

BMJ Open A cross-sectional study on the relationship of age, gestational age and HIV infection to bacterial vaginosis and genital mycoplasma infection

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ABSTRACT

Objectives: Pregnant women are especially at risk of developing complications when infected with reproductive tract infections (RTIs). The objective of this study was to determine the prevalence of bacterial vaginosis (BV) and genital mycoplasmas in pregnant women and investigate the associations between BV, genital mycoplasmas, HIV infection, age and gestational age.

Design: Cross-sectional study with descriptive and analytical components.

Setting: Antenatal clinic of a tertiary academic hospital in South Africa.

Participants: 220 pregnant women older than 18 were included in the study and provided self-collected vaginal swabs.

Primary and secondary outcomes: BV and genital mycoplasma colonisation and/or infection in women of differing age, gestational period and HIV status.

Results: The prevalence of BV was 17.7% (39/220) (95% CI 12.9 to 23.4), intermediate vaginal flora (IVF) 15% (33/220) (95% CI 10.56 to 20.42), and the overall prevalence of genital mycoplasmas was 84% (185/220) (95% CI 78.47 to 88.58). BV was significantly associated with HIV infection with an OR of 2.84 (95% CI 1.08 to 7.46 and p value=0.034). However, BV was inversely associated with gestational age with an OR of 0.08 (95% CI 0.01 to 0.42 and p value=0.003) for second trimester pregnancies and an OR of 0.03 (95% CI 0.01 to 0.17 and p value<0.001) for third trimester pregnancies using the first trimester as reference. IVF was significantly associated with HIV infection with an OR of 2.7 (95% CI 1.07 to 6.79 and p value=0.035) but not with age or gestational age. Genital mycoplasmas were not significantly associated with age, gestational age, HIV status, BV flora or IVF.

Conclusions: The high infection rate of genital mycoplasmas and the association of BV with HIV found in this study reiterate the importance of screening for these RTIs in high-risk groups such as pregnant women.

INTRODUCTION

Bacterial vaginosis (BV) is a dysbiosis of the vagina and is the most common cause of

Strengths and limitations of this study

- This study reports the prevalence of and associations between vaginal flora, genital mycoplasmas, age and gestational age in women attending antenatal care in an HIV endemic setting.
- The large sample size included women from a wide age range and gestational age range.
- No patient follow-up was performed to record downstream infection and/or colonisation status to investigate the dynamics of such infections during the course of pregnancy; hence, pregnancy outcome was also not measured.

vaginal discharge in women of childbearing age.¹ The prevalence of BV ranges from 10% to 50% during pregnancy across the world.^{2–4} Genital mycoplasmas, including *Mycoplasma hominis*, *M. genitalium*, *Ureaplasma parvum* and *U. urealyticum* may reach colonisation rates of even up to 80% in healthy, sexually active women.⁵ Mycoplasma infections are often elusive or subclinical in nature, and it is not always possible to prove the pathogenic nature of these bacteria.⁶ Genital mycoplasmas are primarily transmitted via sexual contact.^{7–8} Transmission may, however, be vertical from mother to offspring, either in utero or during the passage through the birth canal.⁹

Genital mycoplasmas may travel from the vagina and penetrate the amniotic membranes to produce inflammation that results in pregnancy complications.^{10–11} These ascending infections may be enhanced by BV.¹² Genital mycoplasmas are the most frequently isolated bacteria in women who present with chorioamnionitis as these are responsible for 45% of such cases.¹⁰ BV, especially when *G. vaginalis* or *M. hominis* and *U. urealyticum* are cultured, is associated with a fivefold increased risk of pregnancy loss.¹³ These reproductive tract infections (RTIs)

are implicated in medical conditions, such as pelvic inflammatory disease (PID), preterm premature rupture of membranes, preterm birth (PTB), pregnancy loss and postpartum and postabortal sepsis.^{4 5 14–16} Infection with BV and genital mycoplasmas may increase the risk of HIV acquisition and the rates of HIV shedding in the genital tract.^{17–21} In this way, BV and genital mycoplasma infections may contribute notably to morbidity and mortality. The clinical course of conventional RTIs may also be modified by HIV infection and this complicates the diagnosis and treatment of these infections.²²

Although effective treatment options are available, the treatment of BV is complicated when infection status is unknown, as screening for BV-related bacteria is not the standard of care and not routinely performed. The lack of screening for these bacteria is a matter of concern in pregnant women who are at risk of developing pregnancy complications, especially in developing countries. In many of these countries, syndromic management is used to treat RTIs, but this approach is complicated by the large number of asymptomatic infections.²³ Even though there is no clear evidence of the advantages from screening or treatment of all women who have asymptomatic BV, evidence suggests that screening and treatment in pregnant women may reduce PTB and low birthweight infants.^{24 25}

The purpose of the study was to determine the prevalence of BV and genital mycoplasmas in a population of HIV-positive and HIV-negative pregnant women attending antenatal care, and to determine the associations between BV, genital mycoplasmas, HIV infection, age and gestational age.

METHODS

Study design, setting and population

This is a descriptive, cross-sectional study with an analytical component. The study was conducted at the Department of Medical Microbiology, University of Pretoria, South Africa. Continuous participant recruitment and sample collection were carried out from July 2012 to December 2012, and laboratory procedures were conducted from July 2012 to March 2013. Samples were obtained from pregnant women attending the antenatal clinic of a tertiary academic hospital in Pretoria, Gauteng, South Africa. Criteria for inclusion into the study were: pregnant woman of any gestational period, older than 18 years of age, who gave informed consent to participate in the study, and who have not taken any antimicrobial agents 30 days prior to sample collection. All the participants gave written informed consent prior to enrolment and sample collection. Available medical information from the participants, such as rapid plasma reagin (RPR) status, current medication, HIV status (as tested by the hospital with a First Response HIV 1–2.0 card rapid test (Premier Medical Corporation Ltd, Daman, India); tests performed twice to confirm results) and current gestational age was

recorded. Gestational age was divided into three stages: first trimester (≤ 12 weeks pregnant), second trimester (13 weeks to 26 weeks pregnant) and third trimester (27–40 weeks pregnant).

Sample collection and processing

Two self-collected vaginal swabs, a dry swab and an ESwab (a flocced nylon swab containing 1 mL modified Amies transport medium (Copan Diagnostics, Inc, Italy) were obtained from every participant. The order in which the swabs were obtained was alternated between women. The women were instructed to swirl the swab once and to keep it inside the vagina for 10 s to absorb vaginal fluid. Immediately after collection, the dry swab was used to make a smear on a glass slide (B&C, Germany) and left to air dry, while the ESwab was used to seed 1 mL of Amies transport medium and stored on ice. The swabs were kept on ice and transported to the laboratory within 1–4 h, and were then processed for further analyses, while the vaginal smears were transported dry.

Microscopy

The dry vaginal smears were fixed to the slides by heating for 5 s using a Bunsen burner, followed by Gram-staining. Gram-stained smears were subsequently graded with the Nugent scoring system as described by Nugent *et al.*²⁶ Based on the resulting Nugent scores, the vaginal flora were categorised as normal vaginal flora (NVF; scores 0–3), intermediate vaginal flora (IVF) (scores 4–6), or BV flora (scores 7–10).

Molecular detection of genital mycoplasmas

DNA extraction from modified Amies transport medium

Bacterial DNA was isolated from the vaginal swabs with the ZR Fungal/Bacterial DNA kit (Zymo Research, USA) according to the manufacturer's instructions. A volume of 150 μ L seeded Amies transport medium (Copan Diagnostics, Inc, Italy) was used to extract bacterial DNA. One hundred microlitres of ultra-pure DNA was eluted and stored at -20°C until further analysis.

Amplification of human β -globin gene

Each sample was subjected to an internal positive control β -globin PCR assay to ensure that amplifiable DNA was successfully extracted from the sample, to monitor for PCR inhibitors, and to exclude false-negative results.²⁷ The oligonucleotide primer sequences used were obtained from Martin *et al.*²⁸ All β -globin gene PCRs were performed using the EmeraldAmp GT PCR Master Mix (Takara Biotechnology, Japan) in a Gradient MasterCycler (Eppendorf, Hamburg, Germany) according to the manufacturer's instructions. The amplification programme comprised an initial denaturation step at 95°C for 15 min, 40 cycles of three steps including denaturation at 95°C for 30 s, annealing at 56°C for 45 s and extension 72°C for 1 min, followed by a final extension step at 72°C for 7 min.

Multiplex-PCR assay for the detection of genital mycoplasmas

The samples that were positive for the β -globin gene were subjected to a multiplex PCR assay to determine the presence of *M. genitalium*, *M. hominis*, *U. parvum* and *U. urealyticum*. The multiplex PCR assay was adapted from the method used by Stellrecht *et al.*²⁹ The primers used in this assay targeted the 140 kDa adhesion protein of *M. genitalium*, the 16S rRNA of *M. hominis* and multiple-banded antigen (MBA) genes of *U. parvum* and *U. urealyticum*. The multiplex PCR assay was validated with the AmpliRun *Mycoplasma genitalium* DNA control (Vircell SL, Spain), a positive *M. hominis* sample isolated with A2 agar and reference strains ATCC 27813 (*U. parvum*) and ATCC 27619 (*U. urealyticum*). The Qiagen multiplex PCR kit (Qiagen, Germany) was used according to the manufacturer's instructions. Amplification was performed in a Gradient MasterCycler (Eppendorf, Hamburg, Germany) using the following conditions: 1 cycle of 15 min at 95°C, followed by 40 three-step cycles at 94°C for 30 s, 60°C for 90 s and 72°C, followed by a final extension of 10 min at 72°C.

Statistical analysis

Categorical data were expressed as percentages. Continuous variables were reported using measures of central tendency and dispersion; for normally distributed data, the mean and SD were used to describe the data. Statistical analyses were performed using STATA V.13.1. Associations were assessed using contingency tables for different variables. A p value of <0.05 was considered significant. Further evaluation was carried out using univariate logistic regression for each dependent outcome with the factors (independent), with a 95% CI. Univariate logistic regression was followed by a multivariable logistic regression analysis to adjust for potential confounders. Only variables that were significant (ie, p value <0.05) in the univariate analyses were included in the multivariable analysis. Multinomial logistic regression was carried out for the vaginal flora categories, including NVF (reference category), IVF and BV. Vaginal flora categories (IVF and BV) and genital mycoplasmas were dependent variables whereas age, gestational age and HIV infection were the independent variables. Furthermore, genital mycoplasmas were used as the independent variable to measure associations between the vaginal flora categories and individual genital mycoplasma species. The test of multicollinearity was also evaluated using the variance inflation factor (vif). In addition, generally STATA has an inbuilt system of assessing multicollinearity in model fitting. Wherever multicollinearity exists, the parameter is dropped out automatically.

RESULTS

Two hundred and twenty pregnant women were included in this study. The mean age of the population

sampld was 30±5.95 years. The mean gestational age of current pregnancies of participating women was 26 ±7.97 weeks. All 220 samples tested positive for the human β -globin gene and all participating women had a negative RPR status. The HIV status of 201 out of 220 women was available and included 36 HIV-positive women. All the HIV-related statistical analyses were carried out according to this subset of women.

A total of 39 (17.7%) (95% CI 12.9% to 23.4%) women had BV flora while 33 (15%) (95% CI 10.56% to 20.42%) women had IVF and 148 (67.3%) (95% CI 60.67% to 73.45%) women had NVF. BV was detected in 9 (25%) of 36 HIV-positive women, and in 26 (15.8%) of 165 HIV-negative women. IVF was also detected in 9 (25%) of 36 HIV-positive women and in 22 (13.3%) of 165 HIV-negative women. BV and IVF were significantly associated with HIV infection with respective ORs of 2.84 (95% CI 1.08 to 7.46 and p value=0.034) and 2.7 (95% CI 1.07 to 6.79 and p value=0.035). Women of age 18 to 24 had the highest proportion of BV infection (20.4%) (95% CI 10.24 to 34.34). In addition, BV was inversely associated with gestational age with an OR of 0.08 (95% CI 0.01 to 0.42 and p value=0.003) for second trimester pregnancies, and an OR of 0.03 (95% CI 0.01 to 0.17 and p value<0.001) for third trimester pregnancies, the first trimester being used as reference. BV and IVF were positively associated with age; however, none of these associations were statistically significant.

The results of the multiplex PCR assay indicated that *M. hominis* was isolated from 111 (50.4%) (95% CI 43.60% to 57.19%) samples, *M. genitalium* from 33 (15%) (95% CI 10.56% to 20.42%) samples, *U. parvum* from 157 (70.2%) (95% CI 63.68% to 76.16%) samples, and *U. urealyticum* from 5 (2.8%) (95% CI 1.05% to 5.94%) samples. The positivity rate for samples with at least one genital mycoplasma species present was 84% (185/220) (95% CI 78.47% to 88.58%). There was no significant difference in mycoplasma colonisation across the three trimesters (table 1) of pregnancy or with increasing age (table 2). *Ureaplasma urealyticum* was not included in the tables because there were not enough observations in the different categories for comparison.

Thirty-eight out of 39 samples (97%) with a high Nugent score ≥ 7 (BV positive), and 27 out of 33 samples (81.8%) with an intermediate Nugent score (4–6) were positive for at least one genital mycoplasma species. In samples with a Nugent score of ≥ 7 , *U. parvum* was detected in 33 (84.6%), *M. hominis* in 28 (71.8%), *M. genitalium* in 7 (17.9%) and *U. urealyticum* in 1 (2.7%) sample. In samples with an intermediate Nugent score, *U. parvum* was detected in 22 (66.7%), *M. hominis* in 18 (54.5%), *M. genitalium* in 5 (15.2%) and *U. urealyticum* in 1 (3%) sample. Fisher's exact test showed no association between BV and individual mycoplasmas (*Ureaplasma parvum* (p value=0.139), *M. hominis* (p value=0.954), *M. genitalium* (p value=0.183) and *U. urealyticum* (p value=0.585)). In the HIV-positive group, *Ureaplasma parvum* was detected in 26 (72.2%),

Table 1 Distribution of BV, IVF and genital mycoplasmas across each trimester of pregnancy in pregnant women attending antenatal care

Reproductive tract infection	Gestational age								Total (n=220)
	Trimester 1 (n=13) (Reference)		Trimester 2 (n=98)			Trimester 3 (n=109)			
	Prevalence (%)	OR	Prevalence (%)	OR and 95% CI	p Value	Prevalence (%)	OR and 95% CI	p Value	
BV	8 (61.5)	1	21 (21.4)	0.08 (0.01 to 0.42)	0.003	10 (9.2)	0.03 (0.01 to 0.17)	<0.001	39
IV flora	1 (7.6)	1	13 (13.3)	0.33 (0.03 to 4.11)	0.396	19 (17.4)	0.44 (0.04 to 5.11)	0.508	33
<i>M. genitalium</i>	1 (7.6)	1	13 (13.3)	1.84 (0.22 to 15.32)	0.575	19 (17.4)	2.53 (0.31 to 20.67)	0.385	33
<i>M. hominis</i>	5 (38.5)	1	51 (52)	1.74 (0.53 to 5.68)	0.362	55 (50.5)	1.63 (0.50 to 5.30)	0.417	111
<i>U. parvum</i>	9 (69.2)	1	67 (67.4)	0.96 (0.27 to 3.36)	0.950	81 (74.3)	1.29 (0.37 to 4.50)	0.694	157

BV, bacterial vaginosis; IVF, intermediate vaginal flora.

Table 2 Distribution of BV, IVF and genital mycoplasmas across three age categories in pregnant women attending antenatal care

Reproductive tract infection	Age category								Total (n=220)
	18–24 years (n=49) (Reference)		25–34 years (n=120)			>35 years (n=51)			
	Prevalence (%)	OR	Prevalence (%)	OR and 95% CI	p Value	Prevalence (%)	OR and 95% CI	p Value	
BV	10 (20.4)	1	20 (16.7)	1.21 (0.42 to 3.48)	0.726	9 (17.6)	1.15 (0.32 to 4.06)	0.831	39
IV flora	6 (12.2)	1	18 (15)	1.25 (0.44 to 3.54)	0.679	9 (17.6)	1.54 (0.47 to 5.15)	0.475	33
<i>M. genitalium</i>	9 (18.4)	1	16 (13.3)	0.78 (0.29 to 2.09)	0.615	8 (15.7)	1.27 (0.41 to 3.93)	0.674	33
<i>M. hominis</i>	26 (53.1)	1	63 (52.5)	0.56 (0.28 to 1.12)	0.102	22 (43.1)	0.69 (0.30 to 1.62)	0.398	111
<i>U. parvum</i>	42 (85.7)	1	81 (67.5)	0.81 (0.38 to 1.73)	0.583	34 (66.7)	1.72 (0.63 to 4.74)	0.291	157

BV, bacterial vaginosis; IVF, intermediate vaginal flora.

M. hominis in 27 (75%), *M. genitalium* in 8 (22.2%) and *U. urealyticum* in 2 (5.5%) women. However, none of these results were significantly different from that respectively observed among HIV-negative women; in the HIV-negative group, *Ureaplasma parvum* was detected in 117 (70.9%), *M. hominis* in 74 (44.8%), *M. genitalium* in 22 (13.3%) and *U. urealyticum* in 2 (1.2%) women.

DISCUSSION

In this study, the prevalence of RTIs in pregnant women was high. BV was present in 17% of the pregnant women and was more strongly associated with the early stages of pregnancy. In addition, BV flora was more common in HIV-positive women. All the mycoplasma species, except for *U. urealyticum*, were present in higher numbers in samples with a high Nugent score (≥ 7) as compared to samples with an intermediate Nugent score (4–6). *Ureaplasma parvum* was the most commonly isolated species and contributed to the high prevalence of genital mycoplasmas found in this study.

This study describes the prevalence of and associations between BV and genital mycoplasmas in women attending antenatal care in an HIV-endemic setting. The study sample size was adequate with a good representation of pregnant women from a wide age range and gestational age range. The investigators failed to discriminate between symptomatic and asymptomatic women with regard to BV. The symptoms that women reported were vague and subjective. Most women could not distinguish between a discharge characteristic of BV or a physiological discharge. Some women described having a discharge that might have been more characteristic of other vaginal infections, such as those caused by *Candida* spp. or *Trichomonas vaginalis*. However, vaginal samples were not assessed for any other RTIs apart from BV and genital mycoplasmas. The prevalence of RTIs found in this study was just a snapshot in time; no follow-up of patients was carried out (which is a problem with cross-sectional studies in general). It was, therefore, difficult to make any causal inferences. Nonetheless, the data available allowed sufficient conclusions to be drawn.

The prevalence of BV found in this study corresponds to the prevalence rates of 5% to 25% in (asymptomatic) pregnant women reported by Tolosa *et al.*³⁰ The association between BV and HIV reported by Myer *et al.*³¹ was confirmed in this study. Bayraktar *et al.*³² reported a prevalence of 29% for genital mycoplasmas in Turkey, using A7 agar to determine the presence of *M. hominis* and *U. urealyticum*. Koh *et al.*³³ used the Mycoplasma IST-2 kit to detect these two species and found a prevalence of 44.2% in South Korea. The overall prevalence of genital mycoplasmas in the present study (as determined by PCR) was high (84%) when compared to these two studies. The higher prevalence obtained with a molecular method is expected as this method is more sensitive than culture tests. However, molecular methods detect viable and non-viable bacteria, and do not

indicate current infection/colonisation. There are contentious reports on the prevalence of *Ureaplasma* spp. De Francesco *et al.*³⁴, Povlsen *et al.*³⁵ and Kataoka *et al.*¹⁵ reported *U. parvum* to be the most prevalent genital mycoplasma in reproductive-age women (prevalence rates of 17–64%), whereas Koh *et al.*³³ and Zdrodowska-Stefanow *et al.*⁷ reported *U. urealyticum* to be the most prevalent genital mycoplasma, with prevalence rates of 22–39%. Many studies that investigated ureaplasmas did not discriminate between the two different species^{5 32} as was carried out in this study. The results reported by these studies may have given a skewed representation of infection rates. Although *U. urealyticum* has been implicated in more pathogenic cases, inconsistent findings exist as to which *Ureaplasma* species is the most pathogenic.^{15 36} *M. hominis* is present in 58–76% of women with BV, and *U. urealyticum* in 62–92% of women with BV.³⁷ The findings of *M. hominis* in the present study are similar to what Hill³⁷ reported as this species was present in 71.7% of women with BV. This association was, however, not significant and is supported by Keane *et al.*³⁸ The present study's finding agrees with the findings of Govender³⁹ in 2010, who reported no association between the colonisation of *M. hominis* and HIV status. The prevalence of *M. genitalium* found in this study corresponded with other studies^{40–43} that reported a prevalence rate of 3–26.3% in women. Although information on the adverse effects of *M. genitalium* in pregnancy is limited, there may be an association between *M. genitalium* infection and preterm birth (PTB) in pregnancy.⁴³

The high prevalence of RTIs found in this study increases the risk for women and their unborn babies to develop health problems. This increased risk is a result of potential ascending infections and eventually poor pregnancy outcomes. Infectious agents involved in ascending infections may be passed on to babies during pregnancy or birth, and result in neonatal death, sepsis, pneumonia and stillbirth.⁴⁴ In addition, undetected and untreated infections can lead to PID, which may lead to infertility or ectopic pregnancies.⁴⁴ These complications will lead to an increased demand for healthcare assistance and an unnecessary downstream of financial burdens. These RTIs also increase the risk of acquiring other infections, including HIV, up to threefold.⁴⁴ Women who are already HIV-positive and have untreated BV infections are at risk of having higher mucosal HIV viral loads (HIV shedding) because BV flora may upregulate viral replication through a heat-stable HIV-inducing factor.^{45–49} The increased shedding of virus particles translates to increased sexual partner infections and babies born with complications.

The potential impacts of highly prevalent BV and genital mycoplasmas should be investigated in more defined populations, focusing on maternal and fetal health. These populations can include women with recent adverse pregnancy outcomes, or a large pregnant and HIV-positive cohort. This study could be improved by conducting an outcome-based study within two or more

different time frames where pregnant women are followed up. Practitioner-collected swabs may be better to correlate symptoms with specific infections, especially if the efficacy of antibiotics and pregnancy outcomes are investigated. The non-significant trend of younger women to be infected with BV and genital mycoplasmas indicates the need to focus on younger age groups to inform and educate them on the risks and preventative measures of RTIs. In resource-poor countries, the potential to screen all women for most RTIs is expensive and not always feasible.⁵⁰ Based on the main study findings, it is recommended that studies assess the advantage of screening pregnant women for BV and genital mycoplasmas, at least at the first antenatal visit to the clinic (ie, targeted screening in a high-risk group). This screening approach could help identify asymptomatic cases and correctly identify symptomatic cases; combined with the correct treatment regimen, this screening may reduce pregnancy complications and HIV genital viral load in HIV-positive women.^{4 51}

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Contributors MJR is the project leader and was involved in concept design, sample procurement, laboratory work, data analysis and writing of the manuscript. MMK is the principal investigator and grant holder. MMK and MME were involved in the conceptual design of the study as well as review of the manuscript. AWD contributed towards the clinical aspect of the study and was involved in review of the manuscript. HL was involved in the conceptual design of the study as well as in overseeing the logistics of sample procurement. SASO was involved in data and statistical analysis. All authors read and approved the final manuscript.

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