

Original article

Prevalence of Candidiasis, Trichomoniasis and Bacterial Vaginosis in Females Attending Out Patient Gynecology Services in a Tertiary Care Hospital

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Abstract:

Candidiasis, Bacterial Vaginosis & Trichomoniasis are common sexually transmitted infections. Furthermore, these infections may amplify HIV transmission. For Trichomoniasis & Bacterial vaginosis, microscopic diagnosis is a challenge as expertise is required for accurate interpretation. Broth Culture for Trichomonas, which is gold standard, requires 2-7 days. Culture for Gardnerella is time consuming. Culture for Candida takes 2-7 days. During this period, patient may continue to transmit these infections. Hence a rapid diagnostic method is an absolute need of the hour. Hence this study was conducted to evaluate a rapid diagnostic DNA probe test from BD AFFIRM against the standard available tests.

Key words- Candidiasis, Vaginosis, Trichomoniasis

Introduction:

Vaginal discharge is a common presenting symptom seen by doctors in many services (primary care, gynaecology, family planning, and departments of genitourinary medicine) which may be physiological or pathological. Many authors have documented vaginal discharge as one of the commonest symptom of genital tract disease reported by women in India^(1, 2, 3). Common conditions among women attending Gynaecology & Genitourinary Medicine clinics are Candidiasis, Bacterial Vaginosis & Trichomoniasis. Vaginal discharge is empirically treated. No efforts or resources are expended towards etiological diagnosis, as methods routinely employed are not specific.

Vulvovaginal candidiasis affects about 75% of women at some time during their reproductive life, with 40-50% having two or more episodes. Bacterial

vaginosis is also very common, but as 50% of cases of bacterial vaginosis are asymptomatic, the true prevalence of this condition in the community is uncertain⁽⁴⁾. As per the published literature, the prevalence of *T. vaginalis* ranges from 0.4-27.4% in women and 0.0-5.6% in men.⁽⁵⁾ Recent literature documents that women having genital tract infections during pregnancy are predisposed to premature rupture of membranes, preterm labour and low birth weight infant.⁽⁶⁾ Further, these infections may amplify HIV transmission.⁽⁷⁾ Therefore appropriate identification of these common infections offer a precious and much needed additional strategy for AIDS prevention.

The traditional diagnosis of vaginitis incorporates patient symptoms, clinical findings observed during vaginal examination, and laboratory analysis of vaginal fluid via conventional methods

like Gram stain, wet mount, KOH mount and culture on appropriate media. These tests need trained technicians or access to a laboratory offering these tests. Therefore, a study was conducted to compare conventional methods against a molecular method of diagnosis. The Affirm™ VP III (Becton Dickinson, San Jose, California), is a nucleic acid probe test that evaluates for *T. vaginalis*, *G. vaginalis*, and *C. albicans* and has been used in this study to diagnose these infections. This test has a quicker turnaround time & sensitivity >83% and a specificity >97%.⁽⁸⁾

Aim:

To evaluate a rapid diagnostic DNA probe test from BD AFFIRM against the standard available tests and find out prevalence of candidiasis, bacterial vaginosis, trichomoniasis.

Materials and Methods:

This study was conducted at Nair Hospital, Mumbai during February 2014 to May 2014. Approval from institutional ethics committee was obtained. Symptomatic women with vaginal discharge were included in this study. Detailed clinical history, history of high risk behavior and examination findings were recorded using a structured questionnaire. Clinical examination was performed to include evaluation of vaginal discharge (if applicable).

Four vaginal swabs were obtained simultaneously from patients under all aseptic precautions. One swab was placed in sterile physiological saline and immediately examined microscopically for the presence of motile trichomonads, clue cells, and/or yeast or hyphae. The

second swab was processed for Grams Stain for Nugent's scoring. The third swab was processed for culture in Sabouraud's dextrose agar.

The fourth swab was placed in the Affirm sample collection tube. After collection of sample, it was immediately placed in the ampoule containing Ambient Temperature Transport System (ATTS). The Affirm swab sample was transported to the testing area within 1 hour at room temperature or 4 hours if refrigerated, and stored in the appropriate ambient temperature transport system. Once it reached the laboratory, 12 drops of lysis solution were added to it and then kept for 10 minutes in a dry bath at 75-80°C. Then 12 drops of buffer solution were added. With a filter cap placed, 5-6 drops were added to the Caddy in the first well and four drops of substrate solution were added in the last well. The card was placed in the first well and the system was run to get the results. The results were read as per positive and negative controls shown on the card.

Results:

Out of 100 Samples, processed by DNA probe analysis, 8 samples were positive for Candida species and 28 for Gardnerella vaginalis. 12 samples out of the 28 positive by DNA probe for Gardnerella vaginalis had a Nugent Score between 4 and 6. 3 swabs out of the 28 positive by DNA probe for Gardnerella vaginalis had a Nugent Score of 2. Out of a total of 8 samples positive for Candidiasis by DNA probe method, 4 did not show yeast cells on microscopy; whereas in 3 samples, yeast cells were demonstrated on microscopy but were negative by DNA Probe. (Table 1, Chart 1)

Table 1: Comparison of Methods

Sr. no.	Infection	Conventional Method		DNA Probe- Positive
		Method	Positive	
1	Bacterial Vaginosis	Nugents score	25	28
2	Candidiasis	KOH mount	7	8
3	Trichomoniasis	Wet mount	Nil	Nil

Chart 1

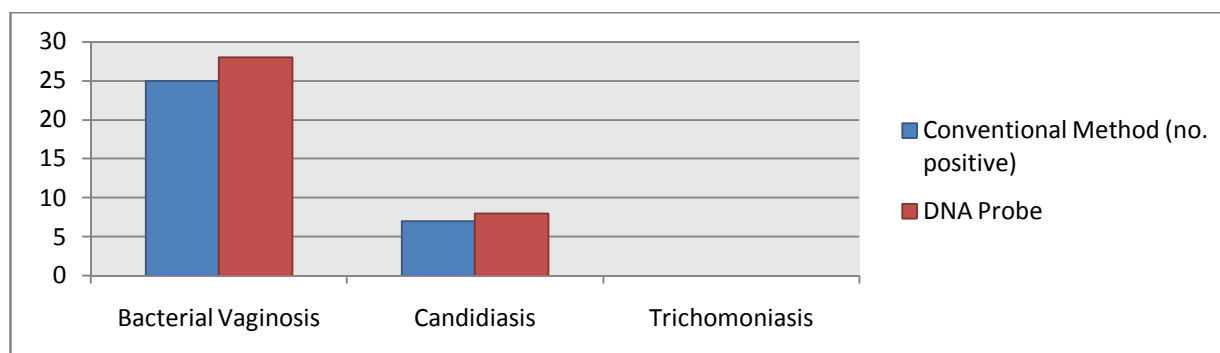


Table 2: Comparison of conventional tests with DNA probe: ^(8,13,14, 15)

Sr. no.	Infection	Sensitivity		Specificity	
		Conventional probe	DNA	Conventional	DNA probe
1	Bacterial Vaginosis	≥97.2%	98.3%	≥88.1%,	100%
2	Candidiasis	39.6	82.3%	90.4	98.4%
3	Trichomoniasis	75	92.8%	96.6	99.9%

Discussion:

Gram stain enables us to determine the relative concentration of lactobacilli (long Gram-positive rods), Gram-negative and Gram-variable rods and cocci (i.e., *G. vaginalis*, *Prevotella*, *Porphyromonas*, and peptostreptococci), and curved Gram-negative rods (*Mobiluncus*) characteristic of BV, and is considered the gold standard laboratory method for diagnosing BV. However, decision of normal and abnormal vaginal flora by microscopy is subjective and hence diagnosis may be missed if microscopy is used as the ‘Only’ diagnostic criterion. Culture of *G. vaginalis* is not recommended as a diagnostic tool because it is not specific.⁽⁹⁾ Commercially available tests that might be useful for the diagnosis of BV

include a card test for the detection of elevated pH and trimethylamine (QuickVue Advance Quidel, San Diego, California) and prolineaminopeptidase (Pip Activity TestCard™, Quidel, San Diego, California) & a DNA probe-based test for high concentrations of *G. vaginalis* (Affirm™ VP III, Becton Dickinson, Sparks, Maryland).

For Trichomoniasis, a positive result requires visualization of characteristic jerky motility in the wet mount preparation. This needs to be immediate and therefore not possible at times due to delay in transport. Also, the viable organisms required to visualize the pathogen by Microscopy are 10,000orgs/ml. One can misinterpret clusters of WBCs as positive for Trichomonads since WBCs are

similar in size and shape. Thus, expertise and competency is required for accurate interpretation. Broth Culture for Trichomonas, which is gold standard, requires 2-7 days^(10,11) In our study, there were no cases of Trichomoniasis, probably because of its exquisite sensitivity to metronidazole. Patients taking metronidazole for other infections like gastrointestinal infections tend to eradicate the parasite. The diagnosis of Vulvo vaginal Candidiasis can be made in a woman who has signs and symptoms of vaginitis when either a wet preparation (saline, 10% KOH) or Gram stain of vaginal discharge demonstrates yeasts or pseudohyphae or a culture or other test yields a positive result for a yeast species. *Candida* vaginitis is associated with a normal vaginal pH (<4.5). Use of 10% KOH in wet preparations improves the visualization of yeast and mycelia by disrupting cellular material that might obscure the yeast or pseudohyphae. Examination of a wet mount with KOH preparation should be performed for all women with symptoms or signs of VVC, and women with a positive result should receive treatment. For those with negative wet mounts, vaginal cultures for *Candida* should be considered for those with any sign or multiple symptoms. Culture for *Candida* also takes 2-7 days.⁽¹²⁾ During this period, patient may continue to transmit these infections.

Besides, diagnosis of the above three causative agents by conventional methods of Gram stain, wet mount & culture requires multiple swabs

from the patient. Primary care clinicians demonstrate a high specificity but low sensitivity when identifying vaginal trichomoniasis and vulvovaginal candidiasis by microscopic techniques.⁽¹³⁾ Correct microscopic diagnosis is difficult for clinicians & need more sensitization in the laboratory diagnosis of vaginitis. If they have access to a microscope, each microscopic slide should be systematically examined for each type of vaginitis, and consider specimen pH and the presence of leukocytes, Lactobacillus organisms, or amine odor as additional clues to infection. The observations made in this study highlight the advantage of DNA probe test in detecting the pathogen and reduced turnaround time of 40 minutes to 1 hour. The sample collected for DNA probe analysis can also be preserved for 72 hours at temperature of 2-8°C. Only one swab from the patient can be processed for all the three agents simultaneously. The sensitivity & specificity of conventional methods have been compared with the DNA probe tests in Table 2.

Conclusion

Few studies are available comparing routine methods of diagnosis versus molecular methods. Molecular diagnosis has a definite advantage due to the reduced turnaround time. Also, species diagnosis by way of genetic identification can be done easily. Therefore etiological diagnosis by molecular methods may be considered in tertiary care settings where specific diagnosis is needed. Larger studies are needed to confirm clinical usefulness of these tests.

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