Bacterial vaginosis and trichomoniasis: prevalence, associated factors, and performance of diagnostic tests

Vaginose bacteriana e tricomoníase: prevalência, fatores associados e desempenho de testes diagnósticos

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ABSTRACT

Introduction: Bacterial vaginosis and vaginal trichomoniasis are frequent causes of health care demand. **Objective:** To estimate the prevalence, identify associated factors, and investigate the performance of diagnostic tests for bacterial vaginosis and trichomoniasis. **Methods:** Cross-sectional study with participants over 18 years old. All of them were submitted to an interview and gynecological examination with evaluation of vaginal secretion, pH verification, collection of material for Pap smear, wet mount test, Whiff test, bacterioscopy, and polymerase chain reaction for trichomoniasis detection. Logistic regression analysis was applied to identify associated factors with bacterial vaginosis. Diagnostic performance for bacterial vaginosis was evaluated following Amsel criteria, the Ison and Hay score, and the Pap smear, considering the Nugent score as the gold standard. As for trichomoniasis, diagnostic performance was evaluated through the Pap smear and the wet mount test, using the polymerase chain reaction as the gold standard. **Results:** The prevalence of bacterial vaginosis was 33.7%, and for trichomoniasis, 0.5%. The complaint of abnormal vaginal secretion was associated with the diagnosis of bacterial vaginosis (odds ratio 2.2). The diagnostic accuracy by Amsel criteria, the Ison and Hay score, and the Pap smear was 35.6, 97.0, and 84.2%, respectively. The sensitivity for trichomoniasis through wet mount test was 0.0%, and through the Pap smear, 100%. **Conclusion:** The prevalence of bacterial vaginosis was high, and trichomoniasis was low. The only associated factor with bacterial vaginosis was the report of abnormal vaginal secretion. The methods with the most accurate diagnostic performance for bacterial vaginosis were the Ison and Hay score and the Pap smear and, for trichomoniasis, the Pap smear.
Keywords: Cross-sectional studies. Sensitivity and specificity. Bacterial vaginosis. Trichomonas infections. Gynecological examination.

RESUMO

Introdução: A vaginose bacteriana e a tricomoníase vaginal constituem causas frequentes de procura por serviços de saúde. **Objetivo:** Estimar a prevalência, identificar os fatores associados e investigar o desempenho dos testes diagnósticos para vaginose bacteriana e tricomoníase. **Métodos:** Estudo de corte transversal, com participantes acima de 18 anos. Todas foram submetidas a entrevista e exame ginecológico com avaliação da secreção vaginal, verificação do pH e coleta de material para Papanicolaou, exame a fresco, teste de Whiff, bacterioscopia e reação em cadeia de polimerase para a detecção de tricomoníase. A análise de regressão logística foi empregada para identificar fatores associados à vaginose bacteriana. O desempenho diagnóstico para vaginose bacteriana foi avaliado pelos seguintes métodos: critérios de Amsel, escore de Ison e Hay e Papanicolaou, tendo como padrão-ouro o escore de Nugent. O desempenho diagnóstico para tricomoníase foi avaliado pelo Papanicolaou e exame a fresco, tendo como padrão-ouro a reação em cadeia de polimerase. **Resultados:** A prevalência da vaginose bacteriana foi de 33,7% e da tricomoníase, de 0,5%. A queixa de secreção vaginal anormal associou-se ao diagnóstico de vaginose bacteriana (*odds ratio* — OR=2,2), a precisão diagnóstica pelos métodos critérios de Amsel, escore de Ison e Hay, e Papanicolaou, foi de 35,6, 97,0 e 84,2%, respectivamente. A sensibilidade para tricomoníase, pelo exame a fresco, foi de 0,0% e, pelo Papanicolaou, de 100%. **Conclusão:** A prevalência da vaginose bacteriana foi elevada e da tricomoníase, baixa. O único fator associado à vaginose bacteriana foi o relato de secreção vaginal anormal. Os métodos com melhor desempenho diagnóstico para vaginose bacteriana foram os escores de Ison e Hay, Papanicolaou e, para tricomoníase, Papanicolaou.
Palavras-chave: Estudos transversais. Sensibilidade e especificidade. Vaginose, bacteriana. Infecções por *trichomonas*. Exame ginecológico.

INTRODUCTION

Sexually transmitted infections (STIs), caused by viruses, bacteria, or protozoa, represent a significant public health problem due to their high incidence, once they remain asymptomatic in most of those infected, and because of the possibility of complications, ranging from reproductive health to carcinogenesis[1,2].

The World Health Organization (WHO) estimated, for 2016, a global 376 million new cases of four curable STIs: *Trichomonas vaginalis* (TV), *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and syphilis, in the 15–49 age group[3]. For TV infection, the estimated incidence was 156 million new cases, the highest in the respective period[3]. Thus, this infection is considered the most prevalent curable STI in the world, showing a peak in the 30–39 age group, with a cosmopolitan distribution among women and men, and in all socioeconomic and racial groups[4].

Bacterial vaginosis (BV) is the most common cause of abnormal vaginal secretion in women of reproductive age[5]. In Brazil, its prevalence ranges from 18.4 to 36.0%[6-8]. Although it is not considered an STI, it shares risk factors with these infections, such as a higher frequency in sexually active women with multiple sexual partners[9].

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The diagnosis of BV can be made according to Amsel, Nugent, and Ison and Hay, as well as oncotic and cervical cytology[10-13]. For the diagnosis of trichomoniasis (TV; from *Trichomonas vaginalis)*, the wet mount examination of vaginal secretion, the Pap smear cytological examination, and polymerase chain reaction (PCR) can be used[13-15].

The importance of correct diagnosis is related to prevention and control necessities, since there is evidence that both BV and TV infestation are associated with increased human immunodeficiency virus (HIV) transmissibility, and gynecological and obstetric complications, such as preterm labor, pelvic inflammatory disease, ectopic pregnancy, and chronic pelvic pain, in addition to postoperative complications[16,17].

The high frequency of BV, TV incidence, the possibility of obstetric and gynecological complications, and the increase in HIV transmissibility, in addition to the range of diagnostic methods, justify this study. Knowing the prevalence, associated factors, and the performance of diagnostic methods in women referred to public health prevention services will provide evidence that may enable the implementation of measures to prevent and control these conditions and reduce aggravations, with benefits for the population's sexual and reproductive health.

OBJECTIVE

The general objective of this study consisted in investigating the prevalence, associated factors, and performance of diagnostic tests for BV and TV in women treated at a gynecological clinic of a public health unit located in the Central-Western region of Brazil.

METHODS

This is a cross-sectional study, conducted from March to September 2018, at the preventive gynecology outpatient clinic of the Emergency Care Unit (UPA, in Portuguese) Dr. Paulo Siqueira Garcia (formerly Cais Chácara do Governador), located in the Southeastern region of Goiânia, Goiás. For the sample size calculation, the formula referenced by Andrade and Zicker was applied, considering the NG infection. The NG is regarded to have the lowest prevalence among the infections investigated in the study, with approximately 2%, with an alpha error of 5% and beta error of 20%, which resulted in 200 participants[18].

Eligible women were those regulated for gynecological consultation at the aforementioned UPA, not pregnant, not immunosuppressed (as far as they were aware), and who had not used antibiotics 15 days before the consultation. The participants comprised women over 18 years old who agreed to be part of the study, signed the informed consent form (IC), agreed to answer the survey, and allowed the collection of the necessary specimens for the gynecological examination and laboratory tests.

The gathering of data on sociodemographic and behavioral characteristics was performed through a questionnaire. All participants underwent a speculum examination with cervicovaginal specimen collection. Speculum examination was used for the clinical evaluation of vaginal secretion considered (1) normal, in the presence of scanty, microfloccular and adherent vaginal secretions; (2) suggestive of BV, in the presence of gray-white, fluid secretion; or (3) suggestive of TV, in the presence of yellow-green, bullous, fluid secretion with an inflammatory reaction of the vaginal mucosa and uterine cervix.

The wet mount test was performed by collecting secretion from the lateral vaginal wall with an Ayre spatula, with the addition of a 0.9% saline solution drop between the slide and the coverslip. We considered the diagnosis of (1) BV, when epithelial cells with cytoplasm covered by coccobacilli (indicator cells) were found; and (2) TV, when small, mobile, piriform cells with eccentric pale nucleus were found. Vaginal pH was assessed by attaching a colorimetric tape to the upper and lateral third of the vaginal wall for 30 seconds. A pH below 4.5 was considered normal and suggestive of BV and/or TV when above 4.5[2,13,14]. The Whiff test consisted of adding a drop of 10% potassium hydroxide to the vaginal secretion deposited on an Ayre spatula. The test was considered positive and suggestive of BV or TV when the samples released an unpleasant amines odor.

According to Amsel, the BV diagnosis was given with at least three of the following criteria:

1. Homogeneous gray-white secretion on speculum examination;
2. Lane cells on wet mount microscopic examination of vaginal secretion;
3. Vaginal pH above 4.5; and
4. Positive Whiff test[10].

The biological specimen for bacterioscopy was collected in the cul-de-sac of the vagina with an Ayre spatula. The material was dry-fixed, stained through the Gram method, analyzed according to the Nugent and the Ison and Hay scores, for the classification of the vaginal flora, and evaluated by the Biodiversity Laboratory of the Pontifical Catholic University of Goiás (PUC-GO)[11,12]. Considering the Nugent score, vaginal flora was classified according to the quantitative bacilli evaluation. The decrease in long Gram-positive bacilli was scored from 4 to 0, the increase in small Gram-variable bacilli was scored from 0 to 4, and curved Gram-variables, from 0 to 2. The sum of points from 7 to 10 was consistent with the BV diagnosis[11]. According to the Ison and Hay score, the vaginal flora was classified as (1) normal, when there was a predominance of long bacilli; (2) intermediate, when there were equivalent amounts of long Gram-positive bacilli and small, curved Gram-variables; and (3) with BV, when there was a predominance of small and curved Gram-variable bacilli[12].

The Pap smear was conducted with material collected from the ectocervix and cervical canal with an Ayre spatula and plastic bristle brushes on a single slide. The material was immediately fixed in an alcohol/ether solution and later stained using the Pap smear technique. The reading of the Pap smear was performed at the Rômulo Rocha Clinical Analysis Center of the Faculty of Pharmacy, at the Federal University of Goiás. The vaginal flora was considered (1) normal, when there was a predominance of long blue-colored bacilli; (2) intermediate, when there were long blue-colored bacilli and short coccobacilli; (3) with BV, when there was a predominance of short coccobacilli; and (4) with TV, when small piriform cells with eosinophilic cytoplasmic granules and pale and eccentric nucleus were detected[12,13].

Molecular detection of TV was performed by amplifying fragments of deoxyribonucleic acid (DNA) using PCR. The specimens collected from the endocervix with a cytobrush were placed in a UCM — Universal Collection Medium QIAGEN Sample & Ensaio

Technologies® buffer solution and sent to the Biodiversity Laboratory at PUC-GO, where they were frozen at -20°C. DNA extraction was performed using the PureLink Invitrogen kit and primers, specific for the amplification of the human constitutive gene and *Glyceraldehyde-3-phosphate dehydrogenase* (GAPDH)[15].

A descriptive analysis of the sociodemographic and behavioral characteristics of the sample studied was performed. The prevalence of BV and genital infections by TV was estimated with a 95%CI. Univariate logistic regression analysis was employed to assess the association between BV, diagnosed according to the Nugent score, and sociodemographic, behavioral and symptom variables. Subsequently, the variables that were associated with *p* values lower than 0.20 were included in the multivariate logistic regression analysis. The odds ratio (OR) was calculated and adjusted with the respective 95%CI and a significance level of 5% ($p<0.05$).

To evaluate the accuracy and degree of agreement of the bacterioscopy (used as the gold standard PCR) for the BV diagnosis, considering Amsel, Ison and Hay, the Pap smear, and Nugent, we applied the Cohen's Kappa coefficient to adjust sensitivity, specificity, positive and negative predictive values, diagnostic precision, and the degree of agreement.

In turn, to evaluate the accuracy and degree of agreement of the fresh tissue examination, the wet mount test for TV diagnosis, having PCR as the gold standard according to the criteria of Nugent, we employed Cohen's Kappa coefficient for adjusting sensitivity, specificity, positive and negative predictive values, diagnostic accuracy, and the degree of agreement.

RESULTS

The sample consisted of 202 women aged 18 to 72 years. The mean age was 43.2, with a standard deviation of 13.7. Among the participants, 138 (68.3%) were married or in a common-law marriage; 158 (79.0%) started sexual activity after the age of 15 years; 163 (80.7%) completed elementary or high school; 119 (58.9%) were self-employed, employed and/or received social security benefits; and 152 (75.2%) had an individual income above minimum wage. Among behavioral habits, 66 (32.7%) were alcoholics, 21 (10.4%) were smokers, and most used no hormonal contraceptives (151; 74.8%). Condom use was reported by 162 (80.2%) participants, and 113 (56.5%) reported having had up to three sexual partners in 2018 (**Table 1**).

The prevalence of BV according to Nugent, Amsel, simplified Amsel, Ison and Hay, and Pap smear was 33.7% (95%CI 27.1–40.6), 37.6% (95%CI 30.9–44.6), 27.7% (95%CI 21.6–34.4), 34.7% (95%CI 28.1–41.6), and 23.8% (95%CI 18.0–30.2), respectively (**Table 2**).

The prevalence of TV observed through the wet mount test was 2.5% (95%CI 1.0–5.6), 0.5% by the Pap smear (95%CI 0.08–2.7), and 0.5% (95%CI 0.08–2.7) by PCR.

BV showed statistically significant association with complaints of abnormal vaginal secretion (OR 2.2; 95%CI 1.1–3.8; p=0.011), and pain during sexual intercourse (OR 1.8; 95%CI 1.0–3.4; p=0.042). After multivariate analyses, BV diagnosis showed statistically significant association with the report of abnormal vaginal secretion (adjusted OR 2.2; 95%CI 1.0–4.5; p=0.039) (**Table 3**).

Table 4 shows the performance of BV diagnosis according to different diagnostic criteria, considering the Nugent score as the gold standard. The sensitivities observed according to Amsel, simplified Amsel, Ison and Hay, and Pap smear were 47.1% (95%CI 35.7–58.8), 60.3% (95%CI 48.4–71.1), 97.1% (95%CI 89.9–99.2), and 61.8% (95%CI 49.9–72.4), respectively. The specificities, also

Table 1. Sociodemographic characteristics and behavioral habits of the 202 participants assisted at the gynecological outpatient clinic in Goiânia (GO), 2018.

Variables	n	%
Age group		
18–43 years old	101	50.0
Over 43 years old	101	50.0
Education		
Elementary level or high school	163	80.7
Higher level	39	19.3
Functional status		
Employee, self-employed or pension beneficiary	119	58.9
Unemployed	83	41.1
Income		
Up to a minimum wage	50	24.8
Above a minimum wage	152	75.2
Smoker		
Yes	21	10.4
No	180	89.6
Alcoholic		
Yes	66	32.7
No	136	67.3
Marital status		
Married or in a stable union	138	68.3
Single or widow	64	31.7
Age of sexual activity onset		
Up to 15 years old	42	21.0
Over 15 years old	158	79.0
Condom use		
Yes	162	80.2
No	40	19.8
Number of sexual partners		
Above 3	87	43.5
Up to 3	113	56.5
Relationship time with last partner		
Up to 11 months	30	14.9
12 months or more	172	85.1

Table 2. Prevalence of bacterial vaginosis based on Nugent, Amsel, simplified Amsel, Ison and Hay, and Pap smear of the 202 participants treated at a gynecological outpatient clinic in Goiânia (GO), 2018.

Diagnostic criteria*	n	Prevalence (95%CI)
Nugent score	68	33.7 (27.1–40.6)
Amsel criteria	76	37.6 (30.9–44.6)
Simplified Amsel criteria	56	27.7 (21.6–34.4)
Ison and Hay score	70	34.7 (28.1–41.6)
Pap smear	48	23.8 (18.0–30.2)

*Amsel criteria: gray secretion; Whiff: foul odor test; pH greater than 4.5; fresh clue cells: 3 positives = positive; Simplified Amsel criteria: gray secretion; Whiff: foul odor test; pH greater than 4.5; Nugent score 3 or 4 = positive; Ison and Hay score: Grade III = positive; Pap smear: short coccobacilli and clue cells.

Table 3. Association between bacterial vaginosis and sociodemographic, behavioral and symptom variables in the 202 participants assisted at a gynecological outpatient clinic in Goiânia (GO), 2018.

Variables	Bacterial Vaginosis		OR* (95%CI)	p	Adjusted OR† (95%CI‡)	p
	Present n (%)	Absent n (%)				
Age (years)						
Over 43	31 (45.6)	70 (52.2)	-	-	-	-
Up to 43	37 (54.4)	64 (47.7)	0.7 (0.4–1.3)	0.372	-	-
Education (level)						
Higher education	54 (79.4)	109 (81.3)	-	-	-	-
Elementary/High school	14 (20.5)	25 (18.6)	0.8 (0.4–1.8)	0.748	-	-
Income (wage)						
Over one	49 (72.0)	103 (76.8)	-	-	-	-
Up to one	19 (27.9)	31(23.1)	1.2 (0.6–2.5)	0.454	-	-
Smoking						
No	60 (88.2)	120 (89.5)	-	-	-	-
Yes	7 (10.2)	14 (10.4)	1.0 (0.3–2.5)	0.372	-	-
Drinking						
No	42 (61.7)	94 (70.1)	-	-	-	-
Yes	26 (38.2)	40 (29.8)	1.4 (0.7–2.6)	0.230		
Marital status						
Married/common-law marriage	46 (67.6)	92 (68.6)	-	-	-	-
Widow/single	22 (32.3)	42 (31.3)	1.0 (0.5–1.9)	0.021	-	-
Age of sexual debut						
Over 15	51 (75.0)	107 (79.8)	-	-	-	-
Up to 15	15 (22.0)	27 (20.1)	1.1 (0.5–2.3)	0.674	-	-
Condom use						
Yes	55 (80.8)	107 (79.8)	-	-	-	-
No	13 (19.1)	27 (20.1)	0.9 (0.4–1.9)	0.862	-	-
Number of sexual partners						
Up to 3	36 (52.9)	77 (57,4)	-	-	-	-
Over 3	32 (47.0)	55 (41,0)	1.2 (0.6–2.2)	0.466	-	-
Complaint of abnormal secretion						
No	31 (45.5)	86 (64.1)	-	-	-	-
Yes	37 (54.4)	48 (35.8)	2.2 (1.1–3.8)	0,011	2.2 (1.0-4.5)	0.039
Complaint of foul odor						
No	48 (70.5)	106 (79.1)	-	-	-	-
Yes	20 (29.4)	28 (20.9)	1.5 (0.8–3.0)	0.179	0.8 (0.3-1.9)	0.635
Complaint of itching						
No	48 (70.5)	103 (76.8)	-	-	-	-
Yes	20 (29.4)	31 (23.1)	1.3 (0.7–2.6)	0.332	-	-
Complaint of pain during intercourse						
No	39 (57.3)	96 (71.6)	-	-	-	-
Yes	29 (42.6)	38 (28.3)	1.8 (1.0–3.4)	0.042	1.7 (0.9-3.2)	0.098

*OR: gross odds ratio; †OR²: adjusted odds ratio; ‡CI: confidence interval.

according to Amsel, simplified Amsel, Ison and Hay, and Pap smear were 29.9% (95%CI 22.8–38.1), 21.6% (95%CI 15, 5–29.4), 97.0% (95%CI 92.6–98.8), and 95.5% (95%CI 90.6–97.9), respectively. The diagnostic accuracy according to Amsel, simplified Amsel, Ison and Hay, and Pap smear was 35.6 (95%CI 29.4–42.5), 34.7 (95%CI 28.4–41.5), 97.0 (95%CI 93.7–98.6), and 84.2 (95%CI 78.5–88.6), respectively.

For the BV diagnosis, considering the Nugent score as the gold standard, the degree of agreement was weak, according to Amsel (k=0.2) and simplified Amsel (k=0.1); strong, through the Pap smear (k=0.6); and almost perfect through Gram-stained bacterioscopy and according to Ison and Hay (k=0.9) (**Table 4**).

Compared to PCR, for the TV diagnosis, the Pap smear showed sensitivity and specificity of 100% (95%CI 20.6–100.0) and 100% (95%CI 98.1–100.0), respectively, with a degree of agreement of 1 (95%CI 0.9–1.1). The wet mount examination of vaginal contents revealed no sensitivity 0.0% (0.0–79.3) and no degree of agreement -0.0% (95% CI: -0.1–0.1) in TV diagnosis through PCR (**Table 4**).

DISCUSSION

The results of this study indicated that the prevalence of BV was high, and the prevalence of TV was low, according to both clinical and laboratory criteria. Most study participants presented no

Table 4. Evaluation of the performance of the diagnosis of bacterial vaginosis and trichomoniasis in the 202 participants attended at a gynecological clinic in Goiânia (GO), 2018.

	Nugent score BV		Estimated performance	
	Positive n (%)	Negative n (%)	% (95%CI)	
Amsel criteria				
			Sensitivity	47.1 (35.7–58.8)
Positive	32 (47.0)	94 (70.1)	Specificity	29.9 (22.8–38.1)
			Positive predictive value	25.4 (18.6–33.7)
			Negative predictive value	52.6 (41.6–63.5)
Negative	36 (52.9)	40 (29.8)	Diagnostic accuracy	35.6 (29.4–42.5)
			Kappa index	0.2 (0.3–0.1)
Simplified Amsel				
			Sensitivity	60,3 (48.4–71.1)
Positive	41 (60.2)	105 (78.3)	Specificity	21.6 (15.5–29.4)
			Positive predictive value	28.1 (21.4–35.9)
			Negative predictive value	51.8 (39.0–64.3)
Negative	27 (39.7)	29 (21.6)	Diagnostic accuracy	34.7 (28.4–41.5)
			Kappa index	0.1 (0.2–0.03)
Ison and Hay				
			Sensitivity	97.1 (89.9–99.2)
Positive	66 (97.0)	4 (2.9)	Specificity	97.0 (92.6–98.8)
			Positive predictive value	94.3 (86.2–97.8)
			Negative predictive value	98.5 (94.6–99.6)
Negative	2 (2.9)	130 (97.0)	Diagnostic accuracy	97.0 (93.7–98.6)
			Kappa index	0.9 (0.8–1.1)
Pap smear				
			Sensitivity	61.8 (49.9–72.4)
Positive	42 (61.7)	6 (4.4)	Specificity	95.5 (90.6–97.9)
			Positive predictive value	87.5 (75.3–94.1)
			Negative predictive value	83.1 (76.4–88.2)
Negative	26 (37.2)	128 (95.5)	Diagnostic accuracy	84.2 (78.5–88.6)
			Kappa index	0.6 (0.5–0.8)
	PCR TV		**Estimated performance**	
	Positive n (%)	**Negative n (%)**	**% (95% CI)**	
Wet mount				
			Sensitivity	0.0% (0.0–79.3)
Positive	0 (0.0)	5 (2.5)	Specificity	97.5% (94.3–98.9)
			Positive predictive value	0.0% (0.0–43.4)
			Negative predictive value	99.5% (97.2–99.9)
Negative	1 (0.5)	196 (97.0)	Diagnostic accuracy	97.0% (93.7–98.6)
			Kappa index	-0.0% (-0.1–0.1)
Pap smear				
			Sensitivity	100% (20.6–10.0)
Positive	1 (0.5)	0 (0.0)	Specificity	100% (98.1–10.0)
			Positive predictive value	100% (20.6–10.0)
			Negative predictive value	100% (98.1–10.0)
Negative	0 (0.0)	201 (99.5)	Diagnostic accuracy	100% (98.1–10.0)
			Kappa index	1 (0.9–1.1)

Amsel criteria: gray discharge; Whiff: foul odor test; pH greater than 4.5; fresh clue cells: 3 positives = positive; Simplified Amsel criteria: gray secretion; Whiff: foul odor test; pH greater than 4.5; Nugent score 3 or 4 = positive; Ison and Hay score: Grade III = positive; Pap smear: short coccobacilli and clue cells.

risk behavior for STIs. The only associated factor with BV was the report of abnormal vaginal secretion. The diagnostic methods with the best performance for BV, compared to the Nugent score, were Pap smear and Gram-stained bacterioscopy, according to the Ison and Hay score. For TV, the best performance method, compared to PCR, was Pap smear.

The prevalence of BV in the five diagnostic methods used was high and similar to previous studies[8,19]. On the other hand, the prevalence of TV through PCR, Pap smear, and wet mount test was low in the area where the study was conducted. This prevalence disagrees with WHO incidence estimates, which point to TV as one of the curable STIs with the highest incidence worldwide[1]. Studies in Africa also

showed low sensitivity (31.6%) and high specificity (100%) of the wet mount test in TV diagnosis with PCR as the gold standard[20].

Other studies conducted in the South and Central-Western regions of Brazil likewise found a low prevalence of TV, ranging from 2 to 9% in women attending a gynecological clinic[8,21].

Corroborating these studies, in a population-based study conducted in the United Kingdom, involving 15,000 women, the prevalence was also low (0.3%)[22]. Nevertheless, considering the low coverage in screening asymptomatic patients for TV, it is necessary to perform molecular tests in clinical settings, taking into account the symptoms and the demographic region[22].

The United States Centers for Disease Control and Prevention (CDC) recommend screening for TV in high prevalence populations[1]. Nonetheless, the high rates of asymptomatic infection, the low sensitivity of the clinical diagnosis, the scarce use of molecular methods, and the lack of population-based studies hinder the prevalence estimate of this infestation in Brazil and, more specifically, in the Central-Western region. The probable regional variation in the distribution of this STI and the evidence that a large proportion of those infected is asymptomatic and, like symptomatic ones, susceptible to complications[1,2] point to the need for a better assessment of its prevalence in different regions of Brazil.

The high prevalence of BV, according to the Nugent score, in the present study was similar to that observed in previous studies. A systematic review study showed a high prevalence of BV in North America, ranging from 5.8 to 29.2%, and in South Africa, ranging from 34 to 58.3%[5]. Likewise, the mean prevalence was high (25.4%) in studies conducted in Brazil, in particular, in Goiânia, it was 25.5%[8,19].

In this study, BV was significantly associated with abnormal vaginal secretion, a finding in accordance with other studies[19,23]. Furthermore, there is evidence that BV is the most frequent cause of visits to a gynecology clinic during reproductive age[24].

Asymptomatic women can also be diagnosed as having BV. However, there is no indication of treatment for these patients, considering the aforementioned diagnosis through laboratory tests[1,2]. There is, nonetheless, in special cases, little evidence indicating treatment for asymptomatic women such as in pregnancy at high risk of preterm birth, invasive gynecological procedures, intrauterine device (IUD) insertion, and gynecological surgeries[2]. Therefore, the indication for treatment, with strong evidence in the literature, would be to relieve the symptoms triggered by this condition[1,2].

On the other hand, BV presented no statistically significant association with other variables studied, like number of partners, non-use of condoms, complaint of foul odor, age of sexual debut, and marital status. Controversially, despite not being considered an STI, the literature demonstrated that associated factors are directly related to sexual activity, such as new and/or multiple partners, high frequency of intercourse, and non-use of condoms[25]. In addition to these factors, there is evidence of an association between BV and behavioral characteristics, such as the use of alcoholic beverages and illicit drugs, as well as sex work[6,26]. Otherwise, the determinants that contribute to the onset and relapse are unprotected sex, sex with a new male partner, and sexual activity in general[9]. Possibly, this association was not mentioned in this study due to memory bias or omission of information from the participants in the questionnaire. Another possibility concerns the sample size, which was sufficient to assess the prevalence and performance of diagnostic tests, but insufficient to assess the associations investigated.

In evaluating the performance of diagnostic methods for BV compared to the Nugent score[11], this study revealed greater diagnostic accuracy for the Ison and Hay score, followed by the Pap smear. Both bacterioscopy, using the Ison and Hay method, and the characterization of the vaginal flora, using the Pap smear, showed high sensitivity and specificity, and a degree of agreement considered almost perfect (k=0.8), similar to another study[12,13,27].

The present study also showed a low diagnostic accuracy of 35.6% (95%CI 29.4–42.5) and a low degree of agreement (k=0.2) for the BV diagnosis, according to Amsel criteria[10]. The lower diagnostic performance of this method may be due to the subjective conditions present in the clinical evaluation. In addition to the inherent subjectivity, compared to laboratory methods, one of the difficulties in using Amsel criteria is associated with the need for microscopic examination to identify indicator cells during outpatient care[10].

Although cytological examination is not considered the ideal test for the diagnosis of genital infections[28], in the present study, it revealed high sensitivity, specificity, and degree of agreement, with correct diagnosis of the only positive TV case and exclusion of the 201 negative cases, compared to PCR. On the other hand, the wet mount test showed no sensitivity or agreement with TV diagnosis, compared to PCR. Thus, the five positive cases of TV through the wet mount test were not detected by PCR, which points to the subjectivity of this assessment, conducted during the gynecological consultation, despite being based on the movement of the protozoan between the slide and the coverslip[14].

Strengths

Among the strengths of this study, we mention, as a gold standard, the use of bacterioscopy, according to the Nugent score for the diagnosis of bacterial vaginosis, and PCR for the diagnosis of TV.

Limitations

Among the possible limitations of the present study are (1) the small sample size; (2) the fact that the participants were selected from only one health unit; (3) the data collection through interviews, which implies memory bias; and (4) subjectivity. The latter must be considered in the clinical evaluation of vaginal secretion, which is one of the four Amsel criteria for BV diagnosis, also used in this study for the TV diagnosis.

CONCLUSION

In conclusion, the study showed a high prevalence of BV and a low prevalence of TV in the samples studied. Moreover, the methods with the best diagnostic accuracy for BV, compared to the Nugent score, were the methods of easy access and greater applicability in the daily clinic, such as bacterioscopy stained by the Gram method, according to the Ison and Hay score, followed by the Pap smear. On the other hand, the method with the best diagnostic performance for TV, compared to PCR, was the Pap smear.

Approval by the Human Research Ethics Committee

This study was approved by the Ethics and Research Committee (CEP) of the Federal University of Goiás (Report n. 2,500,433).

Participation of each author

KCC: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. RRFA: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. VAS: Laboratory analysis, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. JEPR: Data curation, Resources, Software, Visualization, Validation. JPL: Data curation, Visualization, Validation. CLS: Data curation, Visualization, Validation. LAB: Data curation, Visualization, Validation. SHRS: Laboratory analysis, Resources, Visualization, Validation, Writing– review & editing. AAR: Laboratory analysis, Resources, Visualization, Validation, Writing – review & editing.

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Conflict of interest

The authors declare no conflicts of interest.

REFERENCES

1. Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, et al Sexually transmitted infections treatment guidelines, 2021. MMWR Recomm Rep. 2021;70(4):1-187. https://doi.org/10.15585/mmwr.rr7004a1
2. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Doenças de Condições Crônicas e Infecções Sexualmente Transmissíveis. Protocolo clínico e diretrizes terapêuticas para atenção integral às pessoas com infecções sexualmente transmissíveis (IST) [Internet]. Brasília: Ministério da Saúde; 2020 [cited on 2021 Apr. 27]. Available from: https://portaldeboaspraticas.iff.fiocruz.br/wp-content/uploads/2020/08/pcdt_ist_final_revisado_020420.pdf
3. World Health Organization. Report on global sexually transmitted infection surveillance, 2018. Geneva: WHO; 2018.
4. Iwueze MO, Ezeanyanwu LN, Okafor FC, Nwaorgu OC, Ukibe SC. Prevalence of Trichomonas vaginalis infection among women attending hospitals/health centres in Onitsha Community, Onitsha North Local Government Area of Anambra State. Bioscientist. 2014;2(1):54-64.
5. Kenyon C, Colebunders R, Crucitti T. The global epidemiology of bacterial vaginosis: a systematic review. Am J Obstet Gynecol. 2013;209(6):505-23. https://doi.org/10.1016/j.ajog.2013.05.006
6. Ignacio MAO, Andrade J, Freitas APF, Pinto GVS, Silva MG, Duarte MTC. Prevalência de vaginose bacteriana e fatores associados em mulheres que fazem sexo com mulheres. Rev Latino-Am Enfermagem. 2018;26:e3077. https://doi.org/10.1590/1518-8345.2491.3077
7. Resende AF, Santos RWF, Gaspar LMAC, Almeida POS. Prevalência de vaginoses bacterianas em pacientes que realizaram bacterioscopia de secreção vaginal. Rev Ciênc Méd Biol. 2019;18(2):190-3. http://dx.doi.org/10.9771/cmbio.v18i2.29698
8. Camargo KC, Alves RRF, Baylão LA, Ribeiro AA, Araujo NLAS, Tavares SBN, et al. Secreção vaginal anormal: sensibilidade, especificidade e concordância entre o diagnóstico clínico e citológico. Rev Bras Ginecol Obstet. 2015;37(5):222-8. https://doi.org/10.1590/SO100-720320150005183
9. Bilardi J, Walker S, Mooney-Somers J, Temple-Smith M, McNair R, Bellhouse C, et al. Women's views and experiences of the triggers for onset of bacterial vaginosis and exacerbating factors associated with recurrence. PLoS One. 2016;11(3):e0150272. https://doi.org/10.1371/journal.pone.0150272
10. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med. 1983;74(1):14-22. https://doi.org/10.1016/0002-9343(83)91112-9
11. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol. 1991;29(2):297-301. https://doi.org/10.1128/jcm.29.2.297-301.1991
12. Ison CA, Hay PE. Validation of a simplified grading of Gram stained vaginal smears for use in genitourinary medicine clinics. Sex Transm Infect. 2002;78(6):413-5. https://doi.org/10.1136/sti.78.6.413
13. Nayar R, Wilbur DC. The Bethesda system for reporting cervical cytology definitions, criteria, and explanatory notes. 3rd ed. New York: Springer International Publishing; 2015.
14. Garber GE. The laboratory diagnosis of Trichomonas vaginalis. Can J Infect Dis Med Microbiol. 2005;16(1):35-8. https://doi.org/10.1155/2005/373920
15. Tayoun ANA, Burchard PR, Caliendo AM, Scherer A, Tsongalis GJ. A multiplex PCR assay for the simultaneous detection of Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis. Exp Mol Pathol. 2015;98(2):214-8. https://doi.org/10.1016/j.yexmp.2015.01.011
16. Cohen CR, Lingappa JR, Baeten JM, Ngayo MO, Spiegel CA, Hong T, et al. Bacterial vaginosis associated with increased risk of female-to-male HIV-1 transmission: a prospective cohort analysis among African couples. PLoS Med. 2012;9(6):e1001251. https://doi.org/10.1371/journal.pmed.1001251
17. Sabour S, Arzanlou M, Vaez H, Rahimi G, Sahebkar A, Khademi F. Prevalence of bacterial vaginosis in pregnant and non-pregnant Iranian women: a systematic review and meta-analysis. Arch Gynecol Obstet. 2018;297(5):1101-13. https://doi.org/10.1007/s00404-018-4722-8
18. Andrade A, Zicker F. Métodos de investigação epidemiológica em doenças transmissíveis: manual do instrutor. Brasília: Organização Pan-Americana de Saúde/Fundação Nacional de Saúde; 1997.
19. Camargo KC, Alves RRF, Saddi VA. Prevalence and factors associated with bacterial vaginosis in women in Brazil: a systematc review. J bras Doenças Sex Transm. 2023;35:e23351223. https://doi.org/10.5327/DST-2177-8264-2023351223
20. Asmah RH, Agyeman RO, Obeng-Nkrumah N, Blankson H, Awuah-Mensah G, Cham M, et al. Trichomonas vaginalis infection and the diagnostic significance of detection tests among Ghanaian outpatients. BMC Womens Health. 2018;18(1):206. https://doi.org/10.1186/s12905-018-0699-5
21. Ambrozio CL, Nagel AS, Jeske S, Bragança GCM, Borsuk S, Villela MM. Trichomonas vaginalis prevalence and risk factors for women in southern Brazil. Rev Inst Med Trop Sao Paulo. 2016;58:61. https://doi.org/10.1590/S1678-9946201658061
22. Field N, Clifton S, Alexander S, Ison CA, Khanom R, Saunders P, et al. Trichomonas vaginalis infection is uncommon in the British general population: implications for clinical testing and public health screening. Sex Transm Infect. 2018;94(3):226-9. https://doi.org/10.1136/sextrans-2016-052660
23. Marconi C, Duarte MTC, Silva DC, Silva MG. Prevalence of and risk factors for bacterial vaginosis among women of reproductive age attending cervical screening in southeastern Brazil. Int J Gynaecol Obstet. 2015;131(2):137-41. https://doi.org/10.1016/j.ijgo.2015.05.016
24. Bautista CT, Wurapa E, Sateren WB, Morris S, Hollingsworth B, Sanchez JL. Bacterial vaginosis: a synthesis of the literature on etiology, prevalence, risk factors, and relationship with chlamydia and gonorrhea infections. Mil Med Res. 2016;3:4. https://doi.org/10.1186/s40779-016-0074-5
25. Bradshaw CS, Walker J, Fairley CK, Chen MY, Tabrizi SN, Donovan B, et al. Prevalent and incident bacterial vaginosis are associated with sexual and contraceptive behaviours in young Australian women. PLoS One. 2013;8(3):e57688. https://doi.org/10.1371/journal.pone.0057688

26. Krauss-Silva L, Almada-Horta A, Alves MB, Camacho KG, Moreira MEL, Braga A. Basic vaginal pH, bacterial vaginosis and aerobic vaginitis: prevalence in early pregnancy and risk of spontaneous preterm delivery, a prospective study in a low socioeconomic and multiethnic South American population. BMC Pregnancy Childbirth. 2014;14:107. https://doi.org/10.1186/1471-2393-14-107
27. Antonucci FP, Mirandola W, Fontana C, Fontana C. Comparison between Nugent's and Hay/Ison scoring criteria for the diagnosis of bacterial vaginosis inWASP prepared vaginal samples. Clin Investig. 2017;7(3):89-93.
28. Posser J, Girardi JP, Pedroso D, Sandri YP. Estudo das infecções cérvicovaginais diagnosticadas pela citologia. Rev Sau Int. 2015;8(15-16):1-9.

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