Section: Microbiology

Original Research Article

A STUDY ON PREVALENCE OF BACTERIAL VAGINOSIS AMONG REPRODUCTIVE AGE GROUP IN A TERTIARY CARE HOSPITAL

Bhavithra P K[1], Dillirani V[2], Ponnammal P[3], Sheeba V[3]

[1]Post Graduate Student, Department of Microbiology, Govt Stanley Medical College, Chennai, Tamil Nadu, India.
[2]Professor and Head, Department of Microbiology, Government Stanley Medical College, Chennai, Tamil Nadu, India
[3]Associate Professor, Department of Microbiology, Government Stanley Medical College, Chennai, Tamil Nadu, India

Received : 12/09/2023
Received in revised form : 10/10/2023
Accepted : 20/10/2023

Keywords:
Bacterial Vaginosis, HIV, Gonorrhoea, Chlamydia, HSV.

Corresponding Author:
Dr. Bhavithra P K,
Email: drbavibala@gmail.com

DOI: 10.47009/jamp.2023.5.5.327

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (5); 1665-1670

Abstract

Background: Bacterial vaginosis (BV) is a polymicrobial infection affecting women of reproductive age group. It is one of the most common vaginal infections worldwide. The objective of this study was to determine the prevalence and risk factors associated with it. The aim is to study the prevalence of Bacterial vaginosis among reproductive age group in a tertiary care centre. **Materials and Methods:** A cross sectional study was conducted over a five months period from August 2022 to December 2022 and a total of 150 sexually active women who presented with complaints of vaginal discharge attending Gynecology OPD and STD clinic in Stanley medical college were included. Three high vaginal swabs were collected from each subject during speculum examination. Clinical findings were recorded and diagnosis of BV made with Amsel's criteria, Nugent's scoring system and bacterial culture techniques. **Result:** Out of 150 female patients, 63(43%)were diagnosed to have BV by Amsel's criteria, 53(35%) by Nugent's scoring system and 31(21%) by bacterial culture techniques. The important risk factors identified were age group between 30-39 years, high risk sexual behaviour, poor socioeconomic status and daily douching practices. Relatively low risk was observed in women using barrier contraceptives. **Conclusion:** If undiagnosed and untreated, bacterial vaginosis may lead to major obstetric and gynecological complications. Regular screening with simple diagnostic methods like Amsel's criteria and Nugent's scoring system helps in the early diagnosis to prevent complications.

INTRODUCTION

Bacterial vaginosis (BV) is a polymicrobial infection characterized by replacement of the normal vaginal lactobacillus flora with an overgrowth of variety of anaerobic bacteria and Gram-negative bacilli with rise in pH above 4.5.[1] It is one of the most common vaginal infections worldwide,[2] affecting women of reproductive age group.[3] The disease prevalence was about 23% to 29% worldwide in 2019,[4] 17.8 to 63.7% n India in 2022 (5)and 24.2% in Tamilnadu in 2022.[5,6]

BV is usually described as sexually enhanced disease caused by mixed flora like Gardnerella vaginalis,[7] Prevotella spp, Peptostreptococcus spp, Fusobacterium spp, Mobilincus spp, Mycoplasma hominis.[1]

In female reproductive tract, vagina has a dynamic ecosystem that is sterile at birth. Under the influence of mother's oestrogen, there is an increase in the glycogen content of vaginal epithelial cells and the infant vagina is colonised by Lactobacilli. In most instance, this flora continues to dominate throughout the individual's lifetime. The vaginal pH in pre-menarchal females is near neutral pH. At the time of puberty under the influence of oestrogen, the vaginal epithelium increases upto 25 cell thickness with increased glycogen levels. The predominant flora changes to Lactobacilli and the vaginal pH decreases to less than 4.5 due to production of lactic acid. Lactobacillus species like L.crispatus, L.jensenii, L.gasseri and L.iners protect the vagina from infection by other pathogenic bacteria by the production of hydrogen peroxide, lactic acid, defensins, and bacteriocins which are antibacterial in nature.[1]

Risk factors and behaviors like smoking, alcohol intake, lower age at first intercourse, multiple sexual partners, STDs, use of IUCD, vaginal douching, recent antibiotic use and race/ethnicity alters the normal vaginal flora and leads to loss of Lactobacillus and the normal ecosystem.[2]

ISSN (O): 2687-5365; ISSN (P): 2753-6556

BV is clinically characterised by a thin homogenous, malodorous adherent vaginal discharge,[3] pruritis, pain during coitus and lower abdominal pain.[1] It also increases the susceptibility to HIV infection by altering the target cells for HIV in the vaginal wall.[1] The presenting symptoms alone are not reliable for the diagnosis of BV since it can co-exist with other STIs. Hence Amsel's criteria are widely used for the clinical diagnosis. It is based on laboratory diagnostic methods including microscopy, culture and serodiagnosis.[8]

The complications include acquisition of sexually transmitted infections (HIV, Gonorrhoea, Chlamydia, HSV), spontaneous PID, second trimester miscarriage, post-abortal endometritis, spontaneous pre-term birth, pre-mature rupture of membranes, low birth weight, post-Cesarean section endometritis, post-hysterectomy cuff cellulitis.[8]

The present study was conducted to determine the prevalence of BV by Amsel's criteria and laboratory diagnostic methods with its associated risk factors stratification.

Aims and Objectives

- To study the prevalence of Bacterial vaginosis among reproductive age group in a tertiary care centre by various methods like calculation of Nugent's score by microscopy, fulfilment of Amsel's diagnostic criteria and Isolation of organisms by culture.
- This study also aimed at observing for risk factors associated with bacterial vaginosis.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Microbiology, Department of Dermato-Venerology, Stanley Medical College in association with Department of Obstetrics and Gynecology, Government RSRM Hospital, Chennai over a five months period from August 2022 to December 2022. A total of 150 sexually active women who presented with complaints of vaginal discharge were the study subjects. Consent for speculum examination was obtained.

The clinical findings were recorded. By using sterile, clean, un-lubricated cusco's bivalve self-retaining speculum thorough per vaginal examination was done. The amount, odour, colour and consistency of the vaginal discharge were noted. pH of Vaginal discharge was obtained by dipping low pH indicator paper in vaginal discharge. The vaginal discharge was collected by three sterile cotton swabs from the posterior fornix of the vagina. First swab was used for wet mount examination, second swab for Gram staining and Whiff's test and the third swab for culture.

Wet mount: The swab was gently mixed in a test tube with 0.5 ml of saline. A drop of the specimen saline mixture was added on a clean glass slide and examined under low power and high-power microscope for the presence of clue cells. Gram staining: Gram staining was performed for the smear prepared from specimen saline mixture and examined under 100x oil immersion microscope for the presence of clue cells, large Gram positive rods, small Gram negative rods, small and curved Gram variable rods to calculate Nugent score. Whiff test: A drop of 10% potassium hydroxide was added to a drop of vaginal secretion saline mixture on the slide and observed for fishy odour. Culture: Each swab was streaked in Brain heart infusion agar , Neomycin blood agar , Blood agar and Mac-conkey agar. Brain heart infusion agar and Neomycin blood agar were incubated under anaerobically at 37◌̊c for maximum of 72hrs in candle jar with 5 to 10% CO2. Blood agar and Mac-conkey agar were incubated aerobically at 37◌̊c for 24 hrs. After the incubation period, bacterial growth was observed and the colonies were identified by Gram staining and biochemical reactions.

AMSELS criteria and NUGENTS SCORING system were used for interpretation of final diagnosis. For AMSELS CRITERIA the findings recorded include 1) An adherent vaginal discharge, 2) Detection of clue cells, 3) Positive Whiff 's test, 4) Vaginal pH > 4. 5)Three out of four criteria is diagnostic of Bacterial Vaginosis. NUGENTS SCORING: Gram stained slide was evaluated for the morphotypes like large Gram positive rods, small Gram negative rods, small Gram variable rods and curved Gram variable rods. Each morphotype was quantified from 0 to 4+ with regard to the number of morphotype per oil immersion field.

Nugent criteria

Bacterial morphotype	**SCORING**				
	NONE	**1+**	**2+**	**3+**	**4+**
Lactobacilli type (large, gram positive rods	4	3	2	1	0
Gardnerella/Prevotella species (small gram negative or variable rods)	0	1	2	3	4
Mobiluncus species (curved gram negative or variable rods)	0	1+ or 2+	3+ or 4+		

Interpretation: < 1 per oil immersion field - 1+ , 1-5 per oil immersion field - 2+ , 6-30 peroil immersion field - 3+ , >30/ oil immersion field - 4+

Score: 0-3 – Normal, 4-6 -Intermediate,7-10-Bacterial vaginosis

Inclusion Criteria

- Women of age 19 to 49 yrs.
- Women with symptoms like vaginal discharge, dysuria, vaginal itching.

Exclusion Criteria

- Women <19 years and > 49 years .
- Those who were not consenting to speculum examination.
- Women who had taken antibiotics or vaginal douching in last 2 weeks.
- Women who were menstruating.
- Women who were pregnant.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Ethical Clearance

Ethical clearance was obtained from the institutional ethics committee of Stanley medical college before the commencement of the study.

Statistical Analysis

The data were entered in Microsoft Excel and analysed. The significance of clinical features and laboratory parameters were determined by calculation of p-value by Fisher-exact test using SPSS software version 17.0. p value <0.05 was considered as statistically significant.

RESULTS

This cross-sectional study was carried out for a period of 5 months in 150 sexually active women who presented with complaints of vaginal discharge, dysuria, vaginal itching attending STD and Gynecology OPD. In this study maximum samples were collected in the age group 30-39 years followed by 40-49 years [Chart 1]. Majority of the study group were from lower middle class [Chart 2]. All the patients presented with vaginal discharge (100%) followed by genital itching (41%) [Chart 3]. 38% of samples were positive for clue cells by Wet mount [Chart 4]. [Table 1] shows that about 42% of females attending the OPD were diagnosed to have Bacterial vaginosis by Amsels criteria. [Table 2] shows about 35% of females in study group were diagnosed as Bacterial vaginosis by Nugent's criteria and [Table 3] shows that 21% of females in study group were culture positive for Bacterial vaginosis.

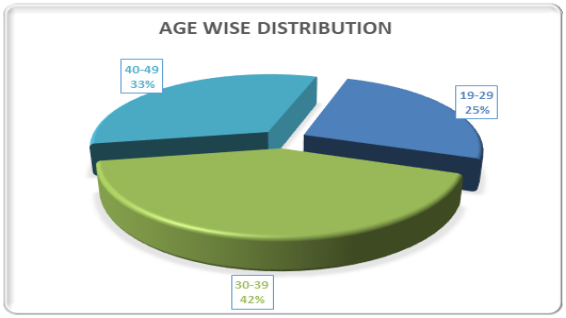

Chart 1: Age wise distribution of samples(n=150)

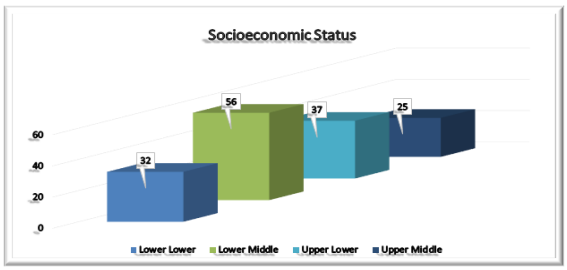

Chart 2: Demography of the study samples(n=150)

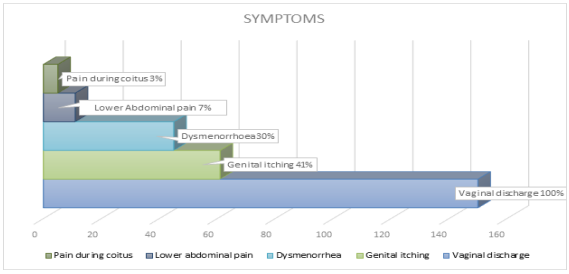

Chart 3: Distribution of symptoms among study group(n=150)

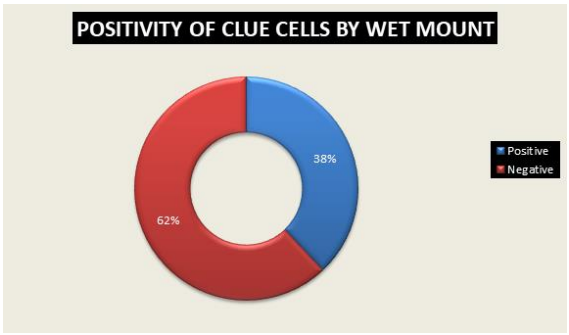

Chart 4: Positivity for clue cells by Wet mount(n=150)

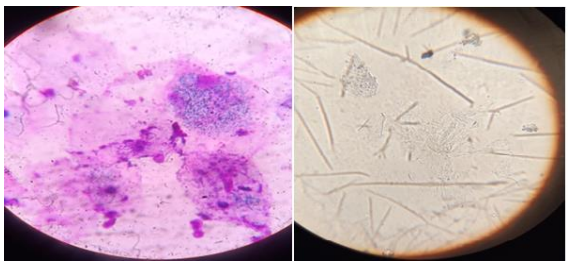

Figure 1 shows Gram-stained CLUE cell(a) and KOH mount showing clue cell(b).

Under oil immersion microscope using 100x magnification the smear is examined for the presence of large gram-positive rods suggestive of Lactobacillus spp, small gram variable rods suggestive of Gardnerella vaginalis, small gram-negative rods suggestive of Bacteroides spp and curved gram variable rods suggestive of Mobiluncus spp.

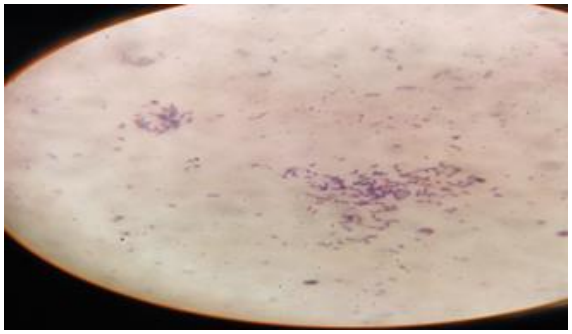

Figure 2: culture smear showing Gardnerella spp.

The bacterial isolates grown on Mac-conkey agar, blood agar, Neomycin blood agar and Brain heart infusion agar were tested biochemically. The

ISSN (O): 2687-5365; ISSN (P): 2753-6556

interpretations of the three bacteria implicated in BV were done as follows

1) Small colonies with beta hemolysis in neomycin blood agar with non-capsulated, non-sporing, non-motile Gram variable coccobacilli, oxidase negative, catalase negative, indole negative, urease negative, MR positive, VP negative, fermented glucose, maltose, sucrose and not fermented mannitol was interpreted as Gardnerella spp.

2) Medium sized, greyish white non haemolytic colonies showing capsulated, non-sporing, non-motile Gram negative bacilli, catalase positive, oxidase negative, indole negative, urease negative, hydrolysed esculin, fermented glucose, lactose, maltose, sucrose but not fermented arabinose and trehalose was interpreted as Bacteriodes spp.

3) Colourless, smooth translucent non haemolytic colonies showed non capsulated, non-sporing, motile Gram-negative curved rods, oxidase negative, catalase negative, indole negative and ferments glucose, maltose, fructose was interpreted as Mobilincus spp.

[Table 5] shows Gardnerella spp (55%) was the commonest organism isolated among the positive samples followed by Mobilinus spp(26%)

Table 1: Positivity by Amsel's criteria(n=150)

Amsel's criteria	No.of cases	Percentage
Positive	63	42
Negative	87	58
Total	150	100

Table 2: Positivity by Nugent criteria(n=150)

Nugent score	No.of samples	Percentage
0-3	74	49
4 – 6	23	16
7-10	53	35
Total	150	100

Table 3: Results of culture(n=150)

Culture	No of samples	Percentage
Positive	31	21
Negative	119	79
Total	150	100

Table 4: Age wise distribution of positive samples(n=150)

Age	Numbers	Bacterial vaginosis		
		Amsel's criteria(%)	Nugent's scoring(%)	culture(%)
19 – 29	38	9(24)	8(21)	5(16)
30-39	63	36(57)	32(51)	17(55)
40 – 49	49	18(37)	13(27)	9(29)
Total	150	63(42%)	53(35%)	31(21%)

Bacterial vaginosis was seen most commonly in the age group of 30 – 39 years according to Amsels criteria(57%), Nugent's scoring system(51%) and culture(21%). [Table 4]

Table 5: Distribution of Organisms isolated in positive samples(n=31)

Name of the organism	No of isolates	Percentage (%)
Gardnerella spp	17	55
Bacteroides spp	6	19
Mobilincus spp	8	26
Total	31	100

Table 6: Demographic Distribution of culture isolates (N=150)

Bacterial vaginosis				
AGE	Culture Positive (%)	Gardnerella sp	Bacteroides	Mobilincus sp
19 – 29	5(13)	3	1	1
30-39	17(27)	10	3	4
40 – 49	9(18)	4	2	3
Marital status				
Unmarried	0	-	-	-
Married	29(21)	6	5	8
Widowed	2(40)	1	1	-
Divorced/Separated	0	-	-	-
Socio economic status				
lower lower	13(41)	8	2	2
lower middle	11(20)	6	2	3
upper lower	4(11)	2	1	2
upper middle	3(12)	1	1	1

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Condom usage				
Daily /sometime	12(38.7)	7	2	3
Never	19(61.3)	10	4	5
DOUCHING				
Never	1(3)	-	-	1
Daily	27(30)	16	5	6
Sometime	3(11)	1	1	1
Multi sexual partner	3(27)	2	-	1
Std in partner	5(71)	3	1	1

Table 7: Risk factors associated with Bacterial vaginosis

Bacterial vaginosis				
AGE	**Total (150)**	**Amsels Criteria**	**Nugent's criteria**	**Culture**
		Positive (%)	**Positive (%)**	**Positive (%)**
19 – 29	38	9(24)	8(21)	5(13)
30-39	63	36(57)	32(51)	17(27)
40 – 49	49	18(37)	13(27)	9(18)
Marital Status				
Unmarried	3	1(33)	1(33)	0
Married	141	60(43)	51(36)	29(21)
Widowed	5	2(40)	1(20)	2(40)
Divorced/Separated	1	0	0	0
Socio Economic Status				
lower lower	32	17(53)	15(47)	13(41)
lower middle	56	25(45)	20(36)	11(20)
upper lower	37	14(38)	12(32)	4(11)
upper middle	25	7(28)	6(24)	3(12)
Condom Usage				
Daily/ sometime	18	4(22)	3(16.7)	2(11)
Never	132	59(44.7)	50(37.9)	29(22)
Douching				
Never	34	9(26)	6(18)	1(3)
Daily	89	43(48)	39(44)	27(30)
Sometime	27	11(41)	8(30)	3(11)
Multiple sexual partner	11	11(100)	9(82)	3(27)
Std in partner	7	7(100)	6(86)	5(71)

Risk factors associated with BV are age, marital status, socio economic status, condom usage, douching, multiple sexual partner, STD in partner. The risk of acquiring Bacterial vaginosis was highest among 30-39yrs age group (57%), married women (43%) lower socioeconomic status (53%), daily douching (48%) and non-usage of condoms (44.7%). The risk is 100% among those with multiple sexual partners and STD in partner [Table 6 and 7]

DISCUSSION

Bacterial vaginosis is the commonest infection among women of reproductive age group. It can cause complications like miscarriage, pre-term delivery, low birth weight baby, pre-mature rupture of membranes, chorioamnionitis, post-partum endometritis, vaginal cuff cellulitis and pelvic inflammatory disease if not identified and diagnosed.[9]

In our study, maximum positivity was seen in the age group of 30-39 years. This was comparable with the study by Eliza Ranjit et al,[3] [2018] and Swapna et al,[9] [2016] where commonest age group affected was 30-39 years. The reason for predominance in 30-39 years age group could be due to increased frequency of sexual activity compared to extremes in reproductive age group like late teenage and near menopausal age. Study by Durga et al,[6] [2022] showed high positivity in 26-30 years age group (50%).

About one third of women in our study (53%) belonged to low socio economic group. This finding emphasizes socio economic status of women plays a vital role in knowledge regarding vaginal hygiene, adoption of protective measures and to overcome the social stigma in treatment seeking behavior. This finding is concordant with Kosambiya et al,[10] [2009] and Durga et al,[6] [2022] which showed 49.2% of affected women to be of low socio-economic status.

In this study, high positivity was seen in married women (43%) compared to unmarried women (33%). This finding is in concordance with Kataria et al,[11] and contradictory to Eliza ranjit et al,[3] [2018] with high positivity in unmarried women. This study shows high positivity among women with daily douching practices (48%) than in women with occasional douching habit (41%) which is similar to Eliza ranjit et al,[3] [2018] and Singh et al,[2] [2015]. Females with multiple sexual partner and with partners having STD showed 100% positivity with BV.

In this study, with Amsel's criteria 42% of females were diagnosed as bacterial vaginosis, [Table 5] similar to a study done by Farheen et al,[12] [2017] where Amsel's criteria were significant in 47% of subjects.

With Nugent scoring, 49% of the subjects were diagnosed as having normal flora, 16% intermediate

ISSN (O): 2687-5365; ISSN (P): 2753-6556

flora and 35% as bacterial vaginosis. [Table 6] This is similar to a study done by Bansal et al,[13] [2019] with 36.92% positivity by Nugent scoring but dissimilar to study by Madhivanan et al,[14] [2008] which showed 19.1% positivity.

About 21% of samples were culture positive in our study [Table 7] which is concordant with the study by Rao et al,[15] [2004] where culture positivity was 17.42%. Gardnerella spp (55%) was the commonest organism isolated among the positive samples. This finding is similar to a study done by Roa et al,[15] [2004].

In our study, among the three methods, Amsel's criteria identified more positives than other two methods with simple clinical criteria and bedside tests.

CONCLUSION

The prevalence of Bacterial vaginosis among reproductive age group women in this study was 42% by Amsel's criteria, 35% by Nugent's scoring system and 21% by culture. The presence of clue cells was proven to be the single most reliable predictor of BV by this study.

BV is associated with infections following gynecological procedures. It is associated with STIs such as chlamydia and gonorrhoea, and is a risk factor for acquisition of HIV in women. In pregnancy, it is a risk factor for late miscarriage, idiopathic pre-term birth, and post-caesarean section endometritis. If undiagnosed and untreated, these conditions may lead to major obstetric and gynecological complications, which considerably increase the treatment costs and hospitalizations. Regular screening with simple diagnostic methods like Amsel's criteria and Nugent's scoring system helps in the early diagnosis to prevent complications. Adolescent medical care requires a multidisciplinary expertise, motivation and enhanced information about STIs and contraceptive methods and emphasizes the need for programs to address these aspects to promote health to adolescents and significantly reduce STIs.

REFERENCES

1. Vinod Sharma, Sexually transmitted diseases and HIV/AIDS, Bacterial vaginosis Page405-414.
2. Singh, Hari & Nain, Sumitra. (2015). Factor Associated to Bacterial Vaginosis in Non-pregnant Women of North Indian Population. Journal of Biotechnology & Biomaterials. 05. 10.4172/2155-952X.1000195.
3. Ranjit E, Raghubanshi BR, Maskey S, Parajuli P. Prevalence of Bacterial Vaginosis and Its Association with Risk Factors among Nonpregnant Women: A Hospital Based Study. Int J Microbiol. 2018;2018:8349601. Published 2018 Mar 5. doi:10.1155/2018/8349601.
4. Peebles K, Velloza J, Balkus JE, McClelland RS, Barnabas RV. High Global Burden and Costs of Bacterial Vaginosis: A Systematic Review and Meta-Analysis. Sex Transm Dis. 2019;46(5):304-311.
5. International institute for population sciences (IIPS) AND ORC Macro. 2000. National Family Health Survey(NFHS-2), Mumbai, India; 1998-99: 307-360.
6. K., Durga, &Karthika K.. "Prevalence of sexually transmitted infections at a tertiary hospital in Tamil Nadu." International Journal of Reproduction, Contraception, Obstetrics and Gynecology, 6.8 (2017): 3573-3580. Web. 20 Jun. 2022K.,
7. Mascarenhas RE, Machado MS, Costa e Silva BF, et al. Prevalence and risk factors for bacterial vaginosis and other vulvovaginitis in a population of sexually active adolescents from Salvador, Bahia, Brazil. Infect Dis Obstet Gynecol. 2012;2012:378640. doi:10.1155/2012/378640
8. Someshgupta, Bhushankumar, Sexually transmitted infections second edition, Bacterial vaginosis Page542 -556
9. Muthusamy, Swapna. (2016). Comparison of Amsel's Criteria, Nugent Score and Culture for the Diagnosis of Bacterial Vaginosis. National Journal of Laboratory Medicine. 10.7860/NJLM/2016/17330.2095.
10. Kosambiya, Desai. RTI/ STI prevalence among urban and RURAL WOMEN OF SURaT: a community based study. Indian J Sex Transm Dis. 2009;30:89-93
11. Usha Kataria, Sunita Siwach, Dinesh Chhillar. Study of vaginal discharge in sexually active females 20- 45 yrs International Archives of Integrated Medicine, Vol.2 Issue 4, April, 2015.
12. Farheen A Humera A. Prevalence and diagnosis of bacterial Vaginosis in tertiary care teaching hospital. Trop J Path Micro 2017;3(3):276-282.doi: 10.17511/jopm.2017.i3.08.
13. BansalR, GargP, GargA.Comparison of Amsel's criteria and Nugent's criteria for diagnosis of bacterial vaginosis in tertiary care centre.Int J Reprod Contracept Obstet Gynecol2019;8:637-40.
14. Madhivanan P, Krupp K, Chandrasekaran V, et al. Prevalence and correlates of bacterial vaginosis among young women of reproductive age in Mysore, India. Indian J Med Microbiol. 2008;26(2):132-137. doi:10.4103/0255-0857.40526
15. Rao PS, Devi S, Shriyan A, Rajaram M, Jagdishchandra K. Diagnosis of bacterial vaginosis in a rural setup: comparison of clinical algorithm, smear scoring and culture by semiquantitative technique. Indian J Med Microbiol. 2004;22(1):47-50.
16. Rajalakshmi R, Kalaivani S. Prevalence of asymptomatic infections in sexually transmitted diseases attendees diagnosed with bacterial vaginosis, vaginal candidiasis, and trichomoniasis. Indian J Sex Transm Dis AIDS. 2016;37(2):139-142. doi:10.4103/0253-7184.192121
17. Vasundhara, V.; Farooq, U.; Najam, R. Comparison Between Different Diagnostic Tests Used for Bacterial Vaginosis. Trop J PatholMicrobiol 2019, 5, 838-843.
18. Money D. The laboratory diagnosis of bacterial vaginosis. Can J Infect Dis Med Microbiol. 2005;16(2):77-79. doi:10.1155/2005/230319

ISSN (O): 2687-5365; ISSN (P): 2753-6556