



VAGINAL DISCHARGE MICROBIOTA AND ITS ANTIMICROBIAL SENSITIVITY PATTERN

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ABSTRACT One of the most common symptoms in women of reproductive age is vaginal discharge. Normal or abnormal vaginal discharge are both possible. It's important to address pathological discharge. The aim of this research was to investigate the microbiological profile of vaginal discharge and antimicrobial susceptibility trends in women of reproductive age. Easy wet laboratory preparation, 10% KOH, Gram staining, and pH paper were used to diagnose the organism, whereas differential agar media such as CHROM agar, Blood agar, and Sabouraud's Dextrose agar were used to identify and categorise the pathogens, followed by biochemical testing and effective antibiotics to observe susceptibility trends. The antimicrobial activity of Clindamycin and Fluconazole against *Gardnerella Vaginalis* and *Candida* bacteria, respectively, was the strongest.

KEYWORDS : Vaginal discharge, laboratory diagnosis, vaginal microbiota, antimicrobial drugs

INTRODUCTION

Vaginal discharge is one of the most frequent signs in women of reproductive age. [1] Vaginal discharge may be either natural or pathological in nature. It is important to treat pathological discharge. Cervicitis or vaginitis may also cause vaginal discharge. *Trichomonas vaginalis*, *Gardnerella vaginalis*, and *Candida albicans* are the organisms that cause vaginitis. [2] Bacterial Vaginosis and Candidiasis, all of which are caused by a disruption in the vaginal bacterial flora. [3] The majority of women suffer in silence, unable to accept advice or medication. [4] That has established syndromic management protocols for the treatment of sexually transmitted infections in a developing world. [5] Normal vaginal discharge is non-irritating and odourless, and it may be clear, cloudy, or white. The volume and colour of regular discharge will vary for a number of causes, and it may be a symptom of a vaginal infection. Trichomonal vaginitis, Candidal vaginitis, and Bacterial vaginosis are the three primary forms of vaginal infections currently known. *Gardnerella vaginalis* causes bacterial vaginosis, *Trichomonas vaginalis* causes Trichomonal vaginitis, and *Candida* genus causes Candidal vaginitis. [6] Vaginal discharge and odour may be affected by vaginitis caused by bacteria, fungi, or protozoa. Bacterial vaginosis is a type of bacterial vaginosis marked by foul-smelling, copious discharge. Curdy white discharge is a symptom of vulvo-vaginal Candidiasis. Greenish frothy discharge is a symptom of vaginal trichomoniasis. A comprehensive history is needed for differential diagnosis of these infections. [7] Vaginal discharge and odour may be impaired by vaginitis caused by bacteria, fungi, or protozoa. Bacterial vaginosis is a type of bacterial vaginosis marked by foul-smelling, copious discharge. Curdy white discharge is a symptom of vulvo-vaginal Candidiasis. Greenish frothy discharge is a symptom of vaginal trichomoniasis. A comprehensive history is needed for differential diagnosis of these infections. [7] Bacterial vaginosis (BV) is the most frequent cause of unexplained vaginal discharge in women of childbearing age, but it may also affect menopausal women and infants. [8] Lactobacilli deficiency is a well-known risk factor for genital infections and pregnancy complications. The frequency is 5-15 percent in Caucasian women and 45-55 percent in African and American black women. The prevalence of Asian women is less well studied, but it is estimated to be between 20 and 30 percent. People who have sex with other women have common Lactobacillary forms and are more likely to contract BV. [9]

Higher rates of BV have been identified in STI clinics and in women who have multiple sexual partners; however, the function of sexual transmission is uncertain, as women who are not sexually involved have been found to be infected; hence, treating a woman with BV's male sexual partner has been indicated to be ineffective. [10] *Candida albicans* is the infecting agent in 80 percent to 90 percent of patients with candidal vaginitis, and *Candida albicans* is the most common cause of vaginitis. However, non-*Albicans* species such as *Candida glabrata* and *Candida tropicalis* have recently become more common,

likely due to the use of over-the-counter antifungal drugs. [11] Women who use oral contraceptives, vaginal diaphragm, spermicidal, or IUCD, as well as those who have asthma, are pregnant, or are taking antibiotics, have an elevated risk of Candidiasis.

T. vaginalis is a protozoan that affects 180 million women worldwide and is responsible for 10 to 25% of vaginal infections. [12] *Trichomonas vaginalis* is a sexually transmitted virus that can be found in an untreated woman's male sexual partner. According to reports, the rate ranges from less than 1% in urban women to more than 20% in underserved populations. [13] Therefore, this study was decided to look into the microbiological profile and anti-biotic susceptibility patterns of vaginal discharge in women of reproductive age who were visiting a tertiary care hospital.

MATERIAL AND METHODS

A total of 120 patients were examined in Gynaecology out patient Department in Obstetrics and Gynaecology, Sharda Hospital, Greater Noida. After taking an informed consent all subject were evaluated by detailed history regarding the duration, amount, consistency, colour, odour any previous episode of discharge and any other associated symptom.

A Descriptive Cross-sectional study was conducted over a period 12 months from January 2015 to December 2015. Patients in reproductive age group attending Gynaecology and Obstetrics Outpatient/ In-Patient Department, with signs and symptoms of vaginitis or vaginal infections. Structured questionnaires were included in the study. Case notes were also used for obtained relevant data. Under strict aseptic condition with full precaution, a per speculum examination of vagina was done and three sterile cotton swabs were used to collect discharge and immediately transported to the laboratory. First swab was used for wet preparation, second swab for gram staining and third swab was used for culture.

• Laboratory diagnosis-

- 1. Wet preparation-** A suspension of vaginal exudate was made on a glass slide using a few drops of isotonic saline and covered with a cover slip. This was examined under high power (40x) lens of microscope. The following features were observed- Clue cells, Budding yeast cells and pseudo hyphae and motile trophozoites of *Trichomonas vaginalis*.
- 2. KOH preparation-** A suspension of vaginal exudate was mixed with 10% KOH solution on a glass slide and covered with a cover slip and it was observed for fishy odour and was examined under high power (40x).
- 3. pH test-** In case of Bacterial vaginosis pH increases to the 4.5-4.7. pH measured with the help of pH paper.
- 4. Diagnosis of bacterial vaginosis-** Vaginal smear was heat fixed and is stained by Gram's method- Gram staining- Gram staining was done as per standard protocol. The slide was examined under oil immersion

(100x). Smear was examined for bacteria of various morphotypes and scored as per Nugent's criteria. Nugent's scoring system is based on the semi quantitative assessment of Lactobacillus morphotypes, Gardnerella vaginalis. A score of 7-10 is considered as diagnostic of bacterial vaginosis.

5. Detection of candida species- The Gram staining were used to observed Budding yeast cells or pseudohyphae. CHROM agar was used for species identification. Suger fermentation test was done using by Andrade's indicator with invert durham's tube to see gas production. The color change in the tube containing the particular sugar indicates the yeast's ability to ferment that carbohydrate.

CANDIDA SPECIES	Glucose	Maltose	Sucrose	Lactose
<i>Candida albicans</i>	+	+	-	-
<i>Candida parasilosis</i>	+	-	-	-
<i>Candida tropicalis</i>	+	+	+	-
<i>Candida krusei</i>	+	-	-	-
<i>Candida glabrata</i>	+	-	-	-

RESULTS AND OBSERVATION

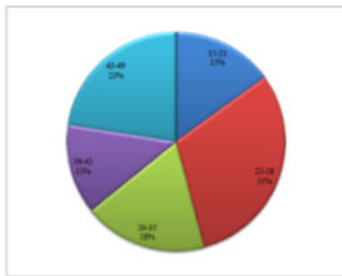


Fig 1. Age- Wise Distribution

Table 1. Distribution of participants according to their douching material

Particulars	Frequency (n=120)	Percentage (%)
Water	66	55
Soap	37	31
Antiseptic	6	5
Local Herb	11	9

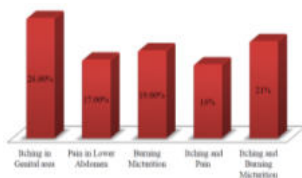


Fig 2. Symptoms with the complaints of vaginal discharge

Table 2.- Distribution of vaginal discharge in participants

Characteristic	Frequency (120)	Percentage (%)
Color		
Whitish	57	47.5
Curdy	54	45
Greenish frothy	9	7.5
TOTAL	120	100
Odour		
Odourless	56	46
Stinky	64	54
TOTAL	120	100

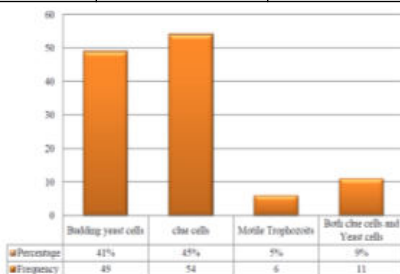


Fig 3. Microscopy Wet mount

Table 3. pH and Direct Gram staining of vaginal discharge

	FREQUENCY (120)	%
<4.3	55	45.83
>4.3	65	54.17
GRAM STAINING		
Budding yeast cells	60	50
Clue cells	65	54

Table 4. Candida species isolated

CANDIDA SPECIES	NO.OF SAMPLES (N=49)	(%)
<i>Candida Albicans</i>	12	24.4
<i>Candida Tropicalis</i>	9	18.3
<i>Candida Parapsilosis</i>	22	44.8
<i>Candida krusei</i>	6	12.4

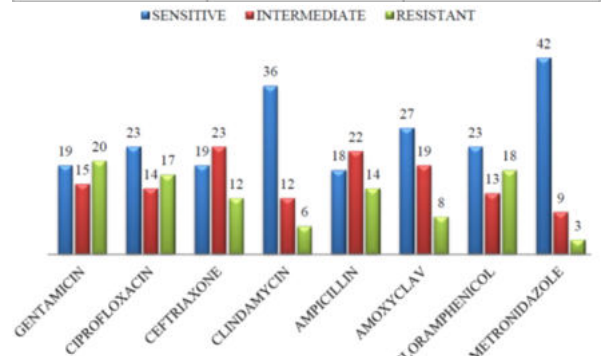


Fig 4. Antibiotic pattern of Bacterial Vaginosis (Gardnerella Vaginalis)

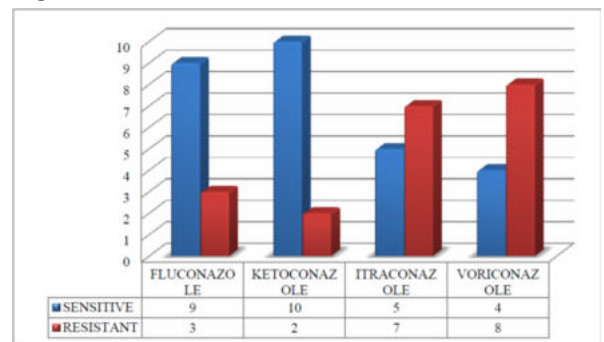


Fig 5. Antifungal pattern of Candida Albicans

Microbiological diagnosis	Frequency (120)	Percentage (%)
Bacterial vaginosis	54	45
Candidiasis	49	41
Trichomoniasis	6	5
Bacterial vaginosis+ Candidiasis	11	9

Table 5. Distribution of causative pathogens for vaginosis

DISCUSSION

Vaginal discharge is a common health issue for women of reproductive age, whether asymptomatic or symptomatic; however, it is mostly ignored by women, making clinical diagnosis more difficult.

Swabs were tested for causative pathogens using normal microbiological procedures (Yeast, bacteria, Trichomonas vaginalis). The findings of this analysis revealed that possible pathogenic agents were isolated in all cases of vaginal discharge seen in the Gynecology OPD. In our sample, the majority of women with vaginal discharge (31%) were between the ages of 25 and 49, while in a related study by R. Saujanya et al (2015), the majority of women with white discharge (34%) were between the ages of 25 and 29. [14]

Vaginal discharge was found to be more common among women aged

28 (55 percent), married women, and housewives in our research. Patel V et al (2005), on the other hand, observed a higher proportion of vaginal discharge in the younger age group, single females, and those who work. [15] This may be attributed to the fact that unmarried and younger age group females with vaginal discharge referred to the hospital less often, and because our research was limited to clinics, their figures were smaller.

The overall incidence of vulval itching (55.9%) and lower abdominal pain (53.2%) in our sample was close to that found by Tanksale et al (2003). [16] In our research, the pH range in bacterial vaginosis cases was 4.7–6.5, which was similar to a study by Dutta et al. (2001) that found that changes in vaginal flora changed surrounding pH, which may contribute to bacterial vaginosis, Trichomoniasis, Candidiasis, and other infections. [17] Bacterial vaginosis (45%) was the most prevalent infection, followed by vulvo vaginal Candidiasis (41%) and Trichomoniasis (5%), as was the case in a report by Sarita et al (2011), in which Bacterial vaginosis (52.6%) was the most common infection, followed by Candidiasis (29.5%) and Trichomoniasis (5%). (1.3 percent). [18] In our research, the prevalence of bacterial vaginosis (45%) was comparable to Mahadani (1998). (44.30 percent). [19] Similarly, our research's frequency of vulvo vaginal Candidiasis is comparable to Kamara's (2000) study (30.7 percent). [20] In their research, Mendling and Brasch (2012) discovered that 22.21 percent of the cases had vulva vaginal Candidiasis. [21]

Gardnerella Vaginalis was shown to be immune to Metronidazole in 29% of cases by Goldstein et al.[22] (2002), and 23% of Gardnerella Vaginalis were resistant to Metronidazole in our study. P Nagaraja (2008) found that Gardnerella Vaginalis strains were immune to Metronidazole in 68 percent of cases,[23], but only 23 percent of Gardnerella Vaginalis strains were resistant in our study. Finally, antibacterial susceptibility monitoring shows that Gardnerella vaginalis is most vulnerable to Metronidazole (77 percent), Clindamycin (66 percent), and Amoxycylav (33 percent) (50 percent). Fluconazole resistance was observed in 85.1 percent of Candida isolates by Salehei Z et al (2012). [24] In comparison, while 18 percent of Candida species were immune to Fluconazole in our research, Quindos et al. observed that 90.2 percent and 91.4 percent isolates of Candida species were sensitive to Fluconazole and Ketoconazole, respectively (81percent), while Fluconazole 82 percent and Ketoconazole 67 percent were considered sensitive for different Candida species in our study. In antifungal susceptibility testing, vaginal isolates of Candida albicans were most susceptible to Fluconazole (82%) followed by Itraconazole (76%) and Voriconazole (6%). (71 percent). According to the findings, factors such as rising age, illiteracy, low socioeconomic status, elevated parity, and general behaviours all play a role in the prevalence of vaginal discharge.

CONCLUSION

In microbial diagnosis, we found that almost half of the patients had Bacterial Vaginosis, slightly over 40% had Candidiasis, and just a few patients had Trichomoniasis and Bacterial Vaginosis and Candidiasis combined infection. Clindamycin exposure was highest in Gardnerella vaginalis, followed by Amoxycylav and Ceftriaxone. Candida Parapsilosis was the most widespread Candida species among the four we isolated, followed by Candida albicans, Candida tropicalis, and Candida krusei. Fluconazole was found to be the drug with the highest sensitivity.

CONFLICT OF INTEREST

No conflict of interest. Each author contributed equally for the study.

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