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Original Research Article

Isolation of Anaerobes in Bacterial Vaginosis

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Abstract: Anaerobic bacteria causing Bacterial Vaginosis (BV) have been linked to a wide variety of upper genital tract infections, particularly in child bearing age. The aim of the study is to know the prevalence of anaerobic organisms in vaginal discharge of women with bacterial vaginosis attending STI clinic at a tertiary care hospital. The material for the study includes vaginaldischarge specimens collected in duplicates using sterile pipettes from 100 women attending STI clinic with the complaint of foul smelling vaginal discharge. BV was diagnosed using clinical composite criteria and Nugent's scoring. Anaerobic culture was done to isolate and identify anaerobes.Out of 100 cases studied, 52 had Nugent's score of ≥7 and 20 had an intermediate score of 4-6. Anaerobic culture was positive in 68 specimens. Gram positive anaerobic bacteria (61.8%) outnumbered the Gram negative isolates (35.3%).Anaerobic bacteria are importantpathogensin causation of Bacterial vaginosis. Isolation of anaerobes from samples with intermediate Nugent's score helps in identifying cases in transitional phase which may develop into frank BV.
Keywords: Bacterial vaginosis, anaerobes, Nugent's score

INTRODUCTION

Microbes co-exist in vaginal ecosystem and are influenced by factors such as hormonal fluctuation, pregnancy, hygiene etc., Microbial colonization of healthy vagina occurs during puberty, 70-90% of which are lactobacilli [1].Bacterial vaginosis (BV) is a clinical entity that is characterized by a change in vaginal ecology where the normal flora of lactobacillus morpho type is replaced by a mixed microbial flora [2]. *Gardnerella vaginalis* initially thought to be associated with bacterial vaginosis actually works synergistically with anaerobic bacteria of the genera *Bacteroides, Peptococcus, and Mobiluncus* to produce the characteristic malodorous discharge. The recovery of *G. vaginalis* in the absence of mixed anaerobic flora and symptoms of BV probably constitutes normal vaginal flora [3]. Bacterial vaginosis often exhibits high prevalence, high relapse rates and associated complications particularly during pregnancy which renders this infection of global importance [1].

Therefore, the present study was undertaken to find out the prevalence of anaerobes in women suffering from Bacterial vaginosis after obtaining clearance from Institutional Ethics Committee.

MATERIAL & METHODS

A total of 100 patients in reproductive age group, clinically suspected of BV were included in the study after taking informed consent from October 2015 to March 2016. Pregnant women, women with bleeding per vagina and those on antibiotic treatment were excluded from the study. For each patient, vaginal discharge was carefully collected in a well lit room from the posterior vaginal fornix with sterile pipettes in duplicates[4].

Color, consistency and odour of vaginal discharge were noted during specimen collection. PH of vaginal discharge was measured by placing the indicator paper directly on the vaginal wall. Amine odour test/whiff test was also performed. Discharge from one pipette was inoculated immediately onto freshly prepared Anaerobic Blood Agar (Hi-media labs) and a Metronidazole 5mcg disc was placed in the well. The plates were placed in GENbag anaer-BIOMERIEUX and incubated for 48-72 hours. Discharge from the second pipette was used for wet mount examination and Gram's staining. Wet mount was used to screen for presence of any motile trichomonads, clue cells, and

yeast cells. In Gram's stain, the presence of epithelial cells, clue cells, polymorphs, budding yeast cells, pseudohyphae, Gram positive & Gram negative morphotypes was noted.

Diagnosis of Bacterial vaginosis was made according to Amsel's clinical composite criteria and Nugent's scoring of Gram's stained smears[5] [Table 1].

Amsel's criteria include -

i) grey white malodorous vaginal discharge
ii) pH of discharge > 4.5
iii) positive whiff test/ amine odour test
IV) presence of clue cells. Presence of 3 out 4 criteria is indicative of BV.

Table 1: Nugents scoring system

Lactobacilli morpho type (Gram positive bacilli	Score	Gardnerella &Bacteroides spp. Morpho type (Gram negative coccobacilli)	*Score*	*Curved Gram variable rods*	*Score*	*Total*
30 or more	0	0	0	0	0	0
5-30	1	<1	1	<1	1	3
1-4	2	1-4	2	1-4	1	5
<1	3	5-30	3	5-30	2	8
0	4	30 or more	4	30 or more	2	10

NUGENT Score Interpretation
1-3: Normal vaginal flora
4-6: Intermediate score
7-10: Indicative of Bacterial Vaginosis

Culture plates were examined for growth at 48 hours and again after 72 hours for slow growers. Gram's staining of the growth was done for observing the morphology of the isolate. Repeated subcultures were done and identification of the organisms was done based on colony characteristics and appearance on Gram stain[5].

RESULTS

Of the 100 patients included in the study, 74% belonged to the age group of 25-44 years and 22% belonged to the age group 20-24 years. Only 42 cases fulfilled 3 out of 4 Amsel's criteria indicating BV [Table 2]. Of the 100 specimens cultured, 68 showed growth. Gram Positive cocci were isolated in 42(61.8%) specimens and Gram negative bacteria were isolated in 24(35.3%) specimens. In 2 specimens mixed isolation of Gram positive and Gram negative anaerobes was observed. Of the 68 culture positive specimens, 75% had a Nugent's score of >7 and 22.05% had an intermediate score of 4-6 [Table 3].

Table 2: Amsel's clinical criteria in the study group

Diagnostic criteria	No. of positives in each Age group				Total (n=100)
	<19 y (n=3)	20-24y (n=22)	25-44y (n=74)	>45y (n=1)	
Grey homogenous discharge	3	20	72	1	96
Amine test positive	1	9	29	1	40
pH more than 4.5	1	8	28	1	38
Clue cells	1	5	20	0	26

Table 3: Comparison between Nugent's score and culture positivity

Nugent's score	No. of patients	No. of cases culture positive for anaerobes
1-3	38	2 (5.26%)
4-6	20	15 (75%)
7-10	52	51 (98.07%)

DISCUSSION

Bacterial vaginosis is a common reproductive tract infection amongst women of reproductive age. Long standing or untreated Bacterial vaginosis may lead to serious sequelae such as endometritis, salpingitis, pelvic inflammatory disease or complications of pregnancy (such as PROM, preterm labour, chorioamnionitis, Low birth weight etc.,) [7].

Gram staining of vaginal secretions is highly reliable with a sensitivity of 89-93% and a specificity of 70-83%[4, 7]. In our study, 42 % of patients were diagnosed as having BV using Amsel's criteria whereas 52 % of patients had a Nugent's score of more than 7 which is diagnostic of BV and 20% had an intermediate score. 75% of specimens from BV cases with intermediate Nugent are scoring showed anaerobic culture positivity. The intermediate stage is considered a transitional phase and the patients may go on to develop frank BV[8].

Anaerobes dominate the microbiota of human skin and mucus membranes and evidence of association of anaerobic bacteria with Bacterial vaginosis is mounting. Aggarwal, *et al.; 2003* [9]observed in their study that Gram positive anaerobes (69.2%) outnumbered the Gram negative ones (30.8%)[9]. In the study by Rosenstein, *et al.;*[8].Anaerobic streptococciwere isolated in 74% of patients with BV, while bacteroidesand other gram negative rods were isolated in 60% of the patients. In a study done by Sumati *et al.;*out of 174 cases studied, 146 were culture positive for anaerobes and the Gram negative anaerobes outnumbered the Gram positives [10]. Rate of isolation of Gram positive anaerobes in the present study was higher than that of Gram negative anaerobes which correlated well with the previous studies done by Aggarwal, *et al.*[9];and Rosenstein *et al.*[10];Anaerobes are important pathogens in the causation of BV. Thus isolation of anaerobes from vaginal discharge using simple media and culture techniques allows for accurate diagnosis and relevant treatment of the cases.

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