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Factors associated with *Trichomonas vaginalis* infection in reproductive-aged women attending cervical screening in southeast of Brazil



Gabriel Vitor da Silva Pinto ^{a,1}, Aline do Nascimento Bolpet ^{a,1},
 Laura Fernandes Martin ^a, Natália Prearo Moço ^a,
 Bruna Ribeiro de Andrade Ramos ^a, Mariana de Castro Silva ^a,
 Marli Teresinha Casamassimo Duarte ^b, Andréa da Rocha Tristão ^c,
 Márcia Guimarães da Silva ^{a,*}, Camila Marconi ^{a,d}

^a Universidade Estadual Paulista (UNESP), Faculdade de Medicina de Botucatu, Departamento de Patologia, Botucatu, SP, Brazil

^b Universidade Estadual Paulista (UNESP), Faculdade de Medicina de Botucatu, Departamento de Enfermagem, Botucatu, SP, Brazil

^c Universidade Estadual Paulista (UNESP), Faculdade de Medicina de Botucatu, Departamento de Ginecologia e Obstetrícia, Botucatu, SP, Brazil

^d Universidade Federal do Paraná (UFPR), Setor de Ciências Biológicas, Departamento de Patologia Básica, Curitiba, PA, Brazil

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ABSTRACT

Background: Sexually Transmitted Infections (STIs) can be caused by viruses, bacteria, and parasites. The World Health Organization estimated more than 300 million new global cases of curable STIs among individuals of reproductive age. Infection by *Trichomonas vaginalis* is one of the most prevalent curable STI. Despite the current treatments available, the diagnosis of *T. vaginalis* can be difficult, and the resistance to the treatment increased concern for the healthcare system.

Objectives: The aim of this study was to determine the prevalence and factors associated with *Trichomonas vaginalis* infection among women of reproductive age attending community-based services for cervical screening.

Patients and methods: A total of 1477 reproductive-aged women attending 18 Primary Health Care Units in Botucatu, Brazil, from September to October 2012, were enrolled. A structured questionnaire was used for individual face-to-face interviews for obtaining data on socio-demographic, gynecologic, and obstetrics history, sexual and hygiene practices, among others. Cervicovaginal samples were obtained for detection of *T. vaginalis* by culture using Diamond's medium and microscopic vaginal microbiota classification according to Nugent. A multivariable logistic regression analysis was carried out to estimate Odds Ratios (OR)

* Corresponding author.

E-mail address: marcia.guimaraes@unesp.br (M.G. da Silva).

¹ These authors have equally contributed the present work.

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and 95% Confidence Intervals (95% CI) for the association between participants' sociodemographic, behavioral factors, and clinical factors with *T. vaginalis* infection.

Results: Median age of study participants was 33 years (ranging from 18 to 50). The overall prevalence of *T. vaginalis* infection was 1.3% ($n = 20$). Several factors were independently associated with *T. vaginalis* infection, such as self-reporting as black or Pardo for ethnicity (OR = 2.70; 95% CI 1.03–7.08), smoking (OR=3.18; 95% CI 1.23–8.24) and having bacterial vaginosis (OR = 4.01; 95%CI = 1.55–10.38) upon enrollment. A protective effect of higher educational level (having high school degree) was observed (OR = 0.16; 95% CI 0.05–0.53).

Conclusions: Our data suggest that screening programs to correctly detect *T. vaginalis* infection can be helpful to guide prevention strategies to the community. Our study supports an association between abnormal vaginal microbiota and *T. vaginalis* infection.

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Introduction

Sexually transmitted infections (STI) are caused by several microorganisms such as viruses, bacteria, and parasites that are transmitted primarily by vaginal, oral, or anal sexual contact.¹ According to the Centers for Disease Control and Prevention (CDC) of the United States, the annual impact of STIs in 2018 on the North American healthcare system was nearly 16 billion in direct medical costs.² The World Health Organization (WHO) estimated 374 million new global cases as one of the most common curable STIs among individuals from 15 to 49 years-old only in 2020. Of these, infection by *Trichomonas vaginalis* is the most prevalent curable STI accounting for nearly half of the cases (156 million).³ Despite the existing treatment regimens with antimicrobial drugs, infections by *T. vaginalis* are frequently a(oligo)symptomatic and diagnosis is not equally available worldwide, being particularly difficult in low resources regions.

T. vaginalis is a typically pyriform and occasionally amoeboid protozoan with primarily anaerobic metabolism that infects the genitourinary tract epithelium gender-independently.⁴ Due to its characteristic morphology, microscopy methods have been largely employed for diagnostic purposes, especially for scrutinizing status of culture of clinical samples in specific media. Most commonly reported signs and symptoms include dyspareunia and vaginal discharge, that may be diffuse, malodorous, or yellow green, with or without vulvar irritation. Also, in clinical examination cervical hemorrhagic punctate may be observed, which is often referred to as a "strawberry cervix".⁵

Due to the occurrence of pelvic inflammatory disease^{6,7} and evidence that the infection reduces the viability of sperm, men and women may have reproductive consequences as a result of infection.⁸ Trichomoniasis may also impact the pregnancy course, causing low birth weight, premature rupture of membranes, and preterm delivery.⁹

Two population-based studies in the United States observed rates of 2.3% among adolescents¹⁰ and 3.1% among women with 14 to 49 years old.¹¹ In Brazil, studies show that prevalence range from 2.6% to 20% in different settings,^{12–15} while the Brazilian Ministry of Health estimates an overall *T. vaginalis* prevalence of 15%.¹⁶

Several factors were already associated with the incidence of *T. vaginalis* infection such as younger ages, sexual activity, increased number of sexual partners, condom use, having other STIs, social demographic, and low economic conditions.^{2,3,17,18} Literature has also shown that *T. vaginalis* infection is associated with changes in the components of the vaginal microbiota, such as in bacterial vaginosis.^{19–24} Additionally, it was demonstrated that the presence of *T. vaginalis* infection increases susceptibility to the Human Immunodeficiency Virus (HIV).²⁵

Studies of prevalence and risk factors for *T. vaginalis* infection are therefore of great importance to the health care system for the adoption of decisions and for the development of new guidelines for routine primary health care. Thus, this study aimed to identify sociodemographic and clinical factors associated with the prevalence of *T. vaginalis* infection in reproductive-aged women in community-based clinics in southeast Brazil.

Materials and methods

Study population and design

This cross-sectional study was carried out in Botucatu, São Paulo, Brazil, from September to October 2012, and included a total of 1477 women of reproductive age who provided informed consent to participate in the study. This study was approved by the Ethics Committee Board of Botucatu Medical School (UNESP), protocol 4121–2012.

The community-based health system in Botucatu comprises 18 Primary Health Care Units which were the enrollment settings for the present study. Primary healthcare units are part of the Brazilian public health system and provide free medical care to the population of Botucatu. The reasons for consultation were 100% cervical screening program with or without genital symptom complaints.

The criteria for eligibility included women who were: non-pregnant, non-menopausal, no hysterectomy, no prior report of seroconversion for HIV, no antibiotic at least four weeks prior to the study or vaginal cream used in the preceding 30 days, and abstinence from sexual intercourse for 72 hours before the visit. A structured questionnaire

containing 58 questions was administered by face-to-face interview to collect information on demographics characteristics, clinical history, and patients' practices that predispose them to *T. vaginalis* infections was applied by trained nursing from Botucatu Medical School of São Paulo State University (FMB-UNESP). Following the interview, women were submitted to a gynecological examination. After inserting a non-lubricated speculum, routine pap smear was collected and cervical cytology was reported according to the Bethesda system. A sample from mid-vaginal wall was collected using a cotton swab to evaluate the vaginal microbiota pattern, and an additional sample from vaginal posterior fornix was taken to assess infection by *T. vaginalis*.

Detection of *Trichomonas vaginalis*

Infection by *T. vaginalis* was examined by culture of vaginal posterior fornix samples in modified Diamond's medium at 37 °C in 5% CO₂. Using a clean glass slide without cover and a sterile pipette, fresh wet-mount microscope slides were prepared with aliquots from the culture and examined microscopically under ×40 objectives.²⁶ Presence of motile protozoan was checked daily, if it was not observed, the culture was incubated again with daily examination for up to 3 days in the same manner for the same trained observer. Whether *T. vaginalis* were not detected, the specimen was considered negative.

Microscopy evaluation of the vaginal microbiota

Vaginal samples were spread onto glass microscope slides to classify vaginal microbiota following Gram-stain according to Nugent's scoring system: normal (scores 0–3), intermediate (4–6), and bacterial vaginosis (7–10), regardless of the presence of symptoms or clinical findings.²⁷ Vaginal candidiasis was based on the presence of yeast blastospores or pseudohyphae on the smears with accompanying inflammatory cells in the presence of vulvovaginal pruritus.²⁸ All smears were evaluated by an experienced microscopist. All women with abnormal vaginal microbiota and/or positive for *Candida* spp. and *T. vaginalis* were treated in accordance with a protocol established by the Health Care Units of Botucatu.

Chlamydia trachomatis and *Neisseria gonorrhoeae* testing

For the evaluation of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection by PCR, DNA extraction was obtained with a previous digestion with proteinase K. The presence and integrity of DNA samples were confirmed by the amplification of β -globin housekeeping gene using primers GH20 (GAA GAG CCA AGG ACA GGT AC) and PCO4 (CAA CTT CAT CCA CGT TCA CC) which target a 268-base pair sequence.²⁹ To verify the presence of *C. trachomatis* infection in the sample, a multiplex PCR was performed using two pairs of specific primers. Primer pairs used were CTP1 (TAG TAA CTG CCA CTT CAT CA); CTP2 (TTC CCC TTG TAA TTC GTT GC) product site: 201 bp sequence, and PL6.1 (5'AGA GTA CAT CGG TCA ACG A 3'); PL6.2 (5'TCA CAG CGG TTG CTC GAA GCA 3') product site: 130 bp sequence, using Taq DNA Polymerase (Platinum,

Invitrogen®). Amplification parameters consisted of 40 cycles of 60 s at 95 °C, 60 s at 55 °C, and 90 s at 72 °C. In all reactions, negative and positive controls were run simultaneously, with respectively sterile water and *C. trachomatis* DNA extracted from infected McCoy cells.³⁰ The evaluation of *N. gonorrhoeae* was performed with specific primers for PCR: HO1 (5'GCT ACG CAT ACC CGC GTT GC 3') and H03 (5'CGA AGA CCT TCG AGC AGA CA 3'). PCR was performed using the Go Taq Green master mix (Promega Corporation, USA). Amplification parameters consisted of 40 cycles of 30 s at 94 °C, 60 s at 55 °C, and 30 s at 74 °C.³¹ Amplicon molecular weight was determined by comparing to a standard size marker, under UV transillumination.

Statistical analysis

All analyses were performed using Stata/SE 17 (StataCorp, College Station, TX). Descriptive statistics for continuous and categorical variables were performed utilizing non-parametric Mann-Whitney and test and Chi-Squared tests according to the status of *T. vaginalis* infection, with p-values <0.05 considered to be statistically significant. Crude univariate logistic regression analysis was performed to estimate the Odds Ratio (OR) and 95% Confidence Intervals (95% CI) for the association between covariates (sociodemographic, gynecologic and obstetrics history, sexual and hygiene practices, and others) and *T. vaginalis* infection. In addition, a multivariable logistic regression analysis was performed using a forward stepwise model (variables were retained at p-value ≤0.15) with positivity for *T. vaginalis* set as the dependent variable.

Results

For this study, 1477 women were enrolled and provided full data available for analysis. Median age of participants was 33 years old (ranging from 18 to 50); the majority self-defined their ethnicity as white (62.0%, *n* = 917) and most of the women were married or living in a steady partner (65.2%, *n* = 964). In relation to educational level, 59.2% (*n* = 875) had completed high school. Microscopic analysis of vaginal smears obtained upon study enrollment showed that bacterial vaginosis was detected in 29.8% (*n* = 440) of the study population (Table 1).

The reported prevalence of *T. vaginalis* was 1.3% (20/1477). Table 2 shows the results comparing population characteristics according to *T. vaginalis* status provided. Most of positive cases of *T. vaginalis* were observed among women that self-reported as black or Pardo (65.0%, *n* = 13), did not complete high school (80.0%, *n* = 16), presented habits as smoking in 50.0%, (*n* = 10) or alcohol consumption in 60.0% (*n* = 12), and had microscopic bacterial vaginosis at the time of enrollment was 35.0% (*n* = 13).

Sociodemographic characteristics, sexual behavior, and clinical history were calculated by the logistic regression model considering the *T. vaginalis* infection as the variable response, estimating each variable's effects as independent. While the prevalence of *T. vaginalis* was neither sociodemographic characteristics nor sexual behavior dependent (*p* > 0.05), abnormal microbiota was independently associated

Table 1 – Sociodemographic, gynecological, and behavioral characteristics of patients according to their *Trichomonas vaginalis* infection status.

	Overall
Characteristics	
Age (y)	33 (18–50)
Ethnicity	
White	917 (62.0)
Black/Pardo	539 (36.4)
Other (Asian, native American)	23 (1.6)
Steady partner	
No (single, divorced, widowed)	515 (34.8)
Yes	964 (65.2)
High school	
No	604 (40.8)
Yes	875 (59.2)
Income (monthly per family member)	
Less than minimum wage	1008 (68.2)
Minimum wage or more	471 (31.9)
Hormonal contraceptive use (past 4 months)	
No	899 (60.8)
Yes	580 (36.2)
Condom (consistent)	
No	1196 (80.9)
Yes	283 (19.1)
Recent genital complains (week prior to enrollment)	
No	651 (44.0)
Yes	828 (56.0)
Previous acknowledged STDs	
No	1349 (91.2)
Yes	130 (8.8)
Age at sexual debut	
15 years-old or younger	921 (62.3)
16 years-old or older	558 (37.7)
Sex with women	
No (only has sex with man)	1455 (98.4)
Yes (sex with women exclusively or not)	24 (1.6)
Has casual sex partners?	
No	1090 (73.7)
Yes	389 (26.3)
Smoker	
No	1201 (81.2)
Yes	278 (18.8)
Alcohol	
No	942 (63.7)
Yes	537 (36.3)
Illicit drugs	
No	1444 (97.6)
Yes	35 (2.4)
Other STIs (current)^c	
No	944 (63.8)
Yes	353 (36.2)
Pap-test	
Normal	1425 (96.3)
ASCUS or AGUS	19 (1.3)
LSIL	26 (1.8)
HSIL	9 (0.6)
Bacterial vaginosis (Nugent classification)	
No	1039 (70.3)
Yes	440 (29.8)

Continuous variables are displayed in median (range) and discrete variables in number (%). ASCUS, Atypical Squamous Cells of Undetermined Significance; AGUS, Atypical Glandular cells of Undetermined Significance; LSIL, Low-grade Squamous Intraepithelial Lesion; HSIL, High-grade Squamous Intraepithelial lesion.

Table 2 – Sociodemographic, gynecological, and behavioral characteristics of patients according to their *Trichomonas vaginalis* infection status.

Characteristics ^b	Trichomonas vaginalis culture		p-value ^a
	Positive	Negative	
Age (y)	35.5 (23–48)	33 (18–50)	0.06
Ethnicity			0.03
White	7 (35.0)	910 (62.4)	
Black/Pardo	13 (65.0)	526 (36.0)	
Other (Asian, native American)	0 (0.0)	23 (1.6)	
Steady partner			0.06
No (single, divorced, widowed)	11 (55.0)	504 (34.5)	
Yes	9 (45.0)	955 (65.5)	
High school			<0.001
No	16 (80.0)	588 (40.3)	
Yes	4 (20.0)	871 (59.7)	
Income (monthly per family member)			0.76
Less than minimum wage	13 (65.0)	995 (68.2)	
Minimum wage or more	7 (35.0)	464 (31.8)	
Hormonal contraceptive use (past 4 months)			0.09
No	16 (80.0)	883 (60.5)	
Yes	4 (20.0)	576 (39.5)	
Condom (consistent)			0.92
No	16 (80.0)	1180 (80.9)	
Yes	4 (20.0)	279 (19.1)	
Recent genital complains (week prior to enrollment)			0.59
No	10 (50.0)	641 (43.9)	
Yes	10 (50.0)	818 (56.1)	
Previous acknowledged STDs			–
No	20 (100.0)	1329 (91.1)	
Yes	0 (0.0)	130 (8.9)	
Age at sexual debut			0.83
15 years-old or younger	12 (60.0)	909 (62.3)	
16 years-old or older	8 (40.0)	550 (37.7)	
Sex with women			–
No (only has sex with man)	20 (100.0)	1435 (98.4)	
Yes (sex with women exclusively or not)	0 (0.0)	24 (1.6)	
Has casual sex partners?			0.71
No	14 (70.0)	1076 (73.8)	
Yes	6 (30.0)	383 (26.2)	
Smoker			<0.01
No	10 (50.0)	1191 (81.6)	
Yes	10 (50.0)	268 (18.4)	
Alcohol			0.03
No	8 (40.0)	934 (64.0)	
Yes	12 (60.0)	525 (36.0)	
Illicit drugs			0.45
No	19 (95.0)	1425 (97.7)	
Yes	1 (5.0)	34 (2.3)	

Table 2 (continued)

	<i>Trichomonas vaginalis</i> culture		p-value ^a
	Positive	Negative	
Other STIs (current)^c			0.56
No	14 (70.0)	930 (63.7)	
Yes	6 (30.0)	529 (36.3)	
Pap-test			0.14
Normal	18 (90.0)	1407 (96.4)	
ASCUS or AGUS	1 (5.0)	18 (1.2)	
LSIL	0 (0.0)	26 (1.8)	
HSIL	1 (5.0)	8 (0.6)	
Bacterial vaginosis (Nugent classification)			<0.01
No	7 (35.0)	1032 (70.7)	
Yes	13 (35.0)	427 (29.3)	

ASCUS, Atypical Squamous Cells of Undetermined Significance; AGUS, Atypical Glandular cells of Undetermined Significance; LSIL, Low-grade Squamous Intraepithelial Lesion; HSIL, High-grade Squamous intraepithelial Lesion.

^a Calculated using Chi-Squared test for categorical and Mann-Whitney non-parametric test for continuous variables.

^b Continuous variables displayed in median (range) and discrete variables in number (%).

^c Positive at molecular testing for *Chlamydia trachomatis*- and/or *Neisseria gonorrhoeae*.

Table 3 – Values of OR and 95% CI were obtained at univariate and multivariable stepwise logistic regression analysis for the population factors according to the status of *Trichomonas vaginalis* infection.

	Crude		Multivariable	
	OR	95% CI	OR	95% CI
Age (y)	1.05	0.99–1.10		
Ethnicity				
White	1.00	–	1.00	–
Black/Pardo	3.21	1.27–8.10	2.70	1.03–7.08
Other ^b	–	–	–	–
Steady partnership				
No (single, divorced, widowed)	1.00	–	1.00	–
Yes	0.43	0.18–1.05	0.37	0.14–0.94
High school				
No	1.00	–	1.00	–
Yes	0.17	0.06–0.51	0.16	0.05–0.53
Income (monthly per family member)				
Less than minimum wage	1.00	–	1.00	–
Minimum wage or more	1.15	0.46–2.91	2.61	0.95–7.17
Hormonal contraceptive (4 months or more)				
No	1.00	–		
Yes	0.38	0.13–1.15		
Condom (consistent)				
No	1.00	–		
Yes	1.06	0.35–3.19		
Recent genital complains (week prior to enrollment)				
No	1.00	–		
Yes	0.78	0.32–1.89		

Table 3 (continued)

	Crude		Multivariable	
	OR	95% CI	OR	95% CI
Previous acknowledged STDs				
No	–	–		
Yes				
Age at sexual debut				
15 years–old or younger	1.00	–		
16 years–old or older	1.10	0.45–2.71		
Sex with women				
No (only has sex with man)	–	–		
Yes (exclusively or not)				
Has casual sex partners?				
No	1.00	–		
Yes	1.20	0.46–3.15		
Smoker				
No	1.00	–	1.00	–
Yes	4.44	1.83–10.78	3.18	1.23–8.24
Alcohol				
No	1.00	–		
Yes	2.67	1.08–6.57		
Illicit drugs				
No	1.00	–		
Yes	2.20	0.29–16.9		
Other STIs (current)^b				
No	1.00	–		
Yes	0.75	0.29–1.97		
Pap-test				
Normal	1.00	–		
ASCUS or AGUS	4.34	0.55–34.30		
LSIL	–	–		
HSIL	9.78	1.16–82.24		
Bacterial vaginosis (Nugent classification)				
No	1.00	–	1.00	–
Yes	4.49	1.78–11.33	4.01	1.55–10.38

ASCUS, Atypical Squamous Cells of Undetermined Significance; AGUS, Atypical Glandular cells of Undetermined Significance; LSIL, Low-grade Squamous Intraepithelial Lesion; HSIL, High-grade Squamous Intraepithelial Lesion.

^aAsian, native American.

^b Positive at molecular testing for *Chlamydia trachomatis*- and/or *Neisseria gonorrhoeae*.

with the infection (OR = 4.49; 95% CI 1.78–11.33) (Table 3). Also, in Table 3, race and ethnicity were categorized as white, black/Pardo and other (Asian, native American). Among all racial/ethnic groups combined, black/Pardo was associated with the infection in both models, univariable (OR = 3.21; 95% CI 1.27–8.10) and multivariable (OR = 2.70; 95% CI 1.03–7.08). Higher education level showed a protective effect also in the two models, univariable (OR = 0.17; 95% CI 0.06–0.51) and multivariable (OR = 0.16; 95% CI 0.05–0.53). In contrast, tobacco smoke was correlated with an increased risk of infection, univariable (OR=4.44; 95% CI 1.83–10.78) and multivariable (OR = 3.18; 95% CI 1.23–8.24). As a result of a multivariable analysis of *T. vaginalis* infection risk factors, being married or living with a partner proved to be protective compared to singles, divorcees, and widows.

Discussion

In this study we observed a prevalence of *T. vaginalis* infection of 1.3% and evidence for a 4-fold increased risk of *T. vaginalis* infection among women who had abnormal vaginal microbiota. The prevalence found here was similar to observed in other studies in Brazil and worldwide.^{32–35} Additionally, sociocultural characteristics should also be considered as different across different populations.³⁶

To establish the infection, *T. vaginalis* must mediate contact with epithelial cells, adhere to, and lyse host epithelial cells, causing tissue damage, generated by a strong infiltration of leukocytes and by high local concentrations of chemokines and pro-inflammatory cytokines.³⁷ Immune responses during infection cause inflammation, which can also contribute to disease establishment, however, *T. vaginalis* has host immunity evasion mechanisms. Subsequently, the parasite lives associated with the vaginal microbiota, leading to pathogenic ruptures of vaginal bacteria.³⁸ Bacterial vaginosis, a microbiome-disturbance prevalent in reproductive-age women, is characterized by a shift from a *Lactobacillus*-dominated to a diverse polymicrobial state, with pro-inflammatory features, combined with an immune response driven by individual variability in the host immune system,³⁹ and occurs commonly in concert with trichomoniasis, and its interaction has been suggested.²⁴

The relation between vaginal microbiota and *T. vaginalis* infection observed in this study corroborates with others.^{40–42} Although several studies have reported an increased risk of *T. vaginalis* acquisition among women with bacterial vaginosis, the precise nature of the relationship between the role of *T. vaginalis* infection in the susceptibility to an unbalance in the vaginal microbial, or even the opposite, a previous imbalance of the microbiota that would favor infection by the protozoan is not well understood.

More longitudinal cohort studies are needed, using accurate detection techniques to demonstrate cause and consequence between vaginal microbiota and *T. vaginalis* infection. As demonstrated, women experiencing abnormal microbiota during a three-month span appear to have significantly increased risk of acquiring *T. vaginalis* infection.⁴²

Prevalence of *T. vaginalis* infection in this study did not differ by economic status, but higher prevalence has been consistently reflected in women who did not complete high school in comparison to having at least completed high school, as demonstrated by Patel et al.,⁴³ and a positive association with infection in participants with lower level of education was demonstrated in previous study.⁴⁴ The importance of identifying the demographic characteristics of populations at higher risk for acquire STIs may impact the need for effective educational strategies, screening of groups at higher risk and treatment for infected people and sexual partners.

The risk factors for *T. vaginalis* infection and the prevalence in our study were similar to those observed by Tompkins et al.⁴⁵ Our studies found risk factors that representing behavioral habits such as smoking were more likely to be infected with *T. vaginalis*, as compared to non-smokers.

According to our results, the infection rate of *T. vaginalis* suggests that screening programs to detect this infection

could be helpful in establishing prevention strategies in our population. Our study supports an association between abnormal vaginal microbiota and *T. vaginalis* infection.

Author contributions

GVSP contributed to conceptualization, investigation, methodology, data analysis, writing.

ANB contributed to investigation LFM contributed to investigation NPM contributed to investigation BRAR contributed to investigation.

MDCS contributed to investigation, writing, reviewing and edition MTCD contributed to investigation, writing, reviewing, and edition ART contributed to investigation, writing, reviewing and edition.

MGS contributed to supervision, conceptualization, writing, editing, reviewing, and funding.

CM contributed to supervision, conceptualization, writing, editing, reviewing, and funding.

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Conflicts of interest

CM has received consultancy fees from Kimberly-Clark outside of the present work. All other authors declare no conflict of interest.

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