

Original article:

Vaginal microbiota and microbiome:- Role in health and disease

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Abstract

Introduction: A “microbiota” is the aggregate of ecological community of commensal , symbiotic and pathogenic microorganisms that literally share our body space: surface and in deep layers of skin, in the saliva and oral mucosa, in the conjunctiva, in the gastrointestinal tracts and in the vagina. Human microbiome refer to their genomes. Microbiome may hold key to better understanding of Bacterial Vaginosis(BV). Study aimed to find out the vaginal microbiota in health and disease in women attending gynaecology and obstetric OPD/IPD at a tertiary care hospital.

Material and Methods: High Vaginal Swabs of two hundred and fifty women of reproductive age(15 – 45 years) group formed the study population. Detail patient history were noted. Gram staining followed by Nugent score elicited .

Observations and results: Bacterial morphotypes observed were Lactobacilli, Gardnerella , Mobiluncus, Gram negative bacilli and Gram positive cocci. *G vaginalis* and Mobiluncus were strongly associated with BV diagnosis by both Nugent's score and Amsel's criteria. In cases with BV, number of Lactobacilli morphotype decreased while number of other bacterial morphotype increased. Multiple bacilli were seen in these cases. In cases without BV, number of lactobacilli morphotype was more while number of other bacterial morphotype was less i.e inversely proportional.

Conclusion: Study concluded that in healthy vagina the vaginal microbiota microscopically shows predominance of Lactobacillus morphotype with or without the Gardnerella morphotype (normal) etc, while in BV it is represented by a mixed flora with few or no Lactobacillus morphotypes (BV).

Keywords: Bacterial vaginosis , Lactobacilli, Microbiota, Microbiome .

Introduction

A “microbiota” is the aggregate of ecological community of commensal , symbiotic and pathogenic microorganisms that literally share our body space: surface and in deep layers of skin (including in mammary glands) in the saliva and oral mucosa, in the conjunctiva, in the gastrointestinal tracts and in the vagina."^{1,2} The human body contains over 10 times more microbial cells than human cells, although the entire microbiome only weighs about 200 grams (7.1 oz), with some weight-estimates ranging as high as 3 pounds (approximately 48 ounces or 1,400 grams). Some regard the microbiome as a

"newly discovered organ" since its existence was not generally recognized until the late 1990s and it is understood to have potentially overwhelming impact on human health³. Modern techniques for sequencing DNA have enabled researchers to find the majority of these microbes - the majority of them cannot be cultured in a laboratory using current techniques. Microbiome may hold key to better understanding of Bacterial Vaginosis(BV). The microbes being discussed are generally non-pathogenic (they do not cause disease unless they grow abnormally); they exist in harmony and symbiotically with their hosts⁴. Some of

these organisms perform tasks that are useful for the human host. However, the majority have been too poorly researched for us to understand the role they play, however communities of microflora have been shown to change their behaviour in diseased individuals. Community population studies using advanced sequencing methodologies are yielding insights into the range of microbial diversity in the human vagina. An unexpected finding was the prevalence of *Prevotella* species, which are known to positively affect the growth of *Gardnerella vaginalis* and *Peptostreptococcus anaerobius*, two species linked to bacterial vaginosis, by providing these disease-associated bacteria with key nutrients⁷. The most abundant vaginal microorganism found in premenopausal women is *Lactobacillus* bacteria. Vaginal flora is influenced by a variety of factors including exogenous and endogenous influences. Bacteria type vary in women depending on the stage of the menstrual cycle⁸. Lactic acid bacteria are predominately found during child-bearing years, otherwise the bacterial flora is mixed. Although the menstrual cycle alters vaginal pH and the growth of various organisms, some research shows *lactobacilli* remain at a constant level regardless of the stage of menstruation⁹ while other research shows that during menstruation, the concentration of vaginal microbiome is observed to decline¹⁰. Race also influences vaginal flora. The occurrence of hydrogen peroxide-producing *lactobacilli* is lower in African American women, and vaginal pH is higher⁹. Other influential factors such as sexual intercourse and antibiotics have been linked to

the loss of *lactobacilli*. Moreover, studies have found that sexual intercourse with a condom does appear to change *lactobacilli* levels, and does increase the level of *Escherichia coli* within the vaginal flora¹¹. Disruption of vaginal flora can lead to infections such as vaginal candidiasis or bacterial vaginosis (BV)¹¹. Our study aimed to find out the vaginal microbiota in health and disease in women attending Gynaecology and Obstetric OPD/IPD at a tertiary care hospital.

Material and Methods

The present observational and prospective study was conducted in the Microbiology department at a Tertiary care Hospital in India. The protocol was reviewed and approved by institutional review board, and each subject gave verbal consent. Age, pregnancy status, parity, ethnicity, mode of contraception, number of sexual partners, presence or absence of symptoms, and a sexually transmitted diseases history were noted. Those who had received systemic antibiotic therapy or local vaginal antimicrobial therapy within the preceding 2 weeks, were menstruating at the time of the examination, cervical cerclage, vaginal bleeding, placenta previa, spermicide use, recent douching, or sexual intercourse within 24 hours were excluded from the study. High Vaginal Swabs of two hundred and fifty women of reproductive age (15 - 45 years) group who attended Gynaecology and Obstetric OPD/IPD at tertiary care hospitals formed the study population. Amsel's Clinical criteria and Nugent's microbiological criteria for diagnosis of BV were used¹².

Results and observations

TABLE 1:- OBSERVATIONS ON THE BASIS OF GRAM/GIEMSA STAINING OF THE VAGINAL SMEAR DONE IN WOMEN SELECTED FOR STUDY

	WOMEN SELECTED FOR STUDY	
	No	%
TOTAL CASES	250	100
NORMAL VAGINAL FLORA	116	47
INTERMEDIATE BACTERIAL VAGINOSIS	43	17
DEFINITE BACTERIAL VAGINOSIS	36	14
VAGINAL CANDIDIASIS	52	21
TRICHOMONAS VAGINITIS	3	1

Statistically the diagnostic inference's of number of cases of Bacterial vaginosis diagnosed by applying Nugent criteria on Gram staining of vaginal smears in above of women were found to be highly significant(P=0.00) on applying ANOVA(one way analysis of variance) test.

FIGURE 1:- OBSERVATIONS ON THE BASIS OF GRAM/GIEMSA STAINING OF THE VAGINAL SMEAR DONE IN WOMEN SELECTED FOR STUDY

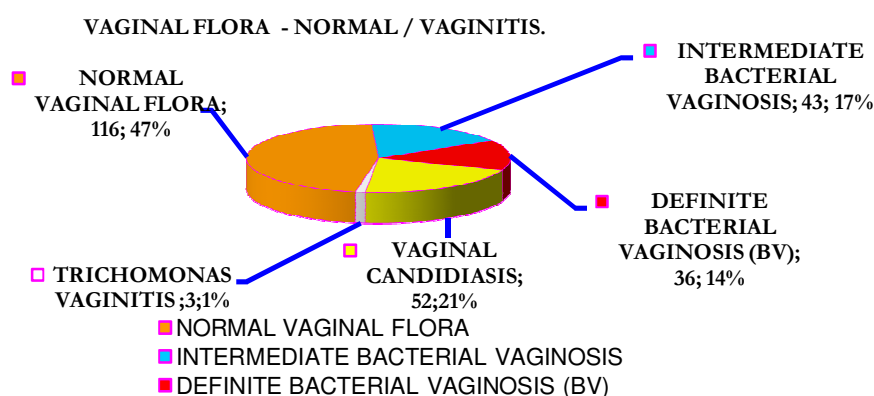


TABLE 2:-BACTERIAL MORPHOTYPE OBSERVED ON GRAM STAINING OF VAGINAL SMEAR

Morphotypes seen	With Bacterial vaginosis (as per Nugent's Scoring) TOTAL CASES - 79.		Without Bacterial vaginosis (as per Nugent's Scoring) TOTAL CASES- 171.	
	No of cases	%	No of cases	%
Lactobacillus (0 -2 +)	43	54	171	100
G.Vaginalis	55	70	103	60
Mobilincus	45	57	0	0
Gram negative bacilli	40	51	0	0
Gram positive cocci	20	25	60	35

FIGURE 2:-.BACTERIAL MORPHOTYPE OBSERVED ON GRAM STAINING OF VAGINAL SMEAR

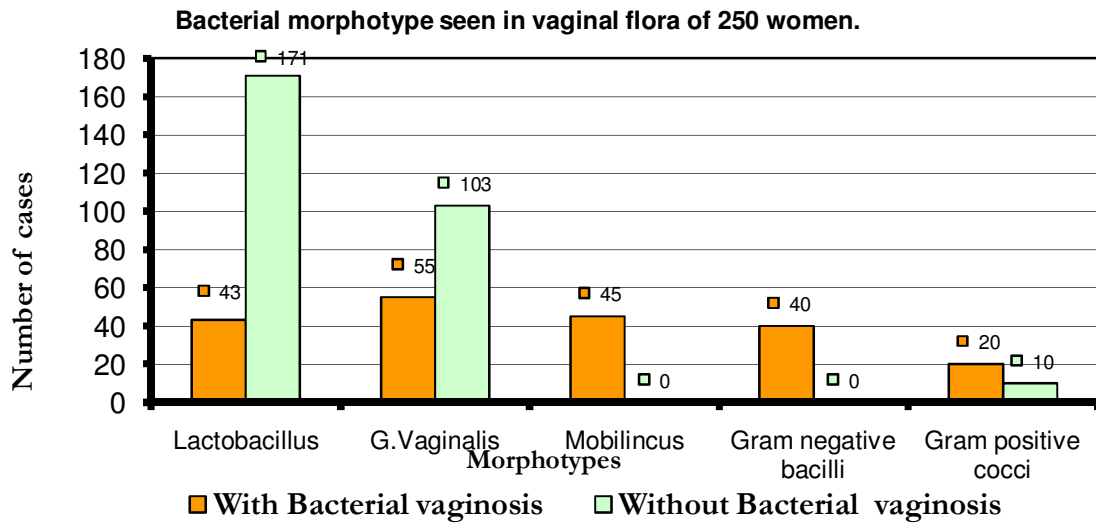


TABLE 3:- MULTIPLE/SINGLE BACTERIAL MORPHOTYPES OBSERVED ON GRAM STAINING OF VAGINAL SMEAR OF 250 WOMEN STUDIED

Number of Morphotypes seen	Women included in study	
	No	%
TOTAL CASES	250	100
Single Morphotype	171	68
Two Morphotype	62	25
Three Morphotype	17	7

FIGURE 3:- MULTIPLE/SINGLE BACTERIAL MORPHOTYPES OBSERVED ON GRAM STAINING OF VAGINAL SMEAR OF 250 WOMEN STUDIED

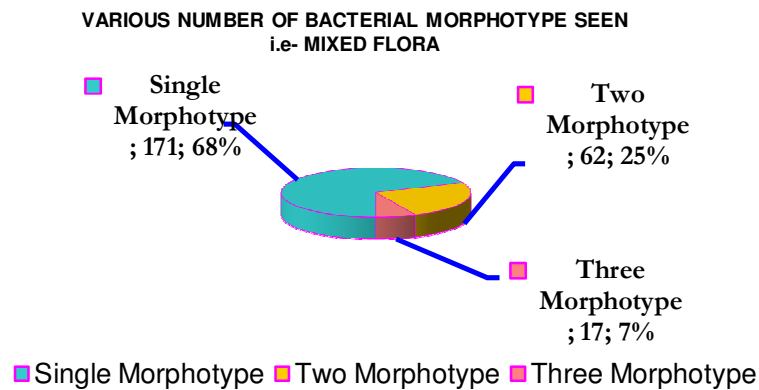
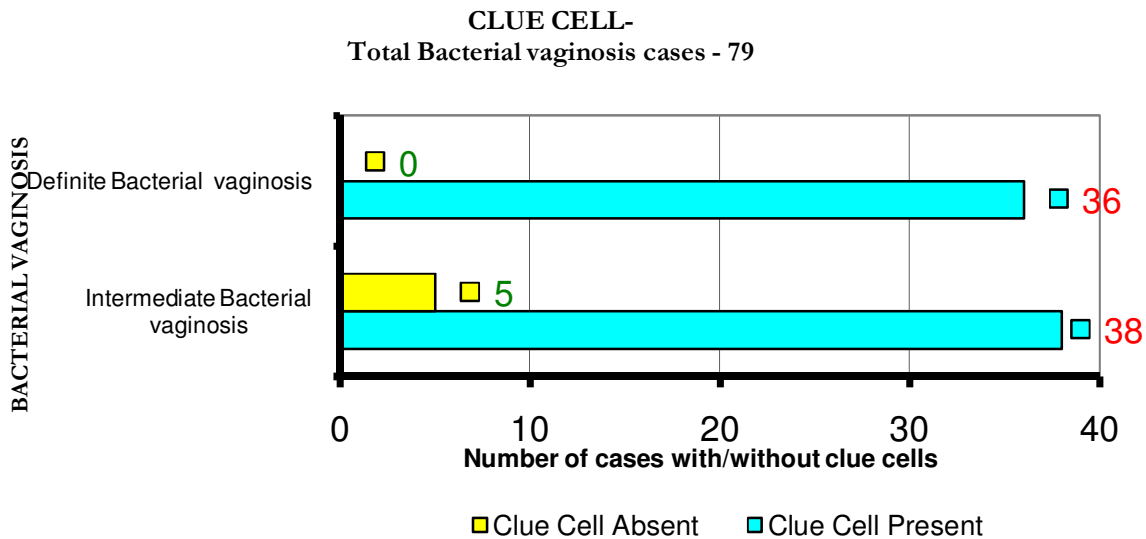


TABLE 4:- CO EXISTENCE OBSERVED BETWEEN BACTERIAL VAGINOSIS AND PRESENCE OF CLUE CELLS ON GRAM STAINING OF VAGINAL SMEAR OF WOMEN DIAGNOSED TO HAVE BACTERIAL; VAGINOSIS

Total Bacterial Vaginosis - 79	Clue Cell Present		Clue Cell Absent	
	No	%	No	%
Intermediate Bacterial vaginosis	38	48	5	6
Definite Bacterial vaginosis	36	45.5	0	0

FIGURE 4:- CO EXISTENCE OBSERVED BETWEEN BACTERIAL VAGINOSIS AND PRESENCE OF CLUE CELLS ON GRAM STAINING OF VAGINAL SMEAR OF WOMEN DIAGNOSED TO HAVE BACTERIAL; VAGINOSIS



Discussion

In our study as shown in Table 2, bacterial morphotypes observed were Lactobacilli, Gardnerella, Mobiluncus, Gram negative bacilli and Gram positive cocci. *G vaginalis* and Mobiluncus were strongly associated with BV diagnosis by both Nugent's score and Amsel's criteria. *G vaginalis* remained significantly associated with BV diagnosis by either method, within the diagnosis categories of the other method. *Coliform* spp, staphylococci and

streptococci were not significantly associated with BV by either Nugent's score or Amsel's criteria. There were significant 2 to 4 fold decreases in colonisation by *G vaginalis* or anaerobes in the presence of lactobacilli. Overall there was no significant difference for *Candida* isolates but the lowest prevalence was observed when no lactobacilli were isolated. In a study on cohort of women with bacterial vaginosis, David A. Eschenbach reported that there is a seven-fold decrease in the prevalence of facultative

Lactobacillus and a corresponding increased prevalence of other bacteria¹³. Symptomatic women with bacterial vaginosis have a 100- to 1000-fold increase in the other bacteria. C A Spiegel et al reported *Gardnerella vaginalis* is almost universally found in high concentration in the vaginal fluid of women with nonspecific vaginitis, but because it is often found in the vaginal flora of normal women, the significance of a positive vaginal culture for this organism in an individual patient is uncertain, even when semiquantitative cultures are done¹⁴. Anaerobic bacteria have also been associated with nonspecific vaginitis¹⁵. As with *G. vaginalis*, interpretation depends upon quantitative

counts and culture is not recommended for the clinical laboratory. In a study conducted by C A Spiegel et al, Gram-positive cocci were seen in 15 of 25 patients with BV and in 3 of 35 patients without BV¹⁴. Curved rods were seen in 11 of 25 patients with BV and in none of 35 patients without BV. The *Gardnerella* morphotype was seen in 25 of 25 patients with BV and in 9 of 35 patients without BV. Small gram-negative bacilli resembling *Bacteroides* spp. were seen in 24 of 25 patients with BV and in none of 35 patients without BV. The *Lactobacillus* morphotype was absent or present only in low quantities (1 to 2+) in 25 of 25 patients with BV and in 5 of 35 patients without BV.

TABLE 5:- MICROBIOTA IN BACTERIAL; VAGINOSIS

Microbiota in Bacterial Vaginosis	
INCREASED PREVALENCE	INCREASED CONCENTRATION (FOLD)
<i>G. vaginalis</i>	17
<i>Bacteroides</i>	15
<i>Peptostreptococcus</i>	10
<i>Mobiluncus</i>	--
<i>M.hominis</i>	15
DECREASED PREVALENCE	DECREASED CONCENTRATION (FOLD)
Facultative <i>Lactobacillus</i>	7
Source: Hillier et al 1993; Martius et al 1988.	

Gardner and Dukes¹⁶ and Dunkelberg¹⁷ described a Gram stain appearance which was characteristic of BV. Normal vaginal fluid contained only *Lactobacillus* morphotypes, whereas fluid from BV patients had many small gram-negative organisms resembling *G. vaginalis* in the absence of *Lactobacillus* morphotypes. In a publication, Balsdon et al¹⁸ again noted a characteristic microscopical appearance of vaginal discharge from patients with BV. In Edward Demba et al's¹⁹ study, *Lactobacillus* spp were isolated in 37.8% of women, and 70% of the isolates were hydrogen-

peroxide (H₂O₂)-producing strains. Prevalence of BV-associated bacteria were: *G vaginalis* 44.4%; *Bacteroides* 16.7%; *Prevotella* 15.2%; *Peptostretococcus* 1.5%; *Mobiluncus* 0%; other anaerobes 3.1%; and *Mycoplasma hominis* 21.4%. In Edward Demba et al's¹⁹ study ,BV was positively associated with isolation of *G vaginalis* and anaerobes , but not with *M hominis*. BV was negatively associated with presence of *Lactobacillus*, and H₂O₂-producing lactobacilli. Presence of H₂O₂-producing lactobacilli was associated with significantly lower prevalence of *G*

vaginalis, anaerobes and *C trachomatis*. In their study Edward Demba et al¹⁹ reported that isolation of lactobacilli and H₂O₂-producing lactobacilli was negatively associated with BV diagnosis by both Nugent's score and Amsel's criteria. Within Amsel's diagnostic categories, the Nugent's diagnosis of BV was still negatively associated with lactobacilli. However, within Nugent's diagnostic categories, Amsel's diagnosis of BV was not associated with lactobacilli. Edward Demba et al¹⁹ found that only two anaerobic isolates, *Bacteroides* spp and *Prevotella* spp, were significantly more common among HIV positive women. In overall crude univariate analysis, there was no any association between douching, menstrual hygiene, genital cutting and HIV. A comparison of vaginal microflora isolates with Nugent's score showed significant positive associations between a diagnosis of BV and the isolation of *G vaginalis*, and a significant negative association with the presence of lactobacilli. The study of Edward Demba et al¹⁹ has demonstrated similar vaginal bacterial isolates to those found in the United States in studies of Hill GB et al, Spiegel CA et al^{20,14}. These studies found a strong association between BV and the isolation of *G vaginalis*, anaerobic gram-negative rods belonging to the genera *Prevotella*, *Porphyromonas* and *Bacteroides*, *Peptostreptococcus* spp, *M hominis*, *Ureaplasma urealyticum*, and often *Mobiluncus* spp. A lower concentration of facultative species of *Lactobacillus* among women with BV in comparison to women with a normal flora was noted in this study. In study of Edward Demba et al¹⁹, a large proportion of lactobacilli isolates (70%) were H₂O₂ producers. These isolates were associated with a significantly lower prevalence of *G vaginalis*, anaerobes and *C trachomatis*, with trends of lower prevalence of *N gonorrhoeae* and *M hominis*, suggesting a protective effect of

vaginal/cervical colonisation conferred by these lactobacilli strains. The absence of H₂O₂-producing lactobacilli was not associated with growth of *Candida* spp. This is different from the findings observed in a study conducted by Hillier et al²¹, in which significant associations between H₂O₂ production and protection against BV and other STI as well as protection against symptomatic candidiasis were reported. Studies among nonpregnant women collecting serial samples of vaginal flora have concluded that some events (either behavioral, hormonal, or environmental) occur that promote a change in the normal flora of the vagina. Studies by Amsel R et al, Hay PE, Morgan DJ et al and Hay PE, Ugwamadu A et al, incorporating repeat measures of vaginal flora concentrations among women throughout the menstrual cycle reported a high rate of BV presentation during the follicular phase of the menstrual cycle and a spontaneous resolution of BV during the luteal phase. These results suggest that endogenous sex hormones may support and assist in sustaining high levels of *Lactobacillus* and illustrate the potential for sex hormones to influence the organisms present in the vagina²². Holst E reported that, bacteria other than *Gardnerella* were positively associated with vaginosis, and evidence of a possible endogenous source of bacteria due to anal-vaginal transfer emerged²³. In previous studies of Mardh PA et al²⁴ and Thorsen P et al²⁵, *M hominis* and *Ureaplasma urealyticum* have been associated with BV, although not in all cases as reported in Arya OP et al's study²⁶.

In our study as shown in Table 3, in cases with BV, number of Lactobacilli morphotype decreased while number of other bacterial morphotype increased. Multiple bacilli were seen in these cases. In cases without BV number of lactobacilli morphotype was more while number of other

bacterial morphotype was less.i.e inversely proportional. Eschenbach DA et al ,Spiegel CA et al reported that BV is a polymicrobial, superficial vaginal infection involving a reduction in the amount of hydrogen-peroxide-producing *Lactobacillus* and an overgrowth of anaerobic and Gram-negative or Gram-variable bacteria ^{13,14}. Although most of these organisms are present in small numbers in the normal vagina, *Mobiluncus* is rarely found and is a sensitive marker for the diagnosis of BV ²⁷. On the other hand, *Gardnerella* has been reported in up to 50 percent of women with no signs or symptoms of BV; therefore, the finding of *Gardnerella* is not a definitive diagnostic of BV²⁸. In fact, it seems that the decrease in *Lactobacillus*, as opposed to the increase in other organisms, influences the vaginal flora and may be the most important predictor in subsequent BV development ²⁹. Bacterial vaginosis is a polymicrobial condition in which a decrease in vaginal acidity and in the concentration of lactobacilli is accompanied by an increase of a 100 fold or more in the concentration of other micro organisms. No single micro-organism is detected in all women with bacterial vaginosis, but *Gardnerella vaginalis*, *Bacteroides* species *Mycoplasma* , *Mobiluncus* have been associated with bacterial vaginosis in most studies. Sophia Yen et al³⁰ stated that Bacterial vaginosis is a clinical syndrome associated with the presence of a group of microorganisms rather than a single etiologic agent. Bacterial vaginosis is characterized by a shift in the vaginal flora from the normal *Lactobacillus*-dominant species to a mixed flora, including *Gardnerella vaginalis*, *Bacteroides* species, *Mobiluncus* species, and *Mycoplasma hominis*. In our study, there was strong inverse relationship between the quantity of *Lactobacillus* and *Gardnerella* morphotypes seen in the Gram-stained smears and when the

smear was scored as 4+ *Lactobacillus*, the *Gardnerella* morphotype was usually absent. When the *Gardnerella* morphotype was scored as 3+ or 4+, the quantity of the *Lactobacillus* morphotype was generally diminished. C. A. Spiegel et al reported that the increased prevalence of gram negative rods, gram positive cocci, and other organisms seen on the smears from BV patients was consistent with the previously reported increase in the prevalence and quantity of *Bacteroides* spp. and butyrate producing *Peptococcus* spp. and an increase in their metabolic products in vaginal fluid from women with BV³¹. The decrease in the prevalence and concentration of the *Lactobacillus* morphotype on Gram stain in women with BV is paralleled by a decrease in the quantity and prevalence of cultivable *Lactobacillus* morphotype and a decrease in lactic acid in vaginal fluid in patients with BV³¹. The presence of curved rods also was correlated with the diagnosis of BV. Motile curved rods have been noted by other investigators³², but the identity of these organisms and their role in BV was not clear at that time. Although vaginal fluids from patients with nonspecific vaginitis have previously been described as yielding pure cultures of *G. vaginalis*³³, such specimens actually contain a mixture of gram-variable *G. vaginalis* and anaerobes , including *Bacteroides* spp., *Peptococcus* spp., curved rods, and *Eubacterium* spp., spp. ³¹.

In our study as shown in Table 4, out of the 79 cases diagnosed as bacterial vaginosis by Gram stain-Nugent's criteria, clue cell's were absent in 6%(5) cases diagnosed as intermediate bacterial vaginosis. While they were present in 48%(38) cases diagnosed as intermediate bacterial vaginosis and 45.5%(36) cases diagnosed as definite bacterial vaginosis. Gardner and Dukes

reported that the appearance of clue cells (i.e., vaginal epithelial cells studded with coccobacillary organisms) in vaginal fluid wet mounts was diagnostic for *Haemophilus vaginalis* vaginitis^{16, 33}. However, Smith et al.³⁴ and Akerlund and Mardh³⁵ subsequently reported that the presence of clue cells on Gram stained vaginal smears and cervical Papanicolaou smears was not useful for the diagnosis of BV.

Conclusion:

Study concluded that in healthy vagina the vaginal microbiota microscopically shows

predominance of *Lactobacillus* morphotype with or without the *Gardnerella* morphotype (normal) etc, while in BV it is represented by a mixed flora with few or no *Lactobacillus* morphotypes (BV) i.e inversely proportional.

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