

PREVALENCE AND CORRELATES OF BACTERIAL VAGINOSIS AMONG YOUNG WOMEN OF REPRODUCTIVE AGE IN MYSORE, INDIA

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Abstract

Purpose: Bacterial vaginosis (BV) is the most common cause of abnormal vaginal discharge among women of childbearing age and is associated with STI/HIV and adverse birth outcomes. The objective of this study was to determine the prevalence and correlates of BV among young women of reproductive age in Mysore, India. **Methods:** Between October 2005 and December 2006, 898 sexually active women of 15-30 years of age were enrolled from two reproductive health clinics in Mysore. The women underwent an interview followed by physical examination, HSV-2 serologic testing, endocervical culture for *Neisseria gonorrhoeae*, and vaginal swabs for diagnosis of BV, *Trichomonas vaginalis* infection and candidiasis. Statistical analyses included conventional descriptive statistics and multivariable analysis using logistic regression. **Results:** Of the 898 women, 391 (43.5%) were diagnosed with ≥ 1 endogenous reproductive tract infection and 157 (17.4%) with ≥ 1 sexually transmitted infection. Only 863 women had Gram-stained vaginal smears available, out of which 165 (19.1, 95% confidence interval [CI]: 16.3%-22.2%) were found to have BV and 133 (15.4, 95% CI: 12.9%-18.3%) were in the 'intermediate' stage. BV was related to concurrent infections with *T. vaginalis* (odds ratio [OR] = 4.07, 95% CI: 2.45-6.72) and HSV-2 seropositivity (OR = 2.22, 95% CI: 1.39-3.53). **Conclusions:** In this population, the prevalence of BV at 19% was relatively low. Coinfection with *T. vaginalis*, however, was common. BV was independently associated with concurrent *T. vaginalis* infection and partner's alcohol use. Muslim women had reduced odds of BV as compared to non-Muslim women. Further research is needed to understand the role of *T. vaginalis* infection in the pathogenesis of BV and the sociocultural context surrounding the condition in India.

Key words: Bacterial vaginosis, correlates, epidemiology, India, prevalence, sexually transmitted infections

Bacterial vaginosis (BV) is the most common cause of abnormal vaginal discharge among women of childbearing age and is associated with low birth weight infants (LBW) and preventable preterm births. Various studies across the world have shown that women with BV are more likely to be co-infected with herpes simplex virus type-2 (HSV-2), *Trichomonas vaginalis*, *Neisseria gonorrhoeae* and HIV.^[1-4] Some studies have found a relationship between BV and high-risk behaviours associated with sexually transmitted infections (STIs) such as early sexual debut and multiple sex partners.^[5] High co-infection rates with other STIs raise the possibility that BV may either increase susceptibility to STI or share a common pathway with other STIs.^[1,4]

BV is of special public health concern in India because of the high burden of reproductive and pregnancy-related morbidity. Research on BV in India is sparse and mainly limited to a few states.^[6-10] To date, there are only two studies on the prevalence of BV in the state of Karnataka.^[9,10]

Because these studies had small sample sizes and one study diagnosed BV using Papanicolaou smears,^[9] it is difficult to interpret the findings. This study examined the prevalence and potentially modifiable risk factors for BV in young women of reproductive age and their male sex partners in Mysore, India.

Materials and Methods

Study population

Female recruiters screened potential participants in the waiting rooms of reproductive health clinics at CSI Holdsworth Memorial Hospital and Chitra's Hospital in Mysore, India. Eligible participants were enrolled into a prospective cohort study investigating the relationship of BV to incident HSV-2 infection. Detailed recruitment methods are described elsewhere.^[11] Women were given a multilingual flier outlining the study and were asked a series of questions to assess eligibility. To be included in the study, participants had to be between the ages of 15 and 30 years, sexually active (defined as having had vaginal intercourse at least once in the three months prior to enrolment), and willing to undergo a pelvic examination. Women who were pregnant or had vaginal bleeding were excluded from the study. Eligible women who expressed interest in participation were directed

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to a study interviewer who explained the study in greater detail, answered questions and obtained informed consent. An interviewer-administered questionnaire in Kannada or *Urdu*, the languages widely spoken, was used to collect socio-demographic and behavioral information about the participants and their sex partners. Women were screened for BV, *T. vaginalis* infection, endocervical gonococcal infection, vaginal candidiasis and HSV-2 antibodies. The study was approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley, and the Asha Kiran Institutional Review Board, and was in compliance with all federal regulations governing the protection of human subjects.

Laboratory methods

Diagnostic testing was carried out in the microbiology laboratories at Holdsworth Memorial Hospital and Vikram Hospital, Mysore. Vaginal swabs from the posterior fornix were used for wet mount examination and preparation of Gram stain for diagnosis of BV using the Nugent scoring system.^[12] Gram stains were interpreted independently by two study personnel, and the inter-observer agreement was excellent. In addition, for treatment purposes, BV was diagnosed based on at least three Amsel criteria:^[13] vaginal fluid pH > 4.5, a positive amine test, homogeneous vaginal discharge and presence of clue cells. In addition, vaginal swabs were cultured for *T. vaginalis* (InPouch Culture Kit; BioMed Diagnostics, White City, OR, USA) and *Candida* spp. (BioMed Diagnostics). Endocervical swabs were cultured for *N. gonorrhoeae* (BioMed Diagnostics). Sera were screened for HSV-2 antibodies using a type-specific ELISA test according to the manufacturer's instructions (Focus Technologies, Cypress, CA, USA). *Chlamydia trachomatis* was not diagnosed due to funding limitations.

Data analysis

Conventional descriptive statistics were used to assess the characteristics of study participants. Univariate associations of baseline characteristics with BV were made using Pearson Chi-square test or Fisher-exact method for categorical or ordinal variables. Continuous variables were compared using Student's *t*-test or the Mann-Whitney test for non-parametric data. Covariates were considered if they were associated with BV in the literature. The following characteristics of the subjects were examined: age, religion, education, years with partner, contraceptive use and sexual risk behaviors. Partner characteristics, such as alcohol and cigarette use and having other sex partners were also examined because we hypothesized that high-risk behavior in men would increase the odds of BV in female sex partners. Variables with *P*-values less than 0.2 were considered for inclusion in multivariable logistic regression models with tests of significance being two-tailed ($P < 0.05$). Adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated. Data were analyzed using Stata 9.0 (Stata Corporation, College Station, TX, USA).

Results

Of the 898 women enrolled, a diagnosis for BV was available among 863 (96%) because 35 slides were unreadable (inadequate material, over-stained or missing). The 35 women for whom a diagnosis of BV was unavailable did not differ from the study participants in age, education level, occupation or marital status.

All women in the study reported being ever married, with 10 women living with another partner, four widowed and four separated. Almost half (49%) of the participants had more than five years of education, but 26% did not receive any schooling. Seventy-four percent reported that their sole occupation was performing household chores and taking care of children. In addition to running a household, 20% worked as unskilled labourers in agriculture, rolling incense sticks or *beedis*, and 6% had other occupations. No women reported being involved in commercial sex work. Among the male partners, 45% were unskilled labourers (e.g., agriculture, construction, truck loading, cleaning, etc.), 43% performed skilled jobs (e.g., electricians, carpenters, technicians, etc.), and another 11% were drivers (lorry, taxi or auto rickshaws). Women reported a mean of five members living in each household, and 82% reported less than or equal to 4000 INR as their total monthly household income.

Sixty percent of the participants had their sexual debut before 18 years of age. Only 18 women (2%) reported more than one sex partner in their lifetime, and only four of those reported a new sex partner in the preceding three months. The median number of times a woman reported having had vaginal intercourse in the prior three months was 24 (IQR = 36-12). Condom use was low, with 94% reporting never having used a condom and only 4% reporting consistent condom use. Thirty-seven (4.3%) women reported ever having had oral sex, of whom 34 had it in the prior three months. Reversible methods of contraception were rarely used. A majority of the participants (63%) had undergone tubal ligation. None of the women were concurrently using multiple contraception methods.

Of the 863 women, 165 were diagnosed with BV (19.1%, 95% CI: 16.5-21.8%), 133 women (15.4%, CI: 13.0-17.9%) had intermediate flora and 565 (65.4%, CI: 62.1-68.6%) had normal flora (Figure). Women with BV were on an average older, non-Muslim, lived with their current partner for more than 10 years, had undergone tubal ligation and reported having had oral sex. These women also reported having partners who had other sex partners and drank alcohol (Table 1). Cigarette use and douching were not reported by any study participant. Unadjusted logistic regression analyses showed several characteristics of participants that were associated with BV (Table 2). Women who were older in age, non-Muslim (Hindu or Christian), having undergone tubal ligation, having partners who used alcohol or had other sex partners had increased odds of being diagnosed with

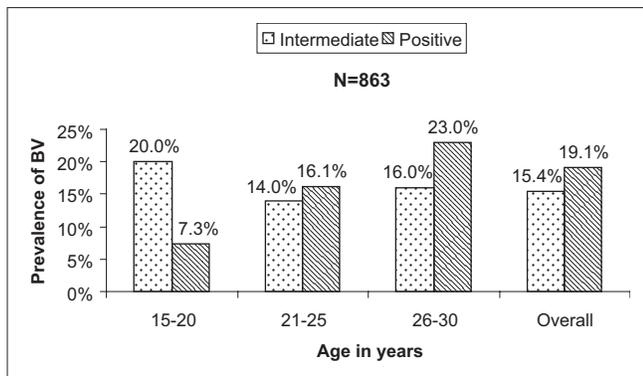


Figure: Prevalence of bacterial vaginosis among young reproductive age women in Mysore, India

BV. BV was also significantly associated with prevalent trichomoniasis and HSV-2 seropositivity. In multivariable analyses, after adjusting for factors that were significant in the unadjusted analyses, only three factors remained significantly associated with the presence of BV. The relative odds of being diagnosed with BV for Muslim women was 0.5 (95% CI: 0.3-0.9) as compared to non-Muslim women. In addition, having prevalent trichomoniasis (AOR: 3.5, 95% CI: 2.0-6.2) or a partner who drank alcohol (AOR: 1.6, 95% CI: 1.1-2.3) was significantly associated with BV.

Discussion

Since India has a high burden of reproductive morbidity, and BV has been documented as a risk factor for both adverse birth outcomes and HIV, this study investigated potentially modifiable behavioural and biological risk factors for BV. The prevalence of BV in this sample, at 19%, was in the range of previous findings for other populations in India.^[6,14]

In unadjusted analyses, BV was associated with older age in this study. Some researchers have previously reported that the condition is more common among younger women,^[15] while others have found that risk for BV increases with age.^[16,17] We hypothesize that, in this population, age is a proxy for cumulative sexual activity, which has been found to be associated with BV. Interestingly, although the proportion of women with BV increased with age, a higher percentage of younger women (15-20 years of age) were found to have intermediate flora by Nugent score (Figure). This seems counterintuitive because one would assume that a higher number of women with intermediate scores should also be reflected in elevated levels of BV among the same age group. It appears that Nugent score may misclassify disturbed vaginal flora as a stage in BV pathogenesis in younger women. In India, this may have important implications because women in the 15-20 year age range are at higher risk for STI and bad birth outcomes. Because we had a relatively small number of women in this age category, more research is needed to understand the

performance of Nugent's score among younger women for the diagnosis of BV.

Religion was the only socio-demographic variable that remained significantly associated with BV after adjusting for other correlates in the multivariable analyses. In our study, non-Muslim women (Hindus and Christians) had 1.9 times the odds of having BV as compared to Muslim women. Another study in Africa showed similar findings.^[18] Since we did not collect information on certain risk factors, such as genital hygiene practices that are known to be associated with BV and may vary by religion,^[15] additional research is needed to better understand the socio-cultural risk factors surrounding this condition. Other socio-demographic known risk factors such as marital status, douching and smoking were not associated with BV in our population.^[19-22]

Consistent with other research, trichomoniasis was associated with BV^[8,18] in our study. The cross-sectional nature of this analysis did not allow us to establish temporality of these infections. It is plausible that *T. vaginalis* infection alters the vaginal ecology and facilitates the development of BV, or that women with BV have lost natural protection against genital tract infections leading to the acquisition of STIs like *T. vaginalis* infection. The mechanisms underlying these relationships are not well understood, and warrant further investigation.

We had hypothesized that high-risk sexual behaviors in men increased the odds of BV in their female sex partners. Although we found that partner age, education, occupation (data not shown) and smoking status were not associated with BV, having a male partner who used alcohol or had other sex partners were found to be significantly associated with BV.^[17] Although our study does not have adequate information on partner sexual behaviours, our findings do suggest that male partner characteristics may play a role in the development of BV.

There are several limitations in our study. Because the analyses were cross-sectional in nature, we cannot infer any causal association between STI and BV. In addition, risk factors were self-reported, and it is possible that there was under-reporting and misclassification of risk behaviors. The survey involved the collection of temporally distant and sensitive sexual behaviors, as well as information on women's sex partners, so there is a possibility of measurement error that may lead to residual confounding obscuring the relationships. Although oral sex was found to be associated with BV in the bivariate analyses, we did not include it in the regression model because we had not collected information that would help us differentiate receptive oral sex from giving oral sex. Because receptive oral sex has been shown to be a risk factor for BV, further research is needed to better understand this association in Indian women. Another limitation of this study was the potential for selection bias as

Table 1: Prevalence of bacterial vaginosis by socioeconomic, demographic, reproductive and sexual risk factors among 863 Indian women

Characteristic	Total		BV present		P-value
	n	%	n	%	
Overall	863		165	19.1	
Demographic characteristics					
Age in years					0.004
15-20	55	6.4	4	7.3	
21-25	348	40.3	56	16.1	
26-30	460	53.3	105	22.8	
Years of education					0.04
≤7 years	349	40.5	78	22.4	
>7 years	513	59.5	86	16.8	
Religion					0.000
Hindu	595	68.9	135	22.7	
Muslim	249	28.9	27	10.8	
Christian	19	2.2	3	15.8	
Current contraception history					
Not using any birth control	223	25.8	38	17.0	0.35
Tubal ligation	546	63.3	116	21.3	0.04
Using IUD	33	3.8	7	21.2	0.76
Oral contraceptive pills	18	2.1	1	5.6	0.14
Condom	35	4.1	2	5.7	0.04
Rhythm or withdrawal	8	0.9	1	12.5	0.50
Sexual behaviors					
Ever had oral sex	37	4.3	14	37.8	0.003
Having sex when partner was under the influence of alcohol	348	40.4	82	23.6	0.007
Number of years with current partner					
≤4 years	140	16.2	25	17.9	
>4 to ≤10 years	417	48.3	67	16.1	
>10 years	306	35.5	73	23.9	
Partner risk behaviors					
Alcohol use by partner	379	44.1	94	24.8	0.000
Cigarette use by partner	425	49.4	86	20.2	0.39
Other sexual partners of spouse					
Yes	65	7.5	15	23.1	
No, not now, in the past	87	10.1	27	31.0	
No, never in the past	335	38.8	56	16.7	
Don't know	376	43.6	66	17.7	
Laboratory tests					
Trichomoniasis	71	8.2	32	45.1	0.000
Vaginal candidiasis	266	30.8	51	19.2	0.97
HSV-2 seropositivity	97	11.2	31	31.9	0.001

a non-random sample was used in the study; and the findings may not be generalizable to other populations.

Despite these limitations, the possible links of BV with trichomoniasis, being a non-Muslim and partner's alcohol use warrant further investigation given the high

burden of reproductive morbidity and poor birth outcomes in India. Furthermore, because there is strong evidence in literature that BV is associated with STIs including HIV, further studies are needed to understand the potential role of screening and treatment of BV in STI/HIV prevention programs.

Table 2: Select socio-demographic, partner characteristics and sexually transmitted infections associated with bacterial vaginosis among 863 young married women in Mysore, India

Characteristic	Crude OR	95% CI	Adj. OR	95% CI
Age in years				
15-20	1.00		1.00	
21-25	2.45	0.84, 7.04	3.29	1.08, 10.0 [†]
26-30	3.77	1.33, 10.7 [†]	3.91	1.28, 11.9 [†]
Religion				
Muslim	0.42	0.27, 0.65 [‡]	0.51	0.31, 0.84 [‡]
Non-muslims (Hindu, Christian)	1.00		1.00	
Partner uses alcohol				
Yes	1.92	1.36, 2.70 [‡]	1.70	0.91, 3.17
No	1.00		1.00	
Sex under the influence of alcohol				
Yes	1.60	1.14, 2.26 [‡]	0.82	0.44, 1.51
No	1.00		1.00	
Tubal ligation				
Yes	1.48	1.02, 2.13 [†]	1.07	0.71, 1.60
No	1.00		1.00	
Ever had oral sex				
Yes	2.72	1.37, 5.40 [‡]	3.62	1.71, 7.67 [‡]
No	1.00		1.00	
Trichomoniasis				
Yes	4.07	2.45, 6.72 [‡]	3.48	2.03, 5.98 [‡]
No	1.00		1.00	
HSV-2 seropositive				
Yes	2.22	1.39, 3.53 [‡]	1.47	0.89, 2.43
No	1.00		1.00	

[†] $P < 0.05$; [‡] $P < 0.001$

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