (4.50%) were coinfected with *M. hominis* and *U. parvum*, and 0.4% were coinfected with *M. hominis* and *M. genitalium* (Table 2).

Table 2: Prevalence of Mycoplasma coinfections in the study participants

Percent (%)	***M. genitalium***	***M. hominis***	***U. urealyticum***	***U. parvum***	**Number of women**
59.1	Neg, 97.3%	Pos, 81.4%	Pos, 89.4%	Pos, 76.9%	156
05.7	Neg, 97.3%	Neg, 18.6%	Pos, 89.4%	Neg, 23.1%	15
13.3	Neg, 97.3%	Pos, 81.4%	Pos, 89.4%	Neg, 23.1%	35
09.1	Neg, 97.3%	Neg, 18.6%	Pos, 89.4%	Pos, 76.9%	24
04.5	Neg, 97.3%	Pos, 81.4%	Neg, 10.6%	Pos, 76.9%	12
00.4	Pos, 02.7%	Neg, 18.6%	Pos, 89.4%	Neg, 23.1%	1
02.3	Neg, 97.3%	Pos, 81.4%	Neg, 10.6%	Neg, 23.1%	6
01.9	Pos, 02.7%	Pos, 81.4%	Pos, 89.4%	Pos, 76.9%	5
01.1	Neg, 97.3%	Neg, 18.6%	Neg, 10.6%	Neg, 23.1%	3
00.4	Pos, 02.7%	Pos, 81.4%	Neg, 10.6%	Neg, 23.1%	1
02.3	Neg, 97.3%	Neg, 18.6%	Neg, 10.6%	Pos, 76.9%	6

4.3 Factors associated with *M. genitalium* positivity

Socio-demographic factors

There was no significant difference in age across the *M. genitalium* negative and positive women (p=0.765). Within the group of women who tested *M. genitalium* positive, a higher proportion of women had attended high school (85.7%) when compared to 14.3% who had attended college/university. However, level of education was not significantly associated with infection status, p=1.000. A higher proportion of positive women were unemployed, 85.7% when compared

to 14.3% who were employed, this was not significant, p=0.677. One hundred percent (100%) of the positive women were unmarried, marital status was not a significant factor (p=1.000) (Table 3).

Behavioral factors

Within the group of women who tested positive for *M. genitalium*, 100% of the women reported having a regular sexual partner, this factor was not significant (p=1.000). A high proportion of the positive women (71.4%) had a partner who was HIV positive when compared to 28.6% who had a HIV negative partner, however, this was not significant, p=0.671. A larger proportion of the positive women (71.4%) were not cohabiting with their partner when compared to 28.6% who were cohabiting, this factor was also not significant, p=0.706. With respect to age of first sex, the majority of the positive women, 85.7%, had experienced first sex between the ages of 15-20 years when compared to 14.3% of the women who had their first sex between the ages of 21-25 years of age. Age of first sex was not a significant factor, p=1.000. A large proportion of the positive women reported having 1 lifetime sex partner (42.9%) when compared to 28.6% who reported having 2-4 and >4 lifetime sex partners. Lifetime number of sex partners was not significantly associated with being positive, p=0.408. Partner having other partners was the only significant behavioral factor in relation with being positive, p=0.031. However, a smaller proportion of positive women reported that their partner had other partners (28.6%) when compared to 57.1% who reported that their partner did not have other partners and 14.3% who did not know if their partner had other partners. With respect to condom use, a larger proportion of positive women did not use a condom at their last sex act (57.1%), when compared to 42.9% who reported using a condom. Condom use was not significantly associated with being positive, p=0.710 (Table 3).

Clinical factors

A higher proportion of the *M. genitalium* positive women, 57.1% reported that their partner was circumcised when compared to 42.9% who had an uncircumcised partner. This factor was not significant, p=0.697. In addition, 42.9% of the women reported that their partner had symptoms of STIs such as testicular pain, pain during urination and discharge from the penis when compared to 57.1% who had a partner without symptoms, this factor was not significant, p=0.196. A higher proportion of positive women, 57.1% had reported having symptoms of STIs such as genital

itching, abnormal vaginal discharge, genital sore/ulcers, and genital warts at enrollment when compared to 42.9% of the women who were asymptomatic. Symptoms of STIs was not significantly associated with being positive, p=0.462. With respect to history of STI treatment, most women were not previously treated for STIs, 57.1% when compared to 42.9% who had been previously treated, this was not significant, p=0.697. One hundred percent (100%) of the positive women did not engage in intravaginal practices, p=1.000. The majority of the positive women were in the second trimester of pregnancy (57.1%), when compared to 42.9% who were in the third trimester of pregnancy. Trimester of pregnancy was not significantly associated with being positive for infection, p=0.285 (Table 3).

Table 3: Characteristics of the study women according to *M.genitalium* status

M.genitalium	**Neg (N=257)**	**Pos (N=7)**	**p-value**	**Overall (N=264)**
Age			0.765	
Mean ±SD (CV%)	30.8±6.61(21.5)	30.0±7.55(25.2)		30.8±6.62(21.5)
Median(Q1-Q3)	31.0(26.0-37.0)	34.0(23.5-35.5)		31.0(26.0-37.0)
Min-Max	18.0-44.0	20.0-38.0		18.0-44.0
Educational level			1.000	
College, University	47 (18.3%)	1 (14.3%)	Fisher's	48 (18.2%)
Did not attend school	1 (0.4%)	0 (0%)		1 (0.4%)
High school	203 (79.0%)	6 (85.7%)		209 (79.2%)
Primary school	6 (2.3%)	0 (0%)		6 (2.3%)
Employed			0.677	
No	182 (70.8%)	6 (85.7%)	Fisher's	188 (71.2%)
Yes	75 (29.2%)	1 (14.3%)		76 (28.8%)
Married			1.000	
No	227 (88.3%)	7 (100%)	Fisher's	234 (88.6%)
Yes	30 (11.7%)	0 (0%)		30 (11.4%)
Regular sex partner			1.000	
No	20 (7.8%)	0 (0%)	Fisher's	20 (7.6%)
Yes	237 (92.2%)	7 (100%)		244 (92.4%)
Partners HIV status			0.671	
Don't know	40 (15.6%)	0 (0%)	Fisher's	40 (15.2%)
Negative	86 (33.5%)	2 (28.6%)		88 (33.3%)
Positive	131 (51.0%)	5 (71.4%)		136 (51.5%)
Cohabiting			0.706	
No	152 (59.1%)	5 (71.4%)	Fisher's	157 (59.5%)

M.genitalium	Neg (N=257)	Pos (N=7)	p-value	Overall (N=264)
Yes	103 (40.1%)	2 (28.6%)		105 (39.8%)
Missing	2 (0.8%)	0 (0%)		2 (0.8%)
Age of 1st sex			1.000	
<15	9 (3.5%)	0 (0%)	Fisher's	9 (3.4%)
>25	6 (2.3%)	0 (0%)		6 (2.3%)
15_20	183 (71.2%)	6 (85.7%)		189 (71.6%)
21 25	59 (23.0%)	1 (14.3%)		60 (22.7%)
Lifetime sex partners			0.408	
>4	52 (20.2%)	2 (28.6%)	Fisher's	54 (20.5%)
1	73 (28.4%)	3 (42.9%)		76 (28.8%)
2 4	132 (51.4%)	2 (28.6%)		134 (50.8%)
Partner has other partners			0.031	
Don't know	137 (53.3%)	1 (14.3%)	Fisher's	138 (52.3%)
No	57 (22.2%)	4 (57.1%)		61 (23.1%)
Yes	63 (24.5%)	2 (28.6%)		65 (24.6%)
Condom used during last sex			0.710	
No	163 (63.4%)	4 (57.1%)	Fisher's	167 (63.3%)
Yes	94 (36.6%)	3 (42.9%)		97 (36.7%)
Partner circumcised			0.697	
No	89 (34.6%)	3 (42.9%)	Fisher's	92 (34.8%)
Yes	168 (65.4%)	4 (57.1%)		172 (65.2%)
Trimester			0.285	
1st	20 (7.8%)	0 (0%)	Fisher's	20 (7.6%)
2nd	78 (30.4%)	4 (57.1%)		82 (31.1%)
3rd	158 (61.5%)	3 (42.9%)		161 (61.0%)
Missing	1 (0.4%)	0 (0%)		1 (0.4%)
Previously treated for STIs			0.697	
No	168 (65.4%)	4 (57.1%)	Fisher's	172 (65.2%)
Yes	89 (34.6%)	3 (42.9%)		92 (34.8%)
Intravaginal practices			1.000	
No	241 (93.8%)	7 (100%)	Fisher's	248 (93.9%)
Yes	16 (6.2%)	0 (0%)		16 (6.1%)
Partner STI symptom			0.196	
No	200 (77.8%)	4 (57.1%)	Fisher's	204 (77.3%)
Yes	57 (22.2%)	3 (42.9%)		60 (22.7%)
Current STIs symptoms			0.462	
No	149 (58.0%)	3 (42.9%)	Fisher's	152 (57.6%)

M.genitalium	Neg (N=257)	Pos (N=7)	p-value	Overall (N=264)
Yes	108 (42.0%)	4 (57.1%)		112 (42.4%)

4.4. Factors associated with *M. hominis* positivity

Socio-demographic factors

There was no significant difference in age across the *M. hominis* negative and positive women (p=0.081). Within the group of women who tested *M. hominis* positive, a higher proportion of women had attended high school (79.5%) when compared to 17.2% who had attended college/university. However, level of education was not significantly associated with infection status, p=0.550. A higher proportion of positive women were unemployed, 70.7% when compared to 29.3% who were employed, this was not significant, p=0.699. A high proportion, 87.4% of the positive women were unmarried, however, marital status was not a significant factor (p=0.200) (Table 4).

Behavioral factors

Within the group of women who tested positive for *M. hominis*, 92.1% of the women reported having a regular sexual partner, this factor was not significant (p=1.000). Approximately, just over half of the positive women (52.1%) had a partner who was HIV positive when compared to 33.5% who had a HIV negative partner, however, this was not significant, p=0.781. A larger proportion of the positive women (61.9%) were not cohabiting with their partner when compared to 37.2% who were cohabiting, this factor was also not significant, p=0.083. With respect to age of first sex, the majority of the positive women, 72.6%, had experienced first sex between the ages of 15-20 years when compared to 22.3 % of the women who had their first sex act between the ages of 21-25 years of age. Age of first sex was not a significant factor, p=0.222. A large proportion of the positive women reported having 2-4 lifetime sex partners (52.1%) when compared to 28.4% who reported having 1 lifetime sex partner and 19.5% who reported having >4 lifetime sex partners. Lifetime number of sex partners was not significantly associated with being positive, p=0.620. Almost a quarter of the positive women reported that their partner had other partners (24.7%) when compared to 20.9 % who reported that their partner did not have other partners. However, the

majority of the positive women 54.4% did not know if their partner had other partners, this factor was not significant (p=0.183). With respect to condom use, a larger proportion of positive women did not use a condom at their last sex act (62.8%), when compared to 37.2% who reported using a condom. Condom use was not significantly associated with being positive, p=0.742 (Table 4).

Clinical factors

A higher proportion of the *M. hominis* positive women, 67.0% reported that their partner was circumcised when compared to 33.0% who had an uncircumcised partner. This factor was not significant, p=0.192. In addition, 20.0% of the women reported that their partner had symptoms of STIs such as testicular pain, pain during urination and discharge from the penis when compared to 80.0% who had a partner without symptoms. Partner having STI symptoms was a significant clinical factor in relation with being positive, p=0.027. A higher proportion of positive women, 52.1% had reported no symptoms of STIs such as genital itching, abnormal vaginal discharge, genital sore/ulcers, and genital warts at enrollment when compared to 47.9% of the women who were symptomatic. Symptoms of STIs was significantly associated with being positive, p<0.001. With respect to history of STI treatment, most women were not previously treated for STIs, 66.0% when compared to 34.0% who had been previously treated, this was not significant, p=0.523. Ninety three percent (93%) of the positive women did not engage in intravaginal practices, whereas 7% engaged in these practices, however this was not significant p=0.319. The majority of the positive women where in the third trimester of pregnancy, 60.9% when compared to 30.7% who were in the second trimester of pregnancy. Trimester of pregnancy was not significantly associated with being positive for infection, p=0.898. Of the women who tested positive for infection, a higher proportion, 91.2% tested positive for *U. urealyticum* infection when compared to 8.8% who were negative and this was significant, p=0.051. In addition, of the *M. hominis* positive women, a higher proportion, 80.5% tested positive for *U. parvum* infection compared to 19.5% who tested negative and this was significant, p=0.004. There was no significant association between *M. hominis* and the other mycoplasmas, p>0.05 (Table 4).

Table 4: Characteristics of the study women according to *M. hominis* status

M. hominis	Neg (N=49)	Pos (N=215)	p-value	Overall (N=264)
Age			0.081	
Mean±SD(CV%)	32.3±6.26(19.4)	30.5±6.67(21.9)		30.8±6.62(21.5)
Median(Q1-Q3)	34.0(27.0-38.0)	30.0(25.0-37.0)		31.0(26.0-37.0)
Min-Max	20.0-42.0	18.0-44.0		18.0-44.0
Educational level			0.550	
College, University	11 (22.4%)	37 (17.2%)	Fisher's	48 (18.2%)
Did not attend school	0 (0%)	1 (0.5%)		1 (0.4%)
High school	38 (77.6%)	171 (79.5%)		209 (79.2%)
Primary school	0 (0%)	6 (2.8%)		6 (2.3%)
Employed			0.699	
No	36 (73.5%)	152 (70.7%)	Chisq.	188 (71.2%)
Yes	13 (26.5%)	63 (29.3%)		76 (28.8%)
Married			0.200	
No	46 (93.9%)	188 (87.4%)	Chisq.	234 (88.6%)
Yes	3 (6.1%)	27 (12.6%)		30 (11.4%)
Regular sex partner			1.000	
No	3 (6.1%)	17 (7.9%)	Fisher's	20 (7.6%)
Yes	46 (93.9%)	198 (92.1%)		244 (92.4%)
Partners HIV status			0.781	
Don't know	9 (18.4%)	31 (14.4%)	Chisq.	40 (15.2%)
Negative	16 (32.7%)	72 (33.5%)		88 (33.3%)
Positive	24 (49.0%)	112 (52.1%)		136 (51.5%)
Cohabiting			0.083	
No	24 (49.0%)	133 (61.9%)	Chisq.	157 (59.5%)
Yes	25 (51.0%)	80 (37.2%)		105 (39.8%)
Missing	0 (0%)	2 (0.9%)		2 (0.8%)
Age of 1st sex			0.222	
<15	1 (2.0%)	8 (3.7%)	Fisher's	9 (3.4%)
>25	3 (6.1%)	3 (1.4%)		6 (2.3%)
15_20	33 (67.3%)	156 (72.6%)		189 (71.6%)
21 25	12 (24.5%)	48 (22.3%)		60 (22.7%)
Lifetime sex partners			0.620	
>4	12 (24.5%)	42 (19.5%)	Chisq.	54 (20.5%)
1	15 (30.6%)	61 (28.4%)		76 (28.8%)
2 4	22 (44.9%)	112 (52.1%)		134 (50.8%)
Partner has other partners			0.183	
Don't know	21 (42.9%)	117 (54.4%)	Chisq.	138 (52.3%)
No	16 (32.7%)	45 (20.9%)		61 (23.1%)

M. hominis	Neg (N=49)	Pos (N=215)	p-value	Overall (N=264)
Yes	12 (24.5%)	53 (24.7%)		65 (24.6%)
Condom used during last sex			0.742	
No	32 (65.3%)	135 (62.8%)	Chisq.	167 (63.3%)
Yes	17 (34.7%)	80 (37.2%)		97 (36.7%)
Partner circumcised			0.192	
No	21 (42.9%)	71 (33.0%)	Chisq.	92 (34.8%)
Yes	28 (57.1%)	144 (67.0%)		172 (65.2%)
Trimester			0.898	
1st	3 (6.1%)	17 (7.9%)	Chisq.	20 (7.6%)
2nd	16 (32.7%)	66 (30.7%)		82 (31.1%)
3rd	30 (61.2%)	131 (60.9%)		161 (61.0%)
Missing	0 (0%)	1 (0.5%)		1 (0.4%)
Previously treated for STIs			0.523	
No	30 (61.2%)	142 (66.0%)	Chisq.	172 (65.2%)
Yes	19 (38.8%)	73 (34.0%)		92 (34.8%)
Intravaginal practices			0.319	
No	48 (98.0%)	200 (93.0%)	Fisher's	248 (93.9%)
Yes	1 (2.0%)	15 (7.0%)		16 (6.1%)
M. genitalium			1.000	
Neg	48 (98.0%)	209 (97.2%)	Fisher's	257 (97.3%)
Pos	1 (2.0%)	6 (2.8%)		7 (2.7%)
U. urealyticum			0.051	
Neg	9 (18.4%)	19 (8.8%)	Chisq.	28 (10.6%)
Pos	40 (81.6%)	196 (91.2%)		236 (89.4%)
U. parvum			0.004	
Neg	19 (38.8%)	42 (19.5%)	Chisq.	61 (23.1%)
Pos	30 (61.2%)	173 (80.5%)		203 (76.9%)
Partner STI symptom			0.027	
No	32 (65.3%)	172 (80.0%)	Chisq.	204 (77.3%)
Yes	17 (34.7%)	43 (20.0%)		60 (22.7%)
Current STIs symptoms			<0.001	
No	40 (81.6%)	112 (52.1%)	Chisq.	152 (57.6%)
Yes	9 (18.4%)	103 (47.9%)		112 (42.4%)

4.5. Factors associated with *U. urealyticum* positivity

Socio-demographic factors

There was no significant difference in age across the *U. urealyticum* negative and positive women (p=0.379). Within the group of women who tested *U. urealyticum* positive, a higher proportion of women had attended high school (78.4%) when compared to 18.6% who had attended college/university. However, level of education was not significantly associated with infection status, p=0.827. A higher proportion of positive women were unemployed, 70.8% when compared to 29.2% who were employed, this was not significant, p=0.640. A higher proportion of the positive women were unmarried (89.0%) whereas 11% were married, marital status was not a significant factor (p=0.538) (Table 5).

Behavioral factors

Within the group of women who tested positive for *U. urealyticum*, 91.9% of the women reported having a regular sexual partner, this factor was not significant (p=0.705). A high proportion of the positive women (51.7%) had a partner who was HIV positive when compared to 33.1 % who had a HIV negative partner, however, this was not significant, p=0.959. A larger proportion of the positive women (60.2%) were not cohabiting with their partner when compared to 39.0% who were cohabiting, this factor was also not significant, p=0.468. With respect to age of first sex, the majority of the positive women, 70.3%, had experienced first sex between the ages of 15-20 years when compared to 23.3% of the women who had their first sex between the ages of 21-25 years of age. Age of first sex was not a significant factor, p=0.724. A large proportion of the positive women reported having 2-4 lifetime sex partners (52.5%) when compared to 28.8% who reported having 1 lifetime sex partner and 18.6% having >4 lifetime sex partners. Lifetime number of sex partners was not significantly associated with being positive, p=0.084. A large proportion of positive women reported that they did not know if their partners had other partners (52.1%) when compared to 22.5% who reported that their partner did not have other partners and 25.4% who reported that their partner had other partners. Partner having other partners was not significant, p=0.607. With respect to condom use, a larger proportion of positive women did not use a condom at their last sex act (62.7%), when compared to 37.3% who reported using a condom. Condom use was not significantly associated with being positive, p=0.593 (Table 5).

Clinical factors

Partner being circumcised was a significant clinical factor in relation with being positive, p=0.028. A higher proportion of the *U. urealyticum* positive women, 67.4% reported that their partner was circumcised when compared to 32.6% who had an uncircumcised partner. In addition, 20.8% of the positive women reported that their partner had symptoms of STIs such as testicular pain, pain during urination and discharge from the penis when compared to 79.2% who had a partner without symptoms, this factor was also a significant clinical factor in relation with being positive, p=0.027. A lower proportion of positive women, 40.7% had reported having symptoms of STIs such as genital itching, abnormal vaginal discharge, genital sore/ulcers, and genital warts at enrollment when compared to 59.3% of the women who were asymptomatic. Symptoms of STIs was not significantly associated with being positive, p=0.096. With respect to history of STI treatment, most women were not previously treated for STIs, 66.9% when compared to 33.1% who had been previously treated, this was not significant, p=0.075. A high proportion of the positive women (93.6%) did not engage in intravaginal practices and only 6.4% of the positive women did, this factor was not significant p=1.000. The majority of the positive women where in the third trimester of pregnancy, 63.6% when compared to 28.8% who were in the second trimester of pregnancy. Trimester of pregnancy was significantly associated with being positive for infection, p=0.040. The majority of positive women were in the third trimester of pregnancy (63.6%) when compared to women in the first (7.2%) and second (28.8%) trimesters of pregnancy. Of the women who tested positive for *U. urealyticum*, a higher proportion of women also tested positive for *M. hominis* (83.1%). This association was significant, p=0.051. There was no significant association between *U. urealyticum* and the other mycoplasmas, p>0.005 (Table 5)

Table 5: Characteristics of the study women according to *U. urealyticum* status

U. urealyticum	Neg (N=28)	Pos (N=236)	p-value	Overall (N=264)
Age			0.379	
Mean±SD(CV%)	31.7±6.85(21.6)	30.7±6.60(21.5)		30.8±6.62(21.5)
Median(Q1-Q3)	33.5(26.0-37.0)	30.0(25.8-37.0)		31.0(26.0-37.0)
Min-Max	19.0-40.0	18.0-44.0		18.0-44.0
Educational level			0.827	
College, University	4 (14.3%)	44 (18.6%)	Fisher's	48 (18.2%)
Did not attend school	0 (0%)	1 (0.4%)		1 (0.4%)

U. urealyticum	Neg (N=28)	Pos (N=236)	p-value	Overall (N=264)
High school	24 (85.7%)	185 (78.4%)		209 (79.2%)
Primary school	0 (0%)	6 (2.5%)		6 (2.3%)
Employed			0.640	
No	21 (75.0%)	167 (70.8%)	Chisq.	188 (71.2%)
Yes	7 (25.0%)	69 (29.2%)		76 (28.8%)
Married			0.538	
No	24 (85.7%)	210 (89.0%)	Fisher's	234 (88.6%)
Yes	4 (14.3%)	26 (11.0%)		30 (11.4%)
Regular sex partner			0.705	
No	1 (3.6%)	19 (8.1%)	Fisher's	20 (7.6%)
Yes	27 (96.4%)	217 (91.9%)		244 (92.4%)
Partners HIV status			0.959	
Don't know	4 (14.3%)	36 (15.3%)	Chisq.	40 (15.2%)
Negative	10 (35.7%)	78 (33.1%)		88 (33.3%)
Positive	14 (50.0%)	122 (51.7%)		136 (51.5%)
Cohabiting			0.468	
No	15 (53.6%)	142 (60.2%)	Chisq.	157 (59.5%)
Yes	13 (46.4%)	92 (39.0%)		105 (39.8%)
Missing	0 (0%)	2 (0.8%)		2 (0.8%)
Age of 1st sex			0.724	
<15	0 (0%)	9 (3.8%)	Fisher's	9 (3.4%)
>25	0 (0%)	6 (2.5%)		6 (2.3%)
15_20	23 (82.1%)	166 (70.3%)		189 (71.6%)
21_25	5 (17.9%)	55 (23.3%)		60 (22.7%)
Lifetime sex partners			0.084	
>4	10 (35.7%)	44 (18.6%)	Chisq.	54 (20.5%)
1	8 (28.6%)	68 (28.8%)		76 (28.8%)
2_4	10 (35.7%)	124 (52.5%)		134 (50.8%)
Partner has other partners			0.607	
Don't know	15 (53.6%)	123 (52.1%)	Chisq.	138 (52.3%)
No	8 (28.6%)	53 (22.5%)		61 (23.1%)
Yes	5 (17.9%)	60 (25.4%)		65 (24.6%)
Condom used during last sex			0.593	
No	19 (67.9%)	148 (62.7%)	Chisq.	167 (63.3%)
Yes	9 (32.1%)	88 (37.3%)		97 (36.7%)
Partner circumcised			0.028	
No	15 (53.6%)	77 (32.6%)	Chisq.	92 (34.8%)
Yes	13 (46.4%)	159 (67.4%)		172 (65.2%)
Trimester			0.040	
1st	3 (10.7%)	17 (7.2%)	Chisq.	20 (7.6%)

U. urealyticum	Neg (N=28)	Pos (N=236)	p-value	Overall (N=264)
2nd	14 (50.0%)	68 (28.8%)		82 (31.1%)
3rd	11 (39.3%)	150 (63.6%)		161 (61.0%)
Missing	0 (0%)	1 (0.4%)		1 (0.4%)
Previously treated for STIs			0.075	
No	14 (50.0%)	158 (66.9%)	Chisq.	172 (65.2%)
Yes	14 (50.0%)	78 (33.1%)		92 (34.8%)
Intravaginal practices			1.000	
No	27 (96.4%)	221 (93.6%)	Fisher's	248 (93.9%)
Yes	1 (3.6%)	15 (6.4%)		16 (6.1%)
M. genitalium			0.548	
Neg	27 (96.4%)	230 (97.5%)	Fisher's	257 (97.3%)
Pos	1 (3.6%)	6 (2.5%)		7 (2.7%)
M. hominis			0.051	
Neg	9 (32.1%)	40 (16.9%)	Chisq.	49 (18.6%)
Pos	19 (67.9%)	196 (83.1%)		215 (81.4%)
U. parvum			0.094	
Neg	10 (35.7%)	51 (21.6%)	Chisq.	61 (23.1%)
Pos	18 (64.3%)	185 (78.4%)		203 (76.9%)
Partner STI symptom			0.027	
No	17 (60.7%)	187 (79.2%)	Chisq.	204 (77.3%)
Yes	11 (39.3%)	49 (20.8%)		60 (22.7%)
Current STIs symptoms			0.096	
No	12 (42.9%)	140 (59.3%)	Chisq.	152 (57.6%)
Yes	16 (57.1%)	96 (40.7%)		112 (42.4%)

4.6 Factors associated with *U. parvum* positivity

Socio-demographic factors

There was no significant difference in age across the *U. parvum* negative and positive women (p=0.698). Within the group of women who tested *U. parvum* positive, a higher proportion of women had attended high school (80.3%) when compared to 16.7% who had attended college/university. However, level of education was not significantly associated with infection status, p=0.122. A higher proportion of positive women were unemployed, 71.9 % when compared to 28.1% who were employed, this was not significant, p=0.643. A high proportion of the positive women were unmarried (89.7%), marital status was not a significant factor (p=0.341) (Table 6).

Behavioral factors

Within the group of women who tested positive for *U. parvum*, 91.6% of the women reported having a regular sexual partner, this factor was not significant (p=0.581). A high proportion of the positive women (53.7%) had a partner who was HIV positive when compared to 29.6% who had a HIV negative partner, and this behavioral factor was significant in relation with being positive, p=0.049. A larger proportion of the positive women (61.6%) were not cohabiting with their partner when compared to 37.4% who were cohabiting, this factor was not significant, p=0.174. With respect to age of first sex, the majority of the positive women, 70.4%, had experienced first sex between the ages of 15-20 years when compared to 22.2% of the women who had their first sex between the ages of 21-25 years of age. Age of first sex was not a significant factor, p=0.225. A large proportion of the positive women reported having 2-4 lifetime sex partners (52.2 %) when compared to 24.6 % who reported having 1 lifetime sex partner and 23.2% reported having >4 lifetime sex partners. Lifetime number of sex partners was significantly associated with being positive, p=0.012. Partner having other partners was also a significant behavioral factor in relation with being positive, p=0.023. A large proportion of positive women reported that their partner had other partners (28.1%) when compared to 20.2% who reported that their partner did not have other partners and 51.7% who did not know if their partner had other partners. With respect to condom use, a larger proportion of positive women did not use a condom at their last sex act (65.0%), when compared to 35.0% who reported using a condom. Condom use was not significantly associated with being positive, p= 0.277 (Table 6).

Clinical factors

A higher proportion of the *U. parvum* positive women, 66.0% reported that their partner was circumcised when compared to 34.0% who had an uncircumcised partner. This factor was not significant, p=0.593. In addition, 22.2% of the women reported that their partner had symptoms of STIs such as testicular pain, pain during urination and discharge from the penis when compared to 77.8% who had a partner without symptoms, this factor was not significant, p=0.692. A lower proportion of positive women, 39.9% had reported having symptoms of STIs such as genital itching, abnormal vaginal discharge, genital sore/ulcers, and genital warts at enrollment when compared to 60.1% of the women who were asymptomatic. Symptoms of STIs was not significantly associated with being positive, p=0.130. With respect to history of STI treatment,

most women were not previously treated for STIs, 66.5% when compared to 33.5% who had been previously treated, this was not significant, p=0.401. A larger proportion of the positive women did not engage in intravaginal practices (93.1%) compared to 6.9% who engaged in such practices, this was not significant, p=0.376. The majority of the positive women were in the third trimester of pregnancy, 59.6% when compared to 33.5% who were in the second trimester of pregnancy. Trimester of pregnancy was not significantly associated with being positive for infection, p=0.171. Of the *U. parvum* positive women, a higher proportion of women (85.2%) tested positive for *M. hominis*. This association was significant, p=0.004. There was no significant association between *U. parvum* and the other mycoplasmas, p>0.005 (Table 6).

Table 6: Characteristics of the study women according to *U. parvum* status

U. parvum	Neg (N=61)	Pos (N=203)	p-value	Overall (N=264)
Age			0.698	
Mean±SD(CV%)	30.6±7.09(23.2)	30.9±6.49(21.0)		30.8±6.62(21.5)
Median(Q1-Q3)	31.0(24.0-37.0)	31.0(26.0-37.0)		31.0(26.0-37.0)
Min-Max	19.0-44.0	18.0-43.0		18.0-44.0
Educational level			0.122	
College, University	14 (23.0%)	34 (16.7%)	Fisher's	48 (18.2%)
Did not attend school	1 (1.6%)	0 (0%)		1 (0.4%)
High school	46 (75.4%)	163 (80.3%)		209 (79.2%)
Primary school	0 (0%)	6 (3.0%)		6 (2.3%)
Employed			0.643	
No	42 (68.9%)	146 (71.9%)	Chisq.	188 (71.2%)
Yes	19 (31.1%)	57 (28.1%)		76 (28.8%)
Married			0.341	
No	52 (85.2%)	182 (89.7%)	Chisq.	234 (88.6%)
Yes	9 (14.8%)	21 (10.3%)		30 (11.4%)
Regular sex partner			0.581	
No	3 (4.9%)	17 (8.4%)	Fisher's	20 (7.6%)
Yes	58 (95.1%)	186 (91.6%)		244 (92.4%)
Partners HIV status			0.049	
Don't know	6 (9.8%)	34 (16.7%)	Chisq.	40 (15.2%)
Negative	28 (45.9%)	60 (29.6%)		88 (33.3%)
Positive	27 (44.3%)	109 (53.7%)		136 (51.5%)
Cohabiting			0.174	
No	32 (52.5%)	125 (61.6%)	Chisq.	157 (59.5%)
Yes	29 (47.5%)	76 (37.4%)		105 (39.8%)
Missing	0 (0%)	2 (1.0%)		2 (0.8%)

U. parvum	Neg (N=61)	Pos (N=203)	p-value	Overall (N=264)
Age of 1st sex			0.225	
<15	0 (0%)	9 (4.4%)	Fisher's	9 (3.4%)
>25	0 (0%)	6 (3.0%)		6 (2.3%)
15 20	46 (75.4%)	143 (70.4%)		189 (71.6%)
21 25	15 (24.6%)	45 (22.2%)		60 (22.7%)
Lifetime sex partners			0.012	
>4	7 (11.5%)	47 (23.2%)	Chisq.	54 (20.5%)
1	26 (42.6%)	50 (24.6%)		76 (28.8%)
2 4	28 (45.9%)	106 (52.2%)		134 (50.8%)
Partner has other partners			0.023	
Don't know	33 (54.1%)	105 (51.7%)	Chisq.	138 (52.3%)
No	20 (32.8%)	41 (20.2%)		61 (23.1%)
Yes	8 (13.1%)	57 (28.1%)		65 (24.6%)
Condom used during last sex			0.277	
No	35 (57.4%)	132 (65.0%)	Chisq.	167 (63.3%)
Yes	26 (42.6%)	71 (35.0%)		97 (36.7%)
Partner circumcised			0.593	
No	23 (37.7%)	69 (34.0%)	Chisq.	92 (34.8%)
Yes	38 (62.3%)	134 (66.0%)		172 (65.2%)
Trimester			0.171	
1st	7 (11.5%)	13 (6.4%)	Chisq.	20 (7.6%)
2nd	14 (23.0%)	68 (33.5%)		82 (31.1%)
3rd	40 (65.6%)	121 (59.6%)		161 (61.0%)
Missing	0 (0%)	1 (0.5%)		1 (0.4%)
Previously treated for STIs			0.401	
No	37 (60.7%)	135 (66.5%)	Chisq.	172 (65.2%)
Yes	24 (39.3%)	68 (33.5%)		92 (34.8%)
Intravaginal practices			0.376	
No	59 (96.7%)	189 (93.1%)	Fisher's	248 (93.9%)
Yes	2 (3.3%)	14 (6.9%)		16 (6.1%)
M. genitalium			0.664	
Neg	59 (96.7%)	198 (97.5%)	Fisher's	257 (97.3%)
Pos	2 (3.3%)	5 (2.5%)		7 (2.7%)
M. hominis			0.004	
Neg	19 (31.1%)	30 (14.8%)	Chisq.	49 (18.6%)
Pos	42 (68.9%)	173 (85.2%)		215 (81.4%)
U. urealyticum			0.094	
Neg	10 (16.4%)	18 (8.9%)	Chisq.	28 (10.6%)
Pos	51 (83.6%)	185 (91.1%)		236 (89.4%)
Partner STI symptom			0.692	

U. parvum	Neg (N=61)	Pos (N=203)	p-value	Overall (N=264)
No	46 (75.4%)	158 (77.8%)	Chisq.	204 (77.3%)
Yes	15 (24.6%)	45 (22.2%)		60 (22.7%)
Current STIs symptoms			0.130	
No	30 (49.2%)	122 (60.1%)	Chisq.	152 (57.6%)
Yes	31 (50.8%)	81 (39.9%)		112 (42.4%)

4.7 Risk factors for *M. genitalium* infection

Table 7 describes the risk factors associated with *M. genitalium* infection.

In the unadjusted analysis, using a condom at last sex act increased the risk of getting infected with *M. genitalium* by 1.3 fold. Similarly in the adjusted analysis, using a condom at last sex act increased the risk of getting infected with *M. genitalium* by 1.2 fold. However, condom use was not significantly associated with the risk of infection, p=0.847. In the unadjusted analysis, previous treatment for STIs increased the risk for infection by 1.4 fold, however this was not significant, p=0.665 and was not sustained in the adjusted analysis. In the unadjusted and adjusted analysis, being *M. hominis* positive increased the risk for *M. genitalium* by 1.39 and 2.00 fold, respectively. Despite the increased risk, this association was not significant, p=0.762 and p=0.580, respectively.

Partner having symptoms of STIs increased the risk for *M. genitalium* by 2.67 and 3.37 fold in the unadjusted and adjusted analysis, however this factor was not significant, p=0.208 and p=0.184, respectively. According to the analysis, women who presented with current symptoms of STIs were at increased risk of acquiring *M. genitalium*. In the unadjusted analysis, current symptoms of STIs increased the risk of infection by 1.8 fold and in the unadjusted analysis, the risk was shown to be approximately 1.7 fold in the adjusted analysis. However, having current symptoms of STIs was not a significant factor, p=0.441 and p=0.581, respectively. Not knowing if their partner had other partners reduced the risk of infection in both unadjusted and the adjusted analyses, and this factor was shown to be significant throughout, $p<0.05$ (Table 7).

Table 7: Risk factors associated with *M. genitalium* infection

Variable	Unadjusted odds ratio (OR), 95% Confidence Interval (CI)	Adjusted odds ratio (OR), 95% Confidence Interval (CI)	Further Adjusted odds ratio (OR), 95% Confidence Interval (CI): Backstep analysis
Age	0.98 (0.87-1.10, p=0.728)	0.99 (0.86-1.14, p=0.895)	-
Employed Yes	0.40 (0.02-2.40, p=0.400)	0.35 (0.01-2.88, p=0.400)	-
Cohabiting Yes	0.59 (0.08-2.80, p=0.533)	0.38 (0.04-2.33, p=0.315)	-
Lifetime sex partners 2-4	0.37 (0.05-2.29, p=0.284)	0.26 (0.03-1.95, p=0.197)	-
Lifetime sex partners >4	0.95 (0.12-5.95, p=0.960)	0.77 (0.07-6.99, p=0.816)	-
Partner has other partners Yes	0.44 (0.06-2.37, p=0.360)	0.34 (0.04-2.42, p=0.291)	0.44 (0.06-2.37, p=0.360)
Partner has other partners Don't know	0.10 (0.01-0.71, p=0.044)	0.07 (0.00-0.59, p=0.032)	0.10 (0.01-0.71, p=0.044)
Condom used during last sex Yes	1.31 (0.25-6.05, p=0.730)	1.20 (0.17-7.56, p=0.847)	-
Partner circumcised Yes	0.71 (0.15-3.70, p=0.665)	0.90 (0.14-6.45, p=0.911)	-
Previously treated for STIs Yes	1.40 (0.27-6.48, p=0.665)	0.95 (0.14-5.37, p=0.950)	-
Partner STI symptom Yes	2.67 (0.51-12.43, p=0.208)	3.37 (0.52-21.68, p=0.184)	-
Current STIs symptoms Yes	1.81 (0.39-9.37, p=0.441)	1.68 (0.26-11.65, p=0.581)	-
M. hominis Pos	1.39 (0.23-26.60, p=0.762)	2.00 (0.24-47.14, p=0.580)	
U. urealyticum Pos	0.71 (0.12-13.68, p=0.756)	1.59 (0.16-45.20, p=0.730)	
U. parvum Pos	0.75 (0.16-5.35, p=0.738)	1.28 (0.17-14.63, p=0.819)	

4.8 Risk factors for *M. hominis* infection

Table 8 describes the risk factors associated with *M. hominis* infection.

In the unadjusted analysis, being employed increased the risk of getting infected with *M. hominis* by 1.47 fold. Similarly in the adjusted analysis, being employed increased the risk of getting infected with *M. hominis* by 2.23 fold. After having performed further adjustments, being

employed was significantly associated with the risk of infection, p=0.012. In the unadjusted analysis, partner having other partners increased the risk for infection by 2.11 fold, however this was not significant, p=0.149 and was not sustained in the adjusted analysis. Whereas not knowing if partners have other partners in the unadjusted analysis increased the risk of getting infected by 2.65 fold and this was significant, p=0.029, in the adjusted analysis it also increased risk by 2.65 fold but it was not significant, p=0.126. In the unadjusted analysis, using a condom at last sex act increased the risk of getting infected with *M. hominis* by 1.24 fold. Similarly in the adjusted analysis, using a condom at last sex act increased the risk of getting infected with *M. hominis* by 2.62 fold. However, condom use was not significantly associated with the risk of infection, p=0.094. In the unadjusted analysis partner being circumcised increased the risk of infection by 1.44 fold, this was not significant p=0.349. According to the analysis, women who presented with current symptoms of STIs were at increased risk of acquiring *M. hominis*. In the unadjusted and adjusted analysis, current STI symptoms increased the risk for *M. hominis* by 27.80 and 95.27 fold, respectively. This association was significant, p=0.001 and p<0.001, respectively. After further adjustments, this association was still significant, p<0.001. In the unadjusted and adjusted analysis, being *U parvum* positive increased the risk for *M. hominis* by 2.98 and 8.19 fold, respectively. This association was significant, p=0.007 and p=0.001, respectively. After further adjustments, this association was still significant, p<0.001. In the unadjusted and adjusted analysis, being *U. urealyticum* positive increased the risk for *M. hominis* by 2.18 and 4.82 fold, respectively. Despite the increased risk, this association was shown to be not significant, p=0.156 and p=0.069, respectively. However, after further adjustments, this association was significant, p=0.039.

Age was shown to be factor associated with reduced risk in both unadjusted and adjusted analyses, p=0.005 and p=0.01, respectively. After further adjustments, this factor was still significant, p=0.002. Cohabiting with their partner reduced the risk of infection in both unadjusted and adjusted analyses, however this factor was shown to be significant in the unadjusted analysis, p=0.015, only. Partner having STI symptoms reduced the risk of infection in both the unadjusted and adjusted analyses, and this factor was shown to be significant in the unadjusted analyses only, p= 0.016 (Table 8).

Table 8: Risk factors associated with *M. hominis* infection

Variable	Unadjusted odds ratio (OR), 95% Confidence Interval (CI)	Adjusted odds ratio (OR), 95% Confidence Interval (CI)	Further Adjusted odds ratio (OR), 95% Confidence Interval (CI): Backstep analysis
Age	0.91 (0.85-0.97, p=0.005)	0.89 (0.81-0.97, p=0.011)	0.89 (0.82-0.95, p=0.002)
Employed Yes	1.47 (0.63-3.85, p=0.398)	2.23 (0.67-8.56, p=0.211)	4.35 (1.48-15.16, p=0.012)
Cohabiting Yes	0.38 (0.17-0.82, p=0.015)	0.54 (0.18-1.58, p=0.265)	-
Lifetime sex partners 2-4	0.91 (0.33-2.31, p=0.844)	1.33 (0.34-4.87, p=0.669)	-
Lifetime sex partners >4	0.48 (0.16-1.35, p=0.166)	0.99 (0.22-4.32, p=0.988)	-
Partner has other partners Yes	2.11 (0.78-6.12, p=0.149)	2.20 (0.52-10.01, p=0.287)	-
Partner has other partners Don't know	2.65 (1.10-6.41, p=0.029)	2.65 (0.76-9.53, p=0.126)	-
Condom used during last sex Yes	1.24 (0.57-2.88, p=0.598)	2.62 (0.89-8.73, p=0.094)	-
Partner circumcised Yes	1.44 (0.66-3.10, p=0.349)	0.98 (0.33-2.81, p=0.972)	-
Previously treated for STIs Yes	0.83 (0.38-1.84, p=0.630)	0.99 (0.32-3.27, p=0.990)	-
Partner STI symptom Yes	0.37 (0.17-0.85, p=0.016)	0.30 (0.08-1.01, p=0.054)	0.41 (0.15-1.13, p=0.081)
Current STIs symptoms Yes	27.80 (5.79-499.56, p=0.001)	95.27 (13.85-2085.67, p<0.001)	77.30 (12.43-1605.61, p<0.001)
U. parvum Pos	2.98 (1.33-6.54, p=0.007)	8.19 (2.51-30.20, p=0.001)	6.67 (2.37-20.12, p<0.001)
U.urealyticum Pos	2.18 (0.67-6.07, p=0.156)	4.82 (0.90-28.78, p=0.069)	5.14 (1.09-26.32, p=0.039)

4.9 Risk factors for *U. urealyticum* infection

In the unadjusted analysis, using a condom at last sex act increased the risk of getting infected with *U. urealyticum* by 1.24 fold. Similarly in the adjusted analysis, using a condom at last sex act increased the risk of getting infected with *U. urealyticum* by 1.35 fold. However, condom use was not significantly associated with the risk of infection, p=0.557. Having between 2-4 lifetime number of sex partners increased the risk for infection in the unadjusted and adjusted analyses by 1.44 and 1.07 fold, respectively. However, this association was not significant with p=0.468 and p=0.905, respectively. In the unadjusted and adjusted analyses partner having other partners

increased the risk for *U. urealyticum* by 1.85 and 1.15 fold, respectively. However, this was not significant, p=0.845. Partner being circumcised increased the risk for *U. urealyticum* in the adjusted and unadjusted analyses by 2.34 and 2.01 fold, respectively. However, this factor was only significant in the unadjusted analysis, p=0.035 and not significant in the adjusted analysis, p=0.134. In the unadjusted and adjusted analysis, being *M. hominis* positive increased the risk for *U. urealyticum* by 2.29 and 2.24 fold, respectively. Despite the increased risk, this association was not significant, p=0.061 and p=0.145, respectively. In the unadjusted and adjusted analysis, being *U. parvum* positive increased the risk for *U. urealyticum* by 1.98 and 2.03 fold, respectively. Despite the increased risk, this association was not significant, p=0.107 and p=0.168, respectively. In the unadjusted analysis, being employed increased the risk for infection by 1.26 fold, however in the adjusted analysis it reduced the risk for infection by 0.96 times. However this factor was not significant throughout, p=0.612 and p=0.941, respectively. According to the analysis, partner having symptoms of STIs reduced the risk for *U. urealyticum* by 0.39 and 0.49 fold in the unadjusted and adjusted analysis, however this factor was only significant in the unadjusted analysis, p=0.025 and not significant in the adjusted analysis p=0.147 (Table 9).

Table 9: Risk factors associated with *U. urealyticum* infection

Variable	**Unadjusted odds ratio (OR), 95% Confidence Interval (CI)**	**Adjusted odds ratio (OR), 95% Confidence Interval (CI)**	**Further Adjusted odds ratio (OR), 95% Confidence Interval (CI): Backstep analysis**
Age	0.98 (0.92-1.04, p=0.466)	1.01 (0.93-1.09, p=0.813)	-
Employed Yes	1.26 (0.54-3.33, p=0.612)	0.96 (0.33-3.02, p=0.941)	-
Cohabiting Yes	0.75 (0.34-1.68, p=0.480)	1.11 (0.42-3.02, p=0.842)	-
Lifetime sex partners 2-4	1.44 (0.53-3.81, p=0.468)	1.07 (0.33-3.34, p=0.905)	1.03 (0.34-3.02, p=0.960)
Lifetime sex partners >4	0.51 (0.18-1.38, p=0.184)	0.35 (0.09-1.26, p=0.116)	0.37 (0.11-1.20, p=0.101)
Partner has other partners Yes	1.85 (0.58-6.44, p=0.307)	1.15 (0.28-4.87, p=0.845)	-
Partner has other partners Don't know	1.24 (0.47-3.04, p=0.645)	0.84 (0.25-2.58, p=0.769)	-
Condom used during last sex Yes	1.24 (0.55-2.98, p=0.621)	1.35 (0.51-3.87, p=0.557)	-
Partner circumcised	2.34 (1.06-5.23, p=0.035)	2.01 (0.81-5.07, p=0.134)	2.07 (0.89-4.88, p=0.092)

Yes			
Previously treated for STIs Yes	0.50 (0.23-1.12, p=0.088)	0.58 (0.23-1.49, p=0.258)	-
Partner STI symptom Yes	0.39 (0.17-0.91, p=0.025)	0.49 (0.19-1.32, p=0.147)	0.50 (0.20-1.28, p=0.138)
Current STIs symptoms Yes	0.52 (0.23-1.14, p=0.102)	0.46 (0.17-1.23, p=0.126)	0.43 (0.16-1.08, p=0.078)
M. genitalium Pos	0.71 (0.12-13.74, p=0.759)	0.95 (0.13-20.13, p=0.965)	-
M. hominis Pos	2.29 (0.93-5.30, p=0.061)	2.24 (0.74-6.60, p=0.145)	0.77-6.20, p=0.130)
U. parvum Pos	1.98 (0.83-4.49, p=0.107)	2.03 (0.73-5.60, p=0.168)	2.14 (0.81-5.52, p=0.115)

4.10 Risk factors for *U. parvum* infection

In the unadjusted and adjusted analyses, age was associated with increased the risk of getting infected with *U. parvum*, by 1.03 and 1.01 fold, respectively. However, age was not significantly associated with the risk of infection, p>0.005. In the unadjusted analysis, having between 2-4 lifetime sex partners increased the risk of infection with *U. parvum* by 2.10 fold and 3.08 fold in the adjusted analysis and was found to be significant, p=0.033 and p=0.013, respectively. After further adjustments, it was still significant, p=0.017. In the unadjusted analysis having >4 lifetime sex partners increased the risk of infection with *U. parvum* by 20.65 fold and in the adjusted analysis by 88.02 fold. This factor was significant, p=0.004 and p<0.001, respectively. After further adjustments, it was still significant, p<0.001. Partner having other partners increased the risk of infection with *U. parvum* in the unadjusted and adjusted analyses by 4.80 fold and 6.72 fold, respectively. This factor was significant, p=0.005 and p=0.008, respectively. After further adjustments, it was still significant, p=0.005. According to the unadjusted and adjusted analysis, not knowing if their partner had other partners increased the risk of infection with *U. parvum* by 1.81 fold and 1.91 fold, respectively. However, this factor was not significant, p=0.114 and p=0.215, respectively. In the unadjusted analysis, partner being circumcised increased the risk for infection by 1.08 fold. Similar in the adjusted analysis, infection risk was increased by 1.55 fold. This factor was however, not significant, p=0.826 and p=0.355, respectively. In the unadjusted and adjusted analysis, being *M. genitalium* positive increased the risk for *U. parvum* by 1.39 and 2.00 fold, respectively. Despite the increased risk, this association was not significant, p=0.906 and p=0.816, respectively. In the unadjusted and adjusted analysis, being *M. hominis* positive increased

the risk for *U. parvum* by 2.53 and 4.33 fold, respectively. This association was significant, p=0.014 and p=0.008, respectively. After further adjustments, it was still significant, p=0.008. In the unadjusted and adjusted analysis, being *U. urealyticum* positive increased the risk for *U. parvum* by 1.96 and 2.62 fold, respectively. However, this association was not significant, p=0.163 and p=0.141, respectively. According to the analysis, women who presented with current symptoms of STIs had a reduced risk of acquiring *U. parvum*. In the unadjusted analysis, current symptoms of STIs reduced the risk of infection by 0.52 fold and in the adjusted analysis, the risk was shown to be approximately 0.24 fold. Having current symptoms of STIs was a significant factor in the analysis, p=0.050 and p=0.004, respectively. After further adjustments, the reduced risk was still significant, p=0.002 (Table 10).

Table 10: Risk factors associated with *U. parvum* infection

Variable	**Unadjusted odds ratio (OR), 95% Confidence Interval (CI)**	**Adjusted odds ratio (OR), 95% Confidence Interval (CI)**	**Further Adjusted odds ratio (OR), 95% Confidence Interval (CI): Backstep analysis**
Age	1.03 (0.98-1.08, p=0.261)	1.01 (0.95-1.09, p=0.696)	-
Employed Yes	0.97 (0.48-2.04, p=0.935)	0.65 (0.24-1.73, p=0.384)	-
Cohabiting Yes	0.70 (0.36-1.35, p=0.278)	0.96 (0.38-2.45, p=0.937)	-
Lifetime sex partners 2-4	2.10 (1.06-4.18, p=0.033)	3.08 (1.29-7.67, p=0.013)	2.77 (1.21-6.50, p=0.017)
Lifetime sex partners >4	20.65 (4.08-377.29, p=0.004)	88.02 (10.85-2157.18, p<0.001)	81.29 (10.91-1914.65, p<0.001)
Partner has other partners Yes	4.80 (1.72-15.68, p=0.005)	6.72 (1.74-29.76, p=0.008)	6.84 (1.92-28.31, p=0.005)
Partner has other partners Don't know	1.81 (0.86-3.75, p=0.114)	1.91 (0.68-5.34, p=0.215)	1.89 (0.72-4.94, p=0.191)
Condom used during last sex Yes	0.65 (0.34-1.27, p=0.205)	0.82 (0.33-2.05, p=0.668)	-
Partner circumcised Yes	1.08 (0.54-2.10, p=0.826)	1.55 (0.61-3.90, p=0.355)	-
Previously treated for STIs Yes	0.65 (0.34-1.27, p=0.205)	0.83 (0.33-2.08, p=0.687)	-
Partner STI symptom Yes	0.91 (0.43-2.09, p=0.822)	0.96 (0.34-2.91, p=0.943)	-
Current STIs symptoms Yes	0.52 (0.27-1.00, p=0.050)	0.24 (0.08-0.61, p=0.004)	0.25 (0.10-0.59, p=0.002)
M. hominis Pos	2.53 (1.18-5.26, p=0.014)	4.33 (1.48-13.10, p=0.008)	4.05 (1.45-11.55, p=0.008)

M. genitalium Pos	1.14 (0.18-22.13, p=0.906)	1.60 (0.06-105.33, p=0.816)	-
U. urealyticum Pos	1.96 (0.72-4.89, p=0.163)	2.62 (0.71-9.56, p=0.141)	2.84 (0.83-9.66, p=0.091)

CHAPTER 5

DISCUSSION

Genital mycoplasmas (*M. genitalium, M. hominis, U. parvum* and *U. urealyticum*) form part of the normal human flora and are found mostly in the respiratory, reproductive and urinary tracts. However, studies have shown that these bacteria are sexually transmitted and can be linked to sexually transmitted diseases and other conditions (1), (2), (81). The prevalence rates for each organism will differ according to respective geographical locations. The detection rates of *Ureaplasma* species and *Mycoplasma* species in women have shown drastic variations across all regions, countries and in different groups when individuals were classified according to age, ethnicity and socioeconomic status (64) (82) (83). Recently, studies have been investigating the association between these pathogens and pregnancy (1) (2) (81). For this study, 264 HIV infected pregnant women attending the King Edward VIII were recruited and tested for vaginal infections. Samples were obtained via self-collected vaginal swabs and DNA was extracted using the PureLink Microbiome kit. The individual pathogens were detected using the TaqMan Real-time PCR assays using commercially available primers and probes on a QuantStudio 5 Real-time polymerase chain reaction (PCR) platform. The statistical data analysis was conducted in a freely available Statistical Computing Environment, R software, version 3.6.3 using the RStudio platform. In this study, the most prevalent infection in the study population was *U. urealyticum,* 236/264 (89.4%), followed by *M. hominis*, 215/264 (81.4%), *U. parvum*, 203/264 (76.9%) and lastly *M. genitalium*, 7/264 (2.70%).

Our prevalence data is similar to previous studies which have reported high prevalence rates for *U. urealyticum*, 79%, 72.4% and 83%, respectively, for pregnant women (25) (84) (85). Studies conducted in developed countries have shown a colonization rate of 50-70% of the endocervix for *U. urealyticum* in pregnant women (86). The women in our study had a coinfection rate of 83.1% for *U. urealyticum* and *M. hominis* which is higher when compared to the results obtained by Luton *et al*. (1994) who reported a coinfection prevalence rate of 17% (84).

In this study, having a circumcised partner was a significant clinical factor in relation with being *U. urealyticum* positive, p=0.028. A systematic review conducted by Morris *et al*. (2019), confirmed that partner medical circumcision reduces women's risk for STIs and vaginal infections

(87), however, the contrary was observed in this study. In addition, partner having symptoms of STIs was also a significant clinical factor in relation with being positive, p=0.027 in this study. According to Mark *et al*. (2019), male partners with STIs and HIV are at high risk of transmitting or re-infecting their female partners and increasing adverse pregnancy outcomes (88).

In this study, the majority of the positive women were in the third trimester of pregnancy and trimester of pregnancy was significantly associated with being positive for *U. urealyticum*, p=0.040. Similarly, a study conducted by Ahmed *et al*. (2020) reported a high prevalence of *U. urealyticum* (33%) in women in the third trimester of pregnancy and this was significant ($p \leq 0.05$) (89). Of the women who tested positive for *U. urealyticum*, a higher proportion of women also tested positive for *M. hominis* (83.1%). A study conducted by Baraïka *et al*. (2020), reported a coinfection rate of 19.4% for *U. urealyticum* and *M. hominis* (90). A more recent study conducted by Paira *et al*. (2021) reported a high coinfection rate of 90% for *Ureaplasma* species and *M. hominis* (91). This is similar to the findings of the current study. In this study, in the unadjusted and adjusted analysis, being *M. hominis* positive increased the risk for *U. urealyticum*. A significant association between *M. hominis* and *Ureaplasma* species infection was reported for female patients (OR: 33.84, 95% CI: 20.25–56.86, $p < 0.0001$) attending a Reproduction Health Center in Argentina (91).

The data obtained with this study is comparable to previous studies conducted by Redelinghuys *et al*. (2013) and Naicker *et al*. (2021) who reported moderately high prevalence data for *M. hominis*, 50.7% and 48% in pregnant women, respectively (24, 25). The prevalence of *M. hominis* in this study is higher when compared to previous studies (81.4%). In general, the rate of colonization of *M. hominis* in the urogenital tract was reported to be between 21 and 54 % in women (92). However, studies conducted by Kim *et al*. 2011 and Christofolini *et al*, 2012 reported low prevalence data for *M. hominis*, 15.4% and 11.3% respectively (22) (93).

In this study, the following factors were associated with testing positive for *M. hominis*; partner having STI symptoms, women having current symptoms of STIs (abnormal vaginal discharge, genital itching) and testing positive for *U. urealyticum* and *U. parvum*. With regards to partner having symptoms of STIs being significantly associated with infection, our findings are similar to a study by Mark et al. (2019), who reported that male partners with STIs are at high risk of transmitting the infection to their female partners (88). A recent study conducted by Plummer et

al. (2021), reported that symptoms of STIs such as abnormal vaginal discharge (adjusted odds ratio [AOR] = 2.70, 95%CI: 1.92–3.79) and vaginal malodor (AOR = 4.27, 95%CI: 3.08–5.91) was associated with *M. hominis* infection (94). In this study, a high coinfection rate was observed between *M. hominis* and *U. urealyticum* (91.2%) and *M. hominis* and *U. parvum* (80.5%). In a South African study conducted by Taku et al. (2021), a high coinfection rate was observed for *U. parvum* and *M. hominis* (95). Amorim et al. (2020), reported a coinfection rate of 16.7% for *M. hominis* and *U. urealyticum* (96). The coinfection rates reported in this study is higher than reported elsewhere. This high rates could be attributed to the type of population sampled. Our study population was a HIV infected population and there is usually a high prevalence of treatable STIs in pregnancy especially in HIV-infected women (97).

In this study, being employed increased the risk of getting infected with *M. hominis*. There have been no published studies which have found a significant association between being employed and *M. hominis* positivity. However, a study conducted on young women in Uganda observed that women who were employed were at greater risk of exposure to STIs than their counterparts who were unemployed. Instead of being a protective factor against the risk of STIs, their employment may have exposed them to risky sexual behavior and STIs (98). In this study, having current STI symptoms increased the risk for *M. hominis* and this association was significant. As mentioned previously, symptoms of STIs such as abnormal vaginal discharge (adjusted odds ratio [AOR] = 2.70, 95%CI: 1.92–3.79) and vaginal malodor (AOR = 4.27, 95%CI: 3.08–5.91) was associated with an increased risk of *M. hominis* infection (94). In the adjusted analysis, being *U. parvum* and *U. urealyticum* positive increased the risk for *M. hominis* in the study women. A significant association between *Ureaplasma* species and *M. hominis* infection was reported by Paira et al. (2021) (91).

In this study, a prevalence of 76.9% was observed for *U. parvum*. Our data is consistent with a previous study conducted by Redelinghuys et al. (2013) who also reported a high prevalence for *U. parvum* (72.4%) amongst South African pregnant women in Gauteng (25). Redelinghuys et al. (2013) also reported that *U. parvum* was also present in 75% of the HIV positive cases (25). Another study conducted by Peretz et al. (2020) reported a low prevalence rate for *U. parvum* with only 4.19% of pregnant women being infected (37).

In this study, lifetime number of sex partners was significantly associated with being *U. parvum* positive. However, studies conducted by Lobão *et al*. (2017) and Karim *et al*. (2020) did not find a significant association between increased number of lifetime sex partners and testing positive for *U. parvum* (99, 100). In the adjusted analysis, having between 2-4 lifetime sex partners increased the risk of infection with *U. parvum* and was found to be significant in the current study. This correlated to findings observed in a study conducted by Silva *et al*. (2018), where an increase in lifetime number of sexual partners was shown to be associated with increased risk of *U. parvum* (101). Having a HIV positive partner was significantly associated with testing positive for *U. parvum* in the study women. A study conducted by Davey *et al*. (2019), found that women who reported being in a concordant HIV-positive partnership had over twice the odds of having a STI (97). In addition, having a partner who had other partners was also a significant factor in relation to testing positive. A study conducted by Abbai *et al*. (2018) found that having a partner that has other partners was significantly associated with genital infections such as BV (102). A combination of vaginal *U. parvum* and BV has been shown to significantly increase the risk for adverse pregnancy outcomes (103). Testing positive for *M. hominis* was significantly associated with testing positive for *U. parvum*. To date, there are a limited number of studies that have investigated the association between testing positive for *M. hominis* being a risk factor for *U. parvum* infection. A past study had reported on the significant association between *Ureaplasma* species and *M. hominis* infection (91) and not on *U. parvum* exclusively. Therefore, the data presented in the current study now fills in this gap in the literature.

A low prevalence of 2.7% for *M. genitalium*, was observed in this study. Our data is consistent with previous studies conducted in Denmark and Australia amongst the general female population reporting prevalence rates between 3‑4% (104). Studies conducted in Western Europe, North America and Australia estimated the prevalence of *M. genitalium* infection in women to range between 1% and 6.4% (105). Studies conducted in high-risk women in Uganda (14%), Kenya (12.9%‑16%) and the United States (11.3%) recorded slightly higher prevalence rates for *M. genitalium* (106-109). In a meta-analysis conducted by Baumann (2018) the prevalence of *M. genitalium* among pregnant women was found to be 0.9% (110). The prevalence estimates of *M. genitalium* reported, differed in developed and developing countries (110). The possible reasons for the variations in *M. genitalium* prevalence across studies can be due to differences in study

methods, study populations, specimen sampling methods and diagnostic assays used (105). In this study, partner having other partners was the only significant factor in relation with being *M. genitalium* positive. However, in the adjusted analysis, not knowing if ones partner had other partners was associated with a reduced risk of infection. Our findings are similar to a recently published study conducted in South African pregnant women who also did not find a significant association between partner having other partners and the risk for *M. genitalium* infection (24).

Limitations and Strengths

The study had the following limitations; this study was conducted at only one hospital clinic in KwaZulu-Natal and is not representative of the entire pregnant population in the region. A wider population will be needed to obtain more accurate prevalence estimates and risk factors for these infections. This study was also cross sectional and therefore this study could not provide data on the impact of these infections on pregnancy outcomes. This study was not designed to investigate the prevalence of the four pathogens in relation to BV and other STIs. This can be a future research endeavor. The strength of the study is that it provides data on the prevalence and risk factors for infections for which data was previously lacking in our setting.

CHAPTER 6

CONCLUSION

In this study, the prevalence, coinfections and risk factors for the genital mycoplasmas, *U. urealyticum, U. parvum, M. genitalium* and *M. hominis* were determined. In this study, prevalence rates were recorded as follows, *U. urealyticum* (89.4%), followed by *M. hominis*, (81.4%), *U. parvum*, (76.9%) and lastly *M. genitalium*, (2.70%). Our data also showed a significant link between *M. hominis* and *Ureaplasma* species. The present study provides information on the risk factors that are associated with these infection. The identification of risk factors provides the foundation for the development of prevention interventions. In this study, clinical and behavioral factors were shown to be significantly associated with the risk for infection. Based on this finding, it is evident that a single prevention strategy will not be sufficient, what will be needed is a combination prevention strategy for this vulnerable population. STI risk reduction counselling will also need to be strengthened in this population since the majority of the women are not using condoms during sex and a large proportion of women are presenting with symptoms of STIs.

CHAPTER 7

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CHAPTER 8

APPENDIX

APPENDIX 1

31 August 2021

Miss Nikita Nundlall (217004859)
School of Clinical Medicine
Medical School

Dear Miss Nundlall,

Protocol reference number: BREC/00003166/2021
Project title: The prevalence and risk factors for genital mycoplasmas in pregnant women
Degree: Masters

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 31 August 2021. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations, see (http://research.ukzn.ac.za/Libraries/BREC/BREC_Amended_Lockdown_Level_3_Guidelines.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

This approval is valid for one year from 31 August 2021. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2020) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 12 October 2021.

Yours sincerely,

[redacted]
Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
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