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Original Research Article

Bacterial vaginosis: impact of treatment and associated risk factors: a cross section study

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ABSTRACT

Background: Bacterial vaginosis (BV) is a condition caused by an overgrowth of normal vaginal flora. Many socio-demographic factors are associated with bacterial vaginosis. Antibiotic resistance to some organism is a challenge in treatment of bacterial vaginosis.
Methods: This cross-sectional study was conducted including 100 women with reproductive age group presenting with complain of vaginal discharge. After clinical examination, vaginal swab was collected of patients who gave consent for same. Socio-demographic characteristics were recorded and risk factors were assessed**.** The slide smears were prepared from vaginal swabs, and the slides were heat-fixed, gram-stained and examined under oil immersion objective. Each slide was then graded as per the standardized quantitative morphological classification method developed by Nugent. Identification of bacteria to genus and/or species level was done by employing an array of routine biochemical tests for Gram-positive bacteria for Gram-negative bacteria. In vitro antibacterial susceptibility testing of bacterial isolates was performed by the Kirby-Bauer disc diffusion method. Sensitivity test results were interpreted according to the Clinical and Laboratory Standards Institute.
Results: High incidence of bacterial vaginosis was found among 35 to 45 years age group (63.8%), married females (63.9%), illiterate (100%), females with more than 3 sexual partners (66.7%), those with any co-morbidity (80%) and females not using any method of contraception (100%). *E coli* (28%) was predominant organism causing bacterial vaginosis followed by *Gardenella vaginalis* (20%) among the gram-negative bacteria. Among gram positive bacterial *S. pyogen* (2%) and *S. agalactiae* (2%) were present. Drug resistance to trimethoprim/sulfamethxazole, erythromycin, cefoxitin, ceftriaxone and gentamycin was detected.
Conclusions: Higher age, illiteracy, multiple sexual partners and absence of contraception use can increase risk of bacterial vaginosis. Gram negative organisms are common cause of bacterial vaginosis and they are resistant to trimethoprim/sulfamethoxazole and erythromycin.

Keywords: Bacterial vaginosis, Resistance, Risk factors, Treatment

INTRODUCTION

Bacterial vaginosis (BV) is a condition caused by an overgrowth of normal vaginal flora.[1] Bacterial vaginosis is the most common vaginal infection found in women of reproductive age and is estimated to occur in anywhere from 5 to 70% of women.[2] BV is a vaginal dysbiosis resulting from replacement of normal hydrogen peroxide and lactic-acid–producing *Lactobacillus* species in vagina with high concentrations of anaerobic bacteria, including *G.vaginalis*, *Prevotella* species, *Mobiluncus* species, *A. vaginae*, and other BV-associated bacteria.[3] Many studies have shown causative agents of bacterial vaginosis to be *Gardenella vaginalis* along with

Lactobacillus prevotella and anaerobes *Mobiluncus*, *Bacteroids*, *Peptostretococcus*, *Fusobacterium*, *Veillonella*, and *Eubacterium*. Other microorganisms associated with BV are *Streptococcus viridians*, *Atopobium vaginae* and *Ureaplasm urealyticum*.[4]

A notable feature is the appearance of a polymicrobial biofilm on vaginal epithelial cells.[5] Most commonly, this presents clinically with increased vaginal discharge that has a fish-like odor.[1] The discharge itself is typically thin and either grey or white.[1] After being diagnosed with bacterial vaginosis, women have an increased risk of acquiring other sexually transmitted infections (STI), and pregnant women have an increased risk of early delivery.[2,6,7] Although bacterial vaginosis is not considered a sexually transmitted infection, the role of transmissibility is yet to be completely understood.[8] The spread of bacteria among individuals through sexual intercourse may alter the natural balance of bacterial flora within the vagina, and this imbalance appears to lead to the development of bacterial vaginosis.[9] (Typically, this condition is caused by a decrease in the number of normal hydrogen peroxide-producing *Lactobacilli* with an overgrowth of anaerobic bacteria.[10]

BV is associated with having multiple male sex partners, female partners, sexual relationships with more than one person a new sex partner, lack of condom use douching and HSV-2 seropositivity male circumcision reduces the risk for BV among women.[3] There is spread of *Gardnerella vaginalis* between women who have sex with women, either via direct contact of mucus membranes or via shared sex toys. Although bacterial vaginosis is associated with numerous health problems and is associated predisposing risk factor need to be evaluated. Therefore, the purpose of current study was to identify the risk factor associated with bacterial vaginosis and treatment outcome in BV.

The diagnosis of bacterial vaginosis can be made using clinical criteria or in the laboratory by scoring bacterial morphotypes from a Gram stain of vaginal fluid, where a score of 0-3 represented normal vaginal flora, a score of 4-6 represented intermediate vaginal flora, and a score of 7-10 was considered as diagnostic for bacterial vaginosis.[11] A few studies have also isolated and characterized aerobic microorganisms identified as major causes of aerobic vaginitis from cultures of vaginal swabs. *E. coli*, *Pseudomonas spp*., *S. aureus*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* have been reported as the most frequently isolated microorganisms from patients with aerobic vaginitis.[12]

METHODS

Study design and study setting

It was a cross-sectional observational study conducted at private obstetrics and gynaecology clinic at Delhi for a duration of one year form January 2021 to January 2022.

Inclusion criteria

Women of reproductive age (18- 45years) with complain of vaginal discharge without any other symptoms with presumptive diagnosis of vaginal infections who attended the gynaecology clinic were included in the study.

Exclusion criteria

Pregnant women with bacterial vaginosis, those who were already on treatment for the BV or any sexually transmitted disease, undiagnosed vaginal bleeding and genitourinary malignancy were excluded from the study.

Sample size

This study included total 100 women of reproductive age group who had complain of vaginal discharge, out of which 50 were diagnosed by laboratory criteria with confirmed bacterial vaginosis whose data were analysed.

Disease definition

Bacterial vaginosis: The diagnosis of bacterial vaginosis can be made using clinical criteria[13] or in the laboratory by scoring bacterial morphotypes from a Gram stain of vaginal fluid.[14]

Clinical diagnosis of BV by Amsel criteria[13] requires at least three of the following four symptoms or signs: 1) Homogeneous, thin discharge (milklike consistency) that smoothly coats the vaginal walls, 2) Clue cells (e.g., vaginal epithelial cells studded with adherent bacteria) on microscopic examination, 3) pH of vaginal fluid >4.5, 4) A fishy odour of vaginal discharge before or after addition of 10% KOH (i.e., the whiff test).

Study procedure

As per inclusion criteria, women of reproductive age (18-45years) who attended gynaecology private clinic during year 2021 -2022 with complain of vaginal discharge were included in the study. Clinical diagnosis and laboratory confirmation of bacterial vaginosis was made (Figure 1).

Clinical examination and collection of base line data

Women of reproductive age (18-45years) who had complained of vaginal discharge with presumptive diagnosis were considered for clinical examination for establishing clinical diagnosis. Data regarding sociodemographic characteristics, age, education, marital status, sexual history (number of lifetime male sex partners, frequency, contraceptive method adopted), comorbidity, genial hygiene previous history of genital tract infection, obstetric history (history of abortion, no. of kids) history of drug allergy was recorded in predefined case record form.

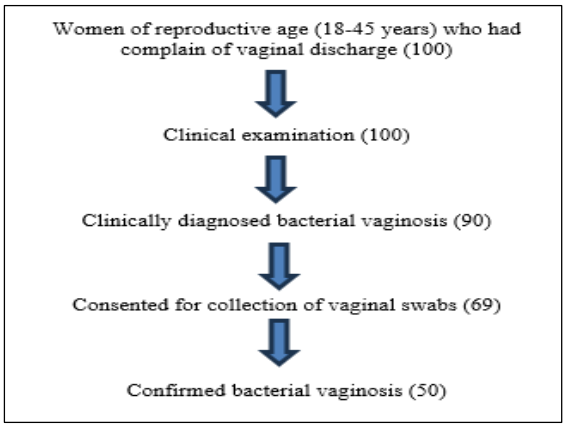

Figure 1: Study vignette.

Specimen collection, handling, and transportation to laboratory

The gynaecologist performed clinical examination of each participant and recorded signs of vaginal discharge and relevant details on case record form. During examinations, vaginal specimens were collected aseptically from the study participants using sterile rayon-tipped applicator stick swabs by experienced nurses. The vaginal swabs were then transferred within 30 minutes to the microbiology laboratory.

Microscopic examination of vaginal swab

The slide smears were prepared from vaginal swabs, and the slides were heat-fixed, gram-stained and examined under oil immersion objective. Each slide was then graded as per the standardized quantitative morphological classification method developed by Nugent et al which assigns a score between 0 and 10 based on the following various 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.[14] Each morphotype was quantitated from 1+ to 4+ with regard to number of morphotypes per oil immersion field and score was generated to reach final diagnosis of bacterial vaginosis as mentioned in following table.

Each vaginal swab was inoculated onto Blood Agar base (Oxoid, Basingstoke, Hampshire, UK) to which 10% sheep blood was incorporated, MacConkey agar and chocolate agar before slide preparation for the isolation and characterization of aerobic bacteria. Blood agar and chocolate agar plates were incubated at 35-37∘C up to 48 hours in a 5% CO2 incubator. MacConkey agar was incubated at 35-37∘C up to 48 hours aerobically. Preparation and performance evaluation of culture media were done as per the instruction of the manufacturer

Aerobic vaginitis (AV) diagnosis was done using Gram staining under dry high power objective (400x) (to determine AV score) and oil immersion magnification (for identification of organisms). AV score was calculated by determining the presence or absence of lactobacilli, type of vaginal flora, the number of leukocytes, and parabasal epithelial cells using 400x magnification, according to a modified Donder's score.[15] An AV score of less than 3 was defined as normal AV, 3 to 4 as light AV, 5 to 6 as moderate AV, and any score >6 as severe AV.[15]

Bacteria identification

Pure isolates of bacterial pathogen were preliminarily characterized based on colony morphology, microscopic examination (gram stain), and haemolytic reaction on blood agar plates. Identification of bacteria to genus and/or species level was done by employing an array of routine biochemical tests for gram-positive bacteria for Gram-negative bacteria.

Antimicrobial susceptibility profile

In vitro antibacterial susceptibility testing of bacterial isolates was performed by the Kirby-Bauer disc diffusion method. The following antimicrobial agents were employed: penicillin (10 μg), amoxicillin (10μg), amoxicillin/clavulanate (20/10μg), cefoxitin (30μg), trimethoprim/sulfamethoxazole (25/23.75 μg), ceftriaxone (30μg), ciprofloxacin (5μg), erythromycin (15μg), gentamycin (10μg), amikacin (30 g), tobramycin (10 μg), vancomycin (10μg), tetracycline (30μg), and clindamycin (2μg). Sensitivity test results were interpreted according to the Clinical and Laboratory Standards Institute.

Data analysis

All data from case record form were entered into Microsoft office excel 2016 and analysed using Jamovi version 2.3.28. Descriptive statistics was to describe the sociodemographic data and logistic regression was used to estimate crude and adjusted crude ratio with 95% confidence interval to the different variables. p value <0.05 was considered significant.

RESULTS

Total 100 women had consulted to the gynaecologist with complain of vaginal discharge during study duration and 90 were clinically diagnosed bacterial vaginosis and 69 study participants consented for collection of vaginal swabs for laboratory confirmation of which 50 women were confirmed through microscopic examination vaginal swab. Majority patients (52.2%) were in age of 35-45 years of group with mean of 35±7.1 (Table 1).

High incidence of bacterial vaginosis was found among 35 to 45 years age group (63.8%), married females (63.9%), illiterate (100%), females with more than 3 sexual partners (66.7%), those with any co-morbidity (80%) and females not using any method of contraception (100%). However, statistically significant association of bacterial vaginosis

was found among married females and illiterate females (Table 2).

Table 1: Quantification of morphotypes and based on scoring for diagnosis of bacterial vaginosis.14

Quantification	Number of morphotypes per oil immersion field
0	No morphotypes
1+	Less than 1 morphotype
2+	1 to 4 morphotypes
3+	5 to 30 morphotypes
4+	30 or more morphotypes
Scoring to diagnose bacterial vaginosis	
Scoring	Type of flora or vaginosis
0 to 3	Normal vaginal flora
4 to 6	Intermediate vaginal flora
7 to 10	Bacterial vaginosis

Total 50 bacterial isolates were identified form vaginal swab. Majority isolates were gram negative (92%) followed by gram positive (8%). *E coli* (28%) was predominant followed by *Gardenella vaginalis* (20%) among the gram-negative bacteria. Among gram positive bacterial *S. pyogen* (2%) and *S. agalactiae* (2%) were present (Table 3).

Drug sensitivity pattern against gram positive bacteria was tested against 12 different antibiotics. Among the gram-positive bacterial isolate sensitive to ceftriaxone, erythromycin, gentamicin, amikacin, vancomycin, tobramycin and resistant to amoxicillin with clavulanate, co-trimoxazole, ciprofloxacin and tetracycline. Among gram positive bacteria causing bacterial vaginosis, *S. agalactiae* was sensitive to 66.7% of antibiotics and *S. pyogenes* was sensitive to 75% of antibiotics (Table 4).

Table 2: Sociodemographic characteristic of study participants (N=90).

Sociodemographic characteristic		Total		Bacterial vaginosis				P value
		No.	%	Yes		No		
				No.	%	No.	%	
Age (years)	18-24	8	8.89	5	62.5	3	37.5	0.54
	25-34	35	38.89	15	42.9	20	57.1	
	35-45	47	52.22	30	63.8	27	57.4	
Marital status	Married	61	67.78	39	63.9	22	36.1	<0.01
	Unmarried	25	27.78	10	40.0	15	60.0	
	Divorced	4	4.44	1	25.0	3	75.0	
Education	Illiterate	2	2.22	2	100.0	0	0.0	<0.01
	Up to primary	10	11.11	6	60.0	4	40.0	
	Up to secondary	40	44.44	15	37.5	25	62.5	
	Graduate	23	25.56	15	65.2	8	34.8	
	Postgraduate	15	16.67	10	66.7	5	33.3	
No. of sexual partner	1	82	91.11	45	54.9	37	45.1	0.82
	2	5	5.56	3	60.0	2	40.0	
	>3	3	3.33	2	66.7	1	33.3	
Co morbidity	Yes	10	11.11	8	80.0	2	20.0	0.4
	No	80	88.89	42	52.5	38	47.5	
Abortion	No	50	55.56	10	20.0	40	80.0	0.38
	Yes	40	44.44	10	25.0	30	75.0	
Type of use of contraception	Not using	12	13.33	12	100.0	0	0.0	0.49
	Hormone contraceptive	5	5.56	4	80.0	1	20.0	
	Barrier contraceptive	73	81.11	34	46.6	39	53.4	

Table 3: Bacterial isolates identified in samples (N=50).

Bacterial isolates	N	%
E. coli	14	28
Gardenella vaginalis	10	20
Lactobacillus prevotella	6	12
Bacteriods	5	10
Enterobacter aerogenes	4	08
Klebsiella pneumoniae	3	06
S. Pyogenes	2	04
Proteus mirabilis	2	04

Continued.

Bacterial isolates	N	%
S. Agalactiae	2	04
Citrobacter freundill	1	02
Citrobacter diversus	1	02

Table 4: Antimicrobial susceptibility testing of gram-positive isolates.

Antibiotics	*S. agalactiae*	*S. pyogenes*
Amoxicillin/clavulanate	R	S
Cefoxitin	S	S
Trimethoprim/ Sulfamethoxazole	R	R
Ceftriaxone	S	S
Ciprofloxacin	R	S
Erythromycin	S	S
Gentamycin	S	R
Amikacin	S	S
Tobramycin	S	S
Vancomycin	S	S
Tetracycline	R	R
Clindamycin	S	S
No. of sensitive (%)	8 (66.7)	9 (75.0)
No. of resistant (%)	4 (33.3)	3 (25.0)

Table 5: Antimicrobial susceptibility testing of gram-negative isolates.

Antibiotics	*E. coli*	*Gardenella vaginalis*	*lactobacillus prevotella*	*Bacteriods*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Enterobacter aerogenes*
Amoxicillin/ clavulanate	R	S	S	S	R	R	S
Cefoxitin	S	S	S	R	S	S	S
Trimethoprim/ Sulfamethoxazole	R	S	R	R	R	R	R
Ceftriaxone	S	S	S	R	S	S	S
Ciprofloxacin	S	S	S	R	R	R	S
Erythromycin	R	S	S	R	R	R	S
Gentamycin	S	S	S	R	S	S	R
Amikacin	S	S	S	S	S	S	S
Tobramycin	S	S	S	R	S	S	R
Vancomycin	S	S	S	S	R	S	S
Tetracycline	R	R	S	R	R	R	S
Clindamycin	S	R	R	R	S	R	S
Metronidazole	-	S	-	S	R	R	R
Secnidazole	-	S	-	S	R	R	R
Tinidazole	-	S	-	S	R	R	R
No. of sensitive (%)	8 (66.7)	13(86.6)	10 (83.3)	7(46.6)	6 (50)	6 (50)	9 (75.0)
No. of resistant (%)	4 (33.3)	02 (13.4)	02 (16.7)	08(53.4)	6(50)	6 (50)	3 (25.0)

Among the gram-negative isolates, antibiotic sensitivity proportion of different organisms were: *E coli* (66.7%), *Gardenella vaginalis* (86.6%), *Lactobacillus prevotella* (83.3%), *Bacteriods* (46.6%), *Klebsiella pneumoniae* (50%), *Proetus mirabilis* (50%) and *Enterobacter aerogenes* (75%). Among the tested antibiotic, highest resistant observed among the gram-negative organism was trimethoprim/sulfamethxazole (85.71%) followed by erythromycin (57.14%) and overall low resistant was observed with cefoxitin (14.28%) and ceftriaxone (14.28%) and gentamycin (14.28%) (Table 5).

DISCUSSION

The overall prevalence rate of bacterial vaginosis as confirmed as by gram-stain Nugent scoring criteria was

55.55% by among the females presenting with vaginal discharge in the present study. The prevalence rate was in the range of the other studies done by Murta et al and Wondemagegn et al.[16,17]

Bacterial vaginosis was more common in age group of 35-45 years in this study. Similarly other studies also reported about occurrence of BV was more in the same age group.[18-21]

Our study shows that there was high incidence of bacterial vaginosis also among, married females (63.9%), illiterate females (100%), females with more than 3 sexual partners (66.7%), those with any co-morbidity (80%) and females not using any method of contraception (100%). However, statistically significant association of bacterial vaginosis was found among married females and illiterate females. Bitew et al reported in their study that he prevalence of bacterial vaginosis varied with education and marital status.[12] Women with a college-level education were less likely to be positive for bacterial vaginosis than those with a high school education or less (35.9% versus 44.7-55.3%).[12] Similarly, the prevalence of bacterial vaginosis was high among unmarried study subjects (53.8%) compared to those who were married (44.8%) or divorced (50.0%).[12] The prevalence of bacterial vaginosis also varied with number of lifetime male sex partners. Women who reported 1-3 lifetime male sex partners had prevalence rate of 43.4%, while those who reported ≥4 lifetime male sex partners had prevalence rate of 58%.[12] The role of sexual activity in the acquisition of bacterial vaginosis is not clear. Bacterial vaginosis' prevalence rates of 18.8%, 18%, and 12% were reported among women who reported that they have never had sex by Koumans et al, Yen et al, and Bump and Buesching, respectively.[22-24] These studies also suggested that condom use may be protective.

BV associated with various factors including vaginal douching by the use of scented soaps or perfumed bubble bath and antiseptics during bath.[25] There is long standing evidence to support the contribution of sexual transmission to the pathogenesis of BV. In the 1950s, Gardner and Dukes first characterised BV and hypothesised that the husbands must be treated simultaneously if recurrences by reinfection are to be prevented.[26] Incident BV has also been strongly associated with sexual practices and women reporting a new sexual partner, or multiple sex partners are more likely to acquire BV.[27-29] Among women with male partners, penile-vaginal sex, inconsistent condom use, and semen exposure have been associated with BV acquisition/incidence. [30-32]

BV is an infection that is associated with a group of pathogenic anaerobic microorganisms rather than a specific pathogen. It is a very common manifestation among the women of reproductive age group.[33] Although the exact causative pathogen was not defined but there is a corresponding decrease in the population of the lactobacilli species in vaginal leads to change in pH of the vaginal lumen because of reduction in the lactic acid production. So the lactobacilli is replaced with the increased population of pathogenic gram-negative anaerobic bacteria such as *E. coli*, *Gardnerella vaginalis*, *Mycoplasma hominis*, and *Mycoplasma curtisii*.[34,35] BV require administration of antibiotics as microbial agents were involved. The recommended therapeutic regimens include oral or intravaginal metronidazole and intravaginal clindamycin. These treatments have god efficacy and are effective for short-term resolution of the infection.[36] Recurrent bacterial vaginosis is a common drawback to current treatment options. Within 6-12 months of finishing antibiotic therapy, 50-80% of women will experience a bacterial vaginosis recurrence.[37-39] Persistent polymicrobial biofilm, which has been more often identified in people with recurrent BV compared to healthy people or those with a single episode, may play a part in antimicrobial resistance.[40] Bacterial biofilm reduces antimicrobial penetrance and even after clinically successful bacterial vaginosis antibiotic therapy, biofilm persists.[41]

Our study demonstrated that amoxicillin/clavulanate, trimethoprim/sulfamethoxazole, ciprofloxacin, tetracyclin and gentamicin are antibiotic to which drug resistance was observed with gram positive bacteria. Among gram negative bacteria, highest resistant observed with trimethoprim/ sulfamethoxazole, followed by erythromycin. The overall drug resistance rates of gram-negative bacterial isolates ranged from 14.3% for amikacin to 77.3% for tetracycline. *E. coli*, the most frequently isolated gram-negative bacterium, showed a high level of resistance to tetracycline and ampicillin. Contrary to this, 86% of *E. coli* were susceptible to amikacin and tobramycin. The drug resistance of level of *K. pneumoniae*, the second frequent isolate, was high against ampicillin, amoxicillin, and tetracycline. Bitew et al in their study demonstrated that highest overall resistance rate of gram-positive bacteria was observed against penicillin (67.4%), followed by tetracycline (58.7%) and erythromycin (45.6%).[12] Tetracycline exhibited the highest overall drug resistance rate (77.3%) against gram-negative bacteria, followed by ampicillin (77.1) and amoxicillin (70.6%).[12]

Proposed reasons for this treatment failure include reinfection via sexual partners, antimicrobial resistance, biofilm, and failure to reestablish a health-optimal vaginal microbiota. A more comprehensive review of the nuances of current antibiotic treatment regimens can be found in two review papers by Muzny et al.[36]

CONCLUSION

Higher age, illiteracy, multiple sexual partners and absence of contraception use can increase risk of bacterial vaginosis. Gram negative organisms are common cause of bacterial vaginosis and they are resistant to trimethoprim/sulfamethoxazole and erythromycin.

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