Identification and Characterization of Pathogenic Microbial Flora Associated with Abnormal Vaginal Discharge: A Microbiological Examination Study

Kharsodiya Kiran[1] and Dhingra Harish Kumar[2]*
1. Department of Biosciences, Mody University of Science and Technology, Lakshmangarh-332311, Dist. Sikar (Rajasthan), INDIA
2. Department of Biosciences, School of Liberal Arts and Science, Mody University of Science and Technology, Lakshmangarh-332311, Dist. Sikar (Rajasthan), INDIA
*hkdhingra.slas@modyuniversity.ac.in

Abstract

Abnormal vaginal discharge (AVD) is a common gynecological issue that affects a large number of women globally. One of the primary causes of this condition is the presence of pathogenic microbes in the vaginal microbiome. It is essential to make a microbiological diagnosis in cases of recurrent or treatment-resistant vaginal discharge. The present study aimed to investigate the pathogenic microbial flora associated with AVD using microbiological and biochemical examination. Total 100 samples were examined for microbiological analysis of abnormal vaginal discharge. The most frequently isolated pathogens were Gardnerella vaginalis (39 cases), Candida albicans (28 cases), Ureaplasma urealyticum (21 cases), Group-B Streptococcus and E.coli (13 cases). Less common pathogens included Trichomonas vaginalis, Mycoplasma hominis, Chlamydia trachomatis and Neisseria gonorrhoeae.

The study found that younger women, particularly in the 25–34 age group, were more frequently affected by infections, especially those caused by Gardnerella vaginalis and Candida albicans. Socioeconomic, lifestyle and personal hygiene factors such as douching with soap, lower education levels and middle-income status, were associated with increased risk of infections. The identification and characterization of these pathogenic microbial flora can provide valuable insights into the etiology of this condition as well as potential therapeutic interventions.

Keywords: Abnormal vaginal discharge, Pathogens, Microbial flora, Bacterial Vaginosis, Candidiasis, Trichomoniasis, Microscopy.

Introduction

Abnormal vaginal discharge is a gynecological condition characterized by an unpleasant, non-bloody discharge from the lower female genital tract. It is a common concern among women of all age groups, regardless of sexual activity. Vaginal discharge can be either normal or abnormal[1]. Normal discharge is physiological, occurring during sexual arousal, pregnancy, or at specific points in the menstrual cycle. It is typically clear or white, non-irritating and either odorless or with a mild odor and it does not lead to infection or complications. In contrast, abnormal vaginal discharge can appear green, yellow, brown, or red and may be accompanied by a foul odor, itching, irritation, painful urination (dysuria), or pain during intercourse (dyspareunia), depending on the infection involved[2].

The causes of abnormal vaginal discharge can be either infectious or non-infectious, with infection being the most common cause[3]. Microbial agents associated with abnormal vaginal discharge include: (a) Cervical infections: *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, Group B Streptococcus, *Ureaplasma urealyticum* and herpes simplex virus. (b) Vaginal infections: *Candida albicans*, *Trichomonas vaginalis* and *Gardnerella vaginalis*[2].

The vaginal flora is a dynamic ecosystem that can be easily disrupted. Abnormal vaginal discharge primarily occurs when the normal vaginal flora is replaced by pathogenic bacteria. It is not a disease in itself but rather a symptom of underlying conditions such as reproductive tract infections and sexually transmitted diseases. If left untreated, abnormal vaginal discharge can lead to severe complications including pelvic inflammatory disease, ectopic pregnancy, congenital abnormalities and an increased risk of genital tract malignancies. Therefore, early detection and treatment are crucial in reducing maternal morbidity and mortality[4].

The majority of cases of abnormal vaginal discharge are due to vulvovaginal infections, particularly bacterial vaginosis (BV), candidiasis and trichomoniasis[5,6]. These three conditions account for approximately 90% of all cases of abnormal vaginal discharge[7]. BV is the most common of the three causes mentioned, accounting for up to 50 % of all infections[8,9]. Multiple infections can also coexist. BV is polymicrobial in nature rather than being caused by a single pathogen. Common organisms involved in BV include *Gardnerella vaginalis*, *Mobiluncus*, *Bacteroides saprophytes* and *Mycoplasma hominis* [10]. Candidiasis is typically caused by *Candida albicans*[11] and may be associated with risk factors such as diabetes, pregnancy, or prolonged antibiotic use. The flagellated protozoan *Trichomonas vaginalis* infection leads to trichomoniasis, a sexually transmitted disease.

Cervicitis, another significant cause of vaginal discharge, is most commonly linked to *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. It is suspected in sexually active

women presenting with purulent endocervical discharge and easily induced cervical bleeding[12]. Non-infectious causes of abnormal vaginal discharge include atrophic vaginitis, contact dermatitis from allergic or irritant reactions, foreign body vaginitis (e.g. retained tampon or condom), cervical polyps, fistulas and genital tumors (e.g. tumors of the vulva, vagina, cervix, fallopian tubes and endometrium)[13].

To ensure appropriate empirical and definitive antibiotic treatment of abnormal vaginal discharge, it is essential to identify the most common infectious agents causing this condition in our region. The prevention of this pathological condition requires a prompt diagnosis and treatment. Therefore, the objective of this study is to identify and to characterize the pathogenic microbial flora associated with abnormal vaginal discharges through microbiological and biochemical analysis and to contribute to the development of improved syndromic treatment guidelines.

Material and Methods

A total of 100 samples from female patients complaining of abnormal vaginal discharge at Endocrine Laboratory, Jodhpur, were included in the study. A detailed history of patient was taken. The nature, colour and consistency of discharge and pH were checked. The pH was measured with pH paper (pH range of 4.0 to 7.0) held with forceps and dipped into the vaginal discharge, care was taken to avoid contamination with cervical secretion as it falsely changes pH. Additionally, Whiff or amine test was performed by mixing vaginal secretion with 10 % KOH on the clean glass slide. Endo-cervical and two high vaginal swabs (HVS) were collected for each patient following aseptic precautions. The swabs were rubbed and rotated in the post-vaginal fornix. Endocervical swabs were used for the diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. The diagnosis was achieved through a nucleic acid amplification test (NAAT).

One swab was used immediately to prepare a wet mount with one to two drops of normal saline on a glass slide and was examined by light microscopy for motility of *Trichomonas vaginali*s. The pus cells, budding yeast cells, pseudohyphal and clue cells were also looked for in the same wet mount. Another swab was used for Gram's staining and culture of *Gardnerella vaginalis*, Group-B *Streptococcus*, *Candida saprophytes*, *Ureaplasma urealyticum*, *Mycoplasma hominis* and other bacteria. For culture, the swabs were inoculated on human blood bilayer tween (HBT) agar, Sabouraud's medium agar, differential agar medium (A7) and blood agar. The colony morphology was determined based on the size, height and form of the colonies[14,15]. In order to identify the microorganisms, Gram-staining technique and biochemical tests were used.

For morphological identification of *Gardnerella vaginalis*, human blood bilayer tween (HBT) agar was used and identified as a clear or diffuse beta-hemolysis growth on HBT agar plate. Biochemically, it was confirmed by negative oxidase and catalase test, positive hippurate and starch hydrolysis test and positive alpha-glucosidase, but negative beta-glucosidase test. Candida saprophytes were cultured on Sabouraud's medium and incubated at 35 ºC±2 ºC for 48 hours and identified by germ-tube test and sugar assimilation test. Yeast cells were later detected in the growth. Infection with *Trichomonas Vaginalis* was identified by characteristic morphology in a wet mount. Group B streptococcus was cultured. For isolation of *Ureaplasma urealyticum*, differential agar medium (A7) was used and identified as deep-dark brown colonies in contrast with a light background of the medium under low power microscope. For other microbes, blood agar was used for morphological identification and characterization.

Results and Discussion

Vaginal discharge is one of the most common symptoms women. It can have various causes, ranging from physiological to pathological. Physiological conditions can be either infective or non-infective in nature. The present study was set to detect common microbial causes of abnormal vaginal discharges in 100 female genital samples in order to improve the syndromic management tool of vaginal discharges. A total of 78 patients were detected as positive cases (78.0 %) while 22 (22.0 %) were undetermined. However, 22 patients complained of abnormal vaginal discharge, but pathogenic microorganisms were not detected in them. The detected pathogenic microbial agents in positive cases were as follows *Gardnerella vaginalis*, *Candida albicans*, *Ureaplasma urealyticum*, Group-B Streptococcus, *E. coli*, *Mycoplasma hominis*, *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae* in decreasing order.

Table 1 reflects the distribution of various pathogens and control cases across different age groups. It was observed that *Gardnerella vaginalis* is most prevalent in the 25–34 age group (21 cases) and the 15–24 age group (14 cases). Its incidence sharply decreases in older age groups, with very few cases in individuals above 45. This may suggest that *Gardnerella vaginalis* is more common in sexually active younger adults. Group-B Streptococcus (GBS) shows its highest incidence in the 25–34 age group (8 cases). *Candida albicans* is more prevalent in younger age groups, with the 25–34 group having 15 cases and the 15–24 group having 12 cases. This pathogen's lower incidence in older groups could be linked to a decline in risk factors, such as hormonal fluctuations and contraceptive use that tend to be more common in younger women. *Ureaplasma urealyticum* shows moderate prevalence, peaking in the 25–34 age group (10 cases) and tapering off significantly in older age groups.

Ureaplasma is often transmitted sexually, which might explain its higher incidence in sexually active younger individuals. *Trichomonas vaginalis*, *Mycoplasma hominis* and *Chlamydia trachomatis* are relatively uncommon across all age groups, with only a few cases recorded.

Table 1
Distribution of pathogens across different age groups

Age groups in years	Pathogens									Control cases
	Gardnerella vaginalis	*Group-B Streptococcus*	*Candida albicans*	*Trichomonas vaginalis*	*Ureaplasma urealyticum*	*Mycoplasma hominis*	*Chlamydia trachomatis*	*Neisseria gonorrhoeae*	*E.Coli*	
15-24	14	2	12	2	8	2	2	0	2	3
25-34	21	8	15	0	10	1	0	1	4	6
35-44	3	1	1	0	2	1	0	0	2	4
45-54	1	0	0	0	1	0	0	0	4	9
55-64	0	2	0	0	0	0	0	0	1	0

These pathogens are linked to sexually transmitted infections and their low presence could suggest either a decrease in risk behaviors or effective prevention and treatment measures in these populations. *Neisseria gonorrhoeae* appears only in one case in the 25–34 age group.

The rarity of gonorrhea cases may indicate good control measures or low transmission rates in this population. *E. coli*, a common pathogen often linked to urinary tract infections, is more prominent in the 45–54 group (4 cases) and 25–34 group (4 cases). Control cases show a steady presence across age groups, though the number of controls increases notably in the older age groups. The control cases may provide a baseline for comparison to demonstrate the overall health of the sampled population.

The distribution of pathogens varies significantly by age group. *Gardnerella vaginalis* and *Candida albicans*, are more prevalent among younger age groups, while other pathogens like *E. coli* tend to affect older individuals more. The lower incidence of certain pathogens in older groups may reflect reduced sexual activity or changes in health practices. Control measures appear effective in preventing widespread infection, particularly for pathogens like *Trichomonas vaginalis*, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, which are minimally represented. This suggests that targeted health interventions are more important for younger women in preventing infections while older women may need broader health care screenings to address other emerging health conditions.

Table 2 and fig. 1 present the frequency of different microorganisms isolated from samples. The aetiologic investigation indicated *Gardnerella vaginalis* in 39, *Candida albicans* in 28, *Ureaplasma urealyticum* in 21, Group-B Streptococcus and *E. coli* in 13, *Trichomonas vaginalis* in 2, *Mycoplasma hominis* in 4, *Chlamydia trachomatis* in 2 and *Neisseria gonorrhoeae* in 1 case. Table 2 and fig. 1 suggest that *Gardnerella vaginalis* is the most frequently isolated microorganism, accounting for 39 cases. This pathogen is commonly associated with bacterial vaginosis, indicating that it is a significant contributor to genital infections in this population. Its dominance in the study emphasizes the need for effective prevention and treatment measures for bacterial vaginosis.

The second most common pathogen indicates that yeast infections caused by *Candida albicans* are also widespread likely contributing to a significant number of vaginal infections. *Ureaplasma urealyticum* is another commonly isolated microorganism. This pathogen is often asymptomatic but can cause infections of the reproductive and urinary tracts, especially in women. Its significant presence suggests that it may be an underappreciated contributor to genital infections. Group-B Streptococcus and *E. coli* are equally prevalent. GBS is often associated with pregnancy-related complications while *E. coli* is linked to urinary tract infections. Their relatively high frequencies highlight the importance of screening and treatment, especially in pregnant women or those prone to urinary infections.

Trichomonas vaginalis, *Mycoplasma hominis* and *Chlamydia trachomatis* are less commonly isolated, yet they remain important due to their association with reproductive tract infections and potential complications like pelvic inflammatory disease or infertility. *Neisseria gonorrhoeae* is the least frequently isolated microorganism with only 1 case. The low frequency of gonorrhea suggests either successful control measures or low transmission rates in this particular population.

Table 2 and fig. 1 show a high prevalence of non-sexually transmitted infections, especially bacterial and fungal infections such as those caused by *Gardnerella vaginalis* and *Candida albicans*. While STIs are present, they occur at much lower rates in this sample. This suggests that interventions such as promoting good hygiene practices, managing bacterial vaginosis and treating yeast infections may be more pressing concerns for this population. Additionally, the notable frequency of *Ureaplasma urealyticum* points to the importance of screening for less commonly discussed pathogens that can cause significant health issues. The relatively lower prevalence of pathogens like *Trichomonas vaginalis*, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* suggests that sexually transmitted infections may be under control while the higher incidence of other microorganisms points to the need for targeted public health strategies including education, screening and treatment. This data emphasizes the need for continuous monitoring and interventions to effectively manage and to reduce the prevalence of these infections.

Table 2
Distribution of isolated pathogens in participants with abnormal vaginal discharge

Microorganism isolated	Frequency
Gardnerella vaginalis	39
Group-B *Streptococcus*	13
Candida albicans	28
Trichomonas vaginalis	2
Ureaplasma urealyticum	21
Mycoplasma hominis	4
Chlamydia trachomatis	2
Neisseria gonorrhoeae	1
E. Coli	13

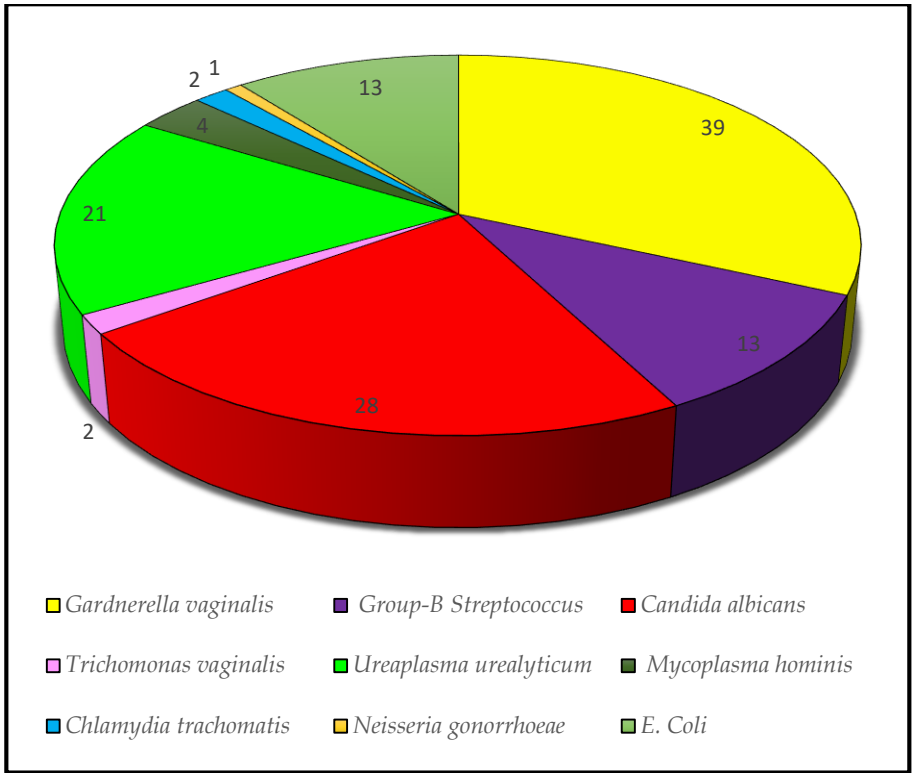

Fig. 1: Distribution of isolated pathogens in participants with abnormal vaginal discharge

Table 3 provides insights into various demographic, socio-economic, personal hygiene and lifestyle factors among the study participants along with the frequency and percentage distribution of these cases. The majority of cases are in the age group 25-34 years (44.87%) followed by 15-24 years (34.61%) which is younger and middle-aged individuals. The prevalence decreases significantly with age with the lowest percentage in the 55-64 age group (3.8%). Women of reproductive age (15-34 years) are more affected by the health conditions under consideration.

A significant proportion of the cases (73.07 %) are married. Married women may have more exposure to factors contributing to reproductive or vaginal health issues, potentially due to sexual activity or other marital responsibilities. The majority of cases fall in the middle-income group (52.56 %), followed by low-income individuals (33.33%). The high-income group accounts for only 14.1%. Economic background seems to play a role, with the middle-income group being more prone to health issues, possibly due to lifestyle or access to healthcare services. Homemakers represent the largest group (42.3%) followed by working women (30.76%) and students (26.92%).

The majority of participants (65.38 %) live in urban areas while 34.61 % are from rural regions. The largest group consists of individuals who are illiterate (30.77%) followed by those with primary education (25.64%). The majority of participants are non-smokers (87.18 %) and non-alcoholic (75.64 %), indicating that risky health behaviors related to smoking and alcohol consumption are relatively uncommon in this population. The most common method of douching is with soap (43.59 %), followed by normal water (35.9 %). Douching with soap may disrupt the natural vaginal flora and may increase the risk of infections, while douching with

normal water is less likely to have harmful effects. Only 6.41 % of participants use salt water and 14.1 % use other products for douching. The choice of products used for personal hygiene can have significant implications for reproductive and general health.

Table 3 highlights several demographic, socio-economic and lifestyle factors that are relevant in understanding health behaviors in this population. The table suggests that reproductive-age women, particularly those in urban areas and with lower education levels, are at a higher risk of health issues. Socioeconomic factors, lifestyle choices and hygiene practices appear to influence health outcomes, with douching practices and economic status playing notable roles. Tailored health interventions focusing on education, lifestyle modifications and improved hygiene practices could be beneficial for this population.

The widespread use of soap for douching raises concerns about potential health risks, as it may lead to infections or other complications. Health education programs should focus on promoting safer hygiene practices, particularly in the areas of personal care and reproductive health. Additionally, interventions aimed at improving access to healthcare and knowledge, especially among rural and lower-income populations, are essential for improving health outcomes in this demographic.

Limitations

Large population should be included to know the most common causes of abnormal vaginal discharge in the community. Also, follow up of the patients was not done to track patient outcomes or to assess the persistence of abnormal vaginal discharge.

Table 3
Distribution of cases according to demographic, socio-economic, personal hygiene and lifestyle factors

Factors	Variables	Cases	Percentage (%)
Age	15-24	27	34.61
	25-34	35	44.87
	35-44	7	8.9
	45-54	6	7.6
	55-64	3	3.8
Marital status	Single	21	26.92
	Married	57	73.07
Economic Background	Low income (<1 lacs per annum)	26	33.33
	Middle income (1-5 lacs per annum)	41	52.56
	High income (>5 lacs per annum)	11	14.1
Occupation	Students	21	26.92
	Working women	24	30.76
	Homemakers	33	42.3
Residence	Rural	27	34.61
	Urban	51	65.38
Education level	Illeterate	24	30.77
	Primary	20	25.64
	Secondary	10	12.82
	Graduate	11	14.1
	Above graduate	13	16.67
Smoking	Smokers	10	12.82
	Nonsmokers	68	87.18
Alcohol Consumption	Alcoholic	19	24.36
	Nonalcoholic	59	75.64
Personal Hygiene			
Douching	Normal water	28	35.9
	Salt water	5	6.41
	Soap	34	43.59
	Other products	11	14.1

Conclusion

Abnormal vaginal discharge is a common concern among women of reproductive age. Simple microbiological and biochemical tests can effectively differentiate between normal and pathological discharge, while also helping to identify the underlying causes. This approach reduces unnecessary drug exposure, minimizing the associated economic, social and psychological burden on affected women. The findings of the present study emphasize the need for targeted health interventions, particularly in educating younger women, especially in urban and middle-income groups, on safe hygiene practices to prevent and manage vaginal infections.

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