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(Research Paper)

Molecular and Morphological Prevalence of Parasitic, Fungal, and Bacterial Vaginitis in Women Referred to Selected Teaching Hospitals in Alborz Province

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Abstract

Vaginitis is a common and increasingly prevalent inflammatory condition in women, characterized by abnormal vaginal discharge, pain, and pruritus. This inflammation typically results from bacterial, fungal, or parasitic infections or disruption of the natural vaginal microbiota. Given the rising prevalence of vaginitis and the shifting patterns of causative agents and drug therapies, this study was designed to investigate the prevalence of parasitic, fungal, and bacterial vaginitis among women with suspected symptoms. A total of 121 patients with vaginitis symptoms were enrolled in this study. Vaginal secretion samples were collected using two sterile swabs and transported in sterile screw-cap tubes. For parasitic detection, both culture on Dorset medium and wet mount preparation were used, followed by microscopic examination. Fungal and bacterial agents were identified through direct microscopy and culture on selective media. Ultimately, species-level identification of parasites and fungi was confirmed by PCR sequencing. The results indicated that 71.9% of the women were positive for vaginitis. Among these, 0.8% had *Trichomonas vaginalis* infection, 34.7% had *Candida* infection, 15.7% had bacterial vaginosis (BV), and 20.7% had mixed *Candida* and bacterial vaginosis. The most prevalent fungal species was *Candida albicans* (33.9%). Although *Lactobacillus* was the most frequently identified bacterial genus, the primary pathogenic bacterium associated with BV was *Streptococcus agalactiae* (15.7%). Based on the results of this study, *Candida* infections were the most common cause of vaginitis in the study population. Furthermore, trichomoniasis, a sexually transmitted infection, demonstrated a low prevalence in this low-risk group. The findings of this study provide valuable insights for guiding preventive and therapeutic measures aimed at promoting women's health.

Keywords: Vaginitis, *Candida* spp., *Trichomonas vaginalis*, B.V, PCR sequencing.

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Introduction

Vaginitis, an infection and inflammation of the vagina, is a common gynecological issue and a frequent reason for clinical visit. In the United States alone, over ten million women seek medical care for this condition annually (1, 2). The three primary causes of vaginitis, accounting for approximately 90% of cases, are *Candida*, *Trichomonas*, and bacterial agents (3-5). Bacterial vaginosis (BV) is the most prevalent, responsible for 40-50% of vaginal infections (6). The second most common cause is vulvovaginal candidiasis, with approximately 75% of women experiencing at least one episode in their lifetime, and half of them experiencing two or more recurrent episodes (7, 8). The third main cause is *Trichomonas vaginalis*, which accounts for 15-30% of vaginal infections. According to World Health Organization (WHO) estimates, nearly 172 million new cases of trichomoniasis are reported globally each year (9, 10). Common symptoms of vaginitis include vaginal irritation, itching, abnormal discharge, odor, dyspareunia (sexual dysfunction), dysuria (painful urination), and inflammation of the labia and perineum (11). Beyond these discomforts, vaginitis is clinically significant because of its association with serious complications, particularly during pregnancy. It can lead to premature labor, premature rupture of membranes, low birth weight, and other adverse pregnancy outcomes, potentially resulting in infertility (12, 13). Several risk factors predispose women to vaginitis, including diabetes, HIV infection, contraceptive use, poor hygiene, low socioeconomic status, multiple sexual partners, sexual history, age, and antibiotic use (14, 15). The management of vaginitis is often complex. In the early stages, the absence of serious clinical symptoms may lead patients to delay seeking care. Furthermore, the lack of proper diagnosis and patient non-adherence to complete treatment regimens contribute to the disease burden. While accurate diagnosis involves assessing patient complaints, clinical examination, and laboratory methods, these tests are often not efficient or economically feasible in developing countries. Additional barriers to effective treatment include limited access to healthcare services, low public awareness, and cultural obstacles. Moreover, resistance to commonly used antifungal and antimicrobial drugs and a high rate of disease recurrence have been widely reported (16-21). Given the points outlined above—the high prevalence, significant complications, diagnostic

challenges, and emerging drug resistance—this study aimed to investigate the frequency of vaginitis in symptomatic women using both morphological and molecular methods.

Materials and Methods

Study Population and Sample Collection

In a cross-sectional study conducted from May to August 2024, 121 adult women (aged ≥ 18 years) with suspected vaginitis who were referred to Imam Ali Alborz Hospital (Karaj, Iran) and met the study eligibility criteria were enrolled.

Vaginal discharge samples were collected under the supervision of a gynecologist using two sterile swabs. A questionnaire was completed by each participant to gather demographic and clinical data, including age, history of pregnancy complications, viral infections, abortion, recurrent vaginitis, contraceptive methods, and underlying medical conditions. Clinical symptoms were also documented for all participants. Women were excluded from the study if they had used any topical or systemic antifungal, antibacterial, or antiprotozoal agents within four weeks prior to sample collection. Additional exclusion criteria included a history of hysterectomy or recent gynecological surgery within three months, active menstruation at the time of sampling, and unwillingness to complete the study questionnaire or provide informed consent.

Ethical Considerations

The study protocol was approved by the Local Ethics Committee of Alborz University of Medical Sciences (Approval No: IR.ABZUMS.REC.1401.022). Written informed consent was obtained from all participants prior to their participation.

Laboratory Identification Techniques

The first swab was inoculated in the liquid phase of Dorset's medium and transported immediately to the parasitology laboratory, where it was incubated at 37 °C for up to 72 h. Cultures were examined daily by light microscopy for the presence of motile *T. vaginalis*, which was considered a positive result (22). The second swab was used to prepare three glass slides. The first slide was used for a wet mount examination using $\times 10$ and $\times 40$ objectives to detect *T. vaginalis* and fungal elements (23). The second and third slides were subjected to Gram staining for bacterial evaluation and Papanicolaou staining for the

identification of *T. vaginalis*, respectively (24, 25).

Additionally, the second swab was inoculated onto Sabouraud Dextrose Agar (SDA). All cultures were incubated at 37 °C for 48 h and subsequently examined for the presence and semi-quantitative growth of fungal and bacterial colonies (26, 27). For the differential identification of yeast colonies, a germ tube test was performed by inoculating a single colony into 0.5 mL of human serum, and isolates were also cultured on CHROM agar™ *Candida* medium (26). The diagnosis of bacterial vaginosis was primarily based on clinical criteria (Amsel's criteria) and Gram stain Nugent scoring. Bacterial isolates were identified using a combination of culture on specific media (Blood agar, EMB agar), Gram staining morphological characteristics, and a comprehensive biochemical profile including catalase reaction, oxidase, CAMP test, Bile esculin, and carbohydrate fermentation patterns. This diagnostic approach allowed for the accurate identification of the key bacterial vaginosis-associated pathogens, focusing on the most prevalent and clinically significant species (27). Finally, for definitive species-level identification, parasitic and fungal isolates were confirmed using the PCR sequencing method.

Molecular Identification

DNA Extraction and Polymerase Chain Reaction (PCR)

DNA from parasite and fungal samples was extracted using the GENET BIO Genomic DNA Extraction Kit (Cat. No: k-3000) and the DNaSol Maxi Kit (Roje-Technologies), respectively, following the manufacturers' instructions. The quality and concentration of the extracted DNA were assessed using a NanoDrop 2000c spectrophotometer (Boeco, Germany). The PCR mixture for all reactions consisted of 13 µL of 2X Master Mix (SinaClon, Iran), 1 µL of each forward and reverse primer (20 pmol/µL), 8 µL of sterile distilled water, and 2 µL of DNA template. The thermal cycling conditions for fungal DNA amplification targeting the ITS-5.8S rDNA region were as follows: initial denaturation at 94 °C for 5 minutes; 45 cycles of denaturation at 95 °C for 40 sec, annealing at 62 °C for 40 sec, and extension at 72°C for 20 seconds; followed by a

final extension at 72 °C for 5 min (28). The conditions for the amplification of the *T. vaginalis* 18S rRNA gene were: initial denaturation at 95 °C for 5 minutes; 45 cycles of denaturation at 94 °C for 40 seconds, annealing at 59 °C for 40 sec, and extension at 72 °C for 40 sec; with a final extension at 72 °C for 5 min. The primers used for amplification were: *T.vaginalis* (18S rRNA): Forward:5'-CGCCATTGCGACTGAGTGACCTA-3', Reverse: 5'-CCCCAGAGCCCAAGAACTATGAT-3' (29,30). Fungi (ITS-5.8S rDNA): ITS4: 5'-TCTCCGCTTATTGATATGC -3', ITS5: 5'-GGAAGTAAAAGTCGTAACAAGG -3' (28). The PCR amplicons were visualized by electrophoresis on a 1% agarose gel.

DNA Sequencing and Analysis

All PCR-amplified products were purified and sequenced in the forward direction using an Applied Biosystems 3730 XL DNA Analyzer (Bioneer Corporation, Korea). The obtained sequences were analyzed using the BLAST algorithm on the NCBI GenBank database (Library of Medicine, Bethesda, MD, USA; <http://www.ncbi.nlm.nih.gov/BLAST/>) to confirm species identification.

Statistical Analysis

All descriptive data were compiled in Microsoft Excel and imported into SPSS software (version 16.0) for analysis. Data were summarized using standard deviation (SD), mean, frequency, and percentage. Quantitative variables are presented as mean ± SD, and qualitative variables were presented as numbers (percentages). Group comparisons were performed using a one-way ANOVA analysis, and a p-value of less than 0.05 was considered statistically significant.

Results

Among the 121 patients with suspected symptoms of vaginitis enrolled in the study, 87 (71.9%) were confirmed positive. The distribution of infections was as follows: 1.15% (1) had a *Trichomonas* infection, 48.3% (42) had a *Candida* infection, 21.8% (19) had bacterial vaginosis (BV), and 28.7% (25) had a mixed infection of BV and candidiasis (Table 1).

Table 1: Prevalence of vaginal infections

Type of vaginal infection	N	(%)	Age
Overall prevalence (any type)	87	71.9	
Vaginal Candidiasis (V.C)	42	48.3	Reng:18-57
Bacterial vaginosis (B.V)	19	21.8	
<i>Trichomonas vaginalis</i> (T.V)	1	1.15	Mean:36.7
Mixed infection (V. C+B.V)	25	28.75	

Analysis of patient demographic information revealed that a history of recurrent vaginitis was the most frequently reported characteristic, present in 63.6% of positive cases. Other recorded factors included pregnancy-related complications (infertility, curettage, stillbirth, and ectopic pregnancy) in 20.7%, a history of abortion in 29.8%, and underlying medical conditions (diabetes, hypertension, and thyroid diseases) in 21.5% of positive patients. All participants were HIV-negative, while 1.7% were positive for HPV. Regarding contraception, 36.4% of participants reported using at least one method (condom, oral pills, surgical, or IUD) (Table 2). No statistically significant relationship was found between vaginitis and pregnancy complications, history of abortion, underlying diseases, viral

infections, or contraceptive methods ($P = 0.07$). Furthermore, pregnancy itself was not identified as a significant risk factor for an increased incidence of vaginitis in this study ($P = 0.09$), (data not shown). The highest prevalence of infection was observed in the women with a mean age of 36 years (Table 1). The most commonly reported clinical symptoms were lower abdominal pain (27%), vaginal discharge (21%), inflammation (11%) genital burring and itching (17% and 24%) respectively (Table 3). Finally, out of 121 vaginal secretion samples, one was confirmed positive for *Trichomonas vaginalis* by wet mount examination, Papanicolaou staining, and culture on Dorset's medium (Table 4).

Table 2: Distribution of demographic and clinical history factors among study participants

Category	Disease History		Reproductive History				Underlying diseases				Contraception Methods		Infections	
Subcategory	Recurrence	History of abortion	Curettage	Infertility	Stillbirth	Ectopic pregnancy	Thyroid	Diabetes	Hypertension	Condom	oral pills	IUD	Surgical	HPV
Frequency (%)	63.6	29.8	13.3	3.2	3	1.2	10.4	6	5.1	15.6	10.5	7	3.3	1.7

Table 3: Frequency (%) of clinical symptoms in the studied groups

Clinical symptoms	Genital itching	abdominal pain	vaginal discharge	inflammation	genital burring
	24%	27%	21%	11%	17%

Table 4: Frequency of *T.vaginalis* in morphological examination and Dorset medium

Parasite species	wet mount	Papanicolaou staining	Dorset culture
<i>T.vaginalis</i>	N (%)	N (%)	N (%)
	1 (1.15)	1 (1.15)	1 (1.15)

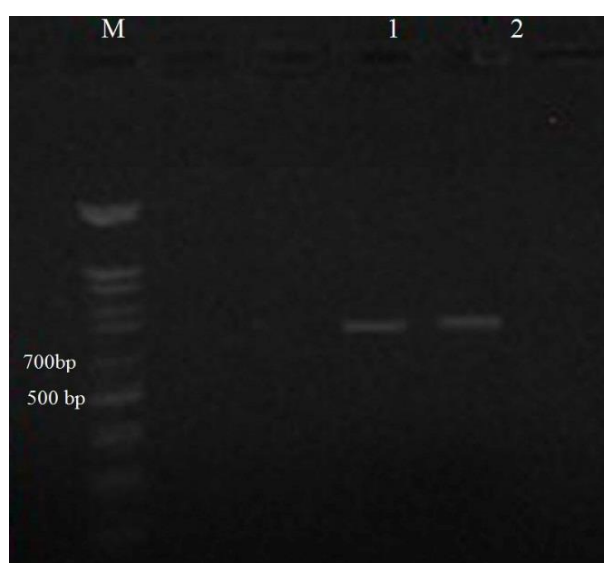


Fig. 1: Gel electrophoresis of polymerase chain reaction products of isolated *Trichomonas vaginalis* (T.V.) from vaginitis on 1% agarose gel; Lane M: 1-kb DNA ladder (100 bp), Lane 1: Standard T.V., and Lane 2: *Trichomonas vaginalis*.

The molecular results for this isolate were consistent with the morphological and culture-based findings (Fig. 1).

Candida strains were isolated using CHROMagar™ *Candida* for initial screening alongside other conventional methods. Among the fungal colonies isolated, *C. albicans* was the most prevalent species (61.19%), followed by *Candida glabrata* (26.84%), *Kluyveromyces marxianus* (formerly known as *Candida kefyr*) (10.46%) and *Candida krusei*

(1.49%) (Table 5). The sequencing results for these fungal strains were consistent with the morphological identification (Fig. 2).

In addition, bacterial isolates were characterized through culture on specific media, Gram staining, and biochemical profiling. This conventional approach enabled the identification of predominant cultivable bacteria. *Lactobacillus* spp. were the most frequently isolated genus (45.5%), followed by the pathogenic bacterium, *Streptococcus agalactiae* (43.18%) (Table 6).

Table 5. Prevalence rate of *Candida* species (Overall prevalence N=67)

<i>Candida</i> species	N	%	Colony color	germ tubes
<i>Candida albicans</i>	41	61.19	Green or light green	+
<i>Candida glabrata</i>	18	26.86	Dark pink	-
<i>Kluyveromyces marxianus</i>	7	10.46	Blue purple	-
<i>Candida krusei</i>	1	1.49	Cream	-

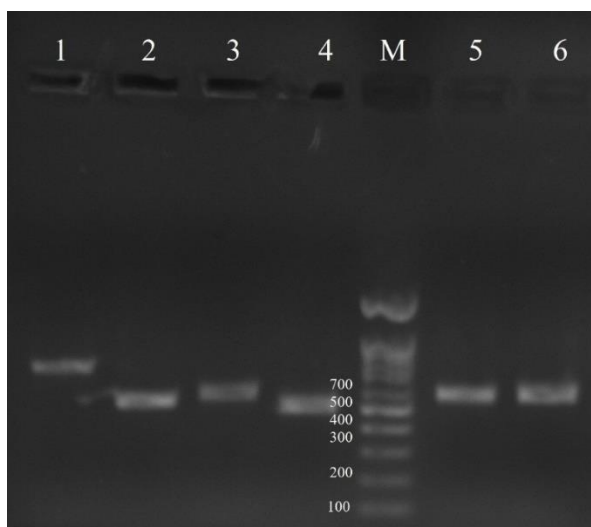


Fig. 2. Gel electrophoresis of polymerase chain reaction products of isolated *Candida* species from vaginitis on 1% agarose gel; Lane M: 1-kb DNA ladder (100 bp), Lanes 3, 5, 6: *C.albicans*, Lane 1: *C. glabrata*, Lane 2: *C.kefir* and Lane 4: *Candida kruzei*

Table 6: Prevalence rate of bacteria species identified by differential culture and biochemical profiling (Total N = 44)

	<i>Streptococcus agalactiae</i>	<i>Lactobacillus</i> spp.	<i>Gram positive bacillus</i>
Overall prevalence (N, %)	N=19 (43.18%)	N=20 (45.45%)	N=5 (11.36%)
Differential tests			
Gram staining	+	+	+
Catalase Test	-	-	+
Oxidase test	-	-	-
CAMP Test	+	-	-
Hemolysis on Blood Agar	Beta-hemolytic	Alpha or non-hemolytic	Beta-hemolytic
Bile Esculin Test	-	Variable	Variable
Salt tolerance (6.5% NaCl)	-	Variable	+
Carbohydrate Fermentation			
Glucose	+	+	+
Lactose	+	+	-
Sucrose	+	Variable	+

Discussion

The findings of this study indicate that the most common vaginal infections were candidiasis (48.3%), bacterial vaginosis (21.8%), and trichomoniasis (1.15%), respectively. This distribution aligns with a previous study in Iran, which reported a prevalence of 47.4% for candidiasis, 38.9% for trichomoniasis, and 13.7% for bacterial vaginosis. Both studies identified candidiasis as the most prevalent cause of vaginitis (31). In the present study, in addition to the more prevalent species such as *C. albicans* and *C. glabrata*, we also isolated *Kluyveromyces marxianus*. It is worth noting that this species is the teleomorph of *Candida kefir*, and the identification was confirmed through PCR sequencing of the ITS region, which is considered the gold standard for differentiating yeast species. While *K. marxianus* is considered an uncommon

cause of vulvovaginal candidiasis, its isolation from symptomatic patients confirms its pathogenic potential in the vaginal environment. This finding highlights the importance of identification at the species level in understanding the full spectrum of etiological agents involved in vaginitis. The significantly lower prevalence of *T.vaginalis* (1.15%) in our study suggests that this infection is more common in high-risk populations compared to the general, low-risk population represented in our cohort. Accordingly, studies conducted in 2004 and 2011 on high-risk populations, namely women in Evin Penitentiary and women with a history of substance use, reported trichomoniasis prevalence of 26.5% and 10.2%, respectively (32, 33). The clinical symptoms reported by infected individuals in the aforementioned studies, such as burning, itching, and vaginal discharge, were

consistent with the presentation in the single case of trichomoniasis identified in our study. Furthermore, a study in Yasuj City reported the highest and lowest rates of infection to be candidiasis (13.13%) and trichomoniasis (1.9%), respectively (34), a pattern that aligns with our results. A study in Cameroon found an overall prevalence of vaginal infections of 49.5%, with candidiasis at 32%, bacterial vaginosis at 27%, and trichomoniasis at 2% (35). This further confirms that candidiasis is the most prevalent and trichomoniasis the least prevalent form of vaginitis, which is consistent with the findings of the present study. An interesting finding of our study was the high prevalence of *Lactobacillus* spp. identified in the samples. While bacterial Vaginosis is classically defined by a sharp decrease in lactobacilli, their over-abundance has been associated with a clinical condition known as Cytolytic Vaginosis (CV) (12,14). CV is characterized by an overgrowth of *lactobacilli* (particularly *L. crispatus*), leading to excessive lactic acid production, cytolysis of epithelial cells, and symptoms including itching, burning, and a thick discharge that can be misdiagnosed as vulvovaginal candidiasis or BV (13). Given that our patient cohort was symptomatic and our methods quantified high lactobacilli loads, it is plausible that a proportion of our cases with dominant *lactobacilli* flora were indeed manifestations of CV. This highlights a diagnostic challenge and suggests that future studies should include microscopic examination for cytolytic patterns (e.g., fragmented epithelial cell nuclei) to better differentiate between BV, CV, and normal flora in symptomatic women. In our study, we observed a notably high rate of co-infections (20.7%) of bacterial vaginosis and candidiasis. This clinically significant finding presents a substantial diagnostic and therapeutic challenge, as the overlapping symptoms of these conditions require distinct antimicrobial treatment strategies. This underscores the necessity for comprehensive diagnostic methods that can simultaneously detect both bacterial and fungal pathogens to guide effective combination therapy and prevent recurrent symptoms. Finally, the robust

identification of a history of recurrent vaginitis as the predominant anamnestic feature in our cohort underscores its critical role in the disease's clinical trajectory, thereby informing both risk stratification and the management of persistent symptoms.

Conclusion

The results of this study demonstrate that *Candida* infections and bacterial vaginosis are the most common causes of vaginitis in the studied population. Based on these findings and their consistency with other research, it is necessary to develop and implement targeted public health education on genital hygiene strategies and raise community awareness to control and prevent these infections. Furthermore, accurate etiologic diagnosis of vaginitis is essential for administering appropriate antimicrobial therapy, which helps prevent the misuse of medications and the emergence of drug resistance.

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Author contributions

All the authors provided their comments and ideas at different stages of the study. E. M. designed the experiments and wrote the final draft of the manuscript. S. GH wrote the first draft of the article and participated in the identification and isolation of *Candida*, parasite, and bacterial species based on morphological and molecular methods.

Conflicts of interest

The authors declare no conflicts of interest regarding the publication of this paper.

Financial disclosure

The authors are responsible for the content of this article.

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