Background: The role of *Mycoplasma hominis* (*M. hominis*) as a genital tract pathogen was still debatable. This study identified the risk factors associated with the prevalence of *M. hominis* in South African pregnant women.

Methods: This was a cross-sectional analysis of *n* = 221 prenatal patients attending a Durban hospital during November 2017 to April 2018. *M. hominis* was detected from urine samples using the quantitative polymerase chain reaction. The population characteristics were described using frequencies stratified by the infection status of *M. hominis*. In addition, a univariate analysis was used to assess the relationship between each risk factor and infection status. The analysis further considered logistic regression to assess the influence of these risk factors univariately and in the presence of other factors. The coinfection rate between *M. hominis* and bacterial vaginosis (BV), *Trichomonas vaginalis* (*T. vaginalis*), *Mycoplasma genitalium* (*M. genitalium*) and *Candida* species was also determined. All the tests were conducted at 5% level of significance.

Results: The prevalence of *M. hominis* in this study population was 48% (106/221). In the univariate analysis, factors significantly associated with *M. hominis* positivity included having past abnormal vaginal discharge (*p* = 0.037), having current abnormal vaginal discharge (*p* = 0.010) and a borderline significance (*p* = 0.052), which were noted for previous pre-term delivery. However, none of these factors were sustained in the multivariate analysis. There was a statistically significant association between *M. hominis* and BV positivity (*p* < 0.001). Similarly, *M. hominis* and *M. genitalium* positivity was significant (*p* = 0.006).

Conclusion: This study showed that *M. hominis* does not share common risk factors with known genital tract pathogens in a population of pregnant women and therefore cannot be considered a genital tract pathogen.

Keywords: *Mycoplasma hominis*; vaginal infections; pregnant women; risk factors; HIV infection; bacterial vaginosis.

Introduction

According to the World Health Organization (WHO), there are more than 376 million incidences of sexually transmitted infections (STIs) reported worldwide annually, thus making these infections an important public health concern.1 Depending on the causative agent, an STI is usually manifested in a skin lesion, secretion, vaginal discharge, wart or blister.2 The vaginal microbiome consists of numerous microorganisms, including members of the class Mollicutes, order Mycoplasmatales (mycoplasmas and ureaplasmas).3

Mollicutes are included in STIs, but they are also found in healthy individuals.2 The most important Mollicutes colonising the female genital tract are *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis* (*M. hominis*) and *Mycoplasma genitalium* (*M. genitalium*).4 Genital mycoplasmas colonise the vaginal tract of up to 80% of pregnant and non-pregnant women.5

*Mycoplasma hominis* is considered to be an important opportunistic pathogen implicated in urogenital infections and complicated pregnancy outcomes.6 These include pelvic inflammatory disease, endometritis, chorioamnionitis and postpartum fever, resulting in complications such as infertility,
spontaneous abortion, stillbirth, preterm birth, low birth weight and perinatal mortality.²⁴,²⁶,²⁷,²⁸,²⁹,³⁰

An earlier study had provided evidence stating that M. hominis is not a vaginal pathogen in adults.¹³ However, a study published in the same year by Arya et al. referred to M. hominis as a vaginal pathogen because of its association with Trichomonas vaginalis (T. vaginalis).¹⁴ There have been limited studies since 2001, which have contributed to resolving the discordance regarding the role of M. hominis as a genital tract pathogen. The aim of the current study was to describe the prevalence and factors associated with M. hominis in pregnant women as well as its likelihood of being considered as a genital tract pathogen.

Materials and methods
Study setting and population
This study was a sub-study of a larger study that investigated the laboratory-based diagnosis of vaginal infections in pregnant women from Durban. The larger study included n = 273 women, 18 years and older from all gestational ages who are willing to provide written informed consent. The study population was recruited from the King Edward VIII hospital in Durban, KwaZulu-Natal between October 2017 and April 2018. All patients were outpatients. There was a 10% refusal rate by the women during screening. A questionnaire was administered to collect data on the women’s demographics, sexual behaviour and clinical information. All interviews were conducted in private, and all study-related information was stored securely. All records and specimens had been identified by study identification numbers only to maintain participant confidentiality.

Only participants who had given written informed consent were included in the study. The study did not collect data on whether the women were experiencing any complications during pregnancy. During the study visit, women were asked to provide self-collected vaginal swab and urine samples. The women were tested for HIV at the clinic as part of routine care. Permission to obtain data on HIV status was obtained from the women. Because of the cross-sectional design of the study, the women were not followed up to collect information on pregnancy outcomes.

For the sub-study, n = 221 urine DNA extracts were available for analysis. Laboratory testing and analyses were performed at the School of Clinical Medicine Laboratory, University of KwaZulu-Natal.

Study procedures
Data collection
At enrolment, a face-to-face questionnaire was administered to collect data on the women’s demographics (age, level of education and marital status), sexual behaviour (condom use, number of lifetime sex partners, age of sexual debut, partner having other partners, intravaginal practices, cohabitation status and recreational habits such as smoking and consuming alcohol) and clinical information (gestational age, history of previous pregnancies and history of STIs).

Detection of Mycoplasma hominis from urine
A sensitivity detection assay was performed on the urine DNA extracts. DNA extraction involved a starting volume of 10 ml of urine. A standardised starting volume was used across all samples. The urine was centrifuged for 45 min at 14 000 × g and the supernatant was discarded. Total DNA was then extracted from recovered sample pellets using the PureLink™ Microbiome DNA Purification Kit (Thermo Fisher Scientific, United States) in accordance with the manufacturer’s instructions. The DNA concentration was measured using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, United States). Resulting DNA concentrations ranged from 3.1 3 ng/µL to 75.3 ng/µL with A₂₆₀/A₂₈₀ ratios in the range of 0.80–1.91.

Mycoplasma hominis was detected using the TaqMan Real-time polymerase chain reaction (PCR) (sensitivity) assay (Thermo Fisher Scientific, United States) using commercially available primers and probes specific for M. hominis (Ba04646255_s1). The assays were run on the Quant Studio 5 Real-time PCR detection system (Thermo Fisher Scientific, USA).

Each PCR reaction was performed in a final volume of 5 uL comprising 0.25 uL of FAM-labelled probe/primer mix, 1.25 uL of Fast Start 4x probe master mix (Thermo Fisher, Part No. 4444434), 1.5 uL of 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 s followed by 45 cycles comprising denaturation at 95°C for 3 s and annealing at 60°C for 30 s. Detection of amplified fluorescent products was carried out at the end of the annealing phase. The raw fluorescence data that included the Cₜ mean values were automatically generated using the Quant Studio 5 Real-time PCR system software.

Detection of Mycoplasma genitalium from urine
Mycoplasma genitalium was detected using the TaqMan Real-time PCR (sensitivity) assay (Thermo Fisher Scientific, United States) using commercially available primers and probes specific for M. hominis (Ba04646249_s1). The reaction and cycling conditions were as per the M. hominis assay conditions.

Detection of bacterial vaginosis, Trichomonas vaginalis and Candida species from vaginal swabs
The presence of BV, T. vaginalis and Candida species was detected using the BD Max™ Vaginal Panel assay (Becton Dickinson, United States) from a single vaginal swab. The assay was performed as per the manufacturer’s recommendations.
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, univariate analysis was used to assess the relationship between each risk factor and the pathogen infection status. The available continuous variable had a skewed distribution calling for a non-parametric test involving a rank-sum test. On the other hand, 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 (ORs). 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.

Ethical considerations

Ethics approval for this study was granted by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (BE214/17).

Results

Characteristics of the population according to Mycoplasma hominis status

The prevalence of M. hominis in the study population was 48% (106/221). Table 1 shows the factors in relation to M. hominis status. There was no significant association (p > 0.05) between the majority of demographic variables and the prevalence of M. hominis. Of the 106 M. hominis positive women, a small proportion of them, 20.8% (22/106) had attended college/university when compared to the majority of women (69.8% [74/106]) who attended high school. When considering the behavioural factors, it was shown that amongst the 106 M. hominis-positive women most women 83.0% (88/106) reported having a regular sex partner when compared to women not reported having a regular sex partner (17%). A majority of positive women engaged in their first sex at an early age of 15 and 20 years, 72.6% (77/106) followed by delayed sex at > 20 years of age constituting 21.7% (23/106). The dominant number of lifetime sex partners amongst the M. hominis-positive women was 2–4 partners 51.9% (55/106) compared to just one lifetime sex partner 22.6% (24/106) and > 4 lifetime sex partners 25.5% (27/106). The results also showed that 71.7% (76/106) of the M. hominis-positive women did not use a condom at their last sex and 90.6% (96/106) did not engage in intravaginal practices. Despite the high proportion of women reporting risky behavioural practices, there was no significant association (p > 0.05) between most of these potential risk factors and the prevalence of M. hominis (Table 1). With respect to the clinical symptoms, it was found that amongst the M. hominis-positive women, almost half did not experience past episodes of abnormal vaginal discharge 50.5% (53/106), which was a significantly (p = 0.037) smaller proportion when compared to 64.3% (74/115) who did not experience past episodes of abnormal vaginal discharge amongst the M. hominis-negative women. Similarly, at the time of enrolment into the study, a significantly (p = 0.010) smaller proportion 56.6% (60/106) of women did not experience current abnormal vaginal discharge amongst the M. hominis-positive women compared to 73% (84/115) within the M. hominis-negative women.

Coinfection between Mycoplasma hominis bacterial vaginosis, Trichomonas vaginalis, Candida species and Mycoplasma genitalium

There was a statistically significant association between M. hominis and BV positivity (p < 0.001) (Table 3). That is, amongst the 106 women who tested positive for M. hominis, 66.0% also tested positive for BV and this was a significantly higher proportion when compared to 27.8% (32/115) BV positives amongst the M. hominis-negative women. Similarly, there was a significant association between M. hominis and M. genitalium positivity (p = 0.006). The coinfection rate between M. hominis and M. genitalium was 4.98% (11/221) constituting 10.4% (11/106) of the M. hominis-positive women (Table 3). Despite high co-infection rates between M. hominis and T. vaginalis (14.2% of the M. hominis positive) and M. hominis and Candida species (59.4% of the M. hominis positive), these associations were not significant (p = 0.130 and p = 0.853, respectively) (Table 3).
### TABLE 1a: Characteristics of the study population by *Mycoplasma hominis* status.

| Variable | Overall (n = 221) | M. hominis | Overall (n = 221) | p-value |
|----------|------------------|------------|------------------|---------|
| Age | | | | |
| Mean ± SD | 28.3 ± 6.04 | 28.0 ± 5.95 | 28.6 ± 6.14 | 0.490 |
| CV | - | 21.3 | 21.5 | - |
| Median | - | 27.0 | 27.0 | - |
| Q1; Q3 | - | 24.0; 33.0 | 24.0; 33.0 | - |
| Min–Max | - | 18.0–43.0 | 18.0–43.0 | - |

### TABLE 1b: Characteristics of the study population by *Mycoplasma hominis* status.

| Variable | Overall (n = 221) | M. hominis | Overall (n = 221) | p-value |
|----------|------------------|------------|------------------|---------|
| Current abnormal vaginal discharge | | | | 0.01 |
| No | 115 | 73.0 | 84 | - |
| Yes | 106 | 56.6 | 102 | - |
| Symptoms of STIs in the past 3 months | | | | 0.08 |
| No | 187 | 84.6 | 102 | - |
| Yes | 34 | 15.4 | 13 | - |
| Level of education | | | | 0.061 |
| Primary and below | 109 | 9.4 | 10 | - |
| High school | 114 | 69.8 | 74 | - |
| College/University | 88 | 31.8 | 38 | - |
| Marital status | | | | 0.191 |
| No | 188 | 85.5 | 94 | - |
| Yes | 32 | 14.5 | 20 | - |
| Has a regular sexual partner | | | | 0.936 |
| No | 102 | 18.7 | 20 | - |
| Yes | 146 | 81.3 | 82 | - |
| Living with sexual partner | | | | 0.116 |
| No | 136 | 61.5 | 67 | - |
| Yes | 85 | 38.5 | 46 | - |
| Age at first sex | | | | 0.71 |
| < 15 | 11 | 11.3 | 5 | - |
| 15–20 | 166 | 75.1 | 77 | - |
| > 20 | 44 | 19.9 | 21 | - |
| Lifetime number of sexual partners | | | | 0.171 |
| 1 | 61 | 27.6 | 14 | - |
| 4-Feb | 113 | 51.1 | 58 | - |
| > 4 | 47 | 21.3 | 20 | - |
| Partner has other partners | | | | |- |
| No/Do not know | 155 | 70.1 | 86 | - |
| Yes | 66 | 29.9 | 29 | - |
| Condom use | | | | 0.47 |
| Never | 76 | 34.4 | 37 | - |
| Always | 145 | 65.6 | 78 | - |
| Condom use at last sexual act | | | | 0.193 |
| No | 149 | 67.4 | 73 | - |
| Yes | 72 | 32.6 | 42 | - |
| Smokes | | | | 0.317 |
| No | 212 | 95.9 | 112 | - |
| Yes | 9 | 04.1 | 3 | - |
| Consumes alcohol | | | | 0.08 |
| No | 198 | 89.6 | 107 | - |
| Yes | 23 | 10.4 | 8 | - |
| Intravaginal practices | | | | 0.973 |
| No | 200 | 90.5 | 104 | - |
| Yes | 21 | 09.5 | 11 | - |
| Trimester of pregnancy | | | | 0.898 |
| 1st | 21 | 09.5 | 10 | - |
| 2nd | 75 | 33.9 | 40 | - |
| 3rd | 125 | 56.6 | 65 | - |

Table 1b continues on the next page
### TABLE 1b (Continues...): Characteristics of the study population by *Mycoplasma hominis* status.

| Variable                          | M. hominis Negative (n = 115) | M. hominis Positive (n = 106) | Overall (n = 221) | p-value |
|-----------------------------------|-------------------------------|-------------------------------|-------------------|---------|
|                                  | N                             | %                            | N                 | %       | N           | %       | p-value |
| Previous pre-term delivery       | -                             | -                            | -                 | -       | 176         | 79.6   | -       |
| No                                | 96                            | 83.5                         | 176               | 79.6    |             |         | 0.052   |
| Yes                               | 15                            | 13.0                         | 40                | 18.1    |             |         |         |
| Missing                           | 4                             | 0.3%                         | 5                 | 2.3     |             |         |         |
| Past miscarriage                 | -                             | -                            | -                 | -       | -           | -       | 0.191   |
| No                                | 80                            | 69.6                         | 162               | 73.3    |             |         |         |
| Yes                               | 35                            | 30.4                         | 59                | 26.7    |             |         |         |
| Past spontaneous abortion         | -                             | -                            | -                 | -       | -           | -       | 0.259   |
| No                                | 107                           | 93.0                         | 201               | 91.0    |             |         |         |
| Yes                               | 8                             | 0.70                         | 40                | 18.1    |             |         |         |
| Previous abnormal vaginal discharge | -                        | -                            | -                 | -       | -           | -       | 0.037   |
| No                                | 74                            | 64.3                         | 127               | 57.7    |             |         |         |
| Yes                               | 41                            | 35.7                         | 93                | 42.3    |             |         |         |
| Previously treated for STIs       | -                             | -                            | -                 | -       | -           | -       | 0.283   |
| No                                | 69                            | 60.0                         | 125               | 56.6    |             |         |         |
| Yes                               | 46                            | 40.0                         | 96                | 43.4    |             |         |         |

STIs, sexually transmitted infections.

### TABLE 2: Univariate and multiple regression analysis of risk factors associated with *Mycoplasma hominis* infection.

| Explanatory                          | OR (Unadjusted) | 95% CI          | p-value | OR (Adjusted) | 95% CI          | p-value |
|--------------------------------------|-----------------|-----------------|---------|---------------|-----------------|---------|
| Current abnormal vaginal discharge   | 2.08            | 1.19–3.67       | 0.011   | 1.58          | 0.77–3.28       | 0.211   |
| No discharge (Referent)              | 1               | -               | -       | 1             | -               | -       |
| STI symptoms                         | 1.94            | 0.93–4.19       | 0.083   | 0.99          | 0.39–2.52       | 0.989   |
| No STI symptoms (Referent)           | 1               | -               | -       | 1             | -               | -       |
| Education (High school)              | 0.51            | 0.15–1.52       | 0.245   | 0.59          | 0.17–1.83       | 0.374   |
| Education (College/University)       | 0.29            | 0.08–0.92       | 0.042   | 0.33          | 0.09–1.11       | 0.080   |
| Education (Primary school) (Referent)| 1               | -               | -       | 1             | -               | -       |
| Consumes alcohol                     | 2.2             | 0.91–5.70       | 0.086   | 1.97          | 0.76–5.54       | 0.175   |
| Does not consume alcohol (Referent)  | 1               | -               | -       | 1             | -               | -       |
| Had a previous pre-term baby         | 2               | 1.00–4.13       | 0.054   | 1.61          | 0.77–3.45       | 0.207   |
| No previous pre-term delivery (Referent) | 1       | -               | -       | 1             | -               | -       |
| Past abnormal vaginal discharge      | 1.77            | 1.03–3.05       | 0.038   | 1.44          | 0.79–2.62       | 0.231   |
| No past discharge (Referent)         | 1               | -               | -       | 1             | -               | -       |

STI, sexually transmitted infection.

### TABLE 3: Coinfection between *Mycoplasma hominis* and genital tract infections.

| *M. hominis* | Negative (N = 115) | Positive (N = 106) | Overall (N = 221) | p-value |
|--------------|--------------------|--------------------|--------------------|---------|
|              | N                  | %                  | N                  | %       | N          | %       | p-value |
| Bacterial vaginosis | -                  | -                  | -                  | -       | < 0.001    |         |         |
| Negative    | 74                 | 64.3               | 29                 | 27.4    | 103        | 46.6    |         |
| Positive    | 32                 | 27.8               | 70                 | 66.0    | 102        | 46.2    |         |
| Missing     | 9                  | 7.8                | 7                  | 6.6     | 16         | 7.2     |         |
| Candida species | -                  | -                  | -                  | -       | -          | 0.853   |         |
| Negative    | 47                 | 40.9               | 42                 | 39.6    | 89         | 40.3    |         |
| Positive    | 67                 | 58.3               | 63                 | 59.4    | 130        | 58.8    |         |
| Missing     | 1                  | 0.9                | 1                  | 0.9     | 2          | 0.9     |         |
| Trichomonas vaginalis | -                  | -                  | -                  | -       | -          | 0.130   |         |
| Negative    | 105                | 91.3               | 90                 | 84.9    | 195        | 88.2    |         |
| Positive    | 9                  | 7.8                | 15                 | 14.2    | 24         | 10.9    |         |
| Missing     | 1                  | 0.9                | 1                  | 0.9     | 2          | 0.9     |         |
| Mycoplasma genitalium | -                  | -                  | -                  | -       | -          | 0.006   |         |
| Negative    | 113                | 98.3               | 95                 | 89.6    | 208        | 94.1    |         |
| Positive    | 2                  | 1.7                | 11                 | 10.4    | 13         | 5.9     |         |
Predicting the risk of Mycoplasma hominis infection in the presence of other genital infections

The results in Table 4 showed that having a prevalent BV infection significantly increased the risk of acquiring M. hominis by 5-fold in both the unadjusted (OR: 5.19, 95% CI: 2.75–10.10, \( p < 0.001 \)) and adjusted analyses (OR: 5.19, 95% CI: 2.75–10.10, \( p < 0.001 \)). The results further revealed that being M. genitalium positive doubled the chances of M. hominis infection as compared to having been BV positive. M. genitalium-positive women had an increased risk of M. hominis infection by 12-fold (OR: 12.28, 95% CI: 2.28–227.76, \( p = 0.018 \)) and 10-fold (OR: 9.54, 95% CI: 1.58–185.73, \( p = 0.041 \)) in the unadjusted and adjusted analyses, respectively. However, the stepwise regression suggested that Candida species and T. vaginalis were not important in predicting the likelihood of M. hominis infection. That is, without taking Candida species and T. vaginalis into consideration, the refined results still show that BV increased the risk for M. hominis infection by close to five-fold (OR: 4.87, 95% CI: 2.61–9.31, \( p < 0.001 \)) and M. genitalium increased the risk for M. hominis infection by close to nine-fold (OR: 8.90, 95% CI: 1.52–170.55, \( p = 0.045 \)).

Predicting the risk of bacterial vaginosis infection in the presence of other genital infections

Table 5 shows that a woman who is M. hominis positive had an increased risk of BV infection by 5-fold both univariately (OR: 5.37, 95% CI: 2.96–9.98, \( p < 0.001 \)) and by controlling for the other genital infections (OR: 5.44, 95% CI: 2.94–10.39, \( p < 0.001 \)). This confirms that the odds of M. hominis infection given BV infection or vice versa are the same (5-fold). Although Candida species infection status was not significantly associated with BV infection, the results showed that it is important to gather data on Candida species alongside that of M. hominis in order to have a better prediction of the BV infection. Unlike for M. hominis, M. genitalium was found to have no leads on the BV infection. However, the T. vaginalis infection status could not indicate the likelihood of infection also for either M. hominis or BV.

### Table 4: Risk of acquiring Mycoplasma hominis in the presence of other genital infections.

| Variable                      | Unadjusted |          |          | Adjusted |          |          | Stepwise |
|-------------------------------|------------|----------|----------|----------|----------|----------|----------|
|                               | OR         | CI       | \( p \)-value | OR      | CI       | \( p \)-value | OR      | CI       | \( p \)-value |
| BV positive                   | 5.24       | 2.84–9.92| < 0.001  | 5.19     | 2.75–10.10| < 0.001  | 4.9      | 2.61–9.31| < 0.001  |
| BV negative (Referent)        | 1          | -        | -        | 1        | -        | -        | -        | -        | -        |
| Candida species positive      | 0.93       | 0.52–1.67| 0.805    | 1.37     | 0.71–2.71| 0.351    | -        | -        | -        |
| Candida species negative (Referent) | 1         | -        | -        | 1        | -        | -        | -        | -        | -        |
| T. vaginalis positive         | 1.69       | 0.69–4.31| 0.254    | 1.99     | 0.73–5.59| 0.181    | -        | -        | -        |
| T. vaginalis negative (Referent) | 1         | -        | -        | 1        | -        | -        | -        | -        | -        |
| M. genitalium positive        | 12.3       | 2.28–227.76| 0.018   | 9.54     | 1.58–185.73| 0.041  | 8.9      | 1.52–170.55| 0.045  |
| M. genitalium negative (Referent) | 1         | -        | -        | 1        | -        | -        | -        | -        | -        |

BV, bacterial vaginosis; T., Trichomonas; M., Mycoplasma.

### Table 5: Risk of acquiring bacterial vaginosis in the presence of other genital infections.

| Variable                      | Unadjusted |          |          | Adjusted |          |          | Stepwise |
|-------------------------------|------------|----------|----------|----------|----------|----------|----------|
|                               | OR         | CI       | \( p \)-value | OR      | CI       | \( p \)-value | OR      | CI       | \( p \)-value |
| Candida species positive      | 0.64       | 0.36–1.13| 0.127    | 0.62     | 0.33–1.16| 0.139    | 0.62     | 0.33–1.15| 0.128    |
| Candida species negative (Referent) | 1         | -        | -        | 1        | -        | -        | -        | -        | -        |
| T. vaginalis positive         | 0.99       | 0.42–2.34| 0.979    | 0.70     | 0.27–1.81| 0.459    | -        | -        | -        |
| T. vaginalis negative (Referent) | 1         | -        | -        | 1        | -        | -        | -        | -        | -        |
| M. hominis positive           | 5.37       | 2.96–9.98| < 0.001  | 5.44     | 2.94–10.39| < 0.001 | 5.43     | 2.98–10.15| < 0.001 |
| M. hominis negative (Referent) | 1          | -        | -        | 1        | -        | -        | -        | -        | -        |
| M. genitalium positive        | 3.16       | 0.91–14.60| 0.091   | 1.32     | 0.34–6.49| 0.703    | -        | -        | -        |
| M. genitalium negative (Referent) | 1         | -        | -        | 1        | -        | -        | -        | -        | -        |

T., Trichomonas; M., Mycoplasma.

Discussion

To the best of our knowledge, this is the first study to provide an estimate on the prevalence of M. hominis in pregnant women from the Durban area in South Africa. We report a prevalence estimate of 48% for M. hominis in this study population. Our data are consistent with a previous study conducted in South Africa where Redelinghuys and colleagues also reported high prevalence data for M. hominis (50.7%) in pregnant women from Gauteng, South Africa.\(^5\)

Going back to the overall study aim, which was to identify risk factors associated with M. hominis as well as to determine if M. hominis shared risk factors with other genital infections, the following factors were significantly associated with the...
prevalence of M. hominis: level of education, current abnormal vaginal discharge, past abnormal vaginal discharge and past pre-term delivery. A high proportion of women in this study had attained a high school level of education. There was a borderline significance between this variable and M. hominis status in this study. Previous studies conducted in women from KwaZulu-Natal, South Africa, have shown that women with a lower level of schooling, that is, less than high school, have more prevalent genital infections.\(^{15,16,17}\) Abbai et al. showed that women with a lower level of education are at high risk of having multiple STIs \((p = 0.034).^{15}\) Similarly, Naidoo et al. also showed that women with the prevalent STIs reported less than high school education \((p < 0.0001).^{16}\) A significant association between low level of education and prevalence of the viral STI, Herpes simplex virus-2 was also found \((p = 0.021).^{17}\)

In this study, current abnormal vaginal discharge was significantly associated with the prevalence of M. hominis. The majority of women who tested positive did not report symptoms of discharge. A similar observation was reported by Dessai et al. for a population of pregnant women from Durban, where the majority of women who tested positive for Candida reported not having a current abnormal vaginal discharge \((p < 0.001).^{18}\) In another recent study conducted by Mabaso et al., it was shown that the majority of women who tested positive for T. vaginalis did not report current symptoms of abnormal vaginal discharge \((p = 0.011).^{19}\) In this study, past abnormal vaginal discharge was also associated with the prevalence of M. hominis. The study conducted by Dessai et al. showed a borderline significance \((p = 0.06)\) for past discharge and prevalent T. vaginalis infections.\(^{18}\) Previous studies have shown an association between pre-term deliveries and M. hominis infection.\(^{7,21}\) However, the present study did not show a positive association between past history of pre-term delivery and the prevalence of M. hominis. An explanation for this could be because of the small overall number of women \((n = 40)\) who reported this event. For future association studies, a larger number of women reporting this event may be needed to see a positive association.

![Prevalence of Mycoplasma hominis amongst HIV infected and uninfected women.](image)

In the current study, a univariate and multivariate analysis was performed in order to determine if the significant variables described thus far were truly risk factors associated with M. hominis. Reported symptoms of abnormal vaginal discharge were shown to be significantly associated \((p < 0.05)\) with prevalent M. hominis in the univariate analysis. Women who presented with a current or previous abnormal discharge were two times and 77\% more likely to develop prevalent M. hominis. These findings are consistent with another study that reported on the prevalence of genital mycoplasma species, especially M. hominis, in patients presenting with vaginal discharge.\(^{22}\) However, this association was not sustained in the multivariable analysis, indicating that the abnormal vaginal discharge may not be a true risk factor for acquiring M. hominis. As shown in this study, there was a high coinfection rate of M. hominis with other infections such as BV, T. vaginalis, M. genitalium and Candida species. These coinfections could have contributed to the discharge and not necessarily M. hominis.

This is not in keeping with another published study conducted in pregnant women, which showed the association of abnormal vaginal discharge as a true risk factor for another genital pathogen.\(^{19}\)

The current study also showed that obtaining a tertiary level of education had significantly reduced the women’s risk of infection by 71\%. This finding is consistent with other previous studies conducted in KwaZulu-Natal.\(^{35,17}\) Abbai and co-workers reported a significant association between HSV-2 infection and women who had received a lower level of education.\(^{17}\) Similarly, Naidoo et al. associated women receiving lower level education with an increased risk of having a prevalent STI.\(^{36}\) However, unlike the findings described by Abbai et al.\(^{17}\) and Naidoo et al.,\(^{36}\) level of education was not significant in the multivariable analysis. Therefore, level of education cannot be deemed as a true low risk factor in this study.

In this study, high coinfection rates were observed for M. hominis with BV, Candida, T. vaginalis, M. genitalium and HIV. However, only coinfection rates between M. hominis and BV and M. hominis and M. genitalium were shown to be significant. In the adjusted analysis, BV was shown to significantly increase the risk for M. hominis by five-fold. A study conducted by Sanchez-Garcia et al. showed that BV was also significantly associated with an increased risk of positivity for M. hominis \((OR: 25.9, 95\% CI: 7.2–93.0; p = 0.001).^{21}\)

According to Panos, the presence of M. genitalium infection was associated with the presence of Mycoplasmataceae family members such as M. hominis and Ureaplasma species, more particularly, Ureaplasma species.\(^{22}\) Testing for the presence of Ureaplasma species in the study cohort is a future research direction.

This study was limited in that samples were collected from pregnant women attending a single antenatal facility. However, the hospital from which the women were sampled in this study serves as a central hospital for women from around the Durban area, thereby making the population...
more generalised. A second limitation is the lack of data on pregnancy outcomes in relation to the prevalent infections. Because of the cross-sectional nature of this study, the data were not collected; however, this limitation will be addressed in future studies. Finally, a full dataset on HIV status was not available for this study, because of the refusal to provide the data by the study women and therefore, this study was unable to draw sound conclusions regarding the association between M. hominis and HIV infections.

Conclusion
To date, there remains uncertainty regarding the role of M. hominis as a genital tract pathogen. The current study has now provided evidence from a South African-based pregnant population, indicating that M. hominis does not share common predisposing risk factors with that of known genital tract pathogens as well as the causative agents of STIs. Based on these study findings, M. hominis cannot be considered a genital tract pathogen. Previous studies have shown a high prevalence of M. hominis in the vaginal compartment. Based on the high prevalence of this pathogen in the vaginal micro-environment, future studies that investigate its explicit role in this environment are needed.

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Competing interests
The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors’ contributions
M.N., F.D., R.S., N.M., PT. and N.S.A. contributed equally to the writing of this article.

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Data availability
The data that support the findings of this study are available from the corresponding author, M.N., upon reasonable request.

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