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Original Article

Mycoplasma hominis increases the risk for *Ureaplasma parvum* infection in Human immunodeficiency virus infected pregnant women

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Abstract

Introduction: *Mycoplasma hominis* and *Ureaplasma parvum* have been recently linked to sexually transmitted diseases and other conditions. There are a limited number of studies conducted on South African pregnant women that have assessed the prevalence and risk factors for genital mycoplasmas.

Methodology: This study included 264 HIV infected pregnant women attending the King Edward VIII antenatal clinic in eThekwini, South Africa. DNA was extracted using the PureLink Microbiome kit and pathogens were detected using the TaqMan Real-time PCR assays. 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 prevalence of *M. hominis* and *U. parvum*, was 215/264 (81.4%), and 203/264 (76.9%), respectively. In the *M. hominis* positive group, a significantly (p = 0.004) higher proportion, 80.5% tested positive for *U. parvum* infection when compared to 61.2% among the *M. hominis* negative. Of the *U. parvum* positive women, a significantly (p = 0.004) higher proportion of women (85.2%) tested positive for *M. hominis* when compared to 68.9% among the *U. parvum* negative. In the unadjusted and adjusted analysis, being *M. hominis* positive increased the risk for *U. parvum* by approximately 3 times more (p = 0.014) and 4-fold (p = 0.008), respectively.

Conclusions: This study showed a significant link between *M. hominis* and *U. parvum* infection. To date, there are a limited number of studies that have investigated *M. hominis* being a risk factor for *U. parvum* infection. Therefore, the data presented in the current study now fills in this gap in the literature.

Key words: *Mycoplasma hominis; Ureaplasma parvum;* human immunodeficiency virus; pregnant women.

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Introduction

The genus *Mycoplasma* is the smallest bacteria to be discovered [1,2]. 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].

M. hominis was first identified and isolated in 1937 as the first *Mycoplasma* of human origin [4]. The role of this bacteria in causing a disease has been researched over the years and is still not yet fully understood. A study conducted by Christofolini *et al.* [5] in a cohort of non-pregnant women observed a prevalence of 11.3% (12/106) for *M. hominis* infection. That same study also reported on coinfections between *M. hominis* and *Chlamydia trachomatis* [5]. A meta-analysis conducted on studies published from 2000-2019, reported a prevalence of 9.68% for *M. hominis* for non-pregnant Iranian women [6]. 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 [7]. A previous study conducted in South Africa by Redelinghuys *et al.* also reported high prevalence data for *M. hominis* (50.7%) in pregnant women from Gauteng, South Africa [8].

Ureaplasma species were only first identified in the last 20 years [9]. In a study conducted by Lee *et al.* (2020) that analyzed 4,035 endocervical swab specimens using a *Mycoplasma* IST2 kit, 1,589 (39.4%) cases were positive for genital mycoplasmas, which included 49 (3.1%) cases of *M. hominis*, 1,243 (78.2%) cases of *Ureaplasma* species and 297 (18.7%) cases of

both *M. hominis* and *Ureaplasma* species [10]. 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) [11], 65.6% and 11.8% by Kweon *et al*. (2016) [12], and 48.8% and 25.3% by Jang *et al*. (2019) [13], respectively. Similar values were reported in Poland [14] and China [15].

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 5 (2.3%) participants testing positive for *M. genitalium*, 9 (4.2%) testing positive for *U. parvum*, and 5 (2.3%) testing positive for *U. urealyticum*. It was found that mothers would pass on these bacteria to their newborns after the newborns were sampled and tested respectively [16].

Currently, there are a limited number of studies conducted on 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* were 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.

Methodology

Study population

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 woman provided self-collected vaginal swabs (dry swabs) for detection of the vaginal infections. The consenting women also completed a questionnaire on socio-demographic, behavioral, and clinical factors. The study was approved by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (UKZN), (BREC/00003166/2021).

DNA isolation and pathogen detection

After collection, the dry swabs were placed in a 2 mL of phosphate buffered saline. The solution was vortexed to dislodge the cells from the swabs and the swab was discarded. DNA was extracted from the vaginal fluid using the PureLink Microbiome kit (Thermofisher Scientific, United States) according to the manufacturer's instructions.

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. hominis* (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 20 µL comprising: 1 µL FAM-labeled probe/primer mix, 5 µL Fast Start 4x probe master mix (Thermofisher, Part No. 4444434), 1.5 µL template DNA, and nuclease-free water. Non-template and positive controls (TaqMan™ Vaginal Microbiota Extraction Control; cat no. A32039) were also included. Amplification was performed at 95°C for 30 seconds followed by 45 cycles comprising of denaturation at 95°C for 3 seconds and annealing at 60°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.

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 the infection status of the pathogens. In addition to the frequencies, regression analysis was used to assess the relationship between each risk factor and the pathogen infection status. This included univariate, multiple, and stepwise logistic regressions to quantify their relationships with the outcome in terms of odds ratios. All the tests were conducted at a 5% level of significance.

Results

Factors associated with M. hominis status in the study population

The following factors were significantly associated ($p \leq 0.05$) with *M. hominis* status: *U. urealyticum* positive status, *U. parvum* positive status, partners STI symptoms and current symptoms of STIs (Table 1). Of the women who tested *U. urealyticum* positive, 91.2% of the women were *M. hominis* positive versus 81.6% who were *M. hominis* negative, $p = 0.051$. Similarly, a

Table 1. Characteristics of the study women according to *M. hominis* status.

***M. hominis* status**	**Negative (N = 49)**	**Positive (N = 215)**	***p* value**	**Overall (N = 264)**
Age				
Median (Q1-Q3)	34.0 (27.0-38.0)	30.0 (25.0-37.0)	0.081 Ranksum	31.0 (26.0-37.0)
Min-Max	20.0-42.0	18.0-44.0		18.0-44.0
Educational level				
College, University	11 (22.4%)	37 (17.2%)	0.550 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				
No	36 (73.5%)	152 (70.7%)	0.699 Chisq.	188 (71.2%)
Yes	13 (26.5%)	63 (29.3%)		76 (28.8%)
Married				
No	46 (93.9%)	188 (87.4%)	0.200 Chisq.	234 (88.6%)
Yes	3 (6.1%)	27 (12.6%)		30 (11.4%)
Regular sex partner				
No	3 (6.1%)	17 (7.9%)	1.000 Fisher's	20 (7.6%)
Yes	46 (93.9%)	198 (92.1%)		244 (92.4%)
Partners HIV status				
Don't know	9 (18.4%)	31 (14.4%)	0.781 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				
No	24 (49.0%)	133 (61.9%)	0.083 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				
< 15	1 (2.0%)	8 (3.7%)	0.222 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 number of sex partners				
> 4	12 (24.5%)	42 (19.5%)	0.620 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				
Don't know	21 (42.9%)	117 (54.4%)	0.183 Chisq.	138 (52.3%)
No	16 (32.7%)	45 (20.9%)		61 (23.1%)
Yes	12 (24.5%)	53 (24.7%)		65 (24.6%)
Condom used during last sex				
No	32 (65.3%)	135 (62.8%)	0.742 Chisq.	167 (63.3%)
Yes	17 (34.7%)	80 (37.2%)		97 (36.7%)
Partner circumcised				
No	21 (42.9%)	71 (33.0%)	0.192 Chisq.	92 (34.8%)
Yes	28 (57.1%)	144 (67.0%)		172 (65.2%)
Trimester				
1st	3 (6.1%)	17 (7.9%)	0.898 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				
No	30 (61.2%)	142 (66.0%)	0.523 Chisq.	172 (65.2%)
Yes	19 (38.8%)	73 (34.0%)		92 (34.8%)
Intravaginal practices				
No	48 (98.0%)	200 (93.0%)	0.319 Fisher's	248 (93.9%)
Yes	1 (2.0%)	15 (7.0%)		16 (6.1%)
M. genitalium				
Neg	48 (98.0%)	209 (97.2%)	1.000 Fisher's	257 (97.3%)
Pos	1 (2.0%)	6 (2.8%)		7 (2.7%)
U. urealyticum				
Neg	9 (18.4%)	19 (8.8%)	0.051 Chisq.	28 (10.6%)
Pos	40 (81.6%)	196 (91.2%)		236 (89.4%)
U. parvum				
Neg	19 (38.8%)	42 (19.5%)	0.004 Chisq.	61 (23.1%)
Pos	30 (61.2%)	173 (80.5%)		203 (76.9%)
Partner STI symptoms				
No	32 (65.3%)	172 (80.0%)	0.027 Chisq.	204 (77.3%)
Yes	17 (34.7%)	43 (20.0%)		60 (22.7%)
Current STIs symptoms				
No	40 (81.6%)	112 (52.1%)	< 0.001 Chisq.	152 (57.6%)
Yes	9 (18.4%)	103 (47.9%)		112 (42.4%)

higher percentage of *U. parvum* positive also tested positive for *M. hominis* (80.5%) when compared to 61.2% who tested negative for *M. hominis*, $p = 0.004$. A higher proportion of women whose partner did not have symptoms of STIs tested negative for *M. hominis* (34.7%) versus 20.0% who tested positive, $p = 0.027$. Of the women who reported having current symptoms of STIs, 47.9% tested positive for *M. hominis* when compared to 18.4% who tested negative for *M. hominis*, $p < 0.001$.

Table 2. Characteristics of the study women according to *U. parvum* status.

***U. parvum* status**	**Neg (N = 61)**	**Pos (N = 203)**	***p* value**	**Overall (N = 264)**
Age				
Median (Q1-Q3)	31.0 (24.0-37.0)	31.0 (26.0-37.0)	0.698 Ranksum	31.0 (26.0-37.0)
Min-Max	19.0-44.0	18.0-43.0		18.0-44.0
Educational level				
College, University	14 (23.0%)	34 (16.7%)		48 (18.2%)
Did not attend school	1 (1.6%)	0 (0%)	0.122 Fisher's	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				
No	42 (68.9%)	146 (71.9%)	0.643 Chisq.	188 (71.2%)
Yes	19 (31.1%)	57 (28.1%)		76 (28.8%)
Married				
No	52 (85.2%)	182 (89.7%)	0.341 Chisq.	234 (88.6%)
Yes	9 (14.8%)	21 (10.3%)		30 (11.4%)
Regular sex partner				
No	3 (4.9%)	17 (8.4%)	0.581 Fisher's	20 (7.6%)
Yes	58 (95.1%)	186 (91.6%)		244 (92.4%)
Partners HIV status				
Don't know	6 (9.8%)	34 (16.7%)		40 (15.2%)
Negative	28 (45.9%)	60 (29.6%)	0.049 Chisq.	88 (33.3%)
Positive	27 (44.3%)	109 (53.7%)		136 (51.5%)
Cohabiting				
No	32 (52.5%)	125 (61.6%)		157 (59.5%)
Yes	29 (47.5%)	76 (37.4%)	0.174 Chisq.	105 (39.8%)
Missing	0 (0%)	2 (1.0%)		2 (0.8%)
Age of 1st sex				
< 15	0 (0%)	9 (4.4%)		9 (3.4%)
> 25	0 (0%)	6 (3.0%)	0.225 Fisher's	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 number of sex partners				
> 4	7 (11.5%)	47 (23.2%)		54 (20.5%)
1	26 (42.6%)	50 (24.6%)	0.012 Chisq.	76 (28.8%)
2-4	28 (45.9%)	106 (52.2%)		134 (50.8%)
Partner has other partners				
Don't know	33 (54.1%)	105 (51.7%)		138 (52.3%)
No	20 (32.8%)	41 (20.2%)	0.023 Chisq.	61 (23.1%)
Yes	8 (13.1%)	57 (28.1%)		65 (24.6%)
Condom used during last sex				
No	35 (57.4%)	132 (65.0%)	0.277 Chisq.	167 (63.3%)
Yes	26 (42.6%)	71 (35.0%)		97 (36.7%)
Partner circumcised				
No	23 (37.7%)	69 (34.0%)	0.593 Chisq.	92 (34.8%)
Yes	38 (62.3%)	134 (66.0%)		172 (65.2%)
Trimester				
1st	7 (11.5%)	13 (6.4%)		20 (7.6%)
2nd	14 (23.0%)	68 (33.5%)	0.171 Chisq.	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				
No	37 (60.7%)	135 (66.5%)	0.401 Chisq.	172 (65.2%)
Yes	24 (39.3%)	68 (33.5%)		92 (34.8%)
Intravaginal practices				
No	59 (96.7%)	189 (93.1%)	0.376 Fisher's	248 (93.9%)
Yes	2 (3.3%)	14 (6.9%)		16 (6.1%)
M. genitalium				
Neg	59 (96.7%)	198 (97.5%)	0.664 Fisher's	257 (97.3%)
Pos	2 (3.3%)	5 (2.5%)		7 (2.7%)
M. hominis				
Neg	19 (31.1%)	30 (14.8%)	0.004 Chisq.	49 (18.6%)
Pos	42 (68.9%)	173 (85.2%)		215 (81.4%)
U. urealyticum				
Neg	10 (16.4%)	18 (8.9%)	0.094 Chisq.	28 (10.6%)
Pos	51 (83.6%)	185 (91.1%)		236 (89.4%)
Partner STI symptom				
No	46 (75.4%)	158 (77.8%)	0.692 Chisq.	204 (77.3%)
Yes	15 (24.6%)	45 (22.2%)		60 (22.7%)
Current STIs symptoms				
No	30 (49.2%)	122 (60.1%)	0.130 Chisq.	152 (57.6%)
Yes	31 (50.8%)	81 (39.9%)		112 (42.4%)

Factors associated with U. parvum status in the study population

The following factors were significantly associated ($p = 0.05$) with *U. parvum* status; partners HIV status, lifetime number of sex partners, partner having other partners, and *M. hominis* positive status (Table 2). A higher proportion of women whose partners were HIV positive were *U. parvum* positive (53.7%) when compared to 44.3% who had an HIV positive partner and tested negative for *U. parvum*, $p = 0.049$. Of the women who reported having between 2 to 4 lifetime sex partners, 52.2% tested positive for *U. parvum* when compared to 45.9% who tested negative for *U. parvum*, $p = 0.012$. A higher percentage of women who reported that their partner had other partners tested positive for *U. parvum* (28.1%) when compared to 13.1% to tested negative, $p = 0.023$. Of the women who tested positive for *M. hominis*, 85.2% tested positive for *U. parvum* versus 68.9% who tested negative for *U. parvum*, $p = 0.004$.

Risk factors for U. parvum infection

In the unadjusted and adjusted analyses, having between 2 to 4 lifetime sex partners increased the risk of infection with *U. parvum* by 2.10-fold and 3.08-fold, $p = 0.033$ and $p = 0.013$, respectively. After further adjustments, it was still significant, $p = 0.017$. Having more than 4 lifetime sex partners increased the risk of infection with *U. parvum* by 20.65-fold in the unadjusted analysis and 88.02-fold in the adjusted analysis, and was significant, $p = 0.004$ and $p < 0.001$, respectively. After further adjustments, it was still significant, $p < 0.001$. In the unadjusted and adjusted analyses partner having other partners increased the risk of infection with *U. parvum* by 4.80-fold and 6.72-fold, respectively. This factor showed to be significant, $p = 0.005$ and $p = 0.008$, respectively. After further adjustments, it was still significant, $p = 0.005$. Testing *M. hominis* positive increased the risk for *U. parvum* by 2.53 in the unadjusted analysis and 4.33-fold in the adjusted analysis. This association was significant, $p = 0.014$ and $p = 0.008$, respectively. After further adjustments, it was still significant, $p = 0.008$ (Table 3).

Discussion

M. hominis and *U. parvum* 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 [1,2,17]. The prevalence rates for each organism will differ according to respective geographical locations. The detection rates of *Ureaplasma* spp. and *Mycoplasma* spp. in women have shown drastic variations across all regions and countries and in different groups when individuals were classified according to age, ethnicity, and socioeconomic status [18-20].

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 [7,8]. The prevalence of *M. hominis* in this study is higher (81.4%) when compared to previous studies. Our study prevalence may be higher than other studies due to socioeconomic factors. In this study, the following factors were associated with testing positive for *M. hominis:* partner having STI symptoms, women having current symptoms of STIs 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 [21]. A recent study conducted by

Table 3. 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. genitalium- Positive	1.14 (0.18-22.13, $p = 0.906$)	1.60 (0.06-105.33, $p = 0.816$)	-
M hominis- Positive	2.53 (1.18-5.26, $p = 0.014$)	4.33 (1.48-13.10, $p = 0.008$)	4.05 (1.45-11.55, $p = 0.008$)
U. urealyticum- Positive	1.96 (0.72-4.89, $p = 0.163$)	2.62 (0.71-9.56, $p = 0.141$)	2.84 (0.83-9.66, $p = 0.091$)

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 [22]. 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* and (26.9%) [23]. Amorim *et al*. (2020), reported a coinfection rate of 16.7% for *M. hominis* and *U. urealyticum* [24]. The coinfection rates reported in this study are higher than those reported elsewhere. These 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 [25].

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 [8]. Redelinghuys *et al*. (2013) also reported that *U. parvum* was also present in 75% of the HIV positive cases [8]. Another study conducted by Peretz *et al*. (2020) reported a low prevalence data for *U. parvum* with only 4.19% of pregnant women being infected [16]. According to this study *Mycoplasma* or *Ureaplasma* infection could be associated with ethnicity and settlement type however further studies are needed [16].

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 the increased number of life-time sex partners and testing positive for *U. parvum* [26,27]. 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 the lifetime number of sexual partners was shown to be associated with an increased risk of *U. parvum* [28]. Having an HIV-positive partner was significantly associated with testing positive for *U. parvum* in the study women. Our findings show that being infected with HIV revealed that individuals were at a higher risk of STI acquisition and other infections. Individuals infected with HIV have compromised immunity which makes it easier to transmit and acquire pathogens [29,30]. To break the cycle of transmission it is fundamental to understand the critical components of STI management. 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 an STI [25]. 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 bacterial vaginosis (BV) [31]. A combination of vaginal *U. parvum* and BV has been shown to significantly increase the risk for adverse pregnancy outcomes [32]. A study conducted by Lendamba *et al*. 2022 found that the prevalence of genital mycoplasma infections such as *M. hominis* and *Ureaplasma* spp. are significantly high in women with bacterial vaginosis as 60.18% of the women were positive for BV and were genital mycoplasma carriers, including 5.19% pregnant women [33]. 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 [34] and not on *U. parvum* exclusively. Therefore, the data presented in the current study now fills in this gap in the literature.

Limitations

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 population. 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 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.

Conclusions

Our data also showed a significant link between *M. hominis* and *U. parvum* infection. The present study provides information on the risk factors that are associated with *U. parvum* 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 high proportion of women are presenting with symptoms of STIs.

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