



MICROBIOLOGICAL PROFILE OF LEUKORRHOEA IN WOMEN OF THE REPRODUCTIVE AGE GROUP

Microbiology

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ABSTRACT

INTRODUCTION: Leukorrhoea develops due to physiological and pathological conditions among women of reproductive age. Due to its presentation, determining the source and cause of discharge is crucial, therefore, differential diagnosis and microbiological profile would aid in its management.

AIM: To identify etiological agents and estimate their proportions in leukorrhoea among women of reproductive age for better treatment of genital tract infection.

MATERIALS AND METHODS: The study was conducted at the OBG Department of a tertiary medical hospital from January 2018 to December 2018. Vaginal swabs were collected sterilely from 152 patients complaining of abnormal vaginal discharge or any other associated ailment. Samples were processed for culture as per standard recommendations. Bacterial vaginosis (BV) was diagnosed by clinical composite criteria (Amsel's criteria) and Gram stain (Nugent's score).

RESULTS: Among the 152 patients, infectious etiology was observed in 65.8%, normal flora in 28.9% & no growth in 5.3% in culture media. BV (44%) had higher prevalence followed by Candidiasis (26%) and Trichomoniasis (2%). Amsel's criteria identified 28.9% of BV compared to Nugent's criteria (25%). A considerable increase in non-*albicans* *Candida* species (53.8%) was observed compared to *Candida albicans* (46.2%). Other organisms isolated included, *Acinetobacter* spp. (3.6%), *Pseudomonas* spp. (14.3%), *E. coli* (39.3%), *Klebsiella pneumoniae* (10.7%), *MSSA* (10.7%), *Enterococcus* spp. (21.4%).

CONCLUSION: Bacterial vaginosis was the prominent cause of infection in leukorrhoea among the women of reproductive age. Timely diagnosis and appropriate management avert serious complications and risks of ectopic pregnancy, infertility, chronic pelvic pain, and abdominal discomfort.

KEYWORDS

Vaginal discharge, *Candida albicans*, Leukorrhea

1. INTRODUCTION

Leukorrhoea is an atypical vaginal discharge which is non-hemorrhagic, whitish to yellowish-green in color associated with irritation. Predisposing elements of leukorrhoea include pH change in vaginal secretions, imbalance of normal bacterial flora, and modification of vaginal epithelium. It is physiological when associated with the various phases of puberty, menstrual cycle, ovulation, and pregnancy. Pathological conditions of leukorrhoea if untreated leads to, atopic vaginitis, cervicitis, desquamative inflammatory vaginitis (DIV), infertility, chronic pelvic pain, and mucoid ectopy.²

Leukorrhoea is commonly associated with four pathological ailments: bacterial vaginosis (BV), candidiasis, aerobic vaginitis (AV), and trichomoniasis. BV is the most widespread atypical vaginal discharge encountered by women, with a prevalence of 45-55% among Black women and 5-15% in Caucasians. BV is dysbacteriosis of the vaginal microflora with overgrowth of anaerobic organisms (*Gardnerella vaginalis*, *Mycoplasma hominis*, *Atopobium vaginae*, *Prevotella* spp., *Mobiluncus* spp.) which replaces the lactobacilli in the vagina thereby increasing vaginal pH. The vaginal discharge appears as, white thin homogenous fluid with a fishy odor. Currently, the etiology of BV is ambiguous, however, research suggests biofilm formation with *G. vaginalis* as a vital change over from the normal vaginal microbiota to BV. Candidiasis is experienced by 75% of all women as a symptomatic episode, while 6-9% suffer from recurring chronic vulvovaginal candidiasis. Vaginal discharge appears curdy & has a non-offensive odor. The condition results predominantly due to *Candida albicans* in 90% of the cases. Presently, the prevalence of non-*albicans* *Candida* (NAC) is reported increasingly and maybe attributed to the continuation of low azole dosage regimes and inappropriate use of non-prescription antimycotics. The predisposing factors may involve, immunosuppression (exogenous & endogenous), pregnancy, antibiotic therapy, immunosuppressive drugs, etc. AV/DIV results in reduced lactobacilli, increased pH and aerobic organisms

(Group B streptococci, *E. coli*, and *Staphylococcus aureus* prevalence). The condition causes purulent discharge. *Trichomonas vaginalis* (TV) in adults is mostly sexually transmitted. Site of infection is intravaginal or intraurethral. Vaginal discharge is offensive in 70% of cases, 10-30% of patients report yellow and frothy discharge.²

Microbiological investigations of the vagina exhibit the presence of microorganisms responsible for infections and thereby analysis of culture-dependent methods to understand the etiology. Antibiotic susceptibility test would further enhance the selection of appropriate antibiotics, thus providing patient-specific treatments. With the current emerging epidemiological data and advanced investigation techniques, it is crucial to be aware of various presentations of abnormal vaginal discharge, investigation techniques and their management corresponding to the symptoms and etiology.

2. AIM:

To acknowledge the microbiological profile which is vital for the diagnosis and treatment of patients with appropriate therapy, thereby preventing recurrence and complications.

3. MATERIALS AND METHODS

This cross-sectional study was conducted at a tertiary medical hospital for a period of 12 months (January 2018 to December 2018). The study protocol was reviewed and approved by the institute's ethical committee. Vaginal swabs were obtained from 152 female patients who attended the Obstetrics and Gynecology (OBG) Department of the hospital complaining of abnormal vaginal flow or any other associated illness. The patients were categorized into two groups: Group 1 – Patients with abnormal vaginal discharge as the chief presenting complaint. Group 2 – Patients with other complaints like lower abdominal pain, infertility, irregular periods, dysmenorrhea and were found to have abnormal vaginal discharge on examination.

3.1. Inclusion Criteria

All patients of the reproductive age (18 to 45 years) presenting with leucorrhoea and attending as out-patients to the OBG Department at the hospital. Patients agreeing to provide informed consent for the study.

3.2. Exclusion Criteria

Patients with bleeding per vagina. Patients with genital tract malignancies (cervical and endometrial carcinomas). Patients on antibiotics and local medications such as, topical ointments and intravaginal pessaries. Non-infective causes of leucorrhoea.

3.3. Sample Collection

A detailed medical history was obtained which included, gynecologic parameters such as, menstrual history, pregnancies, contraception, sexual preference, past and current sexual relationships, vaginal hygiene practices (douching), and prior genitourinary infections.

A sterilized Cosco's speculum was inserted into the vagina (without antibacterial agent creams) to visualize the vagina and cervix. The vaginal discharge was observed for: mode of onset, duration, presence of itching/irritation (localized or generalized, provoked, intermittent, or chronic), color, consistency, odor of discharge, relation to menstrual cycle, associated abdominal pain, dysuria and dyspareunia. Vaginal pH was recorded using pH strips within a range of pH 2.0 - 10.5 from the mid-lateral vaginal wall.

Samples were examined for odor through Whiff test (Amine test). Sample were placed on a microscope slide, a drop of 10% potassium hydroxide (KOH) was added, and the resultant mixture was smelled. Normal secretions have no odour and a fishy odour is suggestive of BV. The Amsel's criteria are thus applied to diagnose BV.

Three vaginal swabs were collected sterilely from the posterior fornix and transported immediately using thioglycollate broth with vitamin K supplement to the microbiology laboratory and processed as per standard recommendations using BSC2 (Biosafety cabinet).

3.4. Microscopic examination

The first high vaginal swab was used to make a wet mount with normal saline and with 10% KOH. The second-high vaginal swab was used for gram staining. The smear was stained by Gram's method and observed. It was evaluated for the presence of pus cells, clue cells, yeasts, vaginal epithelial cells & various bacterial morphotypes. Large Gram-positive rods were measured as lactobacillus morphotypes, smaller Gram-negative to Gram-variable rods were considered as *G. vaginalis* and Bacteroides spp. Nugent's score was applied for the diagnosis of bacterial vaginosis.

The third swab was used to inoculate 5% sheep blood agar, MacConkey's agar, vaginalis base agar. For fungal isolation, modified peptone starch (peptone starch dextrose agar PSD), sabouraud's dextrose agar, CHROMagar, cornmeal agar for dalmat plate technique was used.

For further identification, various biochemical tests were performed (bile esculin test, coagulase test, catalase test, citrate utilization test, indole test, mannitol fermentation test, oxidase test, triple sugar iron test, urease test, sugar assimilation test, sugar fermentation test, and germ tube test).⁴⁶ All cases of BV were identified by, microscopy, Amsel's criteria and Nugent scoring.^{3,7}

3.5. Sample size calculation

Prevalence of bacterial vaginosis to be 32%, in the present study considering absolute precision to be 7.4 % and desired confidence level of 95%, sample size was calculated to be 152.

$$n = \frac{\left(\frac{z_{\alpha/2}}{E}\right)^2 p(1-p)}{1} = \frac{(1.96)^2 \cdot 0.32 \cdot 0.68}{(0.074)^2} = 152$$

Where,

p = prevalence of Bacterial Vaginosis = 32% = 0.32

(1-p) = (1-0.32) = 0.68

$z_{\alpha/2}$ = 1.96 at 95 % confidence level

E = Absolute precision at 7.4%

1.1. Statistical analysis

Data is scrutinized using statistical software R version 3.6.3. The association between any two categorical variables Chi-square/ Fisher's exact test was performed and a descriptive statistic is provided for categorical variables.

Table 1: Analysis of etiological agents based on microscopy and culture findings and distribution of cases based on infectious etiology.

Microscopy and cultural findings n (%)	Isolated infectious organisms n (%)
Pathogens identified 100 (65.8%)	Bacterial vaginosis - 44 (44%) Vulvo-vaginal candidiasis - 26 (26%) Trichomoniasis - 2 (2%) Other bacterial isolates - 28 (28%) • <i>E. coli</i> - 11 (7.23%) • <i>Enterococcus spp.</i> - 6 (3.94%) • <i>Pseudomonas spp.</i> - 4 (2.63%) • <i>Klebsiella pneumoniae</i> - 3 (1.97%) • <i>MSSA</i> - 3 (1.97%) • <i>Acinetobacter spp.</i> - 1 (0.65%)
Normal vaginal flora 44 (28.9%)	<i>Coagulase negative Staphylococcus (CoNS)</i> - 13 (8.54%) <i>Micrococcus</i> - 11 (7.23%) <i>Diphtheroids</i> - 20 (13.15%)
No growth 8 (5.3%)	-
Total 152 (100%)	-

Table 2: Bacterial Vaginosis based on Amsel's Criteria

Amsel's criteria	No. of patients, n (%)
3 or more positive criteria	44 (28.9%)
Less than 3 positive criteria	108 (71.1%)
Total	152 (100%)

Table 3: Bacterial Vaginosis based on Nugent's Scoring

Nugent's score	No. of patients n (%)
0-3 (Normal flora)	91 (59.86%)
4-5 (Intermediate)	25 (16.44%)
7-10 (Bacterial Vaginosis) BB 1 (0.65%)	35 (23.02%)
Total	152 (100%)

Table 4: Distribution of *Candida* species

<i>Candida</i> species	No. of patients n (%)
<i>C. albicans</i>	12 (46.15%)
<i>C. tropicalis</i>	8 (30.76%)
<i>C. krusei</i>	4 (15.38%)
<i>C. guilliermondii</i>	2 (7.69%)

2. RESULTS

The study involved 152 patients of which abnormal vaginal discharge was observed in 65.1% (n=99). The remaining 34.9% (n=53) presented complaints of infertility/lower abdominal pain/irregular menstruation. The studied population was between the age groups of 18-45 years. Abnormal vaginal discharge was observed the highest among the age groups of 18-29 years (n=64; 42.1%), which was closely followed by 30-39 years of age (38.8%). The mean age among the cases was 31.6±7.2 years. Maximum number of patients (n=140; 92.1%) were married in the study. Most of the patients presenting with symptoms were multiparous (n=86; 56.6%), followed by primiparous (30.3%) and nulliparous (13.1%).

Whiff test was positive for 13.15% of the cases. Nearly, 74% of cases reported pH<4.5. Wet mount test showed prevalence of normal cells (n=120; 78.9%), followed by clue cells (19.1%), 1% *Trichomonas vaginalis* and 1.3% of budding yeast cells and pseudohyphae. The rate of infection was common amid (n=43; 28.2%) patients not using any contraceptive methods; whereas, barrier methods (condom), oral contraceptives pills, intrauterine contraceptive device and permanent sterilization (tubectomy) was 17.7%, 19.1%, 15.8% and 19.1% respectively.

Analysis of etiological agents based on microscopy and cultural findings showed the presence of epithelial cells, lactobacilli and a few pus cells which did not satisfy Nugent's score and therefore were included under normal vaginal flora. Infectious etiological results predicted higher number of patients with BV (Table 1). Among the BV and Candidiasis cases, 4 patients showed a combined infection of BV and *Candida* spp.

As per Amsel's criteria, if 3 out of 4 criteria are fulfilled, it is suggestive of BV. Amsel's criteria identified 28.9% of patients as having BV

(Table 2). BV was identified based on the microscopy (clue cells) and Amsel's criteria since there was no growth on the culture media specific for *Gardnerella vaginalis*.

Nugent's score identified 23 % of samples to be BV (Table 3).

The non-*albicans Candida* (NAC) (53.8%) were the most isolated compared to *C. albicans* (n=12;46.2%) of the total *Candida spp.* (Table 4). Among the NAC, *C. tropicalis* (5.3%) was the most isolated.

Antifungal susceptibility testing results of all *Candida* isolates was 100% susceptible to amphotericin B, ketoconazole, clotrimazole, nystatin, fluconazole (except *C. krusei* isolates which exhibited 100% resistance to fluconazole).

The overall patients pregnant during the study was 22 (14.5%), of which 8 women showed the presence of infectious organism and 14 women had normal vaginal flora. Among the 8 women with infection, BV was seen in 7 cases and co-infection with BV and *Candida albicans* was seen in 1 case.

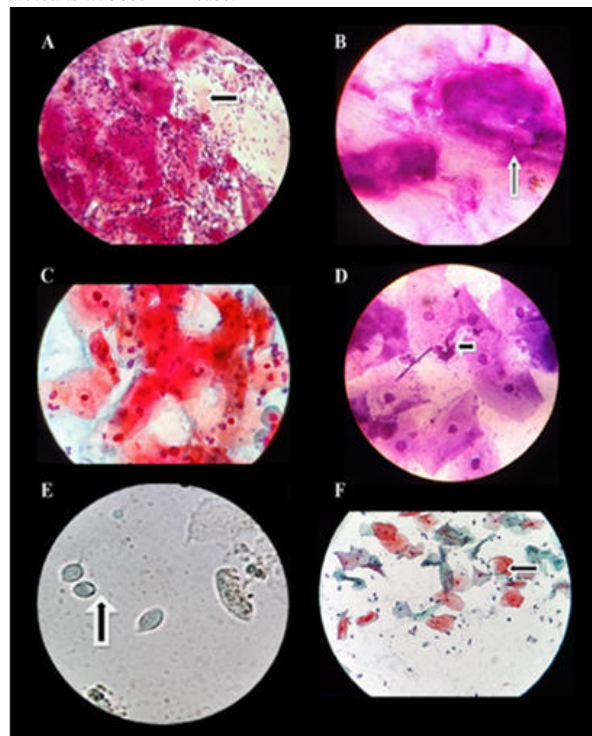


Figure 1: A: Gram staining showing normal vaginal flora (arrow shows Lactobacilli > 30/oil immersion – Nugent score 0); B: Gram staining showing bacterial vaginosis (arrow shows clue cell); C: PAP smear positive for bacterial vaginosis; D: Gram staining showing *Candida* infection (arrow shows *Candida* with pseudo hyphae); E: Wet mount showing *Trichomonas vaginalis*; F: PAP smear positive for *Trichomonas vaginalis*.

5. DISCUSSION

Reproductive tract infection is an overlooked concern among the reproductive age group women of rural as well as urban areas. Prevalence rate of leucorrhoea is projected to be 30% in India.⁸ With the present study we evaluated the microbiological profile of leucorrhoea which would aid in patient-specific management plan and prevention of over-the-counter drugs and antibiotic resistance.

Our study included 152 cases with mean age of 31.6 ± 7.2 years. Atypical vaginal discharge was reported by 65.1% of the cases, whereas, 34.9% presented with other complaints like lower abdominal pain, infertility, irregular menstrual cycle, and dysuria and were found to have abnormal vaginal discharge on examination. Similar findings were reported by Das et al.⁹ where, 68% of patients had symptomatic abnormal vaginal discharge and 32 % were asymptomatic. However, Modak Tet al.¹⁰ study found 21% of cases with abnormal vaginal discharge. Among the women of reproductive age (18 – 45 years) studied, highest prevalence was observed amid the 18-25 years of age. Our observations were in par with other studies conducted by Masand

D.L et al., Sumati et al., and Khamees S.S et al.¹¹⁻¹³ Maximum incidence of leukorrhoea was reported in married women (92.8%), these findings were supported by other studies as well.^{14,15} Increased sexual activity and childbirth could be the reason for higher prevalence in this variable. No contraceptive methods were used by majority of the subjects (28.3%), Moussa B et al. reported comparable results with 25.3% prevalence.¹⁶ This observation focuses the need for the partners to be examined as, their genital tract could be the reason of infections/reinfections, preventing women from healing.

Based on microscopic findings, infectious etiology was predominant in 65.6% of women with BV being the most common followed by Candidiasis and Trichomoniasis. Prevalence of BV, could be due to presence of anaerobic bacteria's and Gardnerella in smaller numbers in the vagina, however, change in pH, normal flora or vaginal epithelium and decreased lactobacillus can lead to higher concentrations of these micro-organisms.¹⁷ Our results were analogous to a study by Bhalla et al.¹⁸ where, the most common infection was BV (32.8%), followed by Candidiasis (16.9%) and Trichomoniasis (2.8%). Other studies conducted by Patel et al., Vishwanath et al., and Puri et al. also support our findings.¹⁹⁻²¹ In contrast to the above studies and our results, the study by Fonck et al., the most prevalent etiology was Candidiasis (46%), followed by Trichomoniasis (21%) while BV (10%) formed the minor group.²² Increased percentage of trichomoniasis could be due to majority of the patients enrolled were from sexually transmitted infection (STI) referral clinics in Kenya. Another study also reported Candidiasis as the most common (35.5%) infection, followed by BV in 23.5% of women and Trichomoniasis in 8.1% women.²³ The emerging cases of Candidiasis as the leading cause of abnormal vaginal discharge could be due to inappropriate usage of broad-spectrum antibiotics in numerous health ailments, which alters the normal vaginal flora resulting in colonization of yeast.²⁴

In our study, Amsel's criteria identified more cases of BV than Nugent's criteria; however, Nugent's scoring is widely accepted as the Gold standard for diagnosis of BV. Sumati et al. study showed prevalence of BV using Nugent criteria, was around 43(19%) and 110 (68.4%) patients were diagnosed as BV using Amsel's criteria.¹³ On the contrary Rangari et al. in their study observed Nugent score having greater sensitivity in diagnosis of BV while Amsel's criteria exhibited lesser sensitivity but higher specificity.²⁵ Their study concluded Amsel's criteria alone without using staining methods for detection of clue cells could be misleading.

In our study, non-*albicans Candida* was more prevalent than *Candida albicans*. This is in accordance with the study by Vijaya Det al., where *C. tropicalis* 14 (26.4%) was common amid the non-*albicans* species isolated from VVC.²⁶ Compared to these recent trends, few older studies showed prevalence of *Candida albicans* more than NAC.²⁷⁻²⁹ The reason for this may be the indiscriminate and injudicious use of over-the-counter usage of antifungals like fluconazole which destroys the more sensitive *Candida albicans* and selects the less sensitive non-*albicans* species. The resistance to the azole group among the Non-*albicans Candida* is increasing and thus their accurate identification becomes mandatory in the present scenarios.

Clinical method using Amsel's criteria in combination with Gram stain (Nugent's scoring) is simple and inexpensive for diagnosis of BV and can be used in peripheral centre where laboratory facilities are few with minimal settings. Public health awareness among the reproductive age groups and sexually active individuals may reduce mortality and morbidity in mother and child. Awareness among clinicians pertaining to emerging epidemiology aids in providing adequate treatments and judicious use of antibiotics, thereby, preventing emergence of resistant strains of pathogens.

6. CONCLUSION

Bacterial Vaginosis was most common etiological agent causing abnormal vaginal discharge followed by Candidiasis which could be due to indiscriminate use of antibiotics resulting in a shift towards fungal pathology. Though *Candida albicans* was the most common species identified, there was a rise in Non-*albicans Candida* (NAC) species. In BV, Amsel's clinical criteria identified a greater number of cases than Nugent's scoring method, however, the laboratory scoring of gram stained smear is considered the gold standard.

7. Source of Funding

None.

8. Conflict of Interest

The authors declare no conflicts of interest.

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