Original Article

Vaginal discharge: The diagnostic enigma

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Abstract

Background: Vaginal discharge is a common clinical problem with varied etiologies, most common being bacterial vaginosis which presents as homogenous gray discharge caused by overgrowth of facultative and anaerobic bacterial species, next common is vulvovaginal candidiasis characterized by pruritus and cottage cheese like discharge followed by vaginal trichomoniasis associated with copious yellow or green and frothy discharge. This necessitates the need to identify the specific cause of vaginal discharge. **Aim:** To determine the etiology of pathological vaginal discharges in women attending tertiary care hospital. **Methodology:** 698 sexually active females in age group of 15 to 65 years with complaints of vaginal discharge attending Department of Dermatology Venereology and Leprosy at a Tertiary care hospital from June 2017 to May 2018 participated in the study. After presumptive clinical diagnosis vaginal discharge was collected. Wet mounts and 10% KOH preparations were examined immediately. Identification of pathogens was done by Gram stain and culture. **Results:** 18.33% of 698 patients showed vulvovaginal candidiasis, 13.75% had bacterial vaginosis, 1.86% showed trichomoniasis. Gold standard was considered to be culture for candidiasis & trichomoniasis whereas for bacterial vaginosis it was Nugent's score. **Conclusions:** Vaginal discharge is of multiple yet specific etiologies hence simple and minimal tests like microscopy available in most laboratories (supported by culture wherever possible) would help in accurate diagnosis without over or under treatment of patient due to the empirical therapy. Syndromic management of STIs (WHO guidelines) should be used only in non-specific cases.

Key words: Bacterial vaginosis, non-*albicans Candida* species, pathological vaginal discharge, trichomoniasis, vulvovaginal candidiasis

INTRODUCTION

Vaginal discharge is a common clinical condition with varied etiologies. Vaginal flora is a dynamic ecosystem that can be easily altered. The vagina, ectocervix, and endocervix are all susceptible to various pathogens, depending on its epithelium and other factors in the microenvironment. The squamous epithelium of the vagina and ectocervix is susceptible to *Candida* species and *Trichomonas vaginalis*, whereas the columnar epithelium of the endocervix is susceptible to *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Herpes simplex virus may infect both types of epithelium. Earlier vaginal discharge was managed under the umbrella of "non-specific vaginitis." At present, knowledge of individual pathogens makes treatment more specific.

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Motile Trichomonas vaginalis observed on wet mount

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Vaginitis resulting from bacterial (bacterial vaginosis [BV]), fungal (vulvovaginal candidiasis [VVC]) or protozoan infections (trichomoniasis) can be associated with altered vaginal discharge, odor, pruritus, vulvovaginal irritation, dysuria, or dyspareunia. The modern management of vaginal discharge demands a specific diagnosis, which is a combination of naked eye examination and laboratory workup of the vaginal discharge.

MATERIALS AND METHODS

A prospective, observational study was undertaken over a period of 12 months from June 2017 to May 2018 in sexually transmitted disease (STD) clinic and STD laboratory, in South India.

Study population

The study population consisted of a total of 698 women with complaints of vaginal discharge who were eligible and consented for the study during the study period after administering inclusion and exclusion criteria.

Inclusion criteria

1. Sexually active reproductive age group women between 15 and 65 years presenting to the STD clinic with complaints of vaginal discharge
2. Willing to be a part of the study with informed consent.

Exclusion criteria

Pregnant and lactating women, postmenopausal women, and women with genital or cervical malignancy and on treatment were excluded from the study.

Method of the study

A consecutive sampling method with zero intervals was followed in the consenting order.[1,2] A detailed clinical history of the patient was obtained;

Cusco's speculum was introduced per vaginally and high vaginal swabs obtained from the posterior fornix for

1. Wet mount of samples with normal saline and 10% of KOH
2. Gram stain
3. Culture: high vaginal swabs were inoculated on
   - Sabouraud's dextrose agar and chromagar for *Candida* at 25°C which were further processed on cornmeal agar for species confirmation
   - Kupferberg medium for *T. vaginalis*
   - Chocolate and blood agar for *N. gonorrhoea*.

RESULTS

Of the total 698 patients, based on history and clinical examination, 120 patients were suspected of candidiasis, 212 patients were suspected of BV, and 9 patients were suspected of trichomoniasis. Ninety-six patients were found to have physiological and 261 were found to have nonspecific vaginitis through clinical diagnosis alone.

Microbiological confirmation was obtained in 237 samples out of 698. One hundred twenty-eight (18.33%) of these had VVC, 96 (13.75%) had BV, and 13 (1.86%) had trichomoniasis [Table 1].

Microscopy

Wet mount findings

The vaginal discharge collected from posterior fornix was mixed with a drop of normal saline and another preparation with 10% KOH and examined for

- Highly refractile round or oval budding yeast-like cells with pseudohyphae seen in case of candidal vaginitis in 102 patients [Figure 1]

Table 1: Percentage of vulvovaginal candidiasis, clue cells, bacterial vaginosis, and trichomoniasis through clinical suspicion, wet mount, Gram stain, and culture

	Clinical diagnosis (%)	Wet mount (%)	Gram stain (%)	Culture (%)
VVC	17.19	14.61	17.9	18.33
BV	30.37	NA	13.7	NA*
TV	1.2	1.43	0.71	1.86

Culture for bacterial vaginosis is not recommended as a routine method (NACO guidelines)[1]. Values provided in bold are based on gold standard techniques (culture for VVC and TV and Nugent's score for BV). VVC=Vulvovaginal candidiasis; TV=*Trichomonas* vaginitis; BV=Bacterial vaginosis; NA=Not significant

Figure 1: *Candida* species on wet mount

- The presence of clue cells which are vaginal epithelial cells with granular surface and blurred margins because of the attached bacteria were found in 60 patients suggestive of BV [Figure 2]
- Jerky movements of flagellate protozoans were observed within 15 min to ½ h in trichomoniasis, in 10 patients [Figure 3 and Video 1].

Gram stain of discharge showed:

- Gram-positive budding yeast cells with pseudohyphae suggestive of *Candida* species in 125 of total Gram stained smears [Figure 4]
- Certain species such as *Candida parapsilosis*, *Candida glabrata* and *Candida krusei* could be presumptively diagnosed on Gram's smear due to the difference in their sizes and shapes. Culture was performed for confirmation
- One hundred and sixteen Gram-stained samples were found to have epithelial cells with granular surface and blurred margins because of the attached bacteria (clue cells) with marked reduction of Gram-positive lactobacilli [Figure 5]
- Nugent scoring system was used to diagnose BV which was consistent with 96 of the total smear.
- Five of the total Gram stains showed *T. vaginalis* [Figure 6]
- Intracellular and extracellular Gram-negative diplococci suggestive of *N. gonorrhoea* were looked for.

Culture growth

Sabouraud's dextrose agar, chromagar, and corn-meal agar

Culture on (Sabaroud's dextrose agar) SDA was identified by colony morphology and Gram stain, showing various *Candida* species. For the confirmation of *C. albicans*, germ tube formation was demonstrated; further, colonies on CHROMagar (Himedia) showing specific colored growth for each species were identified [Figure 7]. Colonies on cornmeal agar were studied under ×10 and ×40 for demonstration of the characteristic

Figure 2: Clue cell on wet mount

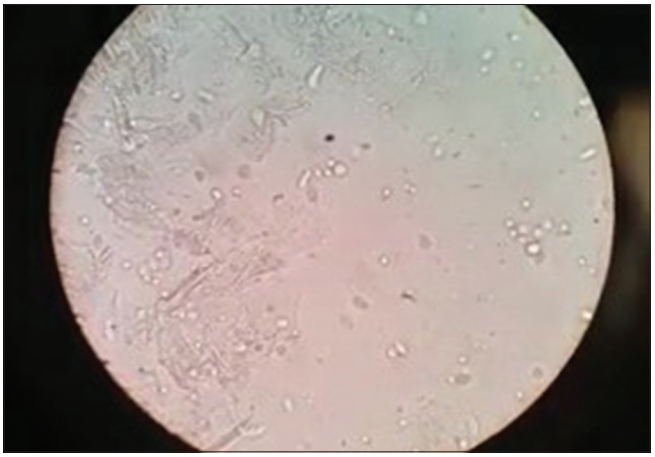


Figure 3: *Trichomonas vaginalis* on wet mount

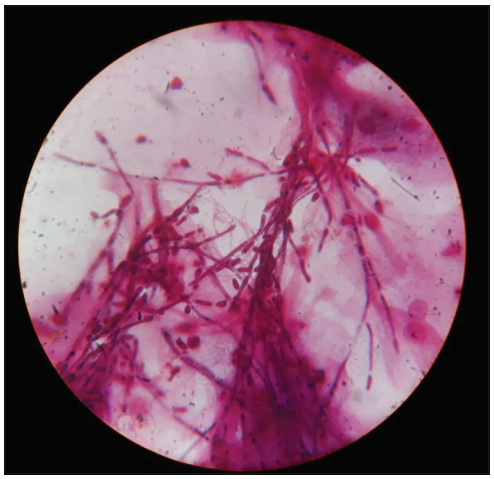


Figure 4: Gram positive budding yeast cells with pseudohyphae on Gram stain

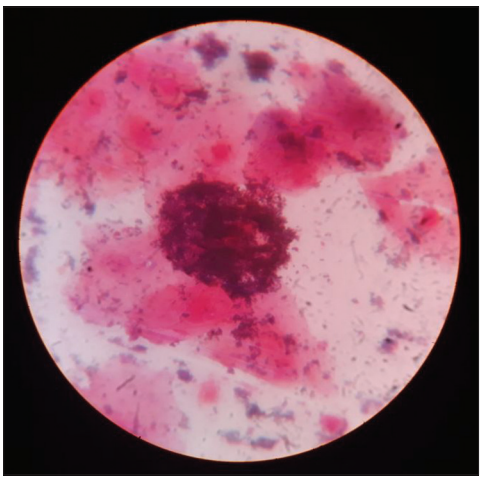


Figure 5: Clue cell on Gram stain

chlamydospores of each species [Table 2] and [Figure 8-12].

In the present study, *C. glabrata* (36%), *C. albicans* (29%), *Candida tropicalis* (28%), *C. krusei* (6%), and *C. parapsilosis* (1%) were found to be the common causes of VVC in the decreasing order of occurrence.

Kupferberg medium

Culture growth on Kupferberg medium [Figure 13] was confirmed by wet mounts performed on day 2, 5, and 7 which were examined for motile trichomonads, and serial subcultures were done for further confirmation. Thirteen of the total samples showed *T. vaginalis*.

Chocolate agar medium

Chocolate agar was examined for translucent to transparent dew-drop-like colonies showing Gram-negative diplococci in chains. In this study group of 698 patients, we could not isolate *N. gonorrhoea*.

DISCUSSION

The etiology of vaginal discharge was identified in all the consenting women aged between 15 and 65 years attending STD clinic and regional STD laboratory of a tertiary hospital, after meeting inclusion and exclusion criteria.

The following observations were made:
In the present study, the diagnosis of BV by clinical

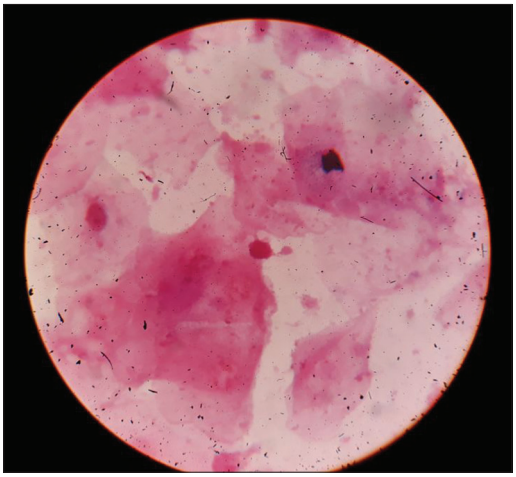


Figure 6: *Trichomonas vaginalis* on Gram stain

Figure 7: Various *Candida* species on CHROMagar showing characteristic color and colony morphology

Table 2: Characteristics of various *Candida* species on wet mount, Gram stain, CHROMagar, and cornmeal agar

Features Number of specific isolates/total number of *Candida*	*Candida glabrata* (46/128)	*Candida albicans* (37/128)	*Candida tropicalis* (36/128)	*Candida krusei* (8/128)	*Candida parapsilosis* (1/128)
Wet mount [Figure 1]	Small budding yeast cells only	Budding yeast cells with pseudohyphae and hyphae	Budding yeast cells with pseudohyphes	Elongated budding yeast cells with pseudohyphae and hyphae	Budding
Gram stain [Figure 4]	Gram-positive budding yeast cells 2-4 μ with no hyphae	Gram-positive budding yeast cells 6-4 μ in size with hyphae and pseudohyphae	Gram-positive budding yeast cells 4-6 μ with hyphae and pseudohyphae	Gram-positive budding yeast cells 6-4 μ in size with hyphae and pseudohyphae with elongated cells i.e., matchstick appearance	Gram-positive budding yeast cells 6-4 μ in size with hyphae and pseudohyphae. Typical sagebrush or spider web appearance
Colour on chromagar [Figure 7]	Moist to mucoid pink-colored colonies	Sea green	Blue green	Rough pink-to-purple-colored colonies	Ivory colored
Chlamydosopores	Absence of pseudohyphae, hyphae, chlamydospores [Figure 8]	Thick-walled terminally arranged. Singly [Figure 9]	Subterminal spores arranged on either side of hyphae and pseudohyphae singly or in clusters [Figure 10]	Scanty or absent chlamydospores [Figure 11]	Chalmydospores absent. Hyphae are seen [Figure 12]

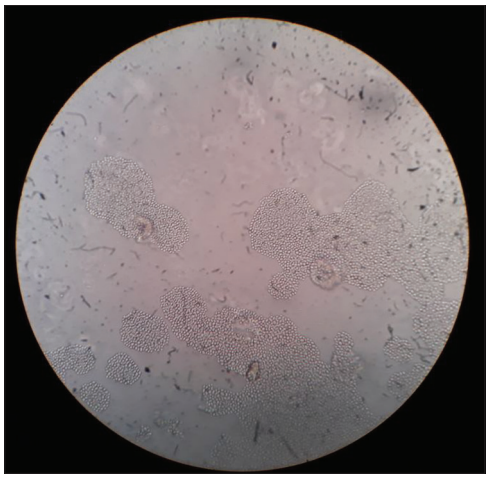


Figure 8: *Candida glabrata* on cornmeal agar characterized by the absence of chlamydospores, hyphae, or pseudohyphae

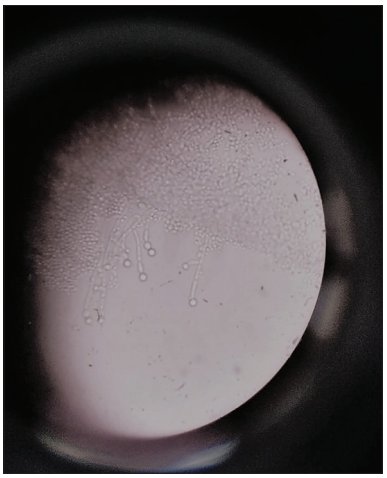


Figure 9: *Candida albicans* on cornmeal agar showing terminal single chlamydospores

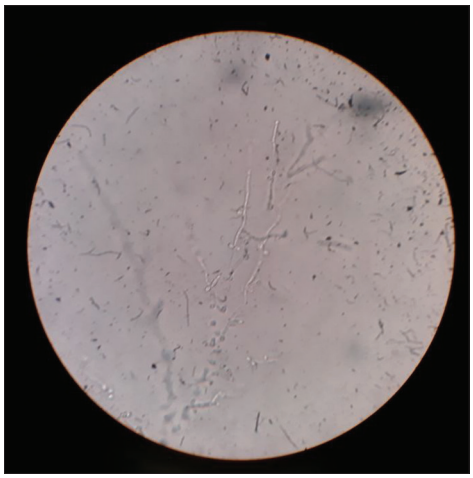


Figure 10: *Candida tropicalis* on cornmeal agar showing sub/nonterminal chlamydospores arranged in clusters or singly

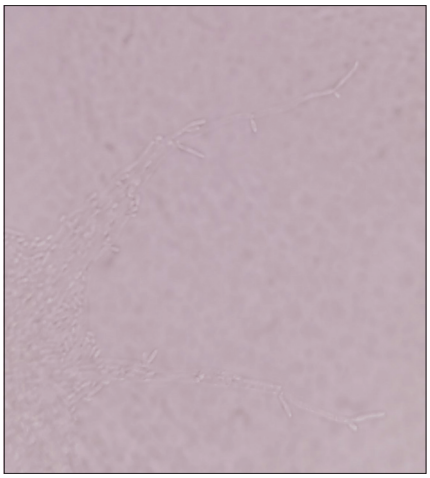


Figure 11: Match sticks appearance of *Candida krusei* on cornmeal agar

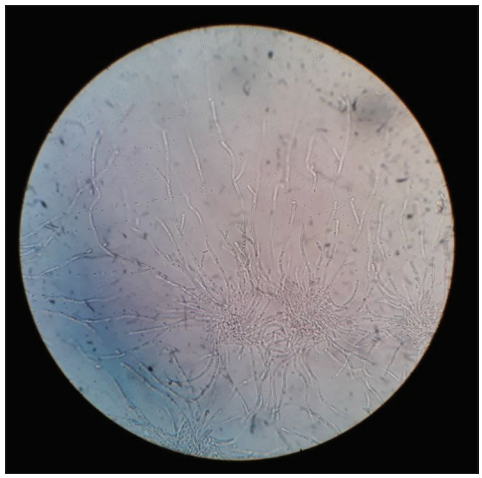


Figure 12: Sage bush/spider web appearance of *Candida parapsilosis* on cornmeal agar

Figure 13: Kupferberg medium uninoculated medium on left, inoculated medium on right

suspicion was higher than microbiological diagnosis, while the prevalence of *Candida* and trichomoniasis was better by microbiological diagnosis versus clinical approach. Thus, clinical diagnosis was found to have higher sensitivity for diagnosing BV and moderate sensitivity for trichomoniasis and candidiasis.[3]

In this study, a maximum number of women showing pathological discharge by microbiological confirmation were found to be in the reproductive and childbearing age group with most of them suffering from VVC followed by BV. In contrast, several studies such as Rao *et al.*,[4] Puri *et al.*,[5] Vijaya *et al.*,[3] Rekha *et al.*,[6] and Ananthula *et al.*[7] showed BV to be more common than candidiasis. This could be due to the use of empirical and over the counter antibiotics in the present study population.

One common finding seen across all these studies and further confirmed by our study is that the highest number of discharges is seen in the initial part of the onset of sexual and reproductive activity age, i.e., between 21 and 25 years and slightly declines during 25–44 years. This study further shows that as the age advances, the number of patients complaining of vaginal discharge decreases [Table 3]. This was attributed to increased awareness on sexual health and experience, thus allowing the women to follow sexual and reproductive hygiene and safer sex practices.

Among the study group, a majority of pathological discharges are seen in the age group of 19–25 years, followed by 25–40 years. The same pattern is seen in both candidal vaginitis and BV but trichomoniasis was seen more in number in elderly patients >45 years [Table 3], which was similar to Ananthula *et al.*[7]

Table 3: Bar graph showing distribution of vulvovaginal candidiasis, bacterial vaginosis and *Trichomonas* vaginitis across various age groups

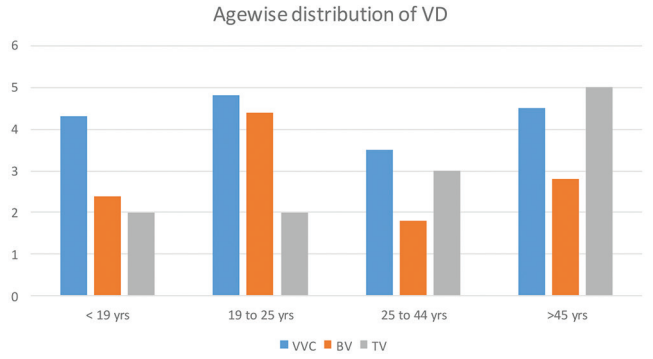
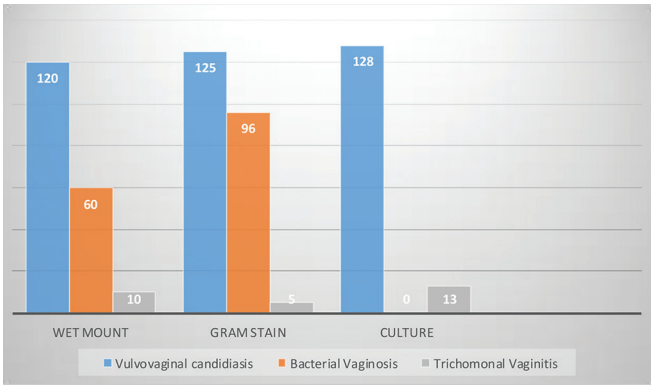


Table 4: Diagnosis of vulvovaginal candidiasis, bacterial vaginosis, *Trichomonas* vaginal by wet mount, Gram stain and culture



Vulvovaginal candidiasis

In this study, 18.33% of the patients were found to have VVC, which was in agreement with Vijaya *et al.*[3] Holland *et al.*,[8] Sowjanya *et al.*,[9] and Mohanty *et al.*[10] The present study showed VVC to be more common than BV unlike Rao *et al.*,[4] Puri *et al.*,[5] Vijaya *et al.*,[3] Rekha *et al.*,[6] and Ananthula *et al.*[7]

The present study showed non-*albicans Candida* species to be more common (71% in total) than Candida albicans (29%) with Candida glabrata (36%) being highest in number, which was similar to Ahmad A *et al*, Kumari *et al* and Swarajya Lakshmi *et al.*[11-13] This was followed by *C. albicans*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis*, which was in close agreement with Kalaisaran *et al.*[14] and Swarajya Lakshmi *et al.*[13] Further, we found Gram stain (17.9%) to be almost as accurate as culture (18.33%) in the diagnosis of VVC. Wet mount (14.6%) was found to be less sensitive compared to Gram stain or culture [Table 4], which was similar to Ahmad and Ahmad and Khan[11] (15.9%). Esmaeilzadeh *et al.*[15] on the other hand showed it to be 27.84%. The difference in sensitivity of direct microscopy may be due to the difference in the concentration of yeast in different vaginal secretions.

Emergence of non-*albicans* species might be due to widespread and inappropriate use of antimycotic treatment in the form of self-medication, long-term maintenance treatments and repeated treatments for candidiasis episodes, and the use of single-dose oral and topical azoles. *C. albicans* eradication by these means results in the selection of species such as *C. glabrata* that are resistant to commonly used agents.[12]

Bacterial vaginosis

About 13.7% of the patients were diagnosed with BV in the present study. This study also confirms that Nugent's scoring is more specific and highly sensitive in diagnosis of BV.[4,16] Rao *et al.*[4] diagnosed 17.4% cases of BV which was similar to our study. Few other studies such as Ananthula

et al.,[7] Puri *et al.*,[5] Vijaya *et al.*,[3] and Rekha *et al.*[6] showed a higher prevalence of BV, this could be due to relatively small sample sizes in all these studies compared to the present study.

The present study shows that Gram stain is better at the detection of clue cells which are characteristic of BV compared to wet mount which gave many false negatives [Table 4]. About 16.61% (116 patients) of all the Gram stains showed clue cells and 96 of them were diagnosed with BV, thus confirming that the presence of clue cells is the single most reliable predictor of BV.[4]

In the present study, clue cells were found to be 100% sensitive and 90% specific in the diagnosis of BV which was similar to Mahadani *et al.*[17] who found clue cells to be 100% sensitive and 95.25% specific, whereas Modak *et al.*[18] found it to be 100% sensitive and 76% specific. Gupta *et al.*[19] found clue cells in only 61% of the cases symptomatic for BV. This study further validates that simple Gram staining of vaginal smears has very good sensitivity of 89%–93%.[20,21]

Trichomonas vaginitis

About 1.86% of the total patients had trichomoniasis which was in agreement with Rao *et al.*,[4] Puri *et al.*,[5] Vijaya *et al.*,[3] and Rekha *et al*;[6] on the other hand, Muthusamy and Elangovan[22] showed it to be 6.15%, Madhivanan *et al.*[23] found it to be 8.5%, and Ananthula *et al.*[7] showed a very high prevalence of 18%.

The present study shows that though culture is the gold standard for diagnosis of *T. vaginalis*, prompt wet mount examination of vaginal discharge within 15 min is highly sensitive showing motile trichomonads after which motility decreases exponentially, similar findings were reported by Muthusamy and Elangovan[22] and Akujobi *et al.*[24]

Taking culture as the gold standard our study found the sensitivity of wet mount to be 77% in the detection of trichomoniasis, which was similar to that found by Ananthula *et al.*[7] (88.8%), Thomason *et al.*[25] (86%), and Akujobi *et al.*[24] (88.6%). Gram stain was found to be a poor tool for the detection of trichomonas.

Culture is more sensitive than wet mount preparation [Table 4] because of the lower minimum concentration of organisms required for a positive result and delay in examining the samples resulting in decreased motility.[26]

CONCLUSION

The present study shows that BV would be overtreated, while candidiasis and trichomoniasis would be under treated if the management was started based on clinical diagnosis alone; thus, it confirms the need to identify the etiological agents in the diagnosis of vaginal discharge whenever possible and instituting accurate treatment rather than giving empirical treatment proposed by the syndromic approach of the WHO which often leads to considerable loss of resources and time due to over management. Indiscriminate use of antibiotics inadvertently causes alteration of normal vaginal flora with the potential of increasing the antibiotic resistance in the community.

In a low-resource setting, primary clinical diagnosis based on simple microscopy, i.e., Gram stain in case of VVC and BV and wet mount in case of trichomoniasis, supported further with culture wherever possible would be a more efficient management strategy.

The present study also points toward an increasing need to speciate *Candida* infections due to an increasing emergence of non-*albicans Candida* species, as these are often resistant to routine drugs and may further affect the management strategy in these patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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