JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Evaluation of diagnostic methods for detection of trichomoniasis in symptomatic women with vaginal discharge

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Abstract

Introduction: Vaginal discharge is a common gynecological condition among reproductive age women. Common infections include bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis. Prevalence of trichomoniasis varies with geographical area and the diagnostic techniques used. This study was conducted to compare various diagnostic methods for detection of trichomoniasis among women of the reproductive age group.

Methodology: The study was conducted from January 2021 to July 2022, and 114 patients were included. Vaginal discharge was collected from the lateral wall of the vagina and posterior fornix using four swabs for bedside culture into Kuperberg media, polymerase chain reaction (PCR), wet mount, Giemsa staining, Gram staining, and culture on blood agar for *Candida spp*. Nugent scoring in Gram stain was used to determine BV.

Result: BV was identified in 21.05% (24/114), VVC in 6.14% (7/114), and *Trichomonas vaginalis* (TV) in 4.4% (5/114) by PCR. However, TV was detected only in three patients by wet mount, Giemsa stain, and culture. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for wet mount and Giemsa stain were 100% for each parameter compared to culture; while sensitivity and specificity were calculated as 100% and 98.2% for PCR. All patients with TV presented with greenish frothy discharge (pH > 4.5) and vaginal wall inflammation.

Conclusions: Culture remains the standard diagnostic approach and is cost effective; but it has major shortcomings such as the need for faster sample transportation and longer turnaround time. PCR can detect non-viable trichomonads and can provide early and accurate diagnosis.

Key words: *Trichomonas vaginalis*; PCR; direct wet mount culture.

J Infect Dev Ctries 2025; 19(1):155-161. doi:10.3855/jidc.18460

(Received 01 May 2023 – Accepted 15 October 2023)

Introduction

Vaginal discharge is one of the most common gynecological conditions in women of the reproductive age group. It accounts for 5–10 million clinic visits per year throughout the world [1]. Symptomatic vaginal discharge can predispose to various complications like pelvic inflammatory diseases, endometriosis, infertility, and increased susceptibility to other sexually transmitted diseases (STD). Moreover, many of these infections often remain underdiagnosed due to social stigma and lack of awareness [2–4]. Detection of infectious etiological agents of vaginal discharge has been challenging because several pathogens may co-exist and limited facilities are available in routine diagnostic laboratories, especially in developing countries like India. The common causes of infection include trichomoniasis (0.1–41%), anaerobic bacteria causing bacterial vaginosis (BV; 11–39%), and vulvovaginal candidiasis (VVC; 3.8–33%) [5–9]. Trichomoniasis is considered the most common cause of non-viral STD. However, it is a neglected STD, especially in developing countries. Globally, the prevalence of trichomoniasis is 1.6–11.7% in reproductive women of age group 15–49 years, which is higher than other non-viral STDs like chlamydia, syphilis, and gonorrhea [10,11]. The clinical manifestations of trichomoniasis in symptomatic women mainly include frothy vaginal discharge; and punctate hemorrhagic spots on the cervix, often referred to as "strawberry vagina/cervix" [12]. However, they may also present with cervicitis, vaginitis, itching, irritation of the vulva, inflammation, painful micturition, pelvic inflammatory diseases (PID), and lower abdominal pain (LAP). *T. vaginalis* (TV) infection is also linked with the adverse outcomes of pregnancy, and increased risk of cervical cancer [13]. Prevalence varies with geographical area, target population, and the diagnostic techniques used. Wet

mount and culture are the most commonly adopted methods for the diagnosis of trichomoniasis. A wet mount is a simple microscopic test and requires a minimum 10^4 organisms/mL in vaginal secretion for detection. However, the time interval between sample collection and microscopy should be a maximum of 10 minutes as the diagnostic yield of the test decreases with time [14]. Around 300–500 organisms/mL of TV should be present in the specimen for a positive culture with available media. Both wet mount and culture require the presence of viable trichomonads and hence the sensitivity of these tests decreases with increase in time between sample collection and processing. In this regard, polymerase chain reaction (PCR) offers the advantage of increased sensitivity and the ability to detect non-viable trichomonads [15].

Syndromic case management (SCM), based on signs and symptoms remains the most reasonable treatment option for clinicians in resource-limited countries like India. Patients presenting with vaginal discharge may experience over diagnosis or overtreatment in certain situations, which in turn may lead to the development of drug resistance [16]. Effective treatment of these conditions requires etiological diagnosis. Trichomoniasis is an underdiagnosed infection in eastern India. Very few studies on the prevalence of trichomoniasis among women of reproductive age group have been conducted so far in eastern India [17,18]. Therefore, the present study was conducted to detect trichomoniasis among symptomatic women of age 15–49 years with vaginal discharge, along with a comparative evaluation of available diagnostic methods such as microscopy, culture, and PCR.

Methodology

This prospective observational study was conducted among symptomatic women of the reproductive age group (15–49 yrs), presenting with vaginal discharge in the outpatient department. A total of 114 patients were included in the study period from Jan 2021 to July 2022. The study was approved by the Institutional Ethics Committee (IEC/AIIMS BBSR/PG THESIS/2020-21/91)

Specimen collection and processing

A total of four sterile swabs were collected from each patient. The processing stages for each vaginal swab are presented in Figure 1. The first swab was immediately inoculated (bedside) into the Kupferberg media for culture of *T. vaginalis* [19]. The second swab was subjected to DNA extraction and subsequent PCR amplification for the identification of TV. The third swab was subjected to a wet mount to check for the motility of TV. The fourth swab was inoculated onto blood agar for culture of *Candida spp.* and further smear preparation for Giemsa stain and Gram stain separately using the same swab. Subsequent identification for *Candida spp.* was done using Hicrome *Candida* differential agar (Himedia, Thane, India). Smears stained with Giemsa stain were screened for trophozoites of TV, while Gram stain was used to determine Nugent scoring for the detection of BV.

PCR for T. vaginalis (TV)

PCR amplification of the *TVK3/TVK7* genes was performed to independently target the TV genome with the forward primer: TVK3: 5′AT TGT CGA ACA TTG GTC TTA CCC TC-3′ and reverse primer: TVK7: 5′-TCT GTG CCG TCT TCA AGT ATG C-3′ [20]. DNA was extracted using 5% weight/volume CHELEX 100 (HI media, Thane, India, MB160-25G), and subsequently amplified using TVK3/K7 primers. Culture-positive TV and DNA-free distilled water were used as positive and negative controls, respectively. The amplification program included an initial one cycle of pre-denaturation at 95 °C for 5 min; followed by 35 cycles of denaturation at 90 °C for 60 s, annealing at 60 °C for 30 s, and extension at 70 °C for 120 s; and a final extension step at 72 °C for 7 min [21]. The PCR product

Figure 1. Workflow for the four high vaginal swabs.

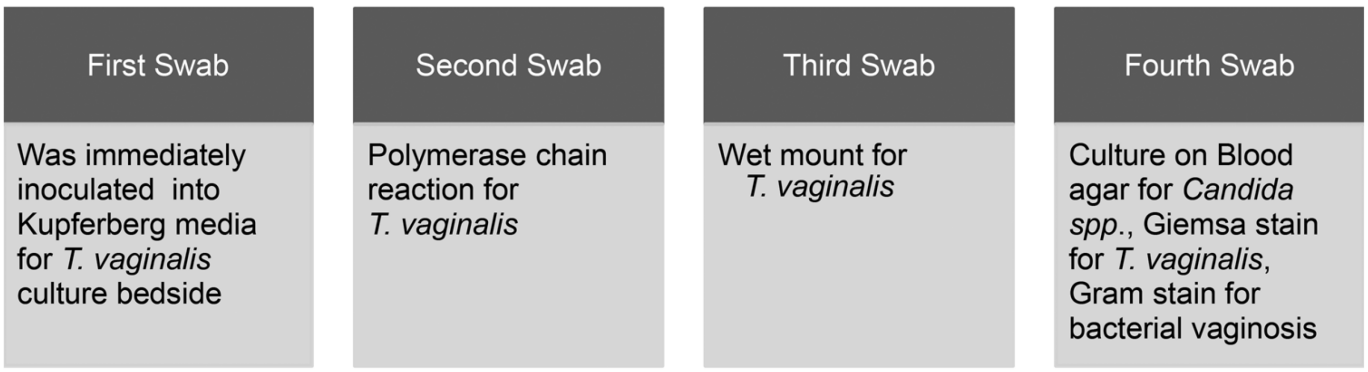
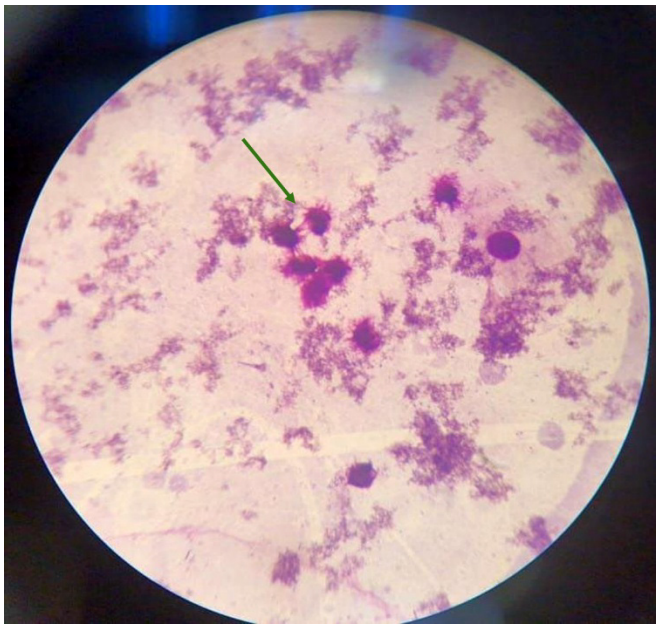


Figure 2. Giemsa Stain: pyriform shaped trophozoites of *Trichomonas vaginalis* with flagella and axostyle (green arrow) (100 X).



amplicon of 300 bp was visualized using 1.5% agarose gel electrophoresis with ethidium bromide staining under ultraviolet illumination.

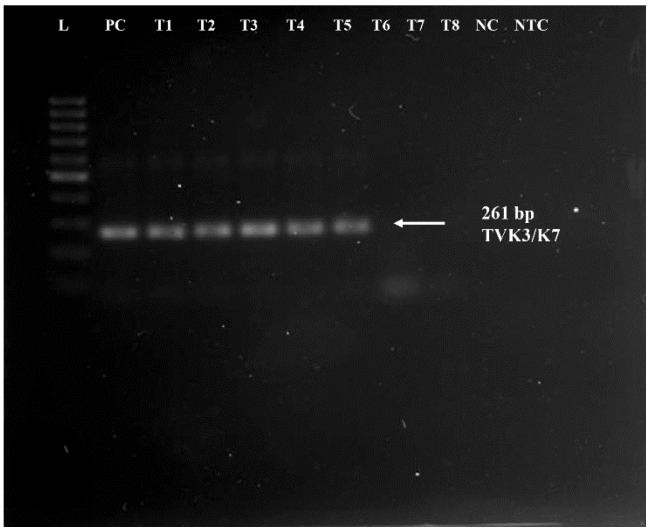
Results

Out of 114 patients, BV was detected in 24 (21.05%), VVC in 7 (6.14%), and TV in 5 (4.4%). Co-infection of TV with BV was seen in only one patient.

All the three commonly used techniques for TV—wet mount, Giemsa staining, and culture—identified 2.63% (3/114) of cases as TV, whereas PCR identified 4.4% of cases (5/114) using the primer set TVK3/TVK7. Figures 2 and 3 show Giemsa stain of TV trophozoites, and the gel electrophoresis image for TVK3 and TVK respectively. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated as 100%, 98.2%, 60%, and 100%, with an accuracy of 98.25% for PCR, considering culture as the gold standard (Table 1).

All TV patients presented with medium to profuse greenish frothy, foul-smelling discharge of high pH. Pruritus and positive whiff test were observed in three patients while two patients presented with vaginal wall inflammation and cervical inflammation. Dysuria was found in four patients while dyspareunia was reported in only two patients. In the case of BV, patients presented with thin discharge (N = 16), pruritus (N = 14), dysuria (N = 14), dyspareunia (N = 8), and positive whiff test (N = 23), pH > 4.5 (N = 17); while all patients of VVC presented with moderate to profuse foul smelling thick curdy white discharge (N = 7), pruritus (N = 06), pH < 4.5 (N = 6), dysuria (N = 3), dyspareunia (N = 1), and positive whiff test (N = 1). Majority of the patients of BV, VVC and TV belonged to the age group 31–40 yrs, had a single sexual partner, had normal menstrual history, and two children with no abortion (Table 2).

Figure 3. Agarose gel electrophoresis of PCR amplified products with primer TVK3/7.



Line 1, 100 bp ladder (L); line 2, positive control (PC); lines 3–7, amplification products of *Trichomonas vaginalis*-positive patients (T1–T5); line 8–10, amplification products samples negative for *T. vaginalis* (T6–T8); line 11 negative control (NC); line 12, no template control (NTC).

Table 1. Performance of diagnostic tests used to detect *T. vaginalis.*

Methods	(Gold standard) Culture		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	+	-				
Wet mount +	03	00	100	100	100	100
Wet mount -	00	111				
Giemsa +	03	00	100	100	100	100
Giemsa -	00	111				
PCR +	03	02	100	98.2	60	100
PCR -	00	109				

NPV: negative predictive value; PCR: polymerase chain reaction; PPV: positive predictive value; *T. vaginalis*: *Trichomonas vaginalis*.

Discussion

Vaginal discharge is a common gynecological condition in many women in the reproductive age group. Etiological diagnosis of vaginal discharge remains challenging because a large number of pathogens can be responsible for this condition. Trichomoniasis has been documented as the most prevalent non-viral STD. The prevalence of TV in our study was 2.3% using all three conventional methods, while PCR was found to be the most sensitive method and prevalence increased to 4.4% after PCR. The prevalence of TV in India is 0.4–35.23% in various geographical regions [18,22]. Very few studies have been conducted in the eastern part of the country. The estimated prevalence of TV in our study using conventional methods of detection is in concordance with the majority of the previous studies [23–25].

However, in a study conducted by Ghosh *et al*. in West Bengal, the prevalence was noted to be higher because the study group consisted of high-risk groups such as female sex workers with a very small sample size of 45 patients [18]. In studies conducted by Anuradha *et al*. and Fule *et al*., all the patients belonged to rural communities which were low socio-economic groups with lack of awareness, poor literacy rates, poor personal hygiene, and most importantly poor treatment-seeking behavior which might have resulted in a higher prevalence [26,27] .

Sensitivity, specificity, PPV, and NPV were found to be 100% each for both wet mount and Giemsa stain; sensitivity and NPV for PCR were 100%, and specificity and PPV of 98.2% and 60% respectively in comparison to culture. The findings were in agreement with that of the study conducted by Singh *et al*. in Delhi where the prevalence of trichomoniasis was identified to be 1.96% by both culture and wet mount, and 2.45% by PCR [25]. Developed countries like the USA and Australia have studied the performance of PCR assays and their studies showed 89–98% sensitivity [28]. In a study by Radonjik *et al*., the PCR method had the highest sensitivity, followed by wet mount and Giemsa, in comparison to culture as the gold standard [29]. In another study conducted in Uganda, similarity in detection potential was observed by culture and PCR, while wet mount yielded lower diagnostic output. [30]

Trichomoniasis can present with a variety of clinical manifestations, and the majority are asymptomatic. All patients of trichomoniasis presented with medium to profuse greenish frothy, foul-smelling discharge of high pH. Pruritus and positive whiff test were observed in three patients, while two patients presented with vaginal wall inflammation and cervical inflammation. Dysuria was found in four patients, and dyspareunia was reported in only two patients. The clinical symptoms are comparable to the study conducted by Vidyasagar *et al.* where almost all patients reported foul smelling abnormal discharge with associated pruritus. The majority of patients also experienced vulvar irritation with dysuria and dyspareunia in 30% [31]. In addition, the majority also presented with high vaginal pH, along with greenish frothy discharge and amine odor with 10% KOH in around 33% and 41% of patients with trichomoniasis respectively [9].

Table 2. Sociodemographic profile of patients who presented with abnormal vaginal discharge.

Parameters	Patients with BV (n = 23)	Patient with VVC (n = 7)	Patient with TV (n = 4)	Patient with BV and TV co-infection (n = 1)
Age group				
≤ 30 (38)	7 (18.4%)*	2 (5.2%)	1 (2.6%)	0
31–40 (51)	11 (21.5%)	4 (7.8%)	2 (3.9%)	0
41–49 (25)	5 (20%)	1 (4%)	1 (4%)	1 (4%)
Menstrual history				
Normal[a] (103)	18 (17.4%)	6 (5.8%)	3 (2.9%)	1 (0.9%)
Abnormal[b] (11)	5 (45.4%)	01(9.1%)	1 (9.09%)	0
Single sexual partner (114)	23 (20.1%)	7 (6.1%)	4 (3.5%)	1 (0.8%)
Parity				
Nulliparous (6)	2 (33.3%)	1 (16.6%)	1 (16.6%)	0
1 (36)	6 (16.6%)	2 (5.5%)	1 (2.7%)	0
2 (59)	13 (22%)	4 (6.7%)	2 (3.3%)	1 (1.69%)
3 (13)	2 (15.3%)	0	0	0
Abortion				
No abortion (55)	14 (25.4%)	4 (7.2%)	3 (5.45%)	0
Single abortion (41)	6 (14.6%)	2 (4.8%)	1 (2.4%)	0
Double abortion (17)	2 (11.7%)	1 (5.8%)	0	0
Triple abortion (1)	0	0	0	1 (100%)

[a] A normal menstrual history refers to parameters such as a frequency of ≥ 24 to ≤ 38 days, a duration of ≤ 8 days, regular (shortest to longest cycle variation ≤ 7–9 days) with normal menstrual volume and no intermenstrual bleeding or unscheduled bleeding in patients taking hormonal contraceptives. [b] Abnormal menstrual history refers to amenorrhoea or frequency < 24 days or > 38 days, duration of bleeding > 8 days, irregular (shortest to longest cycle variation ≥ 8–10 days) with heavy flow and random or cyclic intermenstrual bleeding and unscheduled bleeding in patients taking hormonal contraceptives. *Numbers in parenthesis indicate percentage. BV: bacterial vaginosis; TV: *Trichomonas vaginalis*; VVC: vulvovaginal candidiasis.

TV and BV are both common infections found among women of the reproductive age group (0.2–14.6%) [32–34]. In our study, 1 out of 5 cases had co-infection with BV, with a Nugent score of 9. Their co-occurrence suggests that one of these conditions may alter a woman's susceptibility to the other [34,35]. TV infections have been seen in BV patients with a score ≥ 4 in previous studies, suggesting that BV alters the vaginal ecology thereby predisposing women to TV infection [36]. Several studies have reported a significant association of BV and an intermediate Nugent score with TV [37,38]. However, other studies such as that conducted by Hillier *et al.* have found a strong association only with intermediate Nugent score in pregnant women [39]. A similar observation was also noted by Martin *et al*. in non-pregnant women [40]. Alteration of vaginal flora with loss of protective lactobacilli and increased vaginal pH provides a favorable environment for the growth of TV [40]. Some bacteriostatic and bactericidal compounds are generated in the presence of low vaginal pH which creates a protective environment for the vagina. TV thrives optimally at a higher vaginal pH, preferably above 4.5 [41]. Therefore, patients with high vaginal pH should be screened for TV and BV infection. In our study, the majority of patients with BV (21.5%) were of 31–40 years of age. Similar findings were observed in a study conducted by Ranjit *et al.* in Nepal where BV was noted to be more prevalent in the 31–40 yrs age group [42]. The highest prevalence in the age group 31–40 years in our study might be due to this age being the most reproductively active age group with high sexual exposure. All the patients in our study had single sex partner. Several studies have also documented the occurrence of BV in sexually inactive females or virgins [43,44]. This provides evidence that sexual activity is not a risk factor for BV.

Strengths and limitations of the study

To the best of our knowledge, very few studies on trichomoniasis have been conducted so far in eastern India. This study has determined the prevalence of TV infection by PCR, which proved to be the most sensitive diagnostic tool compared to all three traditional methods, i.e., wet mount, staining, and culture. However, the traditional methods are cost effective and can be performed without much expertise. This study was conducted with a very small sample where only non-pregnant women were enrolled. Further studies can be conducted with large sample sizes and other populations for better understanding of the epidemiology.

Conclusions

Abnormal vaginal discharge is one of the most commonly encountered problems among women of the reproductive age group. The majority of these patients are treated by SCM. However, SCM is based on signs and symptoms, and subjective judgment can lead to over-diagnosis and treatment. Effective treatment of these conditions requires etiological diagnosis. Trichomoniasis is the most prevalent curable sexually transmissible infection (STI) worldwide. The combination of wet mount and culture methods remains the standard approach for diagnosis of trichomoniasis, However, culture may take 7 days for growth. Lack of timely management of the infection may lead to complications in infected individuals and also increase the possibility of transmission of the disease to others. PCR is a promising diagnostic tool; it can detect both viable and non-viable organisms, and can enable early and accurate treatment in these groups of patients.

Authors' contributions

GM: sample collection and analysis, data analysis, writing manuscript; KG: study conception and design, literature search, supervision, manuscript writing; SuM: assistance with sampling and data collection; AKS: data analysis tools; SrM: manuscript writing. All authors reviewed and approved the final version of the manuscript for publication.

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Conflict of interests

No conflict of interests is declared.

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