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Original Research Article

Etiological Profile of Infective Vaginitis Among Women of Reproductive Age Attending a Tertiary Care Hospital in Western Nepal: A Cross-sectional Study

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ABSTRACT:

Introduction: Infective vaginitis, including bacterial vaginosis, vulvovaginal candidiasis, trichomoniasis, and aerobic vaginitis, is a common reproductive health issue. Hospital-based etiological data from western Nepal remain scarce. This study aimed to determine the etiological profile of infective vaginitis among women attending a tertiary care hospital in this region. **Methods**: A descriptive cross-sectional study was conducted at Lumbini Medical College and Teaching Hospital (ethical approval: IRC-LMC-05/S-24). High vaginal swabs were collected from 378 symptomatic women aged 15–49 years attending the gynecology OPD over one year (April 2024–March 2025). Samples underwent physical, chemical, microscopic, and cultural examinations. Data were analyzed using PSPP and $p<0.05$ was considered statistically significant. **Results**: Of 378 women, 213 (56.35%) were diagnosed with infective vaginitis. Aerobic vaginitis was the most common (26.98%), followed by candidiasis (17.72%) and bacterial vaginosis (10.58%); 43.91% were non-infective. Candida albicans (31.75%) and Gardnerella vaginalis (18.78%) were the predominant isolates. Significant associations were found with occupation ($p = 0.02$), contraceptive use ($p<0.001$), smoking ($p=0.08$), and discharge character ($p<0.001$). **Conclusions**: Infective vaginitis was highly prevalent in this population, with aerobic vaginitis and candidiasis as leading causes. Laboratory-based diagnosis should be prioritized over syndromic management in this setting.

Keywords: Aerobic vaginitis; Bacterial vaginosis; Trichomonas vaginalis; Vulvovaginal candidiasis.

INTRODUCTION:

Infective vaginitis, comprising bacterial vaginosis, vulvovaginal candidiasis, trichomoniasis and aerobic vaginitis, is one of the most common gynecological problems among women of reproductive age worldwide.[1,2] Vaginal discharge caused by these conditions accounts for a substantial proportion of outpatient gynecological visits and, if left untreated, has been associated with pelvic inflammatory disease, infertility and adverse pregnancy outcomes.[3]

Aerobic vaginitis, described as an entity distinct from bacterial vaginosis, is characterized by disturbed vaginal flora with predominance of aerobic pathogens such as Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus together with an associated inflammatory response.[4,5] Bacterial vaginosis, in contrast, is a polymicrobial condition traditionally diagnosed using Amsel's clinical criteria or the Nugent Gram-stain scoring system.[6,7]

Studies from South Asia and sub-Saharan Africa have reported wide variation in the etiological spectrum of infective vaginitis, ranging from bacterial vaginosis and candidiasis as the leading causes to a predominance of aerobic pathogens reflecting differences in study population, diagnostic

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criteria, and hygiene practices.[8-13]

In the context of Nepal, hospital-based studies on the etiological profile of infective vaginitis, particularly from tertiary care centers in the western region, remain limited.[14-16] A clear understanding of the local etiological pattern is essential to guide rational antimicrobial use and to move clinical practice from syndromic management toward laboratory-supported etiology-based diagnosis and treatment.[17]

This study was therefore conducted to determine the etiological profile of infective vaginitis among women of reproductive age attending the gynecology outpatient department of a tertiary care hospital in western Nepal. The specific objectives were to determine the proportion of women with vaginal discharge who had an identifiable infective etiology, to describe the distribution of causative organisms, and to determine the association between selected socio-demographic and behavioral factors and infective vaginitis.

METHODS:

A descriptive cross-sectional study was conducted in the Microbiology Department in coordination with the Department of Obstetrics and Gynecology, Lumbini Medical College and Teaching Hospital (LMCTH) from 3rd April 2024 to 4th March 2025. Ethical clearance was obtained from the Institutional Review Committee (LMCIRC 05/S-24). Written informed consent was obtained from all participants. Questionnaires were used to collect sociodemographic and behavioral characteristics as well as medical, reproductive, and sexual history information.

Women aged 15-49 years presenting with a chief complaint of vaginal discharge to the gynecology outpatient department during the study period were included. While women who had used antibiotics, antifungals, or vaginal douching within the preceding two weeks, pregnant women, and those unwilling to give consent were excluded. Participants were enrolled by convenience sampling technique, and the sample size was calculated using the formula: $\mathbf{n=Z_{\alpha/2}p(1-p)/d^2}$ where Z=1.96 at 95% confidence level, p=prevalence rate, q=1-p, d=margin of error. Using a prevalence (p) of 24.4% based on a hospital-based study of bacterial vaginosis among symptomatic non-pregnant women in Nepal,[18] and a 5% margin of error, the minimum required sample size was calculated to be 283. However, a total of 378 high vaginal swab samples were collected from non-pregnant women who met the inclusion criteria, using consecutive enrolment during the study period. This exceeded the calculated minimum sample size which increases the precision of the overall prevalence estimate and improves the statistical power available for sub-group association analyses.

A semi-structured proforma was developed based on a review of relevant literature and was reviewed for content and clarity by faculty of the department of Microbiology and Obstetrics/Gynecology before use: as the proforma consisted of general, closed-ended items, formal pilot pretesting on a sample of patients was not additionally performed. The proforma was used to record socio-demographic information such as age, marital status, ethnicity, occupation and educational status, and relevant behavioral history such as smoking, alcohol consumption, dietary habit and method of contraception through direct interview.

A sterile speculum was inserted into the vagina to collect specimens. Gross examination of vaginal discharge was performed to note its color, consistency, and odor, followed by chemical examination, i.e., vaginal pH. Two vaginal specimens were collected by the gynecologist with the help of sterile cotton swabs from the posterior fornix aseptically. Normal saline was added to the second swab immediately after collection to prevent the swab from drying and to preserve the motility of Trichomonas vaginalis for wet mount examination. Both swabs were transported to the Microbiology Laboratory at ambient temperature and processed within 15-20 minutes of collection.

The first swab was inoculated onto MacConkey agar and blood agar, incubated aerobically at 37°C for 24 hours, along with Sabouraud's dextrose agar to isolate yeasts. Chocolate agar medium was used under increased carbon dioxide tension for fastidious organisms where clinically indicated. Anaerobic organisms associated with bacterial vaginosis such as Gardnerella vaginalis were identified by Gram's stain and Amsel's criteria rather than anaerobic culture. Aerobic bacterial isolates were identified by standard microbiological techniques such as study of colony morphology, Gram's staining and conventional biochemical tests, while yeasts were identified by Gram staining and germ tube test. Candida albicans showed germ tube formation when incubated in 0.5 ml serum for 2-4 hrs. at 37°C.

The second swab was used to measure vaginal

pH by using pH paper strips and then proceeded for wet mount preparation in normal saline to observe Trichomonas vaginalis and stained by Giemsa stain, and 10% potassium hydroxide solution was added to perform Whiff's test, and Gram's staining was performed to observe clue cells and different morphotypes of bacteria.

Bacterial vaginosis was diagnosed by using Amsel's criteria.[6] Amsel's criteria for diagnosis of bacterial vaginosis are:

- Thin homogeneous greyish white discharge
- pH of discharge > 4.5
- Wet mount shows masses of bacteria coating epithelial cells (clue cells)
- On addition of a few drops of 10% KOH to the discharge, a strong fishy odor was noted, i.e., Whiff's test positive.

At least three of the four criteria are required for diagnosis. Aerobic bacterial vaginitis was diagnosed based on wet mount and Gram stain findings showing disturbed lactobacilli with predominance of aerobic pathogens and an associated inflammatory reaction; in keeping with the criteria described by Donders et al.[4] The original Donders scoring system for aerobic vaginitis is based on phase-contrast microscopy (400x magnification) assessing lactobacillary grade, leukocyte count, proportion of toxic leukocytes, and background flora; as phase-contrast microscopy was not available at our center, diagnosis in this study relied on conventional bright-field wet mount and Gram stain examination rather than the formal Donders scoring system, which is acknowledged as a limitation. Vulvovaginal candidiasis was diagnosed by demonstration of budding yeast cells and pseudo-hyphae on 10% potassium hydroxide preparation and cultured on SDA medium. Trichomoniasis was diagnosed by demonstration of motile trichomonas on saline wet mount and stained with Giemsa stain.

Data were entered in Microsoft Excel and analyzed using PSPP 2.1.1. Descriptive statistics were expressed as frequency and percentage. The Chi-square (or Fisher's exact) test was used for categorical data. A p-value of <0.05 was considered statistically significant.

RESULTS:

Among the 378 women, 199 (52.65%) were aged 21-30 years, 82 (21.69%) were aged 31-40 years, 62 (16.40%) were aged 15-20, and 35 (9.26%) were aged 41-49 years. Most participants were married, 334 (88.36%), and engaged as housewives, 250 (66.14%). The largest ethnic group was Janajati, 139 (36.77%), followed by Brahmin, 106 (28.04%), Dalit, 75 (19.84%), and Chhetri, 42 (11.11%). Regarding educational status, 126 (33.33%) women had completed secondary-level education, and 84 (22.22%) were illiterate.

Etiological profile of vaginal discharge is presented in **Table 1**. Aerobic vaginitis was the most common abnormal finding followed by candidiasis and bacterial vaginosis. Normal vaginal flora was reported in 43.65 % cases.

Causative organisms of vaginal discharge is presented in **Table 2**. Candida albicans was the predominant organism identified by KOH wet mount and confirmed by culture on Sabouraud's dextrose agar with a positive germ tube test,

Table 1: *Etiological profile of vaginal discharge among study participants (N=378)*

Diagnostic category	n (%)
Aerobic vaginitis	102 (26.98)
Candidiasis	67 (17.72)
Bacterial vaginosis	40 (10.58)
Bacterial vaginosis + Candidiasis	2 (0.53)
Candidiasis + Trichomoniasis	1 (0.26)
Trichomoniasis	1 (0.26)
Normal vaginal flora	165 (43.65)
Total	378 (100)

Table 2: *Distribution of specific causative organisms among women with infective vaginitis (n = 213)*

Causative organism	n (%)
Candida albicans	67 (31.46)
Gardnerella vaginalis	40 (18.78)
Escherichia coli	32 (15.02)
Staphylococcus aureus	23 (10.80)
Klebsiella pneumoniae	16 (7.51)
Acinetobacter spp.	8 (3.76)
Pseudomonas aeruginosa	8 (3.76)
Enterococcus faecalis	4 (1.88)
Staphylococcus saprophyticus	4 (1.88)
Citrobacter spp.	2 (0.94)
Mixed/polymicrobial infections*	7 (3.29)
Others (Proteus spp., Trichomonas vaginalis	2 (0.94)
Total	**213 (100)**

**Candida albicans + Trichomonas vaginalis (1), Escherichia coli + Enterococcus faecalis (1), Gardnerella vaginalis + Candida albicans (2), Klebsiella pneumoniae + Enterococcus faecalis (1), Pseudomonas aeruginosa + Escherichia coli (1), Staphylococcus aureus + Escherichia coli (1).*

followed by Gardnerella vaginalis, Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae. Polymicrobial or mixed growth was noted in 3.29% of cases.

Association between infective vaginitis and socio-demographic, behavioral and clinical characteristics of the study participants is presented in **Table 3**. Infective vaginitis was significantly associated with occupation, being more frequent among agricultural workers and other manual workers. Age group, marital status, and ethnicity were not significantly associated with infective vaginitis.

Contraceptive method was significantly associated with infective vaginitis; positivity was lowest among intrauterine device users and highest among users of oral contraceptive pills and condom users. Smoking was also significantly associated; given the small number of smokers, this finding should be interpreted with caution. Alcohol consumption and food habits were not significantly associated, although the proportion of positive cases was numerically higher among occasional and regular alcohol users than among non-users.

The character of vaginal discharge was strongly associated with the underlying diagnosis. Curdy white discharge was almost uniformly associated with a positive result, as were bluish-green, greyish-white, and yellow discharge, whereas non-curdy white discharge was almost always associated with a negative result.

Fig. 1 illustrates the representative microscopic findings of high vaginal smear examinations. Germ tube production by Candida albicans is shown in **Fig. 1(a)**, Gram-positive budding yeast cells in a Gram-stained vaginal smear in **Fig. 1(b)**, clue cells in a Giemsa-stained vaginal smear in **Fig. 1(c)**, and clue cells in a wet mount preparation of vaginal discharge with normal saline in **Fig. 1(d)**.

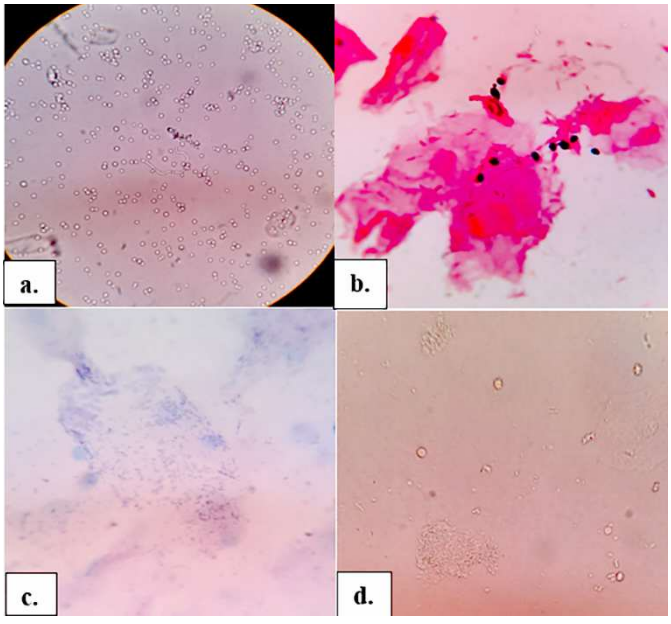

Fig.1: Microscopic images of high vaginal smear preparations

DISCUSSION :

This descriptive cross-sectional study, conducted among 378 women of reproductive age attending a tertiary care hospital in western Nepal, found that a little more than half of the women presenting with vaginal discharge had a laboratory-confirmed infective etiology, with aerobic bacterial vaginitis, candidiasis, and bacterial vaginosis as the leading diagnoses.

This prevalence is broadly comparable with a recent primary care-based study from Nepal,[14] and differs from the pattern reported in a tertiary care Nepalese cohort that focused specifically on bacterial vaginosis.[15] The predominance of aerobic vaginitis over classical bacterial vaginosis in the present study is a notable shift from many South Asian and African reports and may reflect increasing recognition of aerobic pathogens such as Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus in vaginal infections, consistent with the concept of aerobic vaginitis first described by Donders et al.[4,5] The comparatively high burden of trichomoniasis reported in a neighboring Nepalgunj cohort was not mirrored in the present study.[16] In our study, Trichomoniasis was rare (2/378, 0.53%) including one mixed infection, possibly reflecting differences in sexual health seeking behavior, partner treatment practices or the sensitivity of wet mount microscopy compared with molecular diagnostic methods.

Candidiasis as the second most common etiology in this study and was similar to reports from Ghana and Ethiopia,[9,11] while studies from Yemen, Uganda and Nigeria reported bacterial vaginosis as the leading cause,[8,10,13] and an Egyptian cohort described a more balanced distribution across bacterial vaginosis, candidiasis and aerobic vaginitis similar to the present findings.[12] These differences likely reflect variation in diagnostic criteria (Amsel's criteria versus Nugent scoring versus wet mount-based diagnosis of aerobic vaginitis), study population characteristics and the background prevalence of risk factors such as antibiotic use, personal hygiene and healthcare-seeking behavior across settings.

Table 3: *Association between infective vaginitis and socio-demographic, behavioral and clinical characteristics of study participants (n = 378)*

Category		Negative, n (%)	Positive, n (%)	Statistics
Age group (years)	**15–20**	31 (50)	31 (50)	χ^2=4.31, p=0.23
	21–30	81 (40.70)	118 (59.30)	
	31–40	41 (50)	41 (50)	
	41–49	12 (34.29)	23 (65.71)	
Marital status	**Married**	140 (41.92)	194(58.08)	χ^2=2.93, p=0.09
	Unmarried	25 (56.82)	19 (43.18)	
Occupation	**Agriculture**	13 (30.95)	29 (69.05)	p=0.02*
	Housewife	104 (41.60)	146 (58.40)	
	Office	13 (72.22)	5 (27.78)	
	Shopkeeper	11 (64.71)	6 (35.29)	
	Student	14 (53.85)	12 (46.15)	
	Tailor	4 (57.14)	3 (42.86)	
	Teacher	2 (66.67)	1 (33.33)	
	Worker	4 (26.67)	11 (73.33)	
Ethnicity	**Brahmin**	44 (41.51)	62 (58.49)	p=0.28*
	Chhetri	24 (57.14)	18 (42.86)	
	Dalit	34 (45.33)	41 (54.67)	
	Janajati	56 (40.29)	83 (59.71)	
	Muslim	2 (25)	6 (75)	
	Others	5 (62.50)	3 (37.50)	
Smoking	**Non-smoker**	164 (44.69)	203 (55.31)	p=0.03*
	Smoker	1 (9.09)	10 (90.91)	
Alcohol consumption	**Non-alcoholic**	148 (45.68)	176 (54.32)	p=0.13*
	Occasional	16 (32.65)	33 (67.35)	
	Regular	1 (20)	4 (80)	
Contraceptive method	**Condom**	76 (41.53)	107 (58.47)	p<0.001*
	Intrauterine device	43 (82.69)	9 (17.31)	
	None	30 (42.86)	40 (57.14)	
	Oral contraceptive pills	16 (23.19)	53 (76.81)	
	Others	0	3 (100)	
	Tubectomy	0	1 (100)	
Food habit	**Non-vegetarian**	147 (42.24)	201 (57.76)	χ^2=2.856, p=0.10
	Vegetarian	18 (60)	12 (40)	
Character of vaginal discharge	**Curdy white**	0	66 (100)	p<0.001*
	White (non-curdy)	165 (98.80)	2 (1.20)	
	Yellow	1 (1.06)	93 (98.94)	
	Greyish white	0	40 (100)	
	Bluish green	0	9 (100)	
	Others	0	3 (100)	

**Fisher Exact*

The significant association between occupation and infective vaginitis with higher positivity among agricultural and other manual workers may reflect differences in access to hygiene facilities, perineal contamination during physically demanding work or limited time available for genital hygiene, although the cross-sectional design precludes causal inference. This observation is consistent with a study done by Durai et al which found that employed women and those married to unskilled manual workers had a higher risk of reproductive tract infection than homemakers

and spouses of skilled workers, attributed to comparatively limited access to personal hygiene facilities.[18] In contrast, a study done by Danjuman found no significant association between bacterial vaginosis and any sociodemographic or behavioral factors, including occupation, suggesting that this relationship may be setting-specific and influenced by the occupational categories and infective etiologies examined.[20]

The association between contraceptive method and infective vaginitis was statistically significant, with the lowest positivity among Copper-T intrauterine device users and the highest among oral contraceptive pill users. The higher positivity among oral contraceptive pill users is consistent with reports that oral contraceptive use may selectively increase the risk of vulvovaginal candidiasis.[21] The comparatively low positivity among Copper-T users in this study is, however, unexpected. Randomized trial data indicate that copper intrauterine devices increase vaginal bacterial diversity, inflammatory anaerobes, and depletion of lactobacilli relative to hormonal contraceptive options.[22] This discrepancy may reflect residual confounding. For example, Copper-T users in this setting may differ systematically in age, parity, or care-seeking behavior from women using other methods rather than a true protective effect of the device itself and merits targeted investigation in future studies that can adjust for these factors.

The higher positivity was observed among smokers, although the statistical significance was based on only 11 smokers, which was similar to the study done by Nelson et al., which states that cigarette smoking is associated with differences in important vaginal metabolites, and women who smoke, and particularly women who are also depauperate for Lactobacillus spp., may have increased susceptibilities to urogenital infections and increased malodor.[23]

Limitations:

The study was limited by its single-center, hospital-based design and convenience sampling, which may restrict generalizability. Its cross-sectional design prevents causal inference. Conventional diagnostic methods were used instead of molecular or nucleic acid amplification methods, which may have underestimated trichomoniasis and other fastidious organisms. Aerobic vaginitis was diagnosed using conventional bright-field wet-mount and Gram-stain findings rather than the formal Donders phase-contrast scoring system because phase-contrast microscopy was unavailable. This may have affected diagnostic precision. The data-collection proforma was reviewed for content and clarity but was not formally pilot-tested. Several subgroups had small sample sizes, limiting the precision of association estimates and increasing the possibility of type II error. Information on partner treatment, sexual behavior, and duration and consistency of contraceptive use was not collected, limiting interpretation of the observed associations. Residual confounding by unmeasured factors cannot be excluded.

CONCLUSIONS:

Infective vaginitis was identified in more than half of reproductive-age women presenting with vaginal discharge at a tertiary care hospital in western Nepal. Aerobic vaginitis and candidiasis were the predominant diagnoses. Occupation, contraceptive method, smoking, and the character of vaginal discharge were significantly associated with infective vaginitis. These findings support consideration of laboratory-supported etiological diagnosis alongside syndromic management of vaginal discharge in this setting. Larger multicenter studies using standardized aerobic vaginitis scoring systems and molecular diagnostic methods are warranted to better characterize the etiological spectrum and associated factors.

Conflict of interest:
The authors declare no conflict of interest.

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