

## Clinical Study

# Prevalence and Risk Factors for Bacterial Vaginosis and Other Vulvovaginitis in a Population of Sexually Active Adolescents from Salvador, Bahia, Brazil

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Bacterial vaginosis, trichomoniasis, and genital candidiasis are considered the main etiologies of vulvovaginitis. Few studies estimate the prevalence of vulvovaginitis among adolescents, especially in Brazil. This study aimed to determine the prevalence and main risk factors associated with bacterial vaginosis and genital infection by *C. albicans* and *Trichomonas vaginalis* among a group of adolescents from Salvador, Bahia, Brazil. One hundred sexually active adolescents followed at an adolescent gynecology clinic were included. Endocervical and vaginal samples were obtained during gynecological examination. Nugent criteria were applied for the diagnosis of bacterial vaginosis. For *Candida albicans* and *Trichomonas vaginalis* detection, culture in Sabouraud agar plates and Papanicolaou cytology were used, respectively. The mean age of participants was  $16.6 \pm 1.6$  years. The prevalence of bacterial vaginosis was 20% (95% CI 12–28) and of genital infection by *Candida* was 22% (95% CI 14–30). Vaginal cytology detected *Trichomonas vaginalis* in one patient. Alcohol, tobacco, and illegal drug use ( $P = 0.02$ ) and multiple lifetime partners were statistically related to bacterial vaginosis ( $P = 0.01$ ). The prevalence of bacterial vaginosis and genital candidiasis was similar to other studies carried out among adolescents worldwide.

## 1. Introduction

Sexually transmitted diseases (STDs) are prevalent, it is estimated that 340 million new cases occur among adults worldwide [1]. These conditions are also regarded as an important public health problem for its medical, social and economic implications [2]. Vaginal infection is one of the most common gynecological affections and vaginal discharge is one of the most common reasons for which women seek medical attention [3]. Vulvovaginitis is a usual cause of genital infection in women. Bacterial vaginosis, *Candida albicans* genital infection and trichomoniasis are considered the main etiologies of vulvovaginitis [4, 5].

Bacterial vaginosis is a dysbiosis, in which a decrease in resident vaginal lactobacilli is associated with a growth

of anaerobic polymicrobial flora. Bacterial vaginosis is not considered a STD, being usually described, however, as a sexually enhanced disease, in which the frequency of intercourse plays a critical role [6]. Furthermore, bacterial vaginosis has been associated with increased susceptibility to HIV-AIDS and other STD [7].

*Candida* genital infection is the leading cause of fungal vulvovaginitis. Pregnancy, broad-spectrum antibiotic use, diabetes mellitus, and immunodeficiency have been described as important risk factors for *Candida* genital infection; however, asymptomatic microorganism colonization can occur in 25 to 50% of the cases [4, 8, 9].

*Trichomonas* vaginitis is often transmitted by sexual intercourse. It is the most common sexually transmitted pathogen, accounting for 180 million infections annually

[10]. The classical symptoms associated with the *T. vaginalis* infection include a yellowish-green frothy discharge, pruritis, dysuria, and the “strawberry” cervix which is characterized by punctuate hemorrhagic lesions.

The specific causes and risk factors associated with bacterial vaginitis are poorly understood; however, associations with sexual activity, use of hygiene products that alter the vaginal ecosystem, and genetic predisposition have been described [11, 12]. If undiagnosed or untreated, these infections might interfere with the women’s reproductive health, being commonly associated with many obstetric conditions such as pelvic inflammatory disease (PID), premature rupture of membranes (PROM), prematurity, and infertility [4, 5] and also increasing the risk of HIV-1 transmission.

Adolescence is a stage of physical and psychological transformation and behavioral experimentation, also being associated with risky sexual behavior in regards to STDs [12, 13]. During this period of life, reproductive hormones cause considerable physiological and tissue changes, which may increase susceptibility to infections. At this stage, the cylindrical epithelium of the endocervical channel is more ectopic and exposed to various agents that commonly infect these tissues [2, 14]. In Brazil, adolescents account for approximately 11% of the population [15]. Few studies have determined the prevalence of vulvovaginitis and associated risk factors among adolescents in Brazil. This population’s health is considered a priority by the National Health Agenda of the Brazilian Ministry of Health.

The goal of this study was to determine the prevalence and main risk factors associated with bacterial vaginosis, *C. albicans* genital infection, and trichomoniasis in a group of female adolescents from Salvador, Bahia, Brazil.

## 2. Materials and Methods

This is a cross-sectional study involving 100 sexually active adolescents followed in the Adolescent Gynecology Clinic (Serviço de Ginecologia da Infância e Adolescência—SEGIA) of Escola Bahiana de Medicina e Saúde Pública (EBMSP—Salvador, Bahia, Brazil), included between September 2008 and August 2010. By the time of enrollment, all participants have had at least one sexual partner in their lifetime, being considered sexually active. SEGIA is an outpatient clinic that as been offering complete gynecological care to 356 patients since 2008. By August 2010, approximately 37% of patients followed in the clinic were sexually active.

Patients were included sequentially at the time of gynecological appointment. Inclusion criteria were female gender, age between 10 and 19, years and sexual activity. Pregnancy, puerperium, and intravaginal medication use were adopted as exclusion criteria. The study was approved by the Institutional Review Board of EBMSP. An informed consent form was applied and signed by all the legally responsible persons for the adolescents prior to their participation in the study. The patients with 18 years of age or older signed it themselves. All principles outlined in the Declaration of Helsinki were followed.

The clinical and demographic data were obtained by applying a semistructured questionnaire. Then, the patients

were submitted to gynecological examination. To analyze the growth of *Candida* species, samples were collected using a vaginal swab dampened with sterile saline, plated aseptically on Sabouraud Dextrose Agar plates (Acumedia Neogene, Lansing, Michigan, USA) and incubated at 35°C for 24 to 48 hours. White, circular, medium-sized, and catalase positive colonies, observed as Gram-positive yeast in Gram staining, were tested for identification of *Candida albicans*. Positive germ tube test and green stain in CHROMagar *Candida* (Difco-Becton Dickinson Microbiology Systems, Maryland, USA) medium are indicative of these species. The strains that have been tested negative for the germ tube test and have not stained green in the CHROMagar medium were identified as *Candida spp.*

Smears prepared with vaginal and endocervical specimens collected during pelvic examination were Gram stained and observed through optical microscopes (1000X magnification) by two microbiologists. In order to make the diagnosis of bacterial vaginosis, the bacterioscopy was analyzed according to the scoring system proposed by Nugent et al. [16]. The total score ranges from 0 to 10. BV was diagnosed when the score was equal or higher than seven points.

The diagnosis of genital *Trichomonas vaginalis* infection was made with vaginal cytology using the Papanicolaou’s technique. Protozoan were identified by their morphological aspects (rounded, pyriform or irregular structures, measuring 10 to 20  $\mu$ m, cyanophilic cytoplasm and eccentric small nuclei).

Descriptive analysis of all variables was performed, including frequency distributions for ethnic groups, family income, conjugal status, education, alcohol, tobacco and illegal drug use, age at first sexual intercourse, age at menarche, sexual abuse, use of contraceptives, condom use, and number of sexual partners. The prevalence was calculated with a confidence interval of 95%. Chi-square or Fisher’s exact tests were used for the analysis of categorical variables. Students *t*-test was used to analyze the numeric data. A value of  $P \leq 0.05$  was considered statistically significant. All data were analyzed using the SPSS 17.0 software (IBM SPSS, Chicago, IL, USA) for Windows.

## 3. Results

One hundred patients were included in this study and all patients invited accepted to participate. The mean age of these individuals was  $16.6 \pm 1.6$  years; 91% were nonwhite and 80% were married or living with a partner; 55% had more than nine years of education; 49% belonged to families with incomes equal to or less than one minimum wage (Table 1).

The prevalence of bacterial vaginosis was 20% (95% CI 12–28) and genital infection by *Candida albicans* or *Candida spp.* was 22% (95% CI 14–30). Only one patient had genital *Trichomonas vaginalis* infection. Coinfections by *Candida* species and *T. vaginalis* and *C. albicans* and bacterial vaginosis were observed in two adolescents.

Alcohol, tobacco, and illegal drug use was associated with the diagnosis of bacterial vaginosis ( $P = 0.02$ ). Patients

TABLE 1: Sociodemographic and behavioral characteristics of the study population, stratified by the presence or absence of bacterial vaginosis or genital *Candida albicans* infection.

	<i>n</i> = 100	Bacterial vaginosis <sup>†</sup>			<i>Candida albicans</i> <sup>§</sup>		
		Negative ( <i>n</i> = 80)	Positive ( <i>n</i> = 20)	<i>P</i>	Negative ( <i>n</i> = 78)	Positive ( <i>n</i> = 22)	<i>P</i>
Age (years)	16.6 ± 1.6	16.6 ± 1.6	16.6 ± 1.6	0.88	16.6 ± 1.7	16.8 ± 1.4	0.49
Ethnic group				1			1
Nonwhite	91	73 (91.2)	18 (90)		71 (91)	20 (90.9)	
White	9	7 (8.8)	2 (10)		7 (9)	2 (9.1)	
Conjugal status				0.54			1
Married/living together	80	65 (81.2)	15 (75)		16 (20.5)	18 (81.8)	
Single	20	15 (18.8)	5 (25)		62 (79.5)	4 (18.2)	
Family income (minimum wages) <sup>¶</sup>				0.64			1
1	49	40 (50)	9 (45)		38 (48.7)	11 (50)	
2 to 4	47	37 (46.3)	10 (50)		36 (46.2)	11 (50)	
≥5	3	2 (2.5)	1 (5)		3 (3.8)	0	
Education <sup>¶</sup>				0.28			0.28
<9 years (primary/intermediate)	44	33 (41.3)	11 (55)		31 (41)	12 (54.5)	
≥9 years (secondary/higher)	55	46 (57.5)	9 (45)		45 (57.7)	10 (45.5)	
Alcohol, tobacco, and illegal drug use	52	37 (46.3)	15 (75)	0.02*	42 (53.8)	10 (45.5)	0.48
Age at menarche (years) <sup>‡</sup>	12.3 ± 1.6	12.4 ± 1.5	12 ± 2.1	0.55	12.4 ± 1.7	12.2 ± 1.4	0.67
Age at first sexual intercourse (years)				0.24			0.80
≤14	34	25 (31.3)	9 (45)		27 (34.6)	7 (31.8)	
≥15	66	55 (68.8)	11 (55)		51 (65.4)	15 (68.2)	
Sexual abuse	3	2 (2.5)	1 (5)	0.49	3 (3.8)	0	1
Contraceptive use	41	33 (41.3)	8 (40)	0.91	33 (42.3)	8 (36.4)	0.61
Condom use				0.84			0.75
Never/sometimes	53	42 (52.5)	11 (55)		42 (53.8)	11 (50)	
Always/Almost always	47	38 (47.5)	9 (45)		36 (46.2)	11 (50)	
Number of sexual partners <sup>¶</sup>				0.01*			0.18
1	55	49 (61.3)	6 (30)		39 (50)	16 (72.7)	
2 to 4	37	26 (32.5)	11 (55)		32 (41)	5 (22.7)	
≥5	7	4 (5)	3 (15)		6 (7.7)	1 (4.5)	

Data are presented as mean ± SD (standard deviation) for numeric variables and *n* (%) for categorical variables.

\*A *P* value ≤0.05 was considered statistically significant.

<sup>†</sup>The Nugent criteria was used for diagnosis.

<sup>§</sup>Vaginal and/or endocervical.

<sup>¶</sup>Data was available for 99 subjects. One minimal wage is approximately 330 US Dollars.

<sup>‡</sup>The specific age at menarche was available for 59 subjects, being ≤16 for all participants.

with bacterial vaginosis reported a higher number of sexual partners when compared to those without the disease (*P* = 0.01) (Table 1).

Signs and symptoms associated with bacterial vaginosis and genital infection by *Candida* species are shown in Table 2. Vaginal discharge was the clinical sign most frequently observed in patients with bacterial vaginosis (45%) and *Candida* infection (68.2%). Genital itching, dysmenorrhea, and genital lesions were found in 10% of patients with bacterial vaginosis. Only 9.1% of patients with *Candida* reported genital itching. 13.3% of these patients also complained about injuries and/or genital hyperemia. The patient infected with *T. vaginalis* presented with a white, odorless, and bullous vaginal discharge (data not shown).

## 4. Discussion

Bacterial vaginosis and genital *Candida* infection were present in almost a quarter of patients. These prevalences were similar to those reported in other studies involving adolescents in Brazil and other countries [2, 8, 17–19]. In our study, the rate of *Trichomonas vaginalis* infection was low, about 1%. In Brazil, the prevalence of *T. vaginalis* was 3.2% in women aged from 18 to 40 years followed at a primary care health service [20]. The literature unveils great variability in the prevalence rates of trichomoniasis, that may be due to heterogeneity regarding the diagnostic methods used and the group evaluated. Despite its clinical applicability, vaginal cytology has lower sensitivity and specificity rates

TABLE 2: Current genitourinary signs and symptoms of the study population, stratified by the diagnosis of bacterial vaginosis (BV) or genital *Candida albicans* infection ( $n = 99$ ).

	Bacterial vaginosis <sup>†</sup> $n = 20$		<i>Candida albicans</i> <sup>§</sup> $n = 22$	
	$n$ (%)	$P$	$n$ (%)	$P$
Vaginal discharge	9 (45)	0.72	15 (68.2)	0.03*
Genital itching	2 (10)	1	2 (9.1)	1
Dysmenorrhea	2 (10)	1	1 (4.5)	0.45
Abdominal/pelvic pain	0	0.34	2 (9.1)	0.61
Dysuria	0	1	0	1
Genital lesions and/or vaginal hyperemia	2 (10)	1	3 (13.6)	0.41

\*A  $P$  value  $\leq 0.05$  was considered statistically significant. The group of patients with bacterial vaginosis and genital candidiasis were compared with the respective groups of uninfected patients.

<sup>†</sup>The Nugent criteria was used for diagnosis.

<sup>§</sup>Vaginal and/or endocervical.

<sup>¶</sup>4 of these 22 patients were diagnosed with *Candida* spp.

for diagnosing *T. vaginalis* when compared to parasitological and molecular diagnosis, such as direct microscopy, the culture, and polymerase chain reaction [21, 22]. Sutton et al. [23], using molecular techniques, reported a prevalence of 2.1% in a population of 1416 adolescents aged 14–19 years from non-hispanic white, nonHispanic black, and Mexican-American ethnicity. On the other hand, Krashin et al. [14] and Crosby et al. [24], also in the United States, reported higher prevalence rates, 12.9% and 14.4%, respectively. Both studies used culture methods to detect *T. vaginalis* and were carried out among socioeconomically disadvantaged adolescents, mostly nonwhite patients, that attended public outpatient clinics, similar to the ones included in our study. However, the individuals included in these studies reported a high frequency of sexual intercourse with nonsteady, older partners [14, 24], significant history of delinquency and illegal drug use [24], and concurrent *Neisseria gonorrhoeae* infection [4], characteristics that differentiate these adolescents from our population.

The presence of bacterial vaginosis was associated with multiple sexual partners ( $P = 0.01$ ). In spite of the fact that bacterial vaginosis is not considered an STD, a greater risk for being diagnosed with this disease has been identified in patients with multiple sexual partners, which is consistent with our study [11]. In this sense, due to the number of sexual partners, in association with the commonly described irregular condom use among this population, one could infer that these adolescents were likely at increased risk for other STDs. Furthermore, male sexual partners may serve as carriers of anaerobic bacteria that cause bacterial vaginosis; the higher incidence of bacterial vaginosis among women with uncircumcised partners reinforces this hypothesis, since the presence of foreskin facilitates the conduction of microorganisms during intercourse [11].

The use of alcohol, tobacco, and illegal drugs was found to be associated with bacterial vaginosis. However, it should be interpreted as an indirect risk factor for bacterial vaginosis, since the use of these substances is usually related to practices such as irregular condoms use and poor genital hygiene, that foster the development of this condition [11, 12]. Genital infection by *Candida albicans* was not associated with any

of the risk factors evaluated, although some studies have reported that oral sex and sexual activity are important risk factors for genital infection by this microorganism [8, 18, 25].

Vulvovaginitis is an important public health problem, not only due to the gynecological and obstetric complications associated with it [4, 5] but also because the inflammation of the vaginal mucosa and the reduction of lactobacilli could facilitate the transmission of STDs, especially HIV infection [26, 27]. Moreover, the studied population showed a low frequency of condom use and a high number of sexual partners, behaviors that could lead to an important increase in the risk for STDs among these adolescents.

Regarding the method for the diagnosis of bacterial vaginosis, although Amsel's criteria are more often used in the clinical setting [28], the Nugent scoring system has a higher reproducibility [29], with comparable sensitivity and specificity to the former criteria [28, 30, 31].

This study is limited by its small sample size and the fact that a less reliable test was used for the diagnosis of *T. vaginalis* and might not reflect the real prevalence of this infection. Additionally, the small sample size can limit inference of results to other similarly aged adolescents from different regions of Brazil and the world.

## 5. Conclusions

In summary, the results obtained demonstrated a high prevalence of bacterial vaginosis and genital candidiasis among adolescents in a disadvantaged social environment of the third largest city in Brazil. If undiagnosed and untreated, these conditions may lead to major obstetric and gynecological complications, which considerably increase treatment costs and hospitalizations and also have a negative impact on female mortality rates. Adolescent medical care requires a multidisciplinary expertise, which guarantees motivation and enhances information quality about STDs and contraceptive methods. Programs that address these aspects could efficiently promote health to adolescents, significantly reducing STDs and unplanned pregnancy rates among this population.

## Conflict of Interests

The authors declare no conflict of interests.

## Acknowledgments

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