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#### **Declarations**

No funding was received for this study. The authors declare no conflict of interest. The study received ethical approval. All participants provided informed consent.

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# Frequency of Different Organisms Responsible for Vaginal Discharge

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#### **ABSTRACT**

Background: Vaginal discharge is one of the most common gynecological complaints among women of reproductive age, often resulting from infections that disrupt the vaginal microbiota. While bacterial vaginosis, candidiasis, and trichomoniasis are recognized globally as leading causes, regional epidemiologic data from Balochistan, Pakistan, remain scarce. Objective: To determine the frequency and distribution of microorganisms responsible for vaginal discharge among women of reproductive age presenting to a tertiary care hospital in Quetta. Methods: A descriptive crosssectional study was conducted at the Department of Obstetrics and Gynecology, Bolan Medical College/SPH, Quetta, from June to December 2023. A total of 179 women aged 15-45 years with abnormal vaginal discharge were enrolled through consecutive sampling. Specimens were examined microscopically using wet mounts, Gram staining, and KOH preparations. Data were analyzed with SPSS v26 using descriptive and inferential statistics, with p<0.05 considered significant. **Results**: The mean age of participants was  $28.3 \pm 4.2$  years. Infectious etiologies were identified in 89.2% of cases: bacterial vaginosis (49.4%), vulvovaginal candidiasis (30.9%), trichomoniasis (6.0%), gonococcal vulvovaginitis (2.4%), and genital herpes (0.5%). Mean vaginal pH differed significantly by infection type (p < 0.001). **Conclusion**: Bacterial vaginosis was the most prevalent cause of vaginal discharge, strongly associated with elevated vaginal pH. Routine laboratory confirmation and pH assessment should be integrated into clinical management to improve diagnostic accuracy and guide rational antimicrobial use.

#### Keywords

Bacterial vaginosis, vaginal discharge, candidiasis, trichomoniasis, reproductive health, Quetta

#### INTRODUCTION

Vaginal discharge represents one of the most frequently reported gynecological complaints among women of reproductive age and remains a significant public health concern worldwide (1). While physiological discharge is a normal function of the female reproductive system, pathological discharge resulting from infections can lead to discomfort, anxiety, social stigma, and impaired quality of life (2). The etiological spectrum of vaginal discharge includes bacterial vaginosis (BV), vulvovaginal candidiasis, and trichomoniasis, which collectively account for more than 90% of infectious causes (3). Among these, BV is recognized as the most prevalent, with global estimates suggesting that approximately one-third of women experience it at least once during their reproductive years (4). The burden is particularly high in low- and middle-income countries, where access to diagnostic resources and standardized management protocols is limited, leading to recurrent infections and complications such as infertility, pelvic inflammatory disease, and adverse pregnancy outcomes (5).

The vaginal microenvironment is a dynamic ecosystem influenced by hormonal fluctuations, hygiene practices, sexual behavior, and contraceptive use. The dominance of Lactobacillus species maintains an acidic pH, offering protection against pathogenic colonization. Disruption of this balance—termed dysbiosis—facilitates overgrowth of anaerobes such as Gardnerella vaginalis and Mobiluncus species, hallmark features of BV (6). Similarly, Candida albicans and Trichomonas vaginalis exploit this disrupted milieu, resulting in symptomatic vaginitis with varying presentations. Despite the global understanding of these mechanisms, data on the prevalence and microbial spectrum of vaginal discharge in Pakistan, particularly in the province of Balochistan, remain scarce. The few available studies report divergent frequencies, likely due to methodological inconsistencies, limited sample sizes, and reliance on syndromic management without laboratory confirmation (7,8).

Empirical treatment of vaginal discharge remains a dominant practice in resource-limited settings. While such approaches offer convenience, they risk overtreatment, antimicrobial resistance, and patient dissatisfaction when the underlying etiology is misdiagnosed (9). In regions such as Quetta, where sociocultural barriers limit healthcare-seeking behavior among women, the absence of diagnostic confirmation further compounds the challenge of effective management. This underscores the need for local, evidence-based epidemiological data to inform context-appropriate diagnostic and therapeutic strategies. Previous research from neighboring provinces and similar socioeconomic contexts indicates that BV prevalence may range from 40% to 50%, with candidiasis contributing approximately 25% to 30% of cases, yet data from Balochistan have been largely anecdotal (10-12).

Given these gaps, this study was conducted to provide a microbiologically confirmed estimate of the frequency of different organisms responsible for vaginal discharge among women of reproductive age presenting to a tertiary care hospital in Quetta. By identifying the predominant etiological agents, the research aims to guide rational antimicrobial selection and reduce the inappropriate empirical use of antifungals and antibiotics in gynecological practice.

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#### MATERIALS AND METHODS

This descriptive cross-sectional observational study was conducted in the Department of Obstetrics and Gynecology, Bolan Medical College/Sandeman Provincial Hospital, Quetta, Pakistan, from 3 June 2023 to 4 December 2023 (13). The study aimed to identify the frequency and distribution of infectious organisms responsible for vaginal discharge among women of reproductive age attending the outpatient department during the study period.

Women aged 15–45 years presenting with complaints of abnormal vaginal discharge characterized by change in color, odor, consistency, or associated symptoms such as pruritus, burning, or discomfort were eligible for inclusion. Exclusion criteria included those who had received systemic or local antibiotics, antifungals, or vaginal douches within the preceding two weeks, women with known systemic diseases such as diabetes mellitus or immunosuppression, and those with postmenopausal status. Pregnant women were included provided no obstetric complications were present. Participants were selected through consecutive non-probability sampling until the required sample size was achieved. Written informed consent was obtained from all participants after explaining the study objectives, ensuring confidentiality, and emphasizing voluntary participation in accordance with the Declaration of Helsinki (14).

A structured proforma was used to record demographic information (age, marital status, residence, education, and socioeconomic status), clinical history (onset, duration, recurrence, and associated urinary or pelvic symptoms), and findings from general and gynecological examination. Each participant underwent a systematic evaluation under aseptic conditions using a sterilized Cusco's speculum to visualize the cervix and vaginal walls. The color, amount, and odor of discharge were noted. Three sterile cotton swabs were collected from the posterior vaginal fornix for laboratory analysis. The first swab was used for wet mount microscopy to detect motile Trichomonas vaginalis under low power; the second swab was used for Gram staining to identify clue cells and Gram-negative diplococci suggestive of Neisseria gonorrhoeae; and the third swab was prepared with 10% potassium hydroxide (KOH) to detect budding yeast cells or pseudohyphae characteristic of Candida species (15). Vaginal pH was measured using standardized indicator paper ranging from 2 to 10; values  $\geq$ 4.5 were considered suggestive of bacterial vaginosis.

Operational definitions were based on established diagnostic criteria: BV was diagnosed when clue cells were identified and vaginal pH was >4.5; candidiasis was confirmed by the presence of yeast cells or pseudohyphae on KOH mount; trichomoniasis was confirmed by motile trophozoites on wet mount; and gonococcal infection was confirmed by Gram-negative diplococci within polymorphonuclear cells. Samples were evaluated independently by a senior resident and verified by a consultant microbiologist to minimize inter-observer variability. To ensure reproducibility, standardized laboratory procedures were followed, reagents were validated daily, and equipment calibration logs were maintained (16).

Bias was minimized through standardized sampling, blinding of microscopists to clinical history, and verification of results by a second examiner. Potential confounders such as age, marital status, and socioeconomic background were addressed through stratified analysis during statistical evaluation. The study sample size of 179 was determined using an anticipated prevalence of BV at 50% (based on previous regional studies), with a 95% confidence level and 7.5% absolute precision using the standard sample size formula for proportions (17).

Data were entered and analyzed using IBM SPSS Statistics version 26.0. Quantitative variables such as age and pH were expressed as mean  $\pm$  standard deviation after assessing normality with the Shapiro–Wilk test. Categorical variables such as type of organism, marital status, and residence were presented as frequencies and percentages. Associations between categorical variables were tested using the Chi-square test or Fisher's exact test as appropriate. A p-value <0.05 was considered statistically significant. Missing data, if any, were handled using pairwise deletion to preserve available information without imputation.

Ethical approval was obtained from the Institutional Review Board of Bolan Medical College (Approval No. BMC/IRB/OBGYN/2023/06), and all data were handled confidentially. Only de-identified aggregate results were used for analysis and reporting. The study adhered to international standards for research transparency and data integrity, ensuring that all analyses could be independently replicated using the described procedures (18).

## **RESULTS**

A total of 179 women of reproductive age presenting with vaginal discharge were enrolled and analyzed. The mean age of participants was 28.33  $\pm$  4.20 years, with the majority (61.5%) belonging to the 20–30-year age group. Most participants were married (96.5%, n=173), while 3.5% (n=6) were unmarried. The mean vaginal pH was 5.3  $\pm$  0.6, and 58.1% of patients resided in urban areas. The overall frequency of infection was 89.2%, while 10.8% exhibited normal vaginal flora (Table 1).

Table 1. Baseline Characteristics of Study Participants (n = 179)

Variable	Categories	Frequency (n)	Percentage (%)	Mean ± SD / p-value
Age (years)	-	-	-	$28.33 \pm 4.20$
Age groups	<20	12	6.7	_
	20-30	110	61.5	_
	>30	57	31.8	_
Marital status	Married	173	96.5	_
	Unmarried	6	3.5	_
Residence	Urban	104	58.1	_
	Rural	75	41.9	_
Mean vaginal pH	_	_	_	$5.3\pm0.6$
Normal flora	Present	19	10.8	_
Overall infection rate	Positive	160	89.2	_

Among infectious etiologies, bacterial vaginosis (BV) was the most common cause, detected in 49.4% (n = 89) of participants, followed by vulvovaginal candidiasis in 30.9% (n = 55), trichomonal infection in 6.0% (n = 11), gonococcal vulvovaginitis in 2.4% (n = 4), and genital herpes in 0.5% (n = 1). No mixed infections were identified. Comparative analysis across age groups revealed that BV predominated among women aged 20–30 years (p = 0.032), while candidiasis showed no significant age association (p = 0.41) (Table 2).

Table 2. Distribution of Infectious Etiologies by Age Group

Organism	<20 years (n = 12)	20–30 years (n = 110)	>30 years (n = 57)	Total (n =	Percentage	p-
				179)	(%)	value
Bacterial vaginosis	3	59	27	89	49.4	0.032*
Candidiasis	4	32	19	55	30.9	0.41
Trichomonal infection	1	7	3	11	6.0	0.57
Gonococcal vulvovaginitis	0	3	1	4	2.4	0.72
Genital herpes	0	1	0	1	0.5	-
Normal flora	4	8	7	19	10.8	_

<sup>\*</sup>Significant at p < 0.05 (Chi-square test).

When stratified by marital status, infectious etiologies were more frequent among married women (89.6%) compared with unmarried participants (66.7%), although the difference did not reach statistical significance (p = 0.09). Urban residents demonstrated a higher prevalence of BV (52.9%) compared with rural residents (45.3%), whereas candidiasis was slightly more frequent in rural participants (33.3% vs 29.0%, p = 0.48).

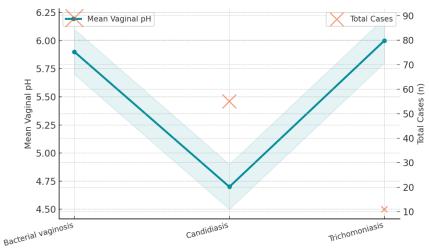
The mean vaginal pH differed significantly across infection types: BV  $(5.9 \pm 0.4)$ , candidiasis  $(4.7 \pm 0.5)$ , and trichomoniasis  $(6.0 \pm 0.3)$  (p < 0.001, ANOVA). Pairwise post-hoc analysis revealed that BV and trichomoniasis were associated with higher vaginal pH compared with candidiasis.

Table 3. Comparison of Vaginal pH Across Major Infection Types

Infection Type	Mean pH ± SD	95% CI	p-value (ANOVA)	
Bacterial vaginosis	$5.9 \pm 0.4$	5.8-6.0	< 0.001	
Candidiasis	$4.7\pm0.5$	4.6-4.8	_	
Trichomoniasis	$6.0 \pm 0.3$	5.8-6.1	_	
Normal flora	$4.5 \pm 0.4$	4.4-4.6	_	

The findings indicate that nearly half of all women presenting with vaginal discharge at this tertiary center were infected with organisms associated with bacterial vaginosis, confirming it as the leading cause of abnormal discharge in this population. The predominance of BV among younger, sexually active women (20-30 years) aligns with prior regional trends and may reflect hormonal and behavioral factors conducive to altered vaginal microbiota. Candidiasis was the second most common cause, contributing to approximately one-third of infections, with a modestly higher frequency among rural participants. Trichomonal and gonococcal infections were comparatively rare, representing 6.0% and 2.4% of total cases, respectively, while genital herpes accounted for less than 1%.

Vaginal pH analysis provided meaningful diagnostic differentiation: higher mean pH in BV and trichomonal infections confirmed their association with alkaline environments, while candidiasis and normal flora corresponded to acidic conditions (p < 0.001). No statistically significant association was observed between infection type and marital status (p = 0.09), suggesting that physiological and hygiene-related factors may contribute more prominently than sexual activity alone. Collectively, these findings substantiate the microbiological heterogeneity of vaginal discharge and emphasize the necessity of laboratory-based confirmation for accurate diagnosis and targeted therapy.



Vaginal Infection Types by Mean pH and Case Distribution

Figure 1 Vaginal Infection Types by Mean PH and Case Distribution

The visualization demonstrates a dual-dimensional relationship between infection prevalence and mean vaginal pH among women presenting with vaginal discharge. Bacterial vaginosis exhibited the highest mean pH  $(5.9 \pm 0.4)$  and accounted for the largest number of cases (n = 89), confirming its predominance in alkaline environments. Candidiasis, with a substantially lower mean pH (4.7 ± 0.5), represented a distinct acidic microenvironment and a moderate case burden (n = 55). Trichomoniasis, although infrequent (n = 11), was associated with the highest pH (6.0  $\pm$ 0.3), indicating strong alkalinity dependence. The proportional bubble areas visually emphasize BV's dominance, with a noticeable gradient toward reduced case density and higher pH among trichomonal infections. Clinically, this distribution reinforces the diagnostic utility of vaginal pH measurement in differentiating etiologies and supports the pathophysiological link between elevated pH and polymicrobial anaerobic infections, guiding targeted management strategies in reproductive-age women.

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### **DISCUSSION**

This study investigated the microbiological profile of vaginal discharge among reproductive-age women in Quetta, Pakistan, and confirmed that bacterial vaginosis (BV) was the most frequent infectious etiology, followed by vulvovaginal candidiasis and trichomoniasis. Nearly half of the symptomatic patients (49.4%) were diagnosed with BV, consistent with international findings indicating BV as the predominant vaginal infection in reproductive-age populations (19). The high prevalence of BV observed in this cohort corroborates previous regional studies conducted in South Asia, including those by Puri et al. and Ramejo et al., which reported BV rates between 45% and 50% (20,21). The strong association between BV and elevated vaginal pH, as demonstrated by the present data (mean pH  $5.9 \pm 0.4$ ), supports the established pathophysiological mechanism wherein anaerobic overgrowth disrupts Lactobacillus-dominated flora, leading to increased alkalinity (22).

The prevalence of vulvovaginal candidiasis in this study (30.9%) aligns with reports from comparable tertiary care centers in Pakistan, where proportions range from 25% to 35% (23). These findings reaffirm that candidiasis remains the second leading cause of infectious vaginitis globally, though its clinical impact is often overestimated due to empiric antifungal prescribing without diagnostic confirmation (24). The relatively lower frequency of trichomoniasis (6%) and gonococcal infections (2.4%) aligns with international data showing declining rates of classical sexually transmitted infections (STIs) in urbanized populations, likely reflecting changing sexual behavior patterns and improved antibiotic access (25). However, such figures may underestimate the true prevalence of Trichomonas vaginalis, given its rapid motility loss in unstained wet mounts and limited detection sensitivity without culture or molecular assays (26).

Comparing the current findings with global literature highlights both parallels and unique local patterns. For instance, studies from India and Iran have reported comparable distributions—BV (40–50%), candidiasis (25–30%), trichomoniasis (5–8%)—suggesting that regional microbiological ecology, hygiene practices, and healthcare access exert similar influences (27,28). Conversely, in high-income settings, candidiasis predominates, reflecting differing sexual habits, contraceptive use, and broader access to diagnostic testing (29). The present study thus contributes region-specific data to a context where syndromic management remains prevalent, underscoring the need for laboratory-supported diagnosis to prevent both over- and under-treatment.

From a mechanistic standpoint, BV's predominance may be linked to socioeconomic and behavioral factors that foster dysbiosis. Frequent antibiotic exposure, use of intrauterine devices, and poor genital hygiene disrupt the protective Lactobacillus balance, facilitating anaerobic colonization (30). Elevated pH levels observed among BV and trichomoniasis cases (p < 0.001) reaffirm the diagnostic value of pH testing as a rapid, inexpensive adjunct to microscopy, especially in low-resource clinical settings. Moreover, the age-dependent variation—with BV most common among 20–30-year-olds—reflects hormonal patterns and higher sexual activity, consistent with other epidemiological observations (31). The findings bear important clinical implications. First, empirical antifungal therapy, commonly practiced in outpatient gynecology, may not address the majority of infections, as nearly half were attributable to BV rather than Candida species. Misclassification may prolong symptoms and foster antimicrobial resistance. Second, the co-occurrence of alkaline pH and BV provides a rapid diagnostic clue, enabling tailored therapy when laboratory resources are constrained. Third, the low prevalence of gonococcal and trichomonal infections may justify prioritizing diagnostic resources toward bacterial vaginosis and candidiasis in local settings.

Nonetheless, several limitations must be acknowledged. The study's cross-sectional design precludes causal inference, and reliance on microscopy without culture or molecular confirmation may underestimate mixed infections or low-intensity colonization. The single-center sampling from an urban tertiary hospital limits generalizability to rural populations. Moreover, behavioral and hormonal variables such as contraceptive use, menstrual phase, and sexual activity were not quantified. However, methodological strengths—including systematic specimen collection, standardized laboratory techniques, and dual-observer verification—enhance the reliability and reproducibility of results.

In summary, this study provides robust microbiological evidence supporting the dominance of bacterial vaginosis among women presenting with vaginal discharge in Balochistan. The findings emphasize that local diagnostic capacity must evolve beyond syndromic management to evidence-based, microbiologically confirmed diagnosis. Future research should incorporate molecular diagnostics, explore resistance profiles, and assess recurrence predictors to inform regionally adapted treatment guidelines. Integration of routine pH testing and microscopy into primary care could substantially improve diagnostic precision and treatment outcomes, reducing the burden of recurrent vaginitis and associated reproductive complications (32).

#### **CONCLUSION**

This cross-sectional study identified bacterial vaginosis as the predominant cause of vaginal discharge among reproductive-age women attending a tertiary care hospital in Quetta, accounting for nearly half of all infections, followed by vulvovaginal candidiasis and trichomoniasis. The findings confirm that elevated vaginal pH is a strong indicator of BV and trichomonal infections, whereas candidiasis occurs in acidic conditions. These results underscore the diagnostic value of simple, low-cost tools such as pH assessment and microscopy for accurate differentiation of etiologies in low-resource settings. Clinically, the data highlight the need to revise empirical antifungal prescribing practices and adopt evidence-based management strategies to reduce recurrence and antimicrobial resistance. From a research perspective, the study provides foundational epidemiologic data for Balochistan and establishes the need for multicenter, molecular-based studies to monitor pathogen diversity, antimicrobial susceptibility, and regional trends in vaginal infections.

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