PREVALENCE AND RISK FACTORS OF BACTERIAL VAGINOSIS AMONG WOMEN OF REPRODUCTIVE AGE ATTENDING RURAL TERTIARY CARE INSTITUTE OF WESTERN UTTAR PRADESH

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ABSTRACT

BACKGROUND

Bacterial vaginosis (BV) is the most common cause of abnormal vaginal discharge among women of child bearing age and is associated with adverse obstetric and gynaecologic outcomes.

OBJECTIVES

The aim of the study was to determine the prevalence of BV by use of Nugent's criteria and to identify modifiable and non-modifiable, the risk factors associated with BV in women of reproductive age.

METHODOLOGY

A descriptive cross sectional study was conducted from January 2013 to December 2013, among women of child bearing age with complaints of vaginal discharge, attending Gynaecology and Obstetric OPD at UPRIMS & R, Saifai, Etawah. Bacterial morphotypes indicative of BV were identified by Nugent's criteria. A pre-coded questionnaire was used to collect demographic and behavioural characteristics (including contraceptive usage, douching practice) in the study participants.

DATA ANALYSIS

Bivariate and multivariate analyses by logistic regression method performed. Crude Odds ratio and Adjusted Odds Ratio for the association between BV and demographic or behavioural characteristics was calculated using Poisson regression. Sensitivity, specificity, PPV and NPV were calculated keeping Nugent score of ≥7 for BV as 'Gold standard.'

RESULTS

A prevalence of 31.5.0%, (95% CI 25.6-38.2) was obtained from the study population. Sensitivity, specificity, PPV and NPV of Amsler's criteria for clinical diagnosis of BV was 67.7%, 89.39%, 74.58% and 85.02% respectively. Low socioeconomic status including occupation, illiteracy, Intrauterine Contraceptive Device (IUCD) usage, douching practice and condom usage were significantly associated with BV.

CONCLUSIONS

The prevalence of BV was 31.5% in this population. Concordance between Amsel's criteria and Nugent's was low (67.67%). Among individual Amsel's parameters, vaginal pH >4.5 had highest sensitivity (84.61%) and demonstration of 'Clue Cell' was most specific (92.2%). Risk factors for BV ought to be evaluated in larger population for development of interventional strategies.

KEYWORDS

Bacterial Vaginosis, Amsel's Criteria, Nugent's Criteria, Reproductive Age Group, Risk Factors.

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INTRODUCTION

Bacterial Vaginosis (BV) is the among the common vaginal condition among women of child bearing age.[1] It is an imbalance in the ecology of the normal vaginal flora that is characterized by the depletion of *lactobacilli* and the proliferation of anaerobic bacteria such as *Gardnerella vaginalis*, *Mobiluncus* species, *Prevotella* species, *Mycoplasma hominis* and the recently identified *Atopobium vaginae*. *Megasphaera* spp., *Lachnospira* spp., and *Sneathia* spp.[2,3] It most often manifests clinically as an increase in thin whitish homogeneous, malodorous vaginal discharge.[4] The pathogenesis of BV remains controversial. It has been hypothesized that G. vaginalis create a biofilm community and successfully competes with lactobacilli for dominance in the vaginal environment.[5] A symbiotic relationship between G. vaginalis and anaerobes exists where G. vaginalis, metabolically produces amino acids through its proteolytic action. These amino acids are utilized by strict anaerobes such as Prevotella bivia as fuel source and as a result produce ammonia, which in turn is used by G. vaginalis.[6] Moreover, this symbiotic relationship with the production of ammonia would cause a shift to a more alkaline pH, which is inhospitable to lactobacilli.[7] Studies have shown that the addition of anaerobes to a G. vaginalis biofilm enhances the growth of G.

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vaginalis.[8] These symbiotic relationships could be responsible for the microbiological findings that define BV as well as the typical vaginal signs (Sloughing of the vaginal epithelium visualized as 'clue cells') and amine odor resulting from metabolic by-products of the increased numbers of BV-associated anaerobes.[9]

BV has been shown to increase the risk of gynaecological morbidity and adverse obstetrical outcomes such as preterm delivery, Pelvic Inflammatory Disease (PID) following surgical procedures, hysterectomy and upper genital tract infections.[10-13]

Bacterial vaginosis has been shown to correlate well with Amsel clinical criteria and is an effective way of screening cases. The four diagnostic elements are: A vaginal fluid of pH>4.5, presence of "clue cells" (Epithelial cells with unclear borders, dotted with bacteria) on microscopic examination of vaginal swab samples in normal saline, milky homogeneous, adherent vaginal discharge and a positive 'whiff' test, which is accentuation of an amine or 'fishy' odor of discharge after addition of 10% potassium hydroxide. The presence of three out of four criteria is recommended by Amsel for diagnosis.[9] However, the Amsel's criteria, has been criticized because two of the four criteria, in particular the appearance of the discharge and the appraisal of the odour are rather subjective and hence may lead to misdiagnosis. By contrast, a pH greater than 4.5 is considered the most sensitive criterion, whereas the presence of clue cells has been considered the single most specific predictor of BV.[14] Nugent et al described a Gram stain scoring system of vaginal smears to diagnose BV.[15] The Nugent score is calculated by assessing for the presence of large gram-positive rods (Lactobacillus morphotypes; decrease in Lactobacillus scored as 0 to 4), small gram-variable rods (G. vaginalis morphotypes; scored as 0 to 4), and curved gram-variable rods (Mobiluncus spp. morphotypes; scored as 0 to 2) and can range from 0 to 10. A score of 7 to 10 is consistent with BV. Compared to the Amsel criteria, the Nugent score allows for assessment of alteration in vaginal flora as a continuum rather than a dichotomy. Because Amsel criteria are dependent on the acumen of the clinician, the Nugent score has been favoured for diagnosing BV due to superior reproducibility and sensitivity.[14]

Previous studies have identified a number of risk factors and behaviour associated with BV including the number of lifetime male sexual partners, recent partner change, lower age of first intercourse, working as sex worker, douching, use of an intrauterine device, non-usage of condoms and smoking.[16-19] Other factors that have been associated with BV are low socio-economic status, poor personal hygiene, marital status, HIV infection, STIs most commonly trichomoniasis.[20]

In India, reported prevalence of BV varies widely among different population. It ranges from 17.8% to 45% among sexually active women with vaginal discharge, 11.53% to 38.5% among pregnant women.[21-24] Many studies have investigated the association of risk factors and bacterial vaginosis elsewhere; however, no such study has been done on population in and around Etawah district of Western Uttar Pradesh. Therefore, the present study was carried out to determine the prevalence of bacterial vaginosis and the associated risk factors.

MATERIALS AND METHODS

A descriptive prospective cross sectional study was conducted at Department of Microbiology and Department of Gynaecology and Obstetrics, UPRIMS and R, Saifai, Etawah. All adult women of child bearing age (18-45) with complaints of vaginal discharge, attending the Gynae and Obs OPD, UPRIMS and R, Saifai from January 2013 to December 2013 were included. Pregnant or puerperal women, those taking medications for STIs or having abnormal uterine/vaginal bleeding were excluded from the study. Sample size of 206 was calculated using the formula[25] $\{n=4pq/ (20\% \text{ of } p)\}^2$ assuming the prevalence of vaginal discharge 32.7%[26] and margin of error 20%.

Ethical clearance was obtained by the Institute. Informed written consent was taken from patient before their enrolment in the study. For illiterate women consent information was read and explained, then thumb print was taken on consent form. A semi-structured schedule was prepared for interviewing women and information about demographic, socio-economic status, obstetric and gynaecological history, douching, contraceptive use was recorded. After history taking, general physical examination, per abdomen, per speculum and per vaginum examinations were done. During per speculum examination vaginal mucosa was inspected for presence of erythema, lesions and discharge. Vaginal material was collected from the posterior fornix with help of sterile cotton-swab. Three swabs per patient were collected.

From the first swab, vaginal pH was determined by touching a pH strip and comparing the change against a reference reader. Then few drops of 10% potassium hydroxide were added to it to determine amine/fishy odor (whiff test). The second swab was smeared on to a glass slide for Gram staining. They were then methanol fixed, coded and sent to the Department of Microbiology, UPRIMS and R for Gram's staining. Gram stained smears were evaluated for bacterial vaginosis as well as for candida spp. (Budding yeast-like cells).

The third swab was placed in screw-cap plastic tubes containing 0.5 mL of 0.9% saline to carry out wet mount microscopy for detection of Trichomonas vaginalis. Swab was vigorously rotated in the saline and pressed against the side of the tube to express as much fluid as possible. A drop of the expressed fluid was placed on glass slide with a cover slip and examined at magnification of 400x within 15 minutes of collection of the sample. The positive result was defined as the presence of one or more Trichomonads with characteristic morphology and jerky motility.[27]

For the purpose of the study, the Nugent's score[15] was taken to be the gold standard. The Nugent's score was assessed as following:

- Small Gram-negative/Gram-variable rods (G. vaginalis morphotypes) more than 30 bacteria per oil immersion field (OIF) was scored as 4; a count of 6-30 bacteria per OIF scored 3; and 1-5 bacteria per OIF scored 2. Less than 1 per OIF scored 1 and their absence scored 0.
- For large Gram-positive rods (Lactobacillus morphotypes), the scoring was reversed with their absence scored as 4, fewer than 1 per OIF scored 3; a count of 1-5 per OIF scored 2; a count of 6-30 per OIF scored 1; and more than 30 per OIF scored 0.
- For curved Gram-variable rods (Mobiluncus spp. morphotypes), the presence of five or more bacteria was scored 2, less than 5 scored 1 and absence of bacteria was scored as 0.

The sum of the 3 scores was taken and a score of 7 or more

was considered as a case of bacterial vaginosis.

DATA ANALYSIS

Conventional descriptive statistics were used to assess the characteristics of study participants. For numerical data, mean±SD was calculated at 95% CI level. 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. Nugent's scores of 0-3 and 4-6 were clubbed and categorized as 0 and category 7-10 as 1 to denote absence and presence of BV respectively. The following characteristics of the subjects were examined: age, education, parity, contraceptive use. Socio-economic status was assessed according to modified BG Prasad classification.[28] and entered into binary logistic regression method to determine the association of various covariates and BV. The association was reported with Crude Odds Ratio (COR). Multivariate analyses were performed on variable with p-values <0.1. Adjusted Odds Ratio (AOR) and respective p-value were calculated with p-values <0.05 considered significant. Data were analysed using IBM SPSS statistics vs 21 software (USA).

RESULTS

Among of 206 adult women of child bearing age enrolled in the study, BV was diagnosed in 65 (31.5%) patients by Nugent's criteria, while 59 (28.64%) patients were positive by Amsel's criteria [Table 1]. Maximum concordance 84.6% with BV was seen for vaginal pH >4.5 and least 52.3% for the characteristic homogeneous vaginal discharge [Table 2]. The sensitivity, specificity, PPV and NPV of overall and individual parameters of Amsel's criteria are shown in Table 3. Vaginal pH >4.5 had the highest sensitivity, while highest specificity was observed for 'clue cells'. Budding yeast like cells (candida spp) were seen in 8 subjects (3.8%) and only 2 were present in BV. No trichomonal co-infection was seen in our study.

The mean age of the individual was 29.5±5.5 years. The age group 25-30 years maximally presented with 38.8%, while least representation 3.9% was in age group 40-45 years. Majority (49%) of respondents had elementary level education with 21.8% had no formal education; 17% had high school education, whereas 12.1% were graduate.

An updated BG Prasad socioeconomic classification with Consumer Price Index, Oct. 2015 (Industrial worker) of 259, was used as an indicator of socioeconomic status. More than half (56.8%) of the respondents earned less than Rs 2000/ month. Over half of the women enrolled were housewife (51.5%), followed by unskilled labourers 21.8%, while 14.6% were salaried and 12.1% were self-employed [Table 4].

The majority 85.9% of women in our study were married; unmarried and widowed/divorced being 9.7% and 4.4% respectively. A small proportion of women were nulliparous, whereas 25.3% had one or two child and 56% had more than two children [Table 4].

There were various family planning methods used and reported by the study participants. These included condoms, IUCD and oral pills. A small proportion 2.9% of participants did not use any method of contraception and/or used rhythm method. Among contraceptives used, oral pills 29.1% were most preferred followed by condom and IUCD, 27.7% and 15.5% respectively. Terminal sterilization by tubal ligation was seen in 12.7% of women. About one-third (33.0%) of the women gave history of regular douching practice. The frequency of planning methods, reproductive characteristics, behaviour and BV prevalence is shown in Table 5.

Overall prevalence of BV was 31.5% (95% CI 25.6-38.2) and the highest proportion 31/65 (47.9%) was observed in age group of 25-30 years compared with the lowest proportion 2/65 (3.07) % in age group 40-45 years. Women with bacterial vaginosis were significantly younger than non-bacterial vaginosis group (mean 27.8±4.7 years vs 30.3±5.8 years, p<0.0001).

The demographics of BV group and non-BV group is compared and shown in Table 4. On performing the bivariate analysis (Chi-square) of various characteristics among BV and non-BV subgroups, age (p=0.48), marital status (p=0.81), parity (p=0.13), non-use of any contraception method (p=0.42), usage of oral pills (p=0.6), tubal ligation (p=0.35), did not give any statistically significant difference (p≤0.05). For characteristics showing significant difference, bivariate and multivariate analysis for their strength of association (Odds Ratio, OR) with BV was calculated. In multivariate analysis after adjusting for factors that were significant in unadjusted analyses, increased odds of being diagnosed with BV were seen among non-literate women, low socioeconomic (BG Class 5), labour class, IUCD usage and history of regular douching practice with adjusted OR of 2.3 (p=0.001), 2.1 (p=0.02), 1.9 (p=0.03), 1.7 (p=0.023) respectively. Condom usage had AOR of 0.26 (p-value 0.029) indicating its protective role in BV [Table 6].

Criteria		Nugent's Criteria	
		BV Present (n=65)	**BV Absent (n=141)**
Amsel's Criteria	Present	44	15
	Absent	21	126

Table 1: Comparison of Amsel's Criteria for BV Diagnosis (Nugent's Criteria)

Parameter	Number (%) of Samples According to Nugent's Criteria			
	Without BV (n=141)		With BV (n=65)	
	n	%	n	%
Vaginal pH>4.5	40	28.36	55	84.61
Clue cells	11	7.8	48	73.84
Positive whiff test	15	10.63	45	69.23
Characteristic Vaginal discharge	26	18.43	34	52.3

Table 2: Frequency of Positivity of Different Amsel's Parameters in Women With and Without BV Vaginosis Diagnosed Through Nugent Criteria

Diagnostic Method	Sensitivity %	Specificity %	PPV %	NPV %
Total Amsel's criteria	67.69	89.36	74.58	85.02
Vaginal pH>4.5	84.61	71.63	57.89	90.99
Clue Cells	73.85	92.20	81.36	88.44
Positive Whiff test	69.23	89.36	75	86.30
Characteristic vaginal discharge	52.31	81.56	56.67	78.77

Table 3: Sensitivities and Specificities, PPV & NPV of Amsel's Parameters and Individual Components in Comparison to Nugent Score for Diagnosis of BV

Characteristic	Total		BV Present		p-Value; X²(df)
	n	%	n	%	
Overall	206		65		
Age in years					0.48,3.44 (4)
18-24	34	16.5	10	29.4	
25-30	80	38.8	31	38.7	
31-35	64	31.1	16	25.0	
36-40	20	9.7	6	30.0	
41-45	08	3.9	2	25.0	
Education					0.03;8.7(3)
Non literate	45	21.9	22	48.9	
Elementary School	101	49	29	28.7	
High School	35	17	9	25.7	
Graduate	25	12.1	5	20	
Socio-economic status* (Per capita income)					<0.03; 10.6 (4)
I (> Rs 6140)	14	6.8	1	7.1	
II (Rs 3070-Rs 6139)	32	15.5	7	21.8	
III (Rs 1842-Rs 3069)	43	20.9	10	23.3	
IV (Rs 921-Rs 1841)	50	24.3	20	40	
V (<Rs 921)	67	32.5	27	40.3	
Occupation					0.04; 8.34(3)
Labourer	45	21.8	21	46.7	
Self employed	25	12.1	10	40	
Service	30	14.6	7	23.3	
Housewife	106	51.5	27	25.5	
Marital status					0.81;0.42 (2)
Unmarried	20	9.7	6	30	
Married	177	85.9	57	33.2	
Divorced/widowed	9	4.4	2	22.2	
Parity					0.13; 4.0 (2)
0	38	18.4	12	31.6	
1-2	52	25.3	22	42.3	
>2	116	56.3	31	26.7	

Table 4: Baseline Social and Demographic Characteristic of the Study Population

*According to updated BG Prasad Socioeconomic criteria, 2015. p-values in bold are statistically significant (p<0.05).

Characteristic	Total (n=206)	%	BV Present (n=65)	%	P-value; X2(df)
Current Contraception History					
No Contraception Use	26	12.7	7	26.9	0.58; 0.29 (1)
Condom Usage	57	27.7	11	19.2	0.019; 5.48 (1)
Oral Contraceptives	60	29.1	19	31.6	0.94; 0.005 (1)
Tubal Ligation	31	15.0	12	38.7	0.35; 0.86 (1)
IUCD	32	15.5	16	50.0	0.014; 5.9 (1)
Regular Douching Practice	68	33.0	29	42.6	0.016,5.78 (1)

Table 5: BV Prevalence, Frequency of Family Planning Methods Used and Douching Behaviour

P-values in bold are statistically significant (p<0.05).

Characteristic	Crude OR	p value	Adj OR	p value
Education				
Non literate	2.9	0.001	2.3	0.03
Graduate	Reference		Reference	
Socio-economic status				
I	Reference		Reference	
V	2.4	0.006	2.1	0.02
Occupation				
Labour	2.3	0.014	1.9	0.03
Housewife	Reference		Reference	
IUCD				
Yes	2.3	0.03	1.7	0.023
No	Reference		Reference	
Condom usage				
Yes	0.24	0.003	0.26	0.029
No	Reference		Reference	
Douching practice				
Yes	2.1	0.017	2.06	0.022
No	Reference		Reference	

Table 6: Select Socio-Demographic, Contraceptive Characteristics and their Odds Ratios

P-values in bold are statistically significant (p<0.05).

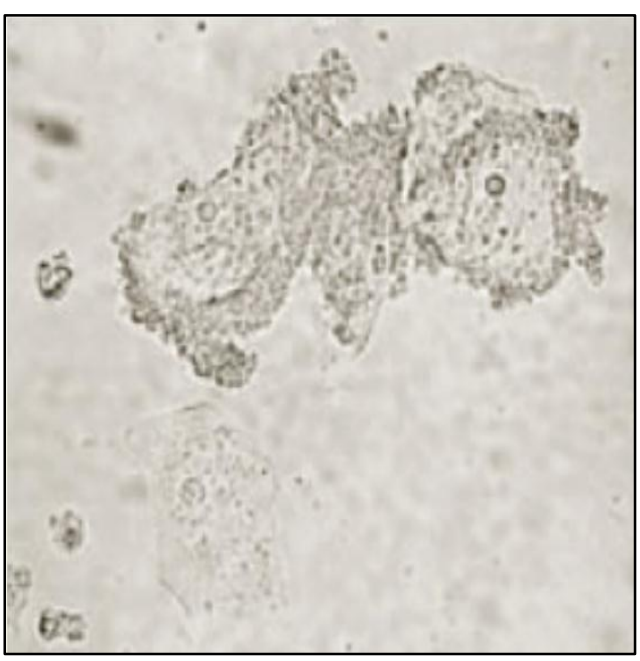

Fig. 1: Wet Mount showing 'Clue Cell'

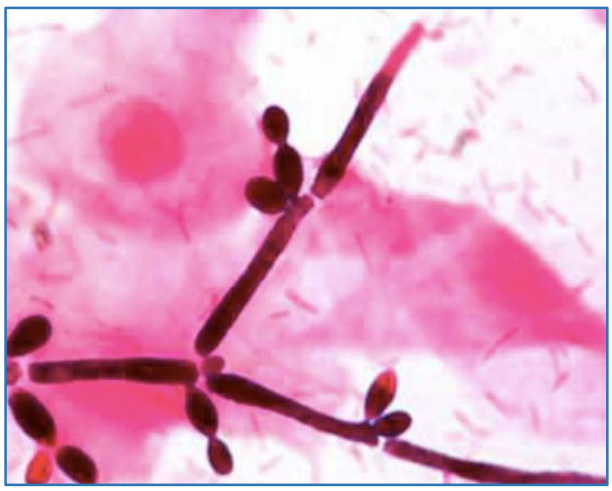

Fig. 2: Candida spp. (BYLC & Pseudohyphae, Gram Stain)

DISCUSSION

Bacterial vaginosis is the most common cause of vaginal symptoms among women.[29] The present study attempts to assess the prevalence of BV in rural setup and the risk factors associated with it. The prevalence of BV among women reproductive age group in our study was 31.5%. Using Nugent's criteria as diagnostic tool, prevalence of BV seems to vary significantly from study to study.[30-32] Our findings were in concurrence with Bhalla et al,[33] who reported 32.8% prevalence of BV among women in Delhi and with Verma et al[26] who observed prevalence of 29.2% among women with vaginal discharge. These variations in the rate could be different to geographical distribution or systematic differences among various population samples. The average age of the bacterial vaginosis group in our study (mean age=27.8±4.7 years) was lower than that of the non-suffering group (Mean age=30.3±5.8 years), and it was statistically significant (p=0.001). Studies have suggested that women of childbearing age are more prone to developing BV.[34] This might be seen as reason for earlier age of presentation of BV in our study.

All parameters of Amsel's clinical criteria were satisfied by 59 subjects; however, concordance for BV by Nugent's criteria was seen in 44 (67.69%) cases only. Thus, overall sensitivity, specificity, PPV and NPV of Amsel's criteria were 67.69% and 89.36%, 74.58% and 85.02%, respectively. Each individual parameter of Amsel was analysed for diagnosis of BV against Nugent's criteria, vaginal pH>4.5 improved the sensitivity of diagnosis but at expense of its specificity [Table 3]. Finding of Clue cells were highly specific (92.2%) for BV. Similar findings have been reported from Sha et al.[35] Nugent score is preferred for diagnosing BV as better reproducibility and sensitivity. However, smear evaluation is also subjective and requires considerable expertise.

We analysed various socio-demographic characters, contraceptive preference, douching practices as risk factors for BV. Multivariate analysis revealed age, marital status, parity, oral contraceptive use and tubal ligation were not associated with BV. Many studies have reported similar findings with age group, parity and marital status, oral pills and tubal ligation.[36,37]

In our study, non-literate woman with low socioeconomic status, working as unskilled labour had almost 2 times the risk of being associated with BV in comparison to literate and economically well to do women. Similar observations were made by various researchers.[38] Ganjoei et al, analysing at the risk factors for bacterial vaginosis found that low education level and low socioeconomic status was a significant risk factor for bacterial vaginosis with OR 3.8 and 2.7 respectively.[39] Literate and economically well-being women are likely to be more health conscious, can afford and maintain good personal hygiene and seek early medical advice in comparison to illiterate women.

Among contraceptives, IUCD usage was significantly associated with BV with crude OR and AOR of 2.3 and 1.7 respectively. This was in congruence with earlier studies. Om HS et al reported significant increase in risk of BV (p=0.017; OR= 1.70) among IUCD users.[37] Joesoef MR et al[40] investigated risk of BV among IUCD user and non-users at Indonesia and concluded BV to be commonly associated with IUCD user with an AOR of 2. However, studies having contradictory finding have been reported from Turkey.[41] and USA.[42] Our data supports the hypothesis that IUCD might change endogenous cervico-vaginal environment, which may lead to vulvovaginal infection and BV.

In our study, condom usage had an AOR of 0.26 (p<0.05), which indicates its protective role against acquisition of BV. Earlier studies had reported association of condom use with decrease in risk for BV.[17,43] Ma L et al investigated the effect of condoms and IUCD on vaginal Lactobacilli colonization and concluded that consistent condom use increases the colonization of Lactobacillus crispatus in the vagina and may protect against BV.[44]

An increased risk for BV prevalence (AOR 2.06, p<0.05) was observed among those subjects who practiced vaginal douching. Earlier studies had reported association between the vaginal cleaning practices using stream of liquid with higher risk of acquisition of BV.[45,46] Frequent douching may alter the vaginal ecology and may enhance the risk for BV. However, only few published studies of the effects of douching on the vaginal environment have been done. Earlier study has reported that douches containing povidone–iodine have a profound inhibitory effect on vaginal Lactobacillus than did douches containing saline or acetic acid. Thus, significant reductions in BV might be achieved by decreasing the frequency of vaginal washing.

CONCLUSION

Bacterial vaginosis prevalence was relatively high in our study population (31.5%). Factors like low socioeconomic status, illiteracy, IUCD usage and douching practices were associated with increased BV, whereas condom usage had protective role for identification of modifiable risk factors could help to develop interventions that could improve vaginal heath and reduce risk of BV and its associated co-morbidities in women.

Study Limitation

Analyses were done on cross-sectional study, hence inference of causal association between risk factor and BV could not be drawn. We did not explore role of multiple sexual partners, sex with women or oro/anal sex, as these sexual behaviours have considerable social stigma attached to them, especially so in our study population which was in rural setting and patients tends to obscure or even refuse to divulge information. Qualitative or quantitative culture of the causative agents for BV were not done as microbiology of BV is heterogeneous and many of them are part of normal vaginal flora. Further, it is technically difficult to culture and speciate all the associated fastidious bacteria of BV.

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