

Microbiological Analysis of Abnormal Vaginal Discharge in Females Attending I. I. M. S. & R., Lucknow”

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Abstract: Vaginal discharge may be a subjective complaint or an objective finding. Patients may complain of excessive secretions, abnormally coloured or textured secretions, or malodorous secretions. In the absence of a complaint, the examiner may note abnormal secretions (symptomatic to the patient). We sought to study prevalence of common aerobic bacteria, parasitic and fungal pathogens in abnormal vaginal discharge. Vaginal discharge was collected in the Department of Obstetrics & Gynecology and processed in the Department of Microbiology, IIMS&R, Lucknow from January 1st 2017 to June 30th 2017. Bacterial vaginosis was diagnosed by Nugent's scoring system and Amsel's criteria. While normal saline and iodine method were used for the diagnosis of Trichomonas and gram's staining used for candida diagnosis. A total of 322 women were included. 147 (45.7%) mixed infection women were diagnosed with Candidiasis, BV, Trichomoniasis. 118 (36.6 %) were diagnosed with AVF with inflammation. 40(12.4%) Vulvovaginal candidiasis and 17(5.3%) of Trichomoniasis. 44(13.7%) Most common bacterial isolate in abnormal vaginal flora was E. coli 44(13.7%), followed by S. aureus 37(11.5%), CoNS 31(9.6%), Pseudomonas spp. 31(9.6%), Enterococcus spp. 28(8.7%), mixed growth 21(6.5%), Acinetobacter spp. 14(4.3%), Citrobacter spp. 11(3.4%), Proteus spp. 6(1.9%), Klebsiella spp. 3(0.9%), Enterobacter spp. and group B Streptococcus in equal number 2(0.6%). The prevalence of mixed infection may be high among symptomatic women and Nugent's scoring system is more sensitive and specific in diagnosing BV cases.

Keywords: Abnormal vaginal flora, Bacterial vaginosis, candidiasis, trichomoniasis, vaginal discharge,

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I. Introduction

The World Health Organization has recommended syndromic management, in which women complaining of discharge are treated for some or all of the five common reproductive tract infections: *Chlamydia trachomatis* infection, gonorrhoea, and Trichomoniasis, which are sexually transmitted infections and bacterial vaginosis and candidiasis, which result from disturbance in the normal bacterial flora of vagina¹. WHO estimates that there are more than 340 million new patients of sexually transmitted infections every year and 75-85% of them from the developing countries and lead to sever complication on the women health². In Symptomatic vaginal discharge cases, Bacterial vaginosis (BV) is the commonest cause followed by Candidiasis and Trichomoniasis. Multiple infections can also coexist but these three conditions account for majority of all etiologies of abnormal vaginal discharge³. BV is the most common cause of abnormal discharge in women of reproductive age. It is a polymicrobial vaginal disorder with a heavily disturbed vaginal microbiota, where the *Lactobacillus* -predominant microbiota is replaced by an over growth of anaerobic bacteria. This condition is most often not associated with clinical signs of inflammation (such as vaginal wall erythema, and leukocytosis), thus the term “vaginosis” is used instead of “vaginitis”⁴.

BV is of special public health concern in India because of the high burden of reproductive and pregnancy-related morbidity. Research on BV in India is sparse and mainly limited to a few states⁵.

There is scarcity of data on microbiological analysis of abnormal vaginal discharge from our region therefore, present study was planned to know the spectrum of pathogens in symptomatic women and try to correlate our finding with bacterial vaginosis.

II. Methods

Ethical considerations: The study was approved by the Institutional Review Committee (IRC) & the Ethical Review Committee (ERC). Before enrolment in the study, written consent was taken from the patients. The risk & benefits had been explained in the consent form.

Study participants: Written informed consent for the study was obtained from 322 female patients who were clinically suspected for the abnormal vaginal discharge, IIMS&R, Lucknow.

Method of collection of data: The cases were studied as per the proforma enclosed. A detailed clinical history including age, sex, marital status, pregnancy status, residence, occupation, socio-economic status, duration of illness and any associated risk factors contributing for the illness were recorded from patients. Vaginal discharge was collected in the Department of Obstetrics & Gynecology and processed in the Department of Microbiology, IIMS&R, Lucknow.

Specimen collection and Microbiological procedure: The swab was inserted in a labeled sterile test tube containing 0.5 ml of normal saline for the culture, wet mount and gram staining. The collected samples was immediately brought to the laboratory and processed according to standard methods.

Vaginal swabs were cultured on the blood agar and CLED agar. The inoculated culture media were incubated at 37°C for 24-48 hrs. The isolated organisms were diagnosed by colonial morphology, cultural characteristics and biochemical tests.

In a BV diagnosis, the bacteria are either classified as *Gardnerellamorphotypes*, which are short bacteria that are either Gram negative or Gram variable, or as *Lactobacillus morphotypes*, which are Gram positive rods.

Diagnosis by Nugent’s scoring: The vaginal discharge was smeared on clean glass slides, air dried, heat fixed and stained by Gram’s staining. Each bacterial morpho-type was quantitated under an oil immersion objective (100x) by using the following scheme: 1+, <1 per field; 2+, 1 to 4 per field; 3+, 5 to 30 per field; 4+, >30 per field. Large Gram-positive rods were taken as *lactobacillus* morphotypes; Small Gram-negative to Gram-variable rods were considered as *G.vaginalis* and *Bacteroides* spp. Morphotypes, curved Gram variable rods were considered as *Mobiluncus* spp. morphotypes.

Diagnosis by Amsel’s criteria: Amsel’s composite criteria includes the presence of a homogeneous vaginal discharge, pH of the vagina being > 4.5, the presence of clue cells in wet mount of the vaginal discharge and a positive whiff test. According to Amsel’s, if 3 of the 4 criteria are positive, the patient has bacterial vaginosis.

Identification of *Candida*: *Candida* was identified as *albicans* and *non-albicans*. A drop of vaginal secretions taken on a clean glass slide and mounted with a cover slip. *Candida* was identified as highly refractile, round or oval budding yeast cells. And in gram staining smears were examined for presence of Gram positive pseudohyphae with budding yeast like cells.

FOR *Trichomonas vaginalis*, specimen which is already mixed with normal saline at the time of collection of sample, will be taken on a clean glass slide & a coverslip should be mounted, examine immediately under microscope & look for flagellate organism under microscope.

Statistics: Comparisons were performed with chi-square test or Fisher’s exact test.

III. Results

A total of 322 female patients with complaints of abnormal vaginal discharge, attending department of Obstetrics and Gynaecology, I.I.M.S. & R., Lucknow, were enrolled in the study. Their vaginal secretions were examined for the microbiological analysis. Pure and mixed both types of infections were detected. Maximum 147(45.7%) cases were found to be of mixed type, followed by abnormal vaginal flora (AVF) in 118(36.6%), Vulvovaginal candidiasis (VVC) in 40(12.4%) and Trichomoniasis (TV) 17(5.3%) cases.

Out of 147(45.7%) cases with mixed infections, majority 72(22.4%) were concurrently infected with abnormal vaginal flora and BV. followed by the predominant mixed type of infection was BV+VVC in 32(10.0%), BV+AF+VVC in 13(4.0%) cases, AVF+VVC in 11(3.4%) cases, AF+TV in 10(3.1%) cases, BV+AF+TV in 6(1.9%) cases and BV+TV in 3(0.9%) cases. (**Table and Figure 1.1**)

Table 1.1: Distribution of cases as type of infection

Type of infection	N (%)
Mixed infection	147 (45.7)
1. BV+ AVF	72 (22.4)
2. BV+VVC	32 (10.0)
3. BV+AVF+VVC	13 (4.0)
4. AVF+VVC	11 (3.4)
5. AVF+TV	10 (3.1)
6. BV+AVF+TV	6 (1.9)
7. BV+TV	3 (0.9)
Abnormal vaginal flora (AVF)	118 (36.6)
Vulvovaginal candidiasis(VVC)	40 (12.4)
Trichomoniasis(TV)	17 (5.3)
Total	322 (100.0)

The results of the present study reveal higher prevalence of Gram negative bacteria which including *Escherichia coli* which were recorded in 44(13.7%) case.

Staphylococcus aureus appear the most common Gram positive bacteria and the predominant organisms isolated from examined specimens, it was isolated from 37(11.5%), in contrast to group B *Streptococcus* which was isolated from only 2 individuals (0.2%) cases.

The most common pathogens isolated from the women with BV was depicted in Table 5.14 and out of 230(71.4%) cases, 91(28.2%) positive for BV were highest abnormal flora was 20(6.2%) of *E. coli*, 15(4.6%) isolates of *Staphylococcus aureus*, 14(4.3%) mixed flora, 11(3.4%) *pseudomonas spp.*, 8(2.4%) CoNS, 6(1.8%) *Acinetobacter spp.*, 5(1.5%) *Enterococcus* 5(1.5%) *Citrobacter spp.*, 4(1.2%) *proteus spp.*, 2(0.6%) *Enterobacter* and 1(0.3%) isolated of *klebsiella spp.* responsible for BV.

Out of 230 isolates of abnormal vaginal flora 139(43.2%) had negative for BV. Bacterial vaginosis (BV) with abnormal vaginal flora was found to be statistically significant with *Enterococcus* associated with BV and BV with mixed growth, but not in other isolates. (Table 1.2)

Table 1.2: Comparative analysis of Bacterial vaginosis cases from abnormal vaginal flora isolates

Abnormal vaginal flora	Bacterial vaginosis			p value
	Total isolate N (%)	Positive N (%)	Negative N (%)	
<i>Staphylococcus aureus</i>	37(11.5)	15(4.6)	22(6.8)	0.894
<i>Coagulase-negative Staphylococci (CoNS)</i>	31(9.6)	8(2.48)	23(7.1)	0.13
<i>Group B Streptococcus</i>	2(0.6)	-	2(0.6)	NA
<i>Enterococcus</i>	28(8.7)	5(1.5)	23(7.1)	0.021
<i>Escherichia coli</i>	44(13.7)	20(6.2)	24(7.4)	0.473
<i>Citrobacter</i>	11(3.4)	5(1.5)	6(1.8)	0.925
<i>Klebsiella</i>	3(0.9)	1(0.3)	2(0.6)	0.824
<i>Enterobacter</i>	2(0.6)	2(0.6)	-	NA
<i>Proteus</i>	6(1.9)	4(1.2)	2(0.6)	0.340
<i>Pseudomonas</i>	31(9.6)	11(3.4)	20(6.2)	0.762
<i>Acinetobacter</i>	14(4.3)	6(1.86)	8(2.4)	0.794
Mixed growth	21(6.5)	14(4.3)	7(2.2)	0.015
Total	230 (71.4)	91(28.2)	139(43.2)	0.0001

N.A- not applicable due to low sample size, ($p \leq 0.05$ =significant), (*=insignificant)

On comparing Amsel's criteria with Nugent's score 79(24.5%) cases were positive by Amsel's criteria out of which only 58(18%) had Nugent's score of >7 and 21(6.5%) had Nugent's score of <6.

While 243(75.5%) cases negative by Amsel's criteria, 68(21.1%) had Nugent's score >7 and 175(54.4%) cases were Nugent's score <6. This difference was had to be highly statistically significant ($p = 0.0001$).

For BV patients on the basis of comparison Amsel's criteria & Nugent's scoring, it was found that sensitivity of test was 46.0 % & Specificity was 89.2% thus, it can be concluded that Nugent's was gold standard method for diagnosis of BV. (Table 1.3)

Table 1.3: Comparison of Amsel's criteria and Nugent scoring for the diagnosis of Bacterial vaginosis cases

Method of diagnosis	Nugent's scoring			p value
	Total N (%)	Nugent's score > 7 N (%)	Nugent's score 0-6 N (%)	
Amsel's criteria	322 (100.0)	126 (39.1)	196 (60.9)	
¾ Positive	79 (24.5)	58 (18.0)	21 (6.5)	0.0001
¾ Negative	243 (75.5)	68 (21.1)	175 (54.4)	0.0001

($p \leq 0.05$ =significant), (*=insignificant)

IV. Discussion

On microbiological analysis of their vaginal discharge, patients were categorized into groups of mixed infection, abnormal vaginal flora (AVF), Vulvovaginal Candidiasis (VVC) and Trichomoniasis. All patients were evaluated for clinical presentation, symptoms, risk factors, duration of illness, age, sex, education, residence and socio-economic status.

Out of total 322 cases, the most common type of infection was mixed infection in 147(45.7%), followed by abnormal vaginal flora in 118(36.6%), VVC in 40(12.4%) and Trichomoniasis in 17(5.3%) cases.

In the present study, mixed infection was most prevalent in 47.7% cases, which is one of the highest rates, as few authors have reported lesser prevalence of 5.5% by Sivaranjini et al⁶ from Puducherry and 3.0% by

Khameeset al⁷ from Libya. Therefore, we are first to report a higher prevalence of mixed infection from India. The reason for detection of high rate might be due to underreporting of this type of presentation, as majority of previous studies have studied one type of presentation only and not in combinations.

Second commonest presentation in our study was Abnormal vaginal flora, which was found in 36.6% cases, higher prevalence rate of 75.8% has been reported in a previous study by Khamees⁷, however, few authors have reported lesser prevalence of 23.9% by Tansarli⁸ from Greece and 15.4% by Gandhiet al⁹ from Gujarat. Therefore, we are first to report a higher prevalence of AVF too from India.

Third commonest presentation in our study was Vulvovaginal candidiasis (VVC) found in 12.4% cases. Similar prevalence rate of 13.6%, 14% and 15.2% has been reported by Khameeset al⁷, Masand et al¹⁰ and Sivaranjini et al⁶ respectively in previous studies. However, few authors have reported a higher prevalence of 45.3%, 44.4%, 42%, 41.7%, 20% and 19% by Rajalakshmi and Kalaivani¹¹, Tansarli⁸, Nwadioha et al¹², Vijayalakshmi et al¹³ and Garg et al¹⁴ respectively. Only one study by Patel et al⁵ have been reported a low prevalence rate of 8.5%. Hence, it can be inferred that, prevalence of VVC varies from one region to other, and has been reported in the range of 8.5% to 45.3%, and our prevalence rate falls within this range.

Least common prevalence of 5.3% for Trichomoniasis was found in our study, which is in accordance with previous studies by Masand et al¹⁰ and Garg et al¹⁴, who reported a prevalence rate of 6% and 4% respectively. However, higher prevalence rates of Trichomoniasis have been reported ranging from 30.5%, 27.8% and 13.5% by Rajalakshmi & Kalaivani¹¹ and Shah et al¹⁵ respectively. Nevertheless, very low prevalence ranging from 3% to 0.8% have been reported by Sivaranjini et al⁶ by Vijayalakshmi et al¹³ (2.5%) from Pune, by Gandhi et al⁹ (1.2%) and by Tansarli et al⁸ (0.8%). Hence, as above mentioned infections, prevalence of *T. vaginalis* also varies with population and our prevalence rate is within the range.

While comparing Amsel's and Nugent's scoring criteria for diagnosing Bacterial Vaginosis (BV), 24.5% cases were diagnosed by Amsel's while 39.1% cases were diagnosed by Nugent's score. The sensitivity and specificity of Amsel's criteria was found to be 46% and 89.2%, respectively. Our findings are similar to the previous studies by Muthusamy et al¹⁶ from Chennai, by Mahajan et al¹⁷ from Jalandhar, who reported a prevalence rate of BV as 35.3% and 34% by Nugent's scoring, whereas some authors have reported a higher prevalence of BV of 98.2% by Rao et al¹⁸ from Hyderabad 31.8% by Banupriya and Geetha¹⁹, from Salem, according to Amsel's criteria. However, due to scarcity of data on the comparative analysis of both the criteria; more studies are needed to confirm findings of our study.

V. Conclusion

To conclude, this study showed that the prevalence of mixed infection may be high among symptomatic women and Nugent's scoring system is more sensitive and specific in diagnosing BV cases. Demographic factors like residence, increasing age, illiteracy, and low socioeconomic status are all contributory factors for frequent occurrence of abnormal vaginal discharge. Therefore, we recommend for creating community awareness about health care facilities and self-concern in women for their own health needs. Nevertheless, further research on the risk factors and treatment schemes is still needed in order to improve the prevention and management of these common vaginal infections.

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