Original article

Bacterial vaginosis: prevalence, risk profile and association with sexually transmitted infections

Vaginose bacteriana: prevalência, perfil de risco e associação com infecções sexualmente transmissíveis

Vaginosis bacteriana: prevalencia, perfil de riesgo y asociación con infecciones de transmisión sexual

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ABSTRACT

Background and Objectives: Bacterial vaginosis (BV) is the most common cause of vaginal discharge in the world. The study aimed to estimate the prevalence and to identify risk factors associated with bacterial vaginosis. **Methods:** A cross-sectional study was conducted in Ouro Preto, Brazil, between February and December 2017. Three hundred and forty-one women aged 18 years or older, users of the Brazilian Unified Health System, participated in this study. Women who used oral or topical antibiotics in the four weeks prior to the sample collection and women who had undergone a total hysterectomy were excluded from the study. After signing the Informed Consent Form and filling out a questionnaire containing sociodemographic, behavioral and sexual data, the participants were directed to the collection room, where the nurse collected the samples for the preventive examination of the cervix and also two vaginal swabs. Vaginal swabs and cervical samples were analyzed for cytological abnormalities and BV using Gram staining and cytology. Pathogens causing sexually transmitted infections (STIs) were identified by Polymerase Chain Reaction (PCR). For the analysis of the data, statistical package STATA version 10.0 was used. This study was approved by the Research Ethics Committee of the Federal University of Ouro Preto (UFOP). **Results:** During the study, 341 women were evaluated. The prevalence of BV using Gram staining (32.5% [CI95% 27.7–37.7%]) and cytology (27.7% [CI95% 23.0–32.8%]) was similar, however, the sensitivity of cytology was lower (77.8%). Risk factors associated with BV were smoking (IRR 1.5 [CI95%: 1.1 – 2.1]), use of an intrauterine device (IRR 2.8 [CI95%: 1.2 – 6.5]), and past medical history of BV (IRR 1.5 [CI95%: 1.1 – 2.1]). Correlation between the presence of BV

and *Trichomonas vaginalis* (TV) infection (r=0.24) was observed. **Conclusion:** The prevalence of BV was affected by life habits and was prevalent in women with TV. Thus, behavioral and social prevention approaches to women with diverse risk profiles may help mitigate TV/BV prevalence and recurrence of BV.
Keywords: bacterial vaginosis. cell biology. molecular biology. prevalence.

RESUMEN

Contexte et objectifs: La vaginose bactérienne (VB) est la cause la plus fréquente de pertes vaginales dans le monde. Le but de cette étude était d'évaluer la prévalence et les facteurs associés à la vaginose bactérienne. **Méthodes:** Il s'agit d'une approche descriptive, transversale et quantitative réalisée à Ouro Preto, Minas Gerais, Brésil, entre février et décembre 2017. 341 femmes ont participé à cette étude, âgées de 18 ans ou plus, utilisatrices du Système de santé unifié. Les femmes ayant utilisé des antibiotiques oraux ou topiques dans les quatre semaines précédant le prélèvement et les femmes ayant subi une hystérectomie totale ont été exclues de l'étude. Après avoir signé le formulaire de consentement éclairé et rempli un questionnaire contenant des données sociodémographiques, comportementales et sexuelles, les participants ont été dirigés vers la salle de collecte, où l'infirmière a prélevé les échantillons pour l'examen préventif du col de l'utérus. et aussi deux écouvillons vaginaux. Les échantillons de frottis vaginaux et cervicaux ont été analysés pour les anomalies cytologiques et VB en utilisant la coloration de Gram et la cytologie. Les agents pathogènes causant des infections sexuellement transmissibles (IST) ont été identifiés par réaction en chaîne par polymérase. Pour l'analyse des données, le progiciel statistique STATA version 10.0 a été utilisé. Cette étude a été approuvée par le Comité d'éthique de la recherche de l'Université fédérale d'Ouro Preto (UFOP). **Résultats:** Au cours de l'étude, 341 femmes ont été évaluées. La prévalence de la VB avec coloration de Gram (32,5% [IC 95% 27,7 - 37,7%]) et de la cytologie (27,7% [IC 95% 23,0 - 32,8%]) était similaire, cependant la sensibilité cytologique était plus faible (77,8%). Les facteurs de risque associés à la VB étaient le tabagisme (IRR 1,5 [IC 95%: 1,1 - 2,1]), l'utilisation d'un dispositif intra-utérin (IRR 2,8 [IC 95%: 1,2 - 6,5]) et antécédents médicaux de VB (IRR 1,5 [IC 95%: 1,1 - 2,1]). Il y avait une corrélation entre la présence d'une infection à VB et Trichomonas vaginalis (TV) (r = 0,24). **Conclusion:** La prévalence de la VB était affectée par le mode de vie et l'infection TV. Ainsi, les approches de prévention comportementale et sociale pour les femmes présentant des profils de risque différents peuvent aider à atténuer la prévalence de la TV / VB et la récurrence de la VB.

Descripteurs: vaginose bactérienne. biologie cellulaire. biologie moléculaire. prévalence.

RESUMO

Justificativa e Objetivos: A vaginose bacteriana (VB) é a causa mais comum de corrimento vaginal no mundo. O objetivo desse estudo foi avaliar a prevalência e os fatores associados à vaginose bacteriana. **Métodos:** Trata-se de um descritivo, de forma transversal e abordagem quantitativa realizado em Ouro Preto, Minas Gerais, Brasil, entre fevereiro a dezembro de 2017. Participaram desse estudo 341 mulheres com idade igual ou superior a 18 anos, usuárias do Sistema Único de Saúde (SUS). Mulheres que usaram antibióticos orais ou tópicos nas quatro semanas anteriores à coleta e mulheres que haviam sido submetidas a uma histerectomia total foram excluídas do estudo. Após a assinatura do Termo de Consentimento Livre e Esclarecido e preenchimento de questionário contendo dados sócio-demográfico, comportamental e sexual, as participantes foram encaminhadas para a sala de coleta, onde a enfermeira realizou a coleta das amostras para o exame preventivo do colo do útero e também de dois *swabs* vaginais. As

amostras de esfregaço vaginal e cervical foram analisadas quanto às anormalidades citológicas e VB usando coloração de Gram e citologia. Patógenos causadores de infecções sexualmente transmissíveis (ISTs) foram identificados por Reação em Cadeia da Polimerase (PCR). Para a análise dos dados foi utilizado o pacote estatístico STATA versão 10.0. O presente estudo foi aprovado pelo Comitê de Ética em Pesquisa da Universidade Federal de Ouro Preto (UFOP). **Resultados:** Durante o estudo, 341 mulheres foram avaliadas. A prevalência de VB com coloração de Gram (32,5% [IC95% 27,7 - 37,7%]) e citologia (27,7% [IC95% 23,0 - 32,8%]) foi semelhante, porém a sensibilidade da citologia foi menor (77,8%). Os fatores de risco associados ao VB foram tabagismo (IRR 1,5 [IC95%: 1,1 - 2,1]), uso de dispositivo intrauterino (IRR 2,8 [IC 95%: 1,2 - 6,5]) e história médica pregressa de VB (IRR 1,5 [IC95%: 1,1 - 2.1]). Observou-se correlação entre a presença de infecção por VB e *Trichomonas vaginalis* (TV) (r = 0,24). **Conclusão:** A prevalência de VB foi afetada por hábitos de vida e infecção por TV. Assim, abordagens de prevenção comportamental e social para mulheres com diversos perfis de risco podem ajudar a mitigar a prevalência de TV / VB e recorrência de VB.
Descritores: vaginose bacteriana. biologia celular. biologia molecular. prevalência.

INTRODUCTION

Bacterial vaginosis (BV) is the leading vaginal disorder in women during their reproductive years and postmenopausal period, contributing to more than 60% of all alterations in the vaginal microbiota. Prevalence rates of BV vary according to the population studied and the Brazilian population presents similar prevalence rates to countries such as Canada (33.0%)[1]. However, as Brazil has a mixed population, Brazilian regions may have different prevalence rates.

In the typical reproductive-aged vaginal environment, BV is categorized by a shift in the vaginal flora from aerobic to predominately pathogenic anaerobic bacteria. Moreover, the postmenopausal vaginal environment, including vaginal atrophy, may also contribute to a shift in the colonization of vaginal microbiota in older woman.[1] BV is characterized by an increase in the vaginal pH, reduction in Lactobacillus flora and an elevated number and type of facultative and anaerobic bacteria.[2]

The acidic environment of the normal vaginal flora inhibits colonization by pathogenic organisms; however, an alkaline environment facilitates the growth of sexually transmissible agents.[2] For the diagnosis of sexually transmitted infections (STIs), traditional methods are laborious and often not very sensitive. However, during recent years, molecular techniques have provided new approaches for the diagnosis of these pathogens.[3]

BV is associated with an increased risk of preterm birth, spontaneous abortion, pelvic inflammatory disease, endometritis, and transmission of pathogens such as HIV. Given the range of reproductive consequences of BV, it is important to know the risk factors for BV among a population so that mechanisms of prevention can be developed.[4]

Sociodemographic and behavioral factors have been associated with the development of BV, including number of sex partners, frequent use of scented soap, and irregular condom use.[5] Furthermore, studies have shown opposite conclusions between BV and cytological abnormalities and association between BV and STIs.[1]

Gram staining *Nugent* scoring method is considered as a gold standard in the diagnosis of BV. However, Papanicolaou staining for cytological investigation is also a practical technique that demonstrates the presence of clue cells indicative of BV.[6] In Brazil, the cytological evaluation should be performed every three years if the first two annual tests were normal.[7]

This study aimed to estimate the prevalence and to identify risk factors and STIs associated with BV in a Brazilian subpopulation.

METHODS

A cross-sectional study was conducted between February and December 2017 in three different Public Health Units in Ouro Preto city, Minas Gerais State, Brazil, during the evaluation of the Extension Program Ambar: *Desafios e Ações em Saúde da Mulher* that mainly aims to expand the structure of care for women, focusing on pharmaceutical attention and quality of life in climacteric. Ouro Preto has nine Public Health Units in its urban area; three units were selected to perform the study. The choice of Public Health Units was based on low to medium socio-economic conditions of different regions in the urban area of the city and for logistical and operational reasons. This study was conducted in collaboration with the Municipality Health Service.

Women who attended the Public Health Units in Ouro Preto city to perform the cervical screening test were invited to be part of the study (convenience sample). Thus, the participants were informed by the doctor or nurse of the health unit about the research objectives and were asked to sign an Informed Consent Form.

Women aged over 18 years old were considered for inclusion. The exclusion criteria included women who were using or who took oral or topical antibiotics within four weeks prior to sample collection and women who had undergone a total hysterectomy.

The sample size calculation was performed based on a population of 6,200 women aged ≥18 years old in three Public Health Units according to the Municipality Health Service. Prevalence of BV estimated at 20%, 95% confidence level, and estimated accuracy of 4% were considered. Thus, it was established that the sample size should be 362 women.

Vaginal and cervical samples were collected from 341 women who filled out a questionnaire [8,9] addressing sociodemographic (educational level, marital status, ethnic origin and average monthly household income of their family), behavioral (smoking, use of alcohol and use of illicit drugs), and sexual (age of first intercourse, history of female sexual partner, pregnancy, abortion, IST, contraceptive use, use of intrauterine device, condom use, history of bacterial vaginosis and age of first menstruation) characteristics. The interviews were conducted individually in a private room.

Cervical samples were collected by nurses from each Basic Health Unit. For collection of the material, a speculum was introduced into the vagina. After the visual inspection of the interior of vagina and cervix, the nurse performed the collection of biological specimens from the external and internal surface of the cervix with an Ayre's spatula and a cervical brush. Collected cells were then placed on a slide, fixed in 95% alcohol and sent to the Exfoliative Cytology of Uterine Cervix Sector at the Pilot Laboratory of Clinical Analysis (LAPAC). The slides were stained by the standard Papanicolaou method[10] and analyzed according to Claus, 1992.[11]

Vaginal samples were also collected by nurses from each Basic Health Unit for Gram stain and nucleic acids amplification tests. With a speculum inserted into the vaginal canal, two sterile cotton swabs (Labor Swab, São Paulo, Brazil) were used to collect vaginal samples. The appearance and type of vaginal secretions were recorded. One swab was used for the preparation of slides that, after drying, were heat-fixed and gram stained and another swab was stored in tubes containing 0.5 mL of 0.9% NaCl solution at -20 ºC for further molecular testing.

Briefly, each stained smear was observed for bacterial morphotypes under oil immersion objective at 100 X magnification and graded as per the standardized quantitative morphological classification method developed by Nugent (1991)[12], which assigns a score between 0 and 10 based on the following bacterial morphotypes: large Gram-positive rods (*Lactobacillus* morphotypes), small Gram-variable rods (*G. vaginalis* morphotypes), small Gram-negative rods (*Bacteroides* spp. morphotypes), curved Gram-variable rods (*Mobiluncus* spp. morphotypes), and Gram-positive cocci.[2,11] .

In the cervical samples, the diagnosis of BV was performed according to the Bethesda System nomenclature[13]. The presence of small coccobacilli coating the surface of squamous cells (*clue cells*) coupled with the absence of lactobacillus was indicative of flora alteration and suggestive of BV.[14-16]

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Samples from forty women with BV detected by the Gram staining and forty women without BV were randomly selected and submitted for *Trichomonas vaginalis* (*T. vaginalis*), *Neisseria gonorrhoeae* (*N. gonorrhoeae*), *Chlamydia trachomatis* (*C. trachomatis*) and Human Papilloma Virus (HPV) detection by Polymerase Chain Reaction (PCR). [3, 17, 18]

Databases were generated using EpiData version 3.2 (EpiDataAssociation, Odense, Denmark) by double entry of the results, and they were subsequently compared, corrected, and analyzed using STATA version 14.0 software (Stata Corp., College Station, TX, USA).

The results of the Gram stain and cytological evaluation were used to calculate the prevalence of BV. Gram stain was used as reference to calculate diagnostic performance of the cytological evaluation. Sensitivity, specificity, accuracy, positive predictive value and negative predictive value of the cytological method were calculated using the Gram stain as a gold standard. Besides that, agreement between these methods was evaluated using agreement percentage and the Kappa coefficient. Interpretation of the results of Kappa coefficient was done according to the following scale: 1.00–0.81 excellent; 0.80–0.61 good; 0.61–0.40 moderate; 0.40–0.21 weak; 0.20–0.0 absence of agreement.

Factors associated with BV (detected by Gram stain) were determined using a univariate analysis performed previously with Poisson regression with estimated robust variance and all the variables of the questionnaire were tested. Variables with p value < 0.25 in univariate analysis were selected for multivariate analysis.

Multivariate analysis was based on a complete model, with all the variables selected in univariate analysis. Non-significant variables were discarded in a step-by-step manner (backward selection). In this step, significance level of 0.05 was used, that is, the variables that presented p > 0.05 were withdrawn from the step-by-step model. The final model consisted only of variables with significance level < 0.05.

The association between the presence of cytological abnormalities and the presence of bacterial vaginosis by Gram staining was evaluated through the chi-square test followed by the univariate analysis using logistic regression. Besides that, the association between non-commensal pathogens (*T. vaginalis*, *N. gonorrhoeae*, *C. trachomatis* and HPV) and presence

of vaginosis was evaluated similarly. It was considered as presence of association when the p value was lower than 0.05.

This study was approved by the Committee of Ethics in Research, protocol no. 1.907.198, **CAAE:** 62883616.8.0000.5150. All procedures in this study were in accordance to the protocol number 466/2012 of the National Health Council. Participants were informed of the research objectives and were required to sign an Informed Consent Form before sample and data collection.

RESULTADOS

Characteristics of the population

From 341 women, the mean age was 40.1±14.0 years old, ranging from 18 to 83 years old. The majority of the women had completed high school (48.7%), were married or lived with a partner (53,4%), self-declared Asian-indigenous or brown (39.4%), had a family income of less than two minimum wages (55,6%) (Table 1).

Table 1 - Sociodemographic characteristics, Ouro Preto, Brazil, 2017.

Variables	Bacterial vaginosis			PR (95%CI)	p
	Total n (%)	Positive n (%)	Negative n (%)		
Age group (years)					
18 to 30	95 (27.9)	30 (27.0)	65 (28.3)		
>30 and ≤ 40	75 (22.0)	26 (23.4)	49 (21.3)	1.1 (0.7–1.6)	0.671
>40 and ≤ 50	82 (24.0)	27 (24.3)	55 (23.9)	1.0 (0.7–1.6)	0.848
>50	89 (26.1)	28 (25.2)	61 (26.5)	1.0 (0.6–1.5)	0.986
Educational level					
Illiterate/Elementary school	118 (34.6)	50 (45.1)	68 (29.6)		
High school	166 (48.7)	44 (39.6)	122 (53.0)	0.9 (0.5–1.4)	0.625
College	57 (16.7)	17 (15.3)	40 (17.4)	1.4 (0.9–2.2)	0.127
Marital status					
Married/Lives with his partner	182 (53.4)	52 (46.9)	130 (56.5)		
Widow/ Separate/Divorced	42 (12.3)	19 (17.1)	23 (10.0)	1.6 (1.0–2.4)	0.026
Single	117 (34.3)	40 (36.0)	77 (33.5)	1.2 (0.8–1.7)	0.302
Ethnic origin*/**					

White	83 (25.8)	24 (23.1)	59 (27.1)		
Afrodescendant	112 (34.8)	38 (36.5)	74 (33.9)	1.2 (0.8–1.8)	0.462
Asian-indigenous/Brown	127 (39.4)	42 (40.4)	85 (39.0)	1.1 (0.7–1.7)	0.530
Family income*					
≥ 5 minimum wages***	72 (21.6)	21 (19.4)	51 (22.7)		
≥ 2 and < 5 minimum wages	76 (22.8)	19 (17.6)	57 (25.3)	0.8 (0.5–1.4)	0.569
≥1 and < 2 minimum wages	114 (34.2)	41 (38.0)	73 (32.4)	1.2 (0.8–1.9)	0.346
< 1 minimum wage	71 (21.4)	27 (25.0)	44 (19.6)	1.3 (0.8–2.1)	0.266

* missing "did not answer"
** Ethnic origin: self-declared
***Brazilian minimum wages (Brazilian monthly minimum wage = U$284).
Univariate analysis

The majority of women were non-smokers (83.0%) or never smoked (74.3%) and 55.6% reported drinking alcoholic beverages and not using illicit drugs (98.5%), oral hormonal contraception (51.6%), injectable hormonal contraception (67.6%), intrauterine device (IUD) (72.7%) or condoms (72.6%)(Table 2).

Table 2. Distribution of the behavioral characteristics of the women interviewed in three different Public Health Units, Ouro Preto, Minas Gerais, Brazil, 2017

Variables	**Bacterial vaginosis**			**PR (95%CI)**	**p**
	Total n (%)	**Positive n(%)**	**Negative n(%)**		
Smoking*					
No	279 (83.0)	85 (78.0)	194 (85.5)		
Yes	57 (17.0)	24 (22.0)	33 (14.5)	1.4 (1.0–2.0)	0.072
Ex smoking*					
No	208 (74.3)	65 (76.5)	143 (73.3)		
Yes	72 (25.7)	20 (23.5)	52 (26.7)	0.9 (0.6–1.3)	0.586
Use of alcoholic beverage*					
No	149 (44.4)	47 (43.1)	102 (44.9)		
Yes	187 (55.6)	62 (56.9)	125 (55.1)	1.0 (0.8–1.4)	0.755
Consumption of alcoholic beverage*					
Not drink	149 (44.7)	47 (43.0)	102 (45.6)		
< 1 time per week	78 (23.4)	26 (23.9)	52 (23.2)	1.0 (0.7–1.6)	0.783
1 time per week	59 (17.7)	15 (13.8)	44 (19.6)	0.8 (0.5–1.3)	0.396
≥2 times per week	47 (14.2)	21 (19.3)	26 (11.6)	1.4 (0.9–2.1)	0.086
Current use of illicit drugs (any)*					
No	330 (98.5)	107 (98.2)	223 (98.7)		

Yes	5 (1.5)	2 (1.8)	3 (1.3)	1.2 (0.4–3.6)	0.705
Current use of oral hormonal contraception*					
No	176 (51.6)	62 (55.9)	114 (49.6)		
Not applicable**	90 (26.4)	26 (23.4)	64 (27.8)	0.8 (0.6–1.2)	0.308
Yes	75 (22.0)	23 (20.7)	52 (22.6)	0.9 (0.6–1.3)	0.492
Current use of injectable hormonal contraception*					
No	230 (67.6)	75 (68.2)	155 (67.4)		
Not applicable**	90 (26.5)	26 (23.6)	64 (23.8)	0.9 (0.6–1.3)	0.526
Yes	20 (5.9)	9 (8.2)	11 (4.8)	1.4 (0.8–2.3)	0.224
Current use of IUD					
No	248 (72.7)	83 (74.8)	165 (71.7)		
Not applicable**	90 (26.4)	26 (23.4)	64 (27.8)	0.9 (0.6–1.2)	0.435
Yes	3 (0.9)	2 (1.8)	1 (0.5)	2.0 (0.9–4.5)	0.100
Consistent condom use*					
No	212 (72.6)	76 (78.4)	136 (69.7)		
Yes	80 (27.4)	21 (21.6)	59 (30.3)	0.7 (0.5–1.1)	0.136

* missing "did not answer"

** Not applicable: concerning menopausal women

Most women were not pregnant at the time of the interview (96.8%) and did not have a history of abortion (79.2%), or STIs (92.0%). Furthermore, 50.8% reported previous BV, 73.3% were not in menopause, 72.9% had a history of pregnancy, 77.6% had an active sex life, 96.0% had only one sexual partner and 97.9% did not have a history of a female sexual partner (Table 3). The mean age and standard deviation (SD) of first menstruation and the first sexual intercourse were 12.8 (SD: 1.8) and 18.3 (SD: 3.5) years old, respectively.

Table 3. Distribution of the sexual behavioral characteristics of the women interviewed in three different Public Health Units, Ouro Preto, Minas Gerais, Brazil 2017

Variables	Bacterial vaginosis			PR (95%CI)	P
	Total n (%)	Positive n (%)	Negative n (%)		
Pregnancy*					
No	328 (96.8)	107 (96.4)	221 (96.9)		
Yes	11 (3.2)	4 (3.6)	7 (3.1)	1.1 (0.5–2.5)	0.790
History of pregnancy*					
No	92 (27.1)	30 (27.3)	62 (27.1)		
Yes	247 (72.9)	80 (72.7)	167 (72.9)	1.0 (0.7–1.4)	0.969
History of abortion					
No	270 (79.2)	90 (81.1)	180 (78.3)		
Yes	71 (20.8)	21 (18.9)	50 (21.7)	0.9 (0.6–1.3)	0.555
History sexually transmitted diseases*					
No	310 (92.0)	98 (89.9)	212 (93.0)		
Yes	27 (8.0)	11 (10.1)	16 (7.0)	1.28 (0.8–2.0)	0.305
Previous bacterial vaginosis*					
No	163 (49.2)	43 (40.6)	120 (53.3)		
Yes	168 (50.8)	63 (59.4)	105 (46.7)	1.4 (1.0–1.9)	0.033

Age of first menstruation*					
≤ 13 years	221 (67.8)	75 (70.1)	146 (66.7)		
> 13 years	105 (32.2)	32 (29.9)	73 (33.3)	1.1 (0.8–1.6)	0.539
Regular menstrual cycle*					
No	78 (32.6)	25 (30.1)	53 (34.0)		
Yes	161 (67.4)	58 (69.9)	103 (66.0)	1.2 (0.7–2.1)	0.545
Are in menopause*					
No	247 (73.3)	84 (76.4)	163 (71.8)		
Yes	90 (26.7)	26 (23.6)	64 (28.2)	0.8 (0.5–1.3)	0.376
Age of first intercourse*					
≤18 years	202 (62.0)	70 (65.4)	132 (60.3)		
>18 years	124 (38.0)	37 (34.6)	87 (39.7)	1.2 (0.8–1.6)	0.375
Active sex life*					
No	76 (22.4)	27 (24.6)	49 (21.3)		
Yes	264 (77.6)	83 (75.4)	181 (78.7)	0.9 (0.6–1.2)	0.496
Has more than 1 sexual partner*					
No	316 (96.0)	103 (95.4)	213 (96.4)		
Yes	13 (4.0)	5 (4.6)	8 (3.6)	1.2 (0.6–2.4)	0.646
History of a female sexual partner					
No	334 (97.9)	108 (97.3)	226 (98.3)		
Yes	7 (2.1)	3 (2.7)	4 (1.7)	1.3 (0.5–3.1)	0.526

* missing “did not answer”

Prevalence of bacterial vaginosis

The prevalence values obtained after the Gram staining and the cytological evaluation were 32.5% (CI95% 27.7–37.7) and 27.7% (CI95% 23.0–32.8), respectively. There was agreement of 90.6% between the two methods (Kappa coefficient= 0.77).

The sensitivity of the cytological evaluation was 77.8% (CI95% 69.1–84.6), the specificity 96.9% (CI95% 93.6–98.5) and the accuracy 90.6% (CI95% 87.0–93.3). The positive predictive value was 92.3% (CI95% 85.0–96.2) and the negative predictive value 90.0% (CI95% 85.5–93.2).

Factors associated with bacterial vaginosis

In order to identify the factors associated with BV, women with and without BV were compared according to Gram staining. From the output of the multivariate model, the variables associated with vaginosis were smoking (PR 1.5 [CI95%: 1.1 – 2.1]), use of IUD (PR 2.8 [CI95%: 1.2 – 6.5]) and previous history of BV (PR 1.5 [CI95%: 1.1 – 2.1]). These results show smoking and previous history of bacterial vaginosis increased the prevalence of BV by 1.5 times when compared to non-smokers and to women who do not have previous history of BV, respectively. Use of IUD increased the prevalence of BV by 2.8 times in comparison with women who do not use this contraceptive method (Table 4).

Table 4. Risk factors for the development of bacterial vaginosis, Brazil 2018

Variable	Crude IRR (95% CI)	Adjusted PR (95% CI)
Smoking		
no *versus* yes	1.4 (1.0–2.0)	1.5 (1.1–2.1)
Use of IUD*		
no *versus* yes	2.0 (0.9–4.5)	2.8 (1.2–6.5)
Previous cases of BV		
no *versus* yes	1.4 (1.0–1.9)	1.5 (1.1–2.1)

* The non-applicable category, which is composed of menopausal women, was also not significant in the multivariate analysis.

Detection of pathogens causing sexually transmissible diseases

The search for the pathogens responsible for STIs was carried out in 80 samples. *C. trachomatis* was the most commonly detected pathogen (20/80 cases), with 11 cases in women without BV and 9 in women with BV. HPV (17/80) was observed in 12 cases in women with BV and 5 in women without BV, followed by *T. vaginalis* (13/80) with 10 cases detected in women with BV and 3 in women without BV. *N. gonorrhoeae* (2/80) was observed in 1 case in a woman with BV and 1 in a woman without BV. The pathogens *T. vaginalis* and HPV were detected with higher frequency in women with BV (25.0 and 30.0% respectively). An association was observed between the presence of BV and the detection of *T. vaginalis* (OR: 4.1 (IC95% 1.1–16.3])

Co-infection was detected in 15% (12/80) of cases among the pathogens studied. The co-infection between *C. trachomatis* and HPV was the most frequent (41.7% [5/12]), followed by *C. trachomatis* and *T. vaginalis* (25.0% [3/12]), *T. vaginalis* and HPV (16.7 % [2/12]), *N. gonorrhoeae* and *C. trachomatis* (8.3% [1/12]) and *C. trachomatis*, HPV and *T. vaginalis* (8.3% [1/12]). Half of the co-infection cases were in women with BV.

DISCUSSION

The results of this study demonstrate a high prevalence of BV. Prevalence rates of vaginosis vary according to the population studied and the method used for diagnosis. In the present study, conducted in a population of women aged ≥18 years old, the prevalence of BV using Gram staining was 32.5%. Studies performed in Lithuania and Ethiopia with women in the same age group and using the same diagnostic method showed prevalence rates of 24.4 and 48.6%, respectively [2,19]. Other countries such as Canada (33.0%) and the United States (30.1%)

showed prevalence rates of 33.0% and 30.1%, respectively [1,4], exemplifying that different populations have different prevalence rates .Variability in the composition of the vaginal flora has been pointed out as an important factor for the difference in BV prevalence among populations. The higher prevalence of BV in African women, for example, can be explained by the low occurrence of *Lactobacillus* species (lactobacillary grade III) producing hydrogen peroxide, which are less frequent in black women and have pathogenic defense activity.[20]

Cervical cytology by Papanicolaou smears was also used to evaluate BV. The prevalence was 27.7%, which is lower than that detected by Gram staining. In a population in Brazil (Olinda, Pernambuco), Peres et al. (2015)[15,] using the same analysis method, observed similar prevalence (28.1%) to the present study, showing that populations within the same country may have similar prevalence rates. Thus, this population's prevalence rate could represent the country's prevalence rate. Although there was a difference in the prevalence rate between the cytological evaluation and the Gram staining, good agreement between them was found (*Kappa* = 0.77). Both methods can be used to diagnose BV [21], however, it is important to emphasize that the cytological evaluation is less sensitive. In the present study, a sensitivity of 77.8% was observed when the cytological technique was used, that is, the ability to correctly diagnose bacterial vaginosis is lower than that of Gram staining.

Gram staining using the Nugent score, despite being considered the gold standard in the diagnosis of BV and having been used in our study, has limitations as the punctuation system can generate confusion in the presence of a more complex vaginal flora, classifying it into only one category. [22] Conversely, cytological evaluation has an important role in the recognition of infectious and inflammatory changes of the female genital tract, designated as reactive cellular changes. Moreover, Anand et al. (2020) [23] have shown that Pap-stained smears can be an effective instrument for BV diagnosis, in addition to being important for the detection of cervical cancer. Papanicolaou is performed routinely for women and can be important in diagnosis because it can lead to an early detection of BV.[24] Despite this, considering the frequency of BV detection, our study showed that the Gram staining method may be more reliable than the Papanicolaou method. Thus, although more studies are needed, we suggest that the combination of the Gram method and cytological analysis can be a good strategy for the correct diagnosis of bacterial vaginosis.

Although no association was found between the presence of BV and the detection of HPV, this pathogen was detected more frequently in women who had BV. Mongelos et al. (2015)[25] showed that a large variety of HPV genotypes was detected in indigenous Paraguayan women with BV. Enzymes produced by anaerobic bacteria involved in the pathogenesis of BV

can potentially alter immune signals and promote the degradation of host factors, rendering women more susceptible to acquiring HPV.[26] HPV screening in this study was performed on vaginal swabs, unlike most studies where cervical specimens were used. Coorevits et al. (2018)[27] compared the sensitivity of HPV detection in cervical and vaginal samples and observed that these samples are equivalent to the detection of this pathogen. Thus, vaginal swabs can be used for the detection of HPV in screening strategies for cervical cancer.

Regarding the factors associated with BV, it was observed that smoking is a factor that increases the prevalence of BV.[28] This association can be explained by the fact that the level of protective Lactobacillus flora in smoker females is lower when compared to that of non-smokers. In addition, some authors have shown that the cigarette has an antiestrogenic effect, which in addition to amine accumulation in the vaginal epithelium, predisposes the women to develop BV.[28]

An association was also found between the use of IUD and the presence of BV. It is believed that the IUD can modify the vaginal flora, favoring the colonization of bacteria associated with BV. The presence of this device could facilitate the rise of cervicovaginal microorganisms, predisposing women to vaginosis and pelvic inflammatory disease.[29]

The recurrence of BV was another factor associated. The previous history of BV associated with new cases was also observed in other studies. [4],[25] Recurrence episodes are often associated with the failure of antibiotic treatment. This is mainly due to biofilm formation in the vaginal mucosa. It is possible that *Gardnerella vaginalis*, through its metabolic pathways and ability to form a biofilm, lowers the reduction-oxidation potential in the vaginal microbiome.[25]

In this study, an association was observed between BV and *T. vaginalis* infection. A study has shown that women with BV can present a higher risk of STIs, especially *Chlamydia trachomatis* and HPV infection.[1] Reasons for this increase are unclear but are believed to be due to elevated vaginal pH and the presence of inflammatory mediators and enzymes such as mucinase, which would provide a favorable environment for the establishment of pathogens. In fact, *T. vaginalis* grows at high pH, an environment provided when the woman has vaginosis.[1]

Besides being the pathogen most frequently detected in this study, *C. trachomatis* was also detected simultaneously with the other pathogens studied and the co-infection with HPV was the most frequent. The presence of *C. trachomatis* may increase the risk of HPV acquisition for two reasons: the presence of these bacteria is associated with disruption of the epithelial barrier, leading to the inflammatory response and *C. trachomatis* may interfere with the immune

response by decreasing the number of antigen-presenting cells and reducing the cell-mediated immunity, thus allowing the persistence of HPV.[30]

We acknowledge several limitations to this study. Only the population assisted in the public health service has participated in the study. However, we believe the associated factors described by our study are apparently not influenced by the socioeconomic profile. Another limitation was the use of a non-probabilistic sample evaluating only three basic health units, despite of the fact these units represent 49% of the female population aged ≥18 years old, living in the urban area of Ouro Preto. Thus, the internal validity of the study was possibly a little impaired.

In conclusion, the present study showed that both Gram staining and cytological evaluation can be used in the diagnosis of BV, despite the lower sensitivity of the cytological method. It is important that physicians and nurses from the Basic Health Units pay particular attention to smoking women, users of IUD and those with a previous history of BV since they have an increased risk for BV. The association found between BV and the detection of *T. vaginalis* signals that socio-educational measures should be taken to reduce the number of cases of BV and STIs.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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REFERENCE

1. Bautista CT, Wurapa E, Sateren WB, et. Bacterial vaginosis: a synthesis of the literature on etiology, prevalence, risk factors, and relationship with chlamydia and gonorrhea infections. Military Med Res 2016; 3:4. https://dx.doi.org/10.1186/s40779-016-0074-5

2. Bitew A, Abebaw Y, Bekele D, et al. Prevalence of bacterial vaginosis and associated risk factors among women complaining of genital tract infection. Int J Microbiol 2017; 4919404. https://dx.doi.org/10.1155/2017/4919404

3. Abou Tayoun AN, Burchard PR, Caliendo AM, et al. A multiplex PCR assay for the simultaneous detection of Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis. Exp Mol Pathol 2015; 98:214-218. https://dx.doi.org/10.1016/j.yexmp.2015.01.011

4.Lokken EM, Richardson BA, Kinuthia J, et al. A prospective cohort study of the association between body mass index and incident bacterial vaginosis. Sex Transm Dis 2019; 46:31-36. https://dx.doi.org/10.1097 / OLQ.0000000000000905

5. Marconi C, Duarte MT, Silva DC, et al. Prevalence of and risk factors for bacterial vaginosis among women of reproductive age attending cervical screening in southeastern Brazil. Int J Gynaecol Obstet 2015; 131:137141. https://dx.doi.org/10.1016/j.ijgo.2015.05.016

6. Isik G, Demirezen Ş, Dönmez HG, et al. Bacterial vaginosis in association with spontaneous abortion and recurrent pregnancy losses. J Cytol 2016; 33: 135-140.

7. Ministério da Saúde. Política Nacional de Atenção Integral à Saúde da Mulher: princípios e diretrizes. Brasília: Editora do Ministério da Saúde; 2011 [10-09-2019]. https://bvsms.saude.gov.br/bvs/publicacoes/politica_nacional_mulher_principios_diretrizes.pdf

8. Mascarenhas RE, Machado MS, Costa e Silva BF, et al. A population of sexually active adolescents from Salvador, Bahia, Brazil. Infect Dis Obstet Gynecol 2012; 2012:378640. https://doi.org/10.1155/2012/378640

9. Bradshaw CS, Walker J, Fairley CK, et al. Prevalent and incident bacterial vaginosis are associated with sexual and contraceptive behaviours in young Australian women. PLoS One 2003; 8(3): e57688. https://doi.org/10.1371/journal.pone.0057688

10. Nayar R, Wilbur DC. The Bethesda System for Reporting Cervical Cytology: A Historical Perspective. Acta Cytol 2017; 61:359 372. https://doi.org/10.1159/000477556

11. Claus, DC. A standardized Gram staining procedure. World J Microbiol Biotechnol 1992; 8:451-452. https://dx.doi.org/10.1007/BF01198764

12. Nugent RP, Krohn MA, Hillier SL. Reliability of Diagnosing Bacterial Vaginosis Is Improved by a Standardized Method of Gram Stain Interpretation. J. Clin Microbial 1991, 297-230. https://dx.doi.org/10.1128/JCM.29.2.297-301.1991

13. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002;287:2114-2119. https://dx.doi.org/10.1001/jama.287.16.2114

14. Boyanova L. Direct Gram Staining and Its Various Benefits in the Diagnosis of Bacterial Infections. Postgrad Med 2018; 130:105110. https://dx.doi.org/10.1080/00325481.2018.1398049

15. Peres AL, Camarotti JRSL, Cartaxo M. Molecular analysis and conventional cytology: association between HPV and bacterial vaginosis in the cervical abnormalities of a Brazilian population. Genet Mol Res 2015; 14: 9497-9505. https://dx.doi.org/10.4238 / 2015.Agosto.14.13

16. Khan Z, Bhargava A, Mittal P, Bharti R, Puri P, Khunger N, Bala M. Evaluation of Reliability of Self-Collected Vaginal Swabs Over Physician-Collected Samples for Diagnosis of Bacterial Vaginosis, Candidiasis and Trichomoniasis, in a Resource-Limited Setting: A Cross-Sectional Study in India. BMJ Open. 2019; 9:e025013. https://dx.doi.org/10.1136 / bmjopen-2018-025013

17. Silva NNT, Sabino AP, Tafuri A, Lima AA. Lack of association between methylenetetrahydrofolate reductase C677T polymorphism, HPV infection and cervical intraepithelial neoplasia in Brazilian women. BMC Medical Genetics 2019; 20:100-106. https://dx.doi.org/10.1186/s12881-019-0831-x

18. Clifford GM, Vaccarella S, Franceschi S, et al. Comparison of Two Widely Used Human Papillomavirus Detection and Genotyping Methods, GP5+/6+-Based PCR Followed by Reverse Line Blot Hybridization and Multiplex Type-Specific E7-Based PCR J Clin Microbiol. 2016; 54: 2031–2038. https://dx.doi.org/10.1128 / JCM.00618-16

19. Janulaitiene M, Paliulyte V, Grinceviciene S, et al. Prevalence and distribution of *Gardnerella vaginalis* subgroups in women with and without bacterial vaginosis. BMC Infect Dis 2017; 17:394. https://dx.doi.org/10.1186/s12879-017-2501-7

20.. Donders G, Bellen G, Donders F. Improvement of abnormal vaginal flora in Ugandan women by self-testing and short use of intravaginal antimicrobials. European J Clin Microbiol 2017; 36:731-738. https://dx.doi.org/10.1007/s10096-016-2856-9

21. Vandana G , Kumar KR , Khan S , Anil S. "Cytological Findings of Bacterial Vaginosis in Routine Pap Smears" A Two Yrs Institutional Study. IOSR-JDMS 2018; 17: 68-78. https://dx.doi.org/10.9790/0853-1701036878

22. Haahr T, Jensen JS, Thomsen L, Duus L, Rygaard K, Humaidan P. Abnormal Vaginal Microbiota May Be Associated With Poor Reproductive Outcomes: A Prospective Study in IVF Patients. Hum Reprod 2016; 31:795-803. https://doi.org/10.1093/humrep/dew026

23. Anand KV, Pimple SA, Mishra GA, et al. Reliability of conventional Papanicolaou smear in diagnosing bacterial vaginosis among women with clinical genital infection. South Asian J Cancer. 2020 Jan-Mar; 9(1): 13–16. https://dx.doi.org/10.4103 / sajc.sajc_421_18

24. Vieira-Baptista P, Lima-Silva J, Pinto C. Bacterial vaginosis, aerobic vaginitis, vaginal inflammation and major Pap smear abnormalities. Eur J Clin Microbiol Infect Dis 2016; 35, 657-664. https://dx.doi.org/10.1007/s10096-016-2584-1

25. Mongelos P, Mendoza LP, Rodriguez-Riveros I, et al. Distribution of human papillomavirus (HPV) genotypes and bacterial vaginosis presence in cervical samples from Paraguayan indigenous. Int J Infect Dis 2015; 39:44-49. https://dx.doi.org/10.1016/j.ijid.2015.08.007

26. Lu H, Jiang PC, Zhang XD. Characteristics of bacterial vaginosis infection in cervical lesions with high risk human papillomavirus infection. Int J Clin Exp Med 2015; 8:21080-21088.

27. Coorevits L, Traen A, Bingé, L. Are vaginal swabs comparable to cervical smears for human papillomavirus DNA testing? J Gynecol Oncol 2018; 29: e8. https://dx.doi.org/10.3802 / jgo.2018.29.e8

28. Nelson TM, Borgogna JC, Michalek RD, et al. Cigarette Smoking Is Associated With an Altered Vaginal Tract Metabolomic Profile. Sci Rep 2018; 8:852-864. https://dx.doi.org/10.1038/s41598-017-14943-3

29. Abdullateef RM, Ijaiya MA, Abayomi,F Adeniran AS, Idris H. Bacterial Vaginosis: Prevalence and Associated Risk Factors Among Non-Pregnant Women of Reproductive Age

Attending a Nigerian Tertiary Hospital. Malawi Med J. 2017; 29:290-293. https://dx.doi.org/10.4314 / mmj.v29i4.2

30. Mancini F, Vescio F, Mochi S, et al. HPV and Chlamydia trachomatis coinfection in women with Pap smear abnormality: baseline data of the HPV Pathogen ISS study. Infez Med 2018; 26:139-144.

Author Contributions:

Pedro Moregola Teixeira contributed to the interpretation of data, performed the experiments and wrote the manuscript.

Nayara Nascimento Toledo Silva performed the experiments for HPV detection.

Wendel Coura Vital contributed to the epidemiological analysis and interpretation of the results.

Glenda Nicioli da Silva, Angélica Alves Lima, Cláudia Martins Carneiro and Luiz Fernando de Medeiros Teixeira contributed to the experimental design, the interpretation of data and the critical reading of the manuscript. All authors have participated in this study and agree with the final version of this manuscript.