

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email editorial.bmjopen@bmj.com

BMJ Open

Evaluation of reliability of self-collected vaginal swabs over physician-collected samples for diagnosis of bacterial vaginosis, candidiasis and trichomoniasis

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-025013
Article Type:	Research
Date Submitted by the Author:	26-Jun-2018
Complete List of Authors:	Khan, Zarine; VMMC & Safdarjung Hospital, Department of Microbiology Bhargava, Aradhana; VMMC and Safdarjung Hospital, Apex Regional STI Training, Research & Reference Laboratory Mittal, Pratima; VMMC & Safdarjung Hospital, Department of Obstetrics & Gynaecology Bharti, Rekha; VMMC & Safdarjung Hospital, Department of Obstetrics & Gynaecology Puri, Poonam; VMMC and Safdarjung Hospital, Department of Dermatology & STD Khunger, Niti; VMMC and Safdarjung Hospital, Department of Dermatology & STD Bala, Manju; VMMC and Safdarjung Hospital, Apex Regional STI Training, Research & Reference Laboratory
Keywords:	Microbiology < BASIC SCIENCES, GENITOURINARY MEDICINE, Diagnostic microbiology < INFECTIOUS DISEASES

SCHOLARONE™
Manuscripts

1
2
3 **Evaluation of reliability of self-collected vaginal swabs over physician-collected samples for**
4 **diagnosis of bacterial vaginosis, candidiasis and trichomoniasis**
5
6
7
8
9

10 **Zarine Khan^{1,2}, Aradhana Bhargava^{1,2}, Pratima Mittal³, Rekha Bharti³, Poonam Puri⁴,**
11 **Niti Khunger⁴, Manju Bala^{1,2*}.**
12
13
14

15
16
17 ¹Department of Microbiology, VMMC & Safdarjung Hospital, New Delhi, India, ²Apex
18 Regional STI Training, Research & Reference Laboratory, VMMC & Safdarjung Hospital, New
19 Delhi, India, ³Department of Obstetrics & Gynaecology, VMMC & Safdarjung Hospital, New
20 Delhi, India, ⁴Department of Dermatology & STD, VMMC & Safdarjung Hospital, New Delhi,
21
22
23
24
25
26 India.
27
28
29
30

31 **Correspondence:**

32
33 Dr. Manju Bala, Consultant and Professor (Microbiology), Apex Regional STI Training,
34 Research & Reference Laboratory, Vardhman Mahavir Medical College & Safdarjung Hospital,
35 New Delhi, India. Telephone number: 91-11-26196740, Fax number: 91-11-26163072, Email:
36 manjubala_2@hotmail.com
37
38
39
40
41
42
43

44 **Key words:** Vaginal discharge; bacterial vaginosis; vulvovaginal candidiasis; trichomonas
45 vaginitis, self-collected vaginal swabs; physician-collected vaginal swabs.
46
47
48
49
50

51 **Word count: 2776**
52
53
54
55
56
57
58
59
60

ABSTRACT

Objectives Self-collected vaginal swabs can facilitate diagnosis of Vaginal discharge (VD) in resource-limited settings, provided reliability of the method is established. The aim of this study was to determine the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of self-obtained over clinician-collected swabs for diagnosis of VD and prevalence of bacterial vaginosis(BV), vulvovaginal candidiasis(VVC) and trichomonas vaginitis(TV).

Methods A total of 550 females (median age:32 years; range:18-45 years) attending two STI/RTI clinics with VD from January 2015 to May 2016 were included in the study after obtaining written informed consent. Swabs were self-collected by patients after instructions and subsequently by a physician under speculum examination. Samples were processed for standard bedside tests, Gram staining, wet mount and culture (gold standard) according to National guidelines. Concordance between the two methods was determined by the Cohen's kappa value.

Results BV, VVC and TV were diagnosed in 79(14.4%), 144(26.2%) and 3(0.5%) patients respectively. VVC coexisted with BV in 58(10.5%) patients. There was no co-infection of TV with BV or VVC. *C. albicans* was isolated in 84(58.3%) VVC cases. Sensitivity, specificity, PPV and NPV of self-collected swabs for diagnosing BV was 91.1%, 100%, 100% and 98.5% respectively while for the *C. albicans* VVC and TV, sensitivity, specificity, PPV and NPV all were 100% as compared to physician-collected swabs. Highly concordant results were obtained between two methods by the Kappa values of 0.95 (BV), 0.99 (VVC) and 1.0 (TV).

Conclusion The comparative performance of self- and physician-collected vaginal swabs establishes self-collection of samples for BV, VVC and TV as a viable alternative for management of STIs/RTIs especially in peripheral and resource-constrained settings. This would

1
2
3 be effective in implementing the diagnostic approaches for STIs/RTIs in community based
4 surveillance studies at national or regional level and therefore strengthening the National
5
6 STI/RTI Control Programme.
7
8

9 10 **ARTICLE SUMMARY**

11 **Strengths and limitations of this study**

- 12
13
14
15
16 • This study determined that if diagnosis based on self-collected vaginal swabs is proven
17 reliable, could contribute to early diagnosis and greatly increase the access to treatment.
- 18
19
20
21 • In this prospective study, specimens were obtained from 550 patients with vaginal
22 discharge attending the two STI/RTI clinics after obtaining the written informed consent.
- 23
24
25 • Standard bedside tests, Gram staining, wet mount and culture (gold standard) were
26 performed on both the self- and physician- collected samples for detecting bacterial
27 vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis (TV).
- 28
29
30
31 • Corresponding findings were compared and analyzed using SPSS statistical analysis tool
32 in terms of Cohen's Kappa values to determine the concordance between findings of self-
33 and physician- collected samples.
- 34
35
36
37 • Limitation is that molecular tests were not performed, although gold standard test culture
38 was performed which is more economical and less labour intensive also.
- 39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

INTRODUCTION

Vaginal discharge is a frequently encountered complaint in women attending Sexually Transmitted Infections (STIs)/Reproductive Tract Infections (RTIs) clinics globally. It is mainly caused by curable bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis (TV).¹ Existing practice to diagnose vaginal discharge includes a speculum examination where the clinician inspects the external genitalia, vagina and cervix, assesses characteristics of the discharge, notes physical signs such as inflammation and lesions, and collects specimens for laboratory testing. The speculum examination requires a trained clinician and a proper setup. The discomfort of a pelvic examination may lead women to delay or avoid seeking care.² Moreover, as frequent sampling by a clinician (weekly or more often) is not practicable, this method may result in lower enrollment rates and higher rates of loss to follow-up.³

Even though high rates of STIs/RTIs are observed in developing countries due to poor hygiene and other factors, reliable detection of these infections are difficult due to poor infrastructure and lack of skilled workforce. Additionally, there is low female STI enrollment rate in developing countries due to women's reluctance to undergo gynecological examination, originating from cultural, religious and socio-economic factors.

Therefore, developing accurate approaches to diagnosing lower genital infections without a speculum examination would be advantageous to both clinicians and patients.² Self-collected vaginal swabs are the only practical and financially feasible method to use for sampling in field-based longitudinal cohort studies.³ As most of the studies comparing the reliability of self-obtained vaginal swabs have been conducted in developed countries such as USA^{3,4} and Australia^{5,6} mostly using advanced molecular techniques such as nucleic acid amplification tests, limited literature on such studies is available from developing countries. In India, there was a

1
2
3 pilot study from Goa, which suggested that self-administered swabs are an acceptable method of
4 collection of vaginal specimens in women attending gynaecological clinics in India.⁷ However,
5
6 the main limitation of the study was a statistically small sample size. Moreover, the samples
7
8 were examined only by Gram's stain for their sensitivity for the diagnosis of STIs and were not
9
10 tested by other diagnostic techniques like wet mount and culture.
11
12
13

14
15 The current study was aimed at establishing reliability of self-obtained vaginal swabs
16 against clinician-obtained swabs by determining its sensitivity, specificity, positive predictive
17 value (PPV) and negative predictive value (NPV) with significant number of samples and quality
18 reliable tests. Additionally, the fungal (*Candida albicans* or non-*albicans Candida* species)
19 bacterial (Bacterial vaginosis) and parasitic (*Trichomonas vaginalis*) etiology of vaginal
20 discharge and prevalence of various types of infections and co-infections were also studied.
21
22
23
24
25
26
27
28
29

30 **METHODS**

31
32
33
34
35 The study was conducted in two National AIDS Control Organization (NACO) designated
36 STI/RTI clinics in the Apex Regional STD Teaching, Training and Research Centre and
37 Department of Obstetrics and Gynaecology, Vardhaman Mahavir Medical College and
38 Safdarjung Hospital, New Delhi, India during January 2015 to May 2016. The thesis protocol
39 was approved by the authors' Institute Ethics Committee (VMMC & SJH). Samples were
40 collected from 550 sexually active females with vaginal discharge. Participants were between 18
41 and 45 years and written informed consent was obtained. Patients with history of antibiotic use
42 or vaginal medication in the previous 14 days, pregnant patients, patients not willing to
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 participate were excluded from this study, patients with human immunodeficiency virus (HIV)
4
5 infection and other serious illness or disability were excluded from this study.
6
7

8 A total of six vaginal swabs, including three self-collected samples, were collected from
9
10 each participant. Participants were given instructions on appropriate specimen collection
11
12 technique before the speculum examination. They were instructed to insert the vaginal swab 1 to
13
14 2 inches into the vagina, twisting the swab to collect material on all sides of the tip, wipe in
15
16 several full circles on the vaginal wall, keep the swab in the vagina for 20 seconds, and then
17
18 carefully remove the swab and place it in a sterile tube. Subsequently, the female clinician
19
20 examined the participants and specimens were collected with gloved hands and vaginal speculum
21
22 in place.
23
24
25

26 The samples were examined by standard bedside tests, Gram staining, wet mount and
27
28 culture (gold standard).⁸ For each patient, the first of the three self-collected swabs was used for
29
30 pH and whiff test, the second for wet mount and Gram staining, whereas the third swab was used
31
32 for Candida and Trichomonas culture. The same tests were repeated for the three physician
33
34 collected swabs. Bacterial vaginosis was diagnosed with the use of Amsel's criteria and Nugent's
35
36 score, whereas candidiasis and trichomoniasis were diagnosed based upon microscopy and
37
38 culture results.
39
40
41

42 **Patient and Public Involvement**

43
44

45 Patients were not involved in this study in developing the design, recruitment or conduction of
46
47 the study. Patients were not informed about the comparative results of the study but were given
48
49 the report of the diagnosis based on physician collected samples as per routine practice. The
50
51 result will be disseminated through this publication.
52
53
54
55
56
57
58
59
60

Statistical analysis

Data was analyzed using Microsoft excel and SPSS software version 21.0. Prevalence of bacterial, fungal and parasitic causative agents of vaginal discharge was studied in patients presenting with vaginal discharge. Sensitivity, specificity, positive predictive value and negative predictive value of self- collected specimens versus clinician-collected specimens were calculated. Concordance between results obtained from self-collected and physician collected swabs was determined by calculating the Cohen's kappa value.

The value of Kappa is defined as

$$\kappa = \frac{p_o - p_e}{1 - p_e},$$

where p_o is the observed level of agreement and p_e is the expected level of agreement. The value of κ lies between -1 and 1 . A value of 1 implies perfect agreement whereas -1 implies perfect disagreement. When the two findings agree purely by chance, the value of kappa will be zero.⁹

RESULTS

Prevalence of various aetiological agents in patients with vaginal discharge using physician collected samples

The results obtained with physician-collected specimens were treated as the "standard results". Prevalence of the three types of vaginal infections, namely BV, VVC and TV, as diagnosed by the standard tests, are summarized in Table 1. Out of the 550 patients presenting with vaginal discharge, 144 (26.2%) cases of VVC, 79 (14.4%) of BV, and 3 (0.5%) cases of TV were detected. Therefore, VVC was the most predominant infection in this population. A significant

number of VVC infections were caused by non-albican species of *Candida* i.e. 60 out of 144 (41.7%) of the total VVC patients. BV and VVC coexisted in 21 (3.8%) patients. None of the TV infected patient had any co-infection with BV or VVC.

Table 1 also shows the prevalence of BV and VVC across different age groups of vaginal discharge patients. In the age group 20 years or less, 9.1% patients were found positive for both BV and VVC. In the 20-30 years range, prevalence of both BV and VVC increased to 14% and 25.7%, respectively. In the age group 30-40 years, the prevalence of BV reduced slightly to 12.8% but the prevalence for VVC continued to increase (29.5%). For the remaining patients aged 40 years or more, the prevalence of BV sharply increased to 19.6% whereas the prevalence decreased to 20.6% for VVC.

Concordance between results obtained from self- and physician- collected samples

Cohen's Kappa was used as the metric of agreement between the two collection methods. The data related to the concordance of the two methods for diagnosis of BV, VVC and TV are depicted in Table 2.

Concordance for BV:

Diagnosis of BV was performed on the basis of Nugent's scoring. Of the total 550 participants, 376 (68.4%) showed a healthy microbiome (Nugent score 0-3), 95 (17.3%) were categorized as "intermediate" (Nugent score 4-6) and the remaining 79 (14.4%) were diagnosed as BV (Nugent score 7-10). For the Nugent score based comparison; the outcome of a diagnosis could be classified in two different ways: (a) Three categories: BV positive or BV intermediate or BV negative and (b) Two categories: BV positive or BV non-positive by putting both intermediate and negative in the same bin of "non-positive".

1
2
3 For the three-category classification, both self- and physician- collection methods agreed
4 on 367 true negative cases, 82 intermediate cases and 72 true positive cases. Out of the BV
5 negative cases, self-collection method found nine cases as intermediate but no false positives.
6
7 For cases diagnosed as intermediate by physician-collected method, 13 were found negative by
8 self-collection method but, again, no false positives were detected. However, there were seven
9 cases of true positive diagnosis which were diagnosed as intermediate by self-collected samples,
10 but no true positive cases were diagnosed as negative by self-collected samples. The kappa value
11 computed for this is 0.890 with 95% confidence interval of 0.851 to 0.928, which indicates
12 excellent agreement between self- and physician- collected samples.
13
14

15
16 For the two-category case, both methods diagnosed 471 non-positive cases and 72
17 positive cases. Self-collection method missed seven cases of BV positive results in this case, but
18 no false positive case was observed by the self-collection method. The kappa value computed for
19 this case is 0.946 with 95% confidence interval of 0.907 to 0.986 suggesting excellent agreement
20 between self- and physician- collected samples.
21
22

23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 *Concordance for VVC:*

39 The numbers of true negative and true positive cases were 406 and 144, respectively for VVC.
40 There was no missed true positive result and only one case of false positive was found with self-
41 collected samples. Very high concordance was observed with the kappa value at 0.994 with 95%
42 confidence interval of 0.982 to 1.000. Table 2 also lists the cases of *Candida albicans* and non-
43 *albicans* separately. For *C. albicans* infections, there was perfect concordance (kappa = 1.000)
44 with 84 true positive and 466 true negative cases. For non-*albicans Candida* cases, there were 60
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 true positive, 489 true negative and 1 false positive cases using self-collected swabs making the
4
5 kappa value to be 0.991 with 95% confidence interval of 0.973 to 1.000.
6
7

8 9 10 *Concordance for TV:*

11
12 This is not shown in the table as only three cases were found positive for TV and the results were
13
14 identical for self- and physician- collected samples yielding perfect concordance (kappa value of
15
16 1.000).
17
18

19 20 21 **Sensitivity, specificity, PPV and NPV of self-collected swabs in comparison to physician-** 22 23 **collected swabs for diagnosis of BV, VVC and TV**

24
25 Table 2 shows the sensitivity, specificity, PPV and NPV of diagnosis using self-collected swabs
26
27 when compared to physician-collected swabs, for BV, VVC and TV. Self-collection method had
28
29 acceptable sensitivity, specificity, PPV and NPV of 91.1%, 100%, 100% and 98.5% for
30
31 diagnosing BV using Nugent score. For VVC, including both *C. albicans* and non-*albicans*
32
33 *Candida* species, the self-collection method had high sensitivity of 100%, specificity of 99.8%,
34
35 PPV of 99.3%, and NPV of 100%. The values for non-*albicans* VVC, were identical to the
36
37 overall VVC cases except that the PPV (98.4%) was less. The sensitivity, specificity, PPV and
38
39 NPV were all 100% for self-obtained swabs as compared to physician-collected swabs for the *C.*
40
41 *albicans* VVC and for TV.
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

DISCUSSION

The prevalence of BV in women presenting with vaginal discharge to STI/RTI clinic was 14.4%. In the literature the prevalence varies widely from 10.7% to 45% [4, 10-11].^{4,10,11} In our study, we excluded all patients above 45 years of age, pregnant women and HIV patients. This difference in the exclusion criteria is one of the causes of the varying prevalence rates particularly because prevalence of BV increases with age and immune-deficient conditions. Additionally, geographical locations and cultural practices often have significant impact on the prevalence rates.

The prevalence for VVC was 26.2% in this study, which agrees well with the prevalence of other studies.^{12,13} However, much smaller prevalence rates of 2.8% to 8.5% were reported in other studies.^{11,14} Out of the 144 VVC positive cases, a significant number of isolates (60 or 41.7%) were due to non-*albican* *Candida* species. This conforms to the increasing trend of non-*albicans* infections observed in the recent studies.¹⁵ Clinical implication of these non-*albican* *Candida* species is their documented decreased susceptibility to azoles, due to the indiscriminate use of azole group of antifungals, especially fluconazole.¹⁶ Thus, early and accurate species identification would be useful for the therapeutic management.

The prevalence of TV was quite low at 0.5% and was close to the values reported in a few studies,^{12,13} but higher prevalence rates ranging from 5.6% to 16.1% were reported in other studies.^{5,11,17} The higher prevalence rates were mostly observed in studies employing molecular techniques such as nucleic acid amplification techniques for detecting TV as compared to conventional culture technique used in this work. Additionally, variation in geographical locations can contribute to the difference, as pointed out in.¹⁸

1
2
3 Prevalence of BV among different age groups showed an increasing trend with age. The
4 vaginal pH is dependent on the amount of lactic acid produced by the vaginal lactobacilli from
5 glycogen. The glycogen production from the superficial cornified cells of the vaginal mucosa is
6 dependent on estrogen stimulation, which is high during the reproductive age group and
7 decreases with age. This increasing vaginal pH increases the susceptibility of aging women to
8 BV.¹⁹ Similar observation was made for VVC up to the age group of 30-40 years. However, the
9 percentage dropped for the population above 40 years. This dip in prevalence can be attributed to
10 normally decreasing sexual activities at this age group.²⁰ The above findings are in agreement
11 with the findings of another study.¹³
12
13
14
15
16
17
18
19
20
21
22
23

24 In the present study, in general the kappa values were significantly higher than the values
25 reported in similar studies. For diagnosis of BV, the Nugent score based method with two and
26 three categories had kappa values of 0.946 and 0.89, respectively, which are significantly higher
27 than kappa values of 0.71 and 0.72, correspondingly, reported in Strauss et al.¹⁰ In a similar
28 study performed by Huppert et al.,⁴ the reliability of self-collected vaginal swabs was
29 established, albeit with a value of kappa of 0.53. This can be attributed to the use of vaginal pH
30 as the only indicator of BV, instead of a more comprehensive Nugent score as used in this work.
31
32
33
34
35
36
37
38
39

40 A near-perfect concordance was observed in cases of VVC, with a kappa value of 0.995.
41 In VVC cases, self-collected samples produced one false positive result as compared to
42 physician-collected sample. This can possibly be attributed to the contamination of the swab with
43 skin commensal flora during self-collection or it may be because of scanty discharge; where after
44 self-collection not enough sample was left for collection by the physician.
45
46
47
48
49
50

51 A perfect match (kappa=1) between two methods was observed in this study for TV infections
52 with a small number of positive cases (three positive cases out of 550).
53
54
55
56
57
58
59
60

1
2
3 The current study findings highlight that near-perfect match was observed between self-
4 collected and physician collected swabs for diagnosis of BV, VVC and TV and it was proven by
5 the high values of kappa (all greater than 0.9). The high concordance can partly be attributed to
6
7 the following reasons:
8
9

- 10
11
12 (1) *Mature patients*: Almost 98% of the patients were of age 20 years or older with average age
13 of 32 years. 96% of the population was married at some point. The maturity helped the
14 patients follow the instructions and insert the swab properly in their genitalia for collecting
15 the sample.
16
17 (2) *Sample collection under supervision*: In the clinic, the patients were under supervision and
18 were provided with clarifications whenever in doubt. However, most of the patients were
19 able to collect the sample without further help post-verbal instructions.
20
21 (3) *No delay in transportation of samples to the laboratory*: The samples were collected by both
22 the methods inside the STI/RTI clinic and were transported to the STI laboratory
23 immediately, which is located very near to the clinic. Specimens were processed immediately
24 in the laboratory. Therefore, no transportation delay, sample labeling errors and sample
25 contamination occurred leading to high concordance between the two methods.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41

42 Even when used in resource constrained peripheral areas, self-collected swabs can perform
43 really well if mislabeling and transport error can be minimized and online or other forms of
44 assistance can be provided in case of doubts. The proliferation of mobile phones even in remote
45 corners of India can facilitate such online assistance. From this study, it was established beyond
46
47 doubt that self-collected swabs yield diagnostic results as accurate as physician-collected swabs
48
49 for almost all practical purposes.
50
51
52
53
54
55
56
57
58
59
60

CONCLUSION

It was demonstrated that with specific instructions and guidance, self-obtained swabs can approximate physician-obtained swabs with a high degree of reliability. Therefore, self-collected vaginal swabs are a viable and accurate method for diagnosing vaginal infections, which may have adverse outcomes including preterm birth, low birth weight, post-operative infections and increased risk of acquisition and transmission of STIs including the HIV infection. Hence, prevention and timely management of curable STIs and RTIs is particularly important. Thus, the findings of this study will help in planning and implementing the diagnostic approaches for STIs/RTIs in community based surveillance studies at national or regional level and also in the effective day-to-day STI/RTI diagnosis and management in the peripheral health settings.

Contributors

MB, ZK, AB designed the study, coordinated the work and finalized the draft of the manuscript. ZK participated in all the data collection, testing, carried out analysis of data and prepared first draft of the manuscript. PM, RB, PP, NK helped in designing the study, sample collection and collaborated in writing of the manuscript. Guarantor of the article: MB.

Ethical approval

The study was approved the Institute Ethics Committee of VMMC & Safdarjung hospital with approval number IEC/VMMC/SJH/Thesis/November-2014/429 and date 25 November 2014.

Acknowledgements

We thank the Medical Superintendent and Principal, VMMC & Safdarjung Hospital for permitting us to carry out this study. The authors are thankful to the Head, Department of

Microbiology and Apex Regional STD Teaching, Training & Research Centre for granting permission to Dr. Zarine Khan to carry out her thesis work. We are grateful to Mrs. Ranjana Gupta and Ms. Hemlata Saxena for their technical assistance.

Competing interests None.

Patient consent Obtained.

Funding There was no funding for the study from any funding agency.

Data sharing statement: There is no additional data available.

REFERENCES

1. **Fule SR**, Fule RP, Tankhiwale NS. Clinical and laboratory evidence of *Trichomonas vaginalis* infection among women of reproductive age in rural area. *Indian J Med Microbiol* 2012;**30**:314.
2. **Singh RH**, Zenilman JM, Brown KM, *et al*. The role of physical examination in diagnosing common causes of vaginitis: a prospective study. *Sex Transm Infect* 2013;**89**:185–90.
3. **Forney LJ**, Gajer P, Williams CJ, *et al*. Comparison of self-collected and physician-collected vaginal swabs for microbiome analysis. *J Clin Microbiol* 2010;**48**:1741–8.
4. **Huppert JS**, Hesse EA, Bernard MC, *et al*. Accuracy and trust of self-testing for bacterial vaginosis. *J Adolesc Health* 2012;**51**:400–5.
5. **Garrow SC**, Smith DW, Harnett GB. The diagnosis of chlamydia, gonorrhoea, and trichomonas infections by self-obtained low vaginal swabs, in remote northern Australian clinical practice. *Sex Transm Infect* 2002;**78**:278–81.
6. **Knox J**, Tabrizi SN, Miller P, *et al*. Evaluation of self-collected samples in contrast to practitioner-collected samples for detection of *Chlamydia trachomatis*, *Neisseria*

- 1
2
3 *gonorrhoeae*, and *Trichomonas vaginalis* by polymerase chain reaction among women living
4 in remote areas. *Sex Transm Dis* 2002;**29**:647–54.
- 5
6
7
8 7. **Tanksale VS**, Sahasrabhojane M, Patel V, *et al*. The reliability of a structured examination
9 protocol and self administered vaginal swabs: a pilot study of gynaecological outpatients in
10 Goa, India. *Sex Transm Infect* 2003;**79**:251–3.
- 11
12
13
14
15 8. **Department of AIDS control**, Ministry of Health and Family Welfare, Government of
16 India. Laboratory Manual for Diagnosis of Sexually Transmitted and Reproductive Tract
17 Infections. February 2014. Available from: URL: [http://www.indiahivinfo.naco.gov.in/sites/
18 default/files/media-gallery/STI_Report.pdf](http://www.indiahivinfo.naco.gov.in/sites/default/files/media-gallery/STI_Report.pdf).
- 19
20
21
22
23
24 9. **Merrill RM**. Fundamentals of Epidemiology and Biostatistics. Jones & Bartlett Publishers,
25 2012. p 167-8.
- 26
27
28
29 10. **Strauss RA**, Eucker B, Savitz DA, Thorp JM. Diagnosis of bacterial vaginosis from self-
30 obtained vaginal swabs. *Infect Dis Obstet Gynecol* 2005;**13**:31–5.
- 31
32
33
34 11. **Passos MR**, Varella RQ, Barreto NA, *et al*. Accuracy of a self-collection kit for the
35 microbiological study of the vaginal content. *Braz J Infect Dis* 2007;**11**:249–53.
- 36
37
38
39 12. **Puri, KJ**, Madan, A, Bajaj, K. Incidence of various causes of vaginal discharge among
40 sexually active females in age group 20-40 years. *Indian J Dermatol Venereol Leprol*
41 2003;**69**:122.
- 42
43
44
45 13. **Gandhi TN**, Patel MG, Jain MR. Prospective study of vaginal discharge and prevalence of
46 Vulvovaginal candidiasis in a tertiary care hospital. *Int J Curr Res Rev* 2015;**7**:34-8.
- 47
48
49
50 14. **Pereira DC**, Backes LT, Calil LN, *et al*. A six-year epidemiological survey of
51 vulvovaginal candidiasis in cytopathology reports in the state of Rio Grande do Sul, Brazil.
52 *Rev Patol Trop* 2012;**41**:163-8.
- 53
54
55
56
57
58
59
60

- 1
2
3 15. **Muzny CA**, Sunesara IR, Kumar R, *et al.* Characterization of the vaginal microbiota among
4 sexual risk behavior groups of women with bacterial vaginosis. *PloS One* 2013;**8**:e80254.
5
6
7
8 16. **Tellapragada C**, Eshwara VK, Johar R, *et al.* Antifungal susceptibility patterns, in vitro
9 production of virulence factors, and evaluation of diagnostic modalities for the speciation of
10 pathogenic candida from blood stream infections and vulvovaginal candidiasis. *J Pathog*
11 2014;**14**:2864.
12
13
14
15
16
17 17. **Holland-Hall CM**, Wiesenfeld HC, Murray PJ. Self-collected vaginal swabs for the
18 detection of multiple sexually transmitted infections in adolescent girls. *J Pediatr Adolesc*
19 *Gynecol* 2002;**15**:307–13.
20
21
22
23
24 18. **Malla NA**, Gupta IN, Mahajan RC. Human trichomoniasis. *Indian J Med Microbiol*
25 2001;**19**:6-13.
26
27
28
29 19. **Padubidri VG**, Daftary SN. Shaw's Textbook of gynecology. 16th ed. New Delhi: Elsevier
30 Health Sciences; 2014:379-90.
31
32
33 20. **Jindal N**, Gill P, Aggarwal A. An epidemiological study of vulvovaginal candidiasis in
34 women of childbearing age. *Indian J Med Microbiol* 2007;**25**:175.
35
36
37

38 **Key messages**

- 39 • This study has shown that VVC was the most prevalent (26.2%) cause of vaginal
40 discharge, followed by BV (14.4%) and TV (0.5%).
41
42
- 43 • High values of Cohen's Kappa were obtained for all three infections: 0.95 (BV), 0.99
44 (VVC) and 1.0 (TV).
45
46
- 47 • High concordance of self-collected swabs with physician-collected swabs proves the
48 efficacy of self-collected swabs in diagnosing the major causes of vaginal discharge, with
49 high sensitivity and specificity.
50
51
52
53
54
55
56
57
58
59
60

- This will also help in early diagnosis and management of patients in resource-constrained and peripheral settings thereby strengthening National STI/RTI control programs worldwide.

For peer review only

Table 1 Prevalence of various types of infections in patients with vaginal discharge

Age group	Number of patients with type of infection					Total N (%)
	BV N (%)	VVC N (%)		TV N (%)		
		<i>C. albicans</i>	Non- <i>albicans</i> <i>Candida</i> species	Total VVC		
<20	1 (9.1)	1 (9.1)	0 (0.0)	1 (9.1)	0 (0.0)	2 (18.2)
20-30	25 (14.0)	32 (17.9)	14 (7.8)	46 (25.7)	1 (0.6)	64 (35.8)
31-40	33 (12.8)	40 (15.5)	36 (14.0)	76 (29.5)	1 (0.4)	101 (39.1)
>40	20 (19.6)	11 (10.8)	10 (9.8)	21 (20.6)	1 (1.0)	38 (37.3)
Total	79 (14.4)	84 (15.3)	60 (10.9)	144 (26.2)	3 (0.5)	205 (37.3)

TABLE 2 Concordance between physician- and self- collected swabs for diagnosis of bacterial vaginosis, vulvovaginal candidiasis and trichomonas vaginitis. Outcomes are categorized with combination of “P” and “S” (representing Physician- and Self- collected, respectively) with “+”, “-” and “i” for positive, negative and intermediate, respectively. Example: P+/Si represents the cases where the diagnosis was positive for physician-collected and intermediate for self-collected samples.

Criterion Used	Number of patients (physician-collected versus self-collected)									Kappa (95% CI)	Prevalence	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	
	P+/S+	P+/Si	P+/S-	Pi/S+	Pi/Si	Pi/S-	P-/S+	P-/Si	P-/S-							
Bacterial vaginosis																
Nugent – 3 category	72	7	0	0	82	13	0	9	367	0.890 (0.851 -0.928)	14.4	91.1	100	100	98.5	
Nugent – 2 category	72	–	7	–	–	–	0	–	471	0.946 (0.907 -0.986)	14.4	91.1	100	100	98.5	
Vulvovaginal candidiasis																
<i>C.albicans</i>	84	–	0	–	–	–	0	–	466	1.000 (1.000 -1.000)	15.3	100	100	100	100	
Non-albicans	60	–	0	–	–	–	1	–	489	0.991 (0.973 -1.000)	10.9	100	99.8	98.4	100	
All	144	–	0	–	–	–	1	–	405	0.995 (0.986 -1.000)	26.2	100	99.8	99.3	100	
Trichomonas vaginitis																
TV culture	3	–	0	–	–	–	0	0	547	1.000 (1.000 -1.000)	0.5	100	100	100	100	
PPV, Positive predictive value; NPV, Negative predictive value.																

Section & Topic	No	Item	Reported on page
TITLE OR ABSTRACT		STARD 2015 Research check-list	
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2-3
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4-5
	4	Study objectives and hypotheses	5
METHODS			
<i>Study design</i>	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
<i>Participants</i>	6	Eligibility criteria	5-6
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	5-6
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5-6
	9	Whether participants formed a consecutive, random or convenience series	5-6
<i>Test methods</i>	10a	Index test, in sufficient detail to allow replication	6
	10b	Reference standard, in sufficient detail to allow replication	6
	11	Rationale for choosing the reference standard (if alternatives exist)	6
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	6
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	6
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	6
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	6
<i>Analysis</i>	14	Methods for estimating or comparing measures of diagnostic accuracy	6-7
	15	How indeterminate index test or reference standard results were handled	N/A
	16	How missing data on the index test and reference standard were handled	N/A
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	6
	18	Intended sample size and how it was determined	5
RESULTS			
<i>Participants</i>	19	Flow of participants, using a diagram	N/A
	20	Baseline demographic and clinical characteristics of participants	7
	21a	Distribution of severity of disease in those with the target condition	NA
	21b	Distribution of alternative diagnoses in those without the target condition	7-8
	22	Time interval and any clinical interventions between index test and reference standard	7-9
<i>Test results</i>	23	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Table 1 & 2 19-20
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	8-10
	25	Any adverse events from performing the index test or the reference standard	Nil
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	10
	27	Implications for practice, including the intended use and clinical role of the index test	11-13
OTHER INFORMATION			
	28	Registration number and name of registry	N/A
	29	Where the full study protocol can be accessed	6
	30	Sources of funding and other support; role of funders	15

BMJ Open

Evaluation of reliability of self-collected vaginal swabs over physician-collected samples for diagnosis of bacterial vaginosis, candidiasis and trichomoniasis

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-025013.R1
Article Type:	Research
Date Submitted by the Author:	08-Apr-2019
Complete List of Authors:	Khan, Zarine; VMMC & Safdarjung Hospital, Department of Microbiology Bhargava, Aradhana; VMMC and Safdarjung Hospital, Apex Regional STI Training, Research & Reference Laboratory Mittal, Pratima; VMMC & Safdarjung Hospital, Department of Obstetrics & Gynaecology Bharti, Rekha; VMMC & Safdarjung Hospital, Department of Obstetrics & Gynaecology Puri, Poonam; VMMC and Safdarjung Hospital, Department of Dermatology & STD Khunger, Niti; VMMC and Safdarjung Hospital, Department of Dermatology & STD Bala, Manju; VMMC and Safdarjung Hospital, Apex Regional STI Training, Research & Reference Laboratory
Primary Subject Heading:	Sexual health
Secondary Subject Heading:	Reproductive medicine, Epidemiology
Keywords:	Microbiology < BASIC SCIENCES, GENITOURINARY MEDICINE, Diagnostic microbiology < INFECTIOUS DISEASES

SCHOLARONE™
Manuscripts

1
2
3 **Evaluation of reliability of self-collected vaginal swabs over physician-collected samples for**
4 **diagnosis of bacterial vaginosis, candidiasis and trichomoniasis, in a resource limited**
5 **setting.**
6
7
8
9

10
11
12 **Zarine Khan^{1,2}, Aradhana Bhargava ^{1,2}, Pratima Mittal³, Rekha Bharti³, Poonam Puri⁴,**
13 **Niti Khunger⁴, Manju Bala ^{1,2*}.**
14
15
16

17
18
19 ¹Department of Microbiology, VMMC & Safdarjung Hospital, New Delhi, India, ²Apex
20 Regional STI Training, Research & Reference Laboratory, VMMC & Safdarjung Hospital, New
21 Delhi, India, ³Department of Obstetrics & Gynaecology, VMMC & Safdarjung Hospital, New
22 Delhi, India, ⁴Department of Dermatology & STD, VMMC & Safdarjung Hospital, New Delhi,
23 India.
24
25
26
27
28
29
30
31
32

33 **Correspondence:**

34
35 Dr. Manju Bala, Consultant and Professor (Microbiology), Apex Regional STI Training,
36 Research & Reference Laboratory, Vardhman Mahavir Medical College & Safdarjung Hospital,
37 New Delhi, India. Telephone number: 91-11-26196740, Fax number: 91-11-26163072, Email:
38 manjubala_2@hotmail.com
39
40
41
42
43
44

45
46
47 **Key words:** Vaginal discharge; bacterial vaginosis; vulvovaginal candidiasis; trichomonas
48 vaginitis, self-collected vaginal swabs; physician-collected vaginal swabs.
49
50
51
52

53 **Word count: 2776**
54
55
56
57
58
59
60

ABSTRACT

Objectives Self-collected vaginal swabs can facilitate diagnosis of Vaginal discharge (VD) in resource-limited settings, provided reliability of the method is established. The aim of this study was to evaluate the concordance between self-obtained and clinician-collected vaginal swabs for etiological diagnosis of VD. and to determine the prevalence of bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis (TV).

Methods A total of 550 females (median age: 32 years; range: 18-45 years) attending two STI/RTI clinics with VD from January 2015 to May 2016 were included in the study after obtaining written informed consent. Swabs were self-collected by patients after instructions and subsequently by a physician under speculum examination. Samples were processed for standard bedside tests, Gram staining, wet mount and culture (gold standard) according to national guidelines. Concordance between the two methods was determined by the Cohen's kappa value.

Results BV, VVC and TV were diagnosed in 79(14.4%), 144(26.2%) and 3(0.5%) patients respectively. VVC coexisted with BV in 58(10.5%) patients. There was no co-infection of TV with BV or VVC. *C. albicans* was isolated in 84(58.3%) VVC cases. Sensitivity, specificity, PPV and NPV of self-collected swabs for diagnosing BV was 91.1%, 100%, 100% and 98.5% respectively while for the *C. albicans* VVC and TV, sensitivity, specificity, PPV and NPV all were 100% as compared to physician-collected swabs. Highly concordant results were obtained between two methods by the Kappa values of 0.95 (BV), 0.99 (VVC) and 1.0 (TV).

Conclusion The comparative performance of self- and physician-collected vaginal swabs establishes self-collection of samples for BV, VVC and TV as a viable alternative for management of sexually transmitted infections/ reproductive tract infections (STIs/RTIs) especially in peripheral and resource-constrained settings. This would be effective in

1
2
3 implementing the diagnostic approaches for STIs/RTIs in community based surveillance studies
4
5 at national or regional level and therefore strengthening the National STI/RTI Control
6
7 Programme.
8
9

11 ARTICLE SUMMARY

14 Strengths and limitations of this study

- 17 • This study determined that if diagnosis based on self-collected vaginal swabs is proven
18 reliable, it could contribute to early diagnosis and greatly increase the access to treatment.
- 21 • Specimens were obtained from 550 patients with vaginal discharge attending the two
22 STI/RTI clinics after obtaining the written informed consent. Concordance of
23 inexpensive standard bedside tests, such as Gram staining, wet mount and culture (gold
24 standard), was evaluated on both the self- and physician- collected samples for detecting
25 bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis
26 (TV).
27
- 28 • Corresponding findings were compared and analyzed using SPSS statistical analysis tool
29 in terms of Cohen's Kappa values to determine the concordance between findings of self-
30 and physician- collected samples.
31
- 32 • Limitation is that molecular tests were not performed, although gold standard test culture
33 was performed which is more economical and less labour intensive also.
34
- 35 • Another limitation was the low positivity of *Trichomonas vaginalis* in patients of vaginal
36 discharge by culture.
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

INTRODUCTION

Vaginal discharge is a frequently encountered complaint in women attending Sexually Transmitted Infections (STIs)/Reproductive Tract Infections (RTIs) clinics globally. It is mainly caused by curable bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis (TV).¹ Existing practice to diagnose vaginal discharge includes a speculum examination where the clinician inspects the external genitalia, vagina and cervix, assesses characteristics of the discharge, notes physical signs such as inflammation and lesions, and collects specimens for laboratory testing. The speculum examination requires a trained clinician and a proper setup. The discomfort of a pelvic examination may lead women to delay or avoid seeking care, which may result in lower enrollment rates and higher rates of loss to follow-up.^{2,3}

Even though high rates of STIs/RTIs are observed in developing countries due to various social and environmental factors such as sex ratio imbalances, urbanization, rural to urban migration and poor hygiene ; reliable detection of these infections are difficult due to poor infrastructure and lack of skilled workforce. Additionally, there is low female STI enrollment rate in developing countries due to women's reluctance to undergo gynecological examination, originating from cultural, religious and socio-economic factors.

Therefore, developing accurate approaches to diagnosing lower genital infections without a speculum examination would be advantageous to both clinicians and patients.² Self-collected vaginal swabs are the only practical and financially feasible method to use for sampling in field-based longitudinal cohort studies.³ As most of the studies comparing the reliability of self-collected vaginal swabs have been conducted in developed countries such as USA^{3,4,5} and Australia^{6,7} mostly using advanced molecular techniques such as nucleic acid amplification tests, limited literature on such studies is available from developing countries. In India, there was a

1
2
3 pilot study from Goa, which suggested that self-collected swabs are an acceptable method of
4 collection of vaginal specimens in women attending gynaecological clinics in India.⁸ However,
5 the main limitation of the study was a statistically small sample size. Moreover, the samples
6 were examined only by Gram's stain for their sensitivity for the diagnosis of STIs and were not
7 tested by other diagnostic techniques like wet mount and culture. Another study was performed
8 in New Delhi,⁹ which showed that self-collected swabs can reasonably approximate physician-
9 collected swabs for BV only. However, this study also was limited by a statistically small sample
10 size.
11
12
13
14
15
16
17
18
19
20
21

22 Hence, the current study was aimed at establishing reliability of self- collected vaginal
23 swabs against physician-collected swabs by determining its sensitivity, specificity, positive
24 predictive value (PPV) and negative predictive value (NPV) with significant number of samples
25 and quality reliable tests. Additionally, the fungal (*Candida albicans* or non-*albicans Candida*
26 species) bacterial (Bacterial vaginosis) and parasitic (*Trichomonas vaginalis*) etiology of vaginal
27 discharge and prevalence of various types of infections and co-infections were also studied.
28
29
30
31
32
33
34
35
36
37

38 METHODS

39
40 The study was conducted in two National AIDS Control Organization (NACO) designated
41 STI/RTI clinics in the Apex Regional STD Teaching, Training and Research Centre and
42 Department of Obstetrics and Gynaecology, Vardhaman Mahavir Medical College and
43 Safdarjung Hospital, New Delhi, India during January 2015 to May 2016. The thesis protocol
44 was approved by the authors' Institute Ethics Committee (VMMC & SJH). Sample size was
45 calculated according to the following formula: $S = \frac{4P(1-P)}{L^2}$ (where S = Sample size, P = Estimated
46 prevalence, L = Margin of error at 5%). Accordingly, samples were collected from 550 sexually
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 active females with vaginal discharge. After explaining the study details to the potentially
4 eligible participants with the help of a pre-designed patient information sheet; a written informed
5 consent was obtained on patient consent form. Both the patient information sheet and the consent
6 form were designed in english and hindi (local language). Patients with history of antibiotic use
7 or vaginal medication in the previous 14 days, pregnant patients, patients not willing to
8 participate were excluded from this study, patients with human immunodeficiency virus (HIV)
9 infection and other serious illness or disability were excluded from this study.
10
11
12
13
14
15
16
17
18

19 A total of six vaginal swabs, including three self-collected samples, were collected from
20 each participant. Participants were given instructions on appropriate specimen collection
21 technique before the speculum examination. They were instructed to insert the vaginal swab 1 to
22 2 inches into the vagina, twisting the swab to collect material on all sides of the tip, wipe in
23 several full circles on the vaginal wall, keep the swab in the vagina for 20 seconds, and then
24 carefully remove the swab and place it in a sterile tube. Subsequently, the female clinician
25 examined the participants and specimens were collected following the same procedure with
26 gloved hands under speculum examination. Proper indexing of the samples were performed and
27 the examining microbiologist was blind regarding the origin (physician- or self- collected) of the
28 swabs.
29
30
31
32
33
34
35
36
37
38
39
40
41

42 The samples were examined by standard bedside tests, Gram staining, wet mount and
43 culture (gold standard).¹⁰ For each patient, the first of the three self-collected swabs was used for
44 pH and whiff test, the second for wet mount and Gram staining, whereas the third swab was used
45 for Candida and Trichomonas culture. The same tests were repeated for the three physician
46 collected swabs. Bacterial vaginosis was diagnosed with the use of Amsel's criteria¹¹ and
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Nugent's score¹¹, whereas candidiasis and trichomoniasis were diagnosed based upon
4
5 microscopy and culture results.
6
7

8 9 **Patient and Public Involvement**

10
11 Patients were not involved in this study in developing the design, recruitment or conduction of
12
13 the study. Patients were not informed about the comparative results of the study but were given
14
15 the report of the diagnosis based on physician collected samples as per routine practice. The
16
17 result will be disseminated through this publication.
18
19
20
21
22
23

24 **Statistical analysis**

25
26 Data was analyzed using Microsoft excel and SPSS software version 21.0. Prevalence of
27
28 bacterial, fungal and parasitic causative agents of vaginal discharge was studied in patients
29
30 presenting with vaginal discharge. Also, their prevalence in various age groups was statistically
31
32 analyzed using t-test (two- tailed). Sensitivity, specificity, positive predictive value and negative
33
34 predictive value of self-collected specimens versus physician-collected specimens were
35
36 calculated. Concordance between results obtained from self-collected and physician collected
37
38 swabs was determined by calculating the Cohen's kappa value.
39
40
41

42 The value of Kappa is defined as

$$43 \kappa = \frac{p_o - p_e}{1 - p_e},$$

44
45 where p_o is the observed level of agreement and p_e is the expected level of agreement. The value
46
47 of κ lies between -1 and 1. A value of 1 implies perfect agreement whereas -1 implies perfect
48
49 disagreement. When the two findings agree purely by chance, the value of kappa will be zero.¹²
50
51
52
53
54
55
56
57
58
59
60

RESULTS

Prevalence of BV, VVC and TV in patients with vaginal discharge using physician collected samples

The results obtained with physician-collected specimens were treated as the “standard results”. Prevalence of the three types of vaginal infections, namely BV, VVC and TV, as diagnosed by the Nugent score, candida culture and trichomonas culture respectively, are summarized in Figure 1. Out of the 550 patients presenting with vaginal discharge, 144 (26.2%) cases of VVC, 79 (14.4%) of BV, and 3 (0.5%) cases of TV were detected. Therefore, VVC was the most predominant infection in this population. A significant number of VVC infections were caused by non-albican species of *Candida* i.e. 60 out of 144 (41.7%) of the total VVC patients. BV and VVC coexisted in 21 (3.8%) patients. None of the TV infected patient had any co-infection with BV or VVC.

Table 1 analyses prevalence of various infections in different age groups. Maximum number of infections were found in the age group of 31-40 years (101/258) followed by 20-30 years (64/179) and >40 years (38/102). Only 2/11 participants less than 20 years of age were infected. In the age group 20 years or less, 9.1% (1/11) patients were found positive for BV and as well as for VVC. Among the 179 participants in the 20-30 years range, prevalence of both BV and VVC increased to 14% and 25.7%, respectively. In the age group 30-40 years (n=258), the prevalence of BV reduced slightly to 12.8% but the prevalence for VVC continued to increase (29.5%). For the remaining patients aged 40 years or more (n=102), the prevalence of BV sharply increased to 19.6% whereas the prevalence decreased to 20.6% for VVC. When the

1
2
3 prevalence of BV and VVC across different age groups of vaginal discharge patients was
4 analyzed by paired t- test (two tailed), it was observed that the difference was not significant.
5
6
7
8
9

10 **Concordance between results from self- and physician- collected samples**

11 Cohen's Kappa was used as the metric of agreement between the two collection methods. The
12 data related to the concordance of the two methods for diagnosis of BV, VVC and TV are
13 depicted in Figure 2 and Table 2.
14
15
16
17
18
19
20
21

22 *Concordance for BV:*

23
24 Diagnosis of BV was performed on the basis of Nugent's scoring. Of the total 550 participants,
25 376 (68.4%) showed a healthy vaginal flora (Nugent score 0-3), 95 (17.3%) were categorized as
26 "intermediate" (Nugent score 4-6) and the remaining 79 (14.4%) were diagnosed as BV (Nugent
27 score 7-10) based upon findings of physician collected swabs. For the Nugent score based
28 comparison; the outcome of a diagnosis could be classified in two different ways: (a) Three
29 categories: BV positive or BV intermediate or BV negative and (b) Two categories: BV positive
30 or BV non-positive by putting both intermediate and negative in the same bin of "non-positive".
31
32
33
34
35
36
37
38
39

40 For the three-category classification, both self- and physician- collection methods agreed
41 on 367 true negative cases, 82 intermediate cases and 72 true positive cases. Out of the BV
42 negative cases, self-collection method found nine cases as intermediate but no false positives.
43 For cases diagnosed as intermediate by physician-collected method, 13 were found negative by
44 self-collection method but, again, no false positives were detected. However, there were seven
45 cases of true positive diagnosis which were diagnosed as intermediate by self-collected samples,
46 but no true positive cases were diagnosed as negative by self-collected samples. The kappa value
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 computed for this is 0.890 with 95% confidence interval of 0.851 to 0.928, which indicates
4 excellent agreement between self- and physician- collected samples. The weighted kappa value
5 was 0.921 for this category.
6
7
8
9

10 For the two-category case, both methods diagnosed 471 non-positive cases and 72
11 positive cases. Self-collection method missed seven cases of BV positive results in this case, but
12 no false positive case was observed by the self-collection method. The kappa value computed for
13 this case is 0.946 with 95% confidence interval of 0.907 to 0.986 suggesting excellent agreement
14 between self- and physician- collected samples.
15
16
17
18
19
20
21
22
23

24 *Concordance for VVC:*

25
26 The numbers of true negative and true positive cases were 406 and 144, respectively for VVC.
27 There was no missed true positive result and only one case of false positive was found with self-
28 collected samples. Very high concordance was observed with the kappa value at 0.994 with 95%
29 confidence interval of 0.982 to 1.000. Table 2 also lists the cases of *Candida albicans* and non-
30 *albicans* separately. For *C. albicans* infections, there was perfect concordance (kappa = 1.000)
31 with 84 true positive and 466 true negative cases. For non-*albicans Candida* cases, there were 60
32 true positive, 489 true negative and 1 false positive cases using self-collected swabs making the
33 kappa value to be 0.991 with 95% confidence interval of 0.973 to 1.000.
34
35
36
37
38
39
40
41
42
43
44
45
46

47 *Concordance for TV:*

48
49 Only three cases were found positive for TV and the results were identical for self- and
50 physician- collected samples yielding perfect concordance (kappa value of 1.000).
51
52
53
54
55
56
57
58
59
60

Sensitivity, specificity, PPV and NPV of self-collected swabs in comparison to physician-collected swabs

Table 2 shows the sensitivity, specificity, PPV and NPV of diagnosis using self-collected swabs when compared to physician-collected swabs, for BV, VVC and TV. Self-collection method had acceptable sensitivity, specificity, PPV and NPV of 91.1%, 100%, 100% and 98.5% for diagnosing BV using Nugent score. For VVC, including both *C. albicans* and non-*albicans Candida* species, the self-collection method had high sensitivity of 100%, specificity of 99.8%, PPV of 99.3%, and NPV of 100%. The values for non-*albicans* VVC, were identical to the overall VVC cases except that the PPV (98.4%) was less. The sensitivity, specificity, PPV and NPV were all 100% for self-collected swabs as compared to physician-collected swabs for the *C. albicans* VVC and for TV.

DISCUSSION

The prevalence of BV in women presenting with vaginal discharge to STI/RTI clinic was 14.4%. In the literature the prevalence varies widely from 10.7% to 45%.^{4,13,14} In our study, we excluded all patients above 45 years of age, pregnant women and HIV patients. This difference in the exclusion criteria is one of the causes of the varying prevalence rates particularly because prevalence of BV increases with age and immune-deficient conditions. More importantly, geographical locations and cultural practices often have significant impact on the prevalence rates.

The prevalence for VVC was 26.2% in this study, which agrees well with the prevalence of other studies.^{15,16} However, much smaller prevalence rates of 2.8% to 8.5% were reported in

1
2
3 In the present study, in general the kappa values were remarkably higher than the values
4 reported in similar studies. For diagnosis of BV, the Nugent score based method with two and
5 three categories had kappa values of 0.946 and 0.89, respectively, which are much higher than
6 kappa values of 0.71 and 0.72, correspondingly, reported in Strauss et al.¹³ In a similar study
7 performed by Huppert et al.,⁴ the reliability of self-collected vaginal swabs was established,
8 albeit with a value of kappa of 0.53. This can be attributed to the use of vaginal pH as the only
9 indicator of BV, instead of a more comprehensive Nugent score as used in this work.

10
11
12 A near-perfect concordance was observed in cases of VVC, with a kappa value of 0.995.
13 In VVC cases, self-collected samples produced one false positive result as compared to
14 physician-collected sample. This can possibly be attributed to the contamination of the swab with
15 skin commensal flora during self-collection or it may be because of scanty discharge; where after
16 self-collection not enough sample was left for collection by the physician. One of the limitation
17 of the study was that the self and physician collection was not alternated for every consecutive
18 patients. But the high agreement in both the methods negates the influence of this factor on the
19 results.

20
21
22 A perfect match (kappa=1) between two methods was observed in this study for TV
23 infections with a small number of positive cases (three positive cases out of 550). In order to
24 maintain the uniformity in data presentation the kappa value was calculated inspite of the low
25 positivity.

26
27
28 Thus, the current study findings highlight that near-perfect match was observed between self-
29 collected and physician collected swabs for diagnosis of BV, VVC and TV and it was proven by
30 the high values of kappa (all greater than 0.9). The high concordance can partly be attributed to
31 the following reasons:

- 1
2
3 (1) *Mature patients*: Almost 98% of the patients were of age 20 years or older with average age
4 of 32 years. 96% of the population was married at some point, which helped the patients to
5 insert the swab properly in their genitalia for collecting the sample.
6
7
8
9
10 (2) *Sample collection under supervision*: In the clinic, the patients were under supervision and
11 were provided with clarifications whenever in doubt. However, most of the patients were
12 able to collect the sample without further help post-verbal instructions.
13
14
15
16
17 (3) *No delay in transportation of samples to the laboratory*: The samples were collected by both
18 the methods inside the STI/RTI clinic and were transported to the STI laboratory
19 immediately, which is located very near to the clinic. Specimens were processed immediately
20 in the laboratory. Therefore, no transportation delay, sample labeling errors and sample
21 contamination occurred leading to high concordance between the two methods.
22
23
24
25
26
27
28
29
30

31 Even when used in resource constrained peripheral areas, self-collected swabs can perform
32 really well if mislabeling and transport error can be minimized and online or other forms of
33 assistance can be provided in case of doubts. The proliferation of mobile phones even in remote
34 corners of India can facilitate such online assistance. From this study, it was established beyond
35 doubt that self-collected swabs yield diagnostic results as accurate as physician-collected swabs
36 for almost all practical purposes.
37
38
39
40
41
42
43
44
45
46

47 When replicated in peripheral resource constrained settings, self-collected swabs would
48 provide alternative method to patients who refrain from getting gynaecological examination
49 either due to social or cultural misconceptions. An early and accurate diagnosis based on
50 inexpensive standard would make testing more approachable, economical and would improve the
51
52
53
54
55
56
57
58
59
60

1
2
3 treatment outcomes. When integrated with proper quality assurance, self-collected swabs may
4 form an important diagnostic tool in community based studies. A limitation of this study is that
5
6 molecular tests were not performed, although gold standard test culture was performed which is
7
8 more economical and less labour intensive also.
9
10

11 12 13 14 **CONCLUSION**

15
16 It was demonstrated that with specific instructions and guidance, self-collected swabs can
17 approximate physician-collected swabs with a high degree of reliability. Therefore, self-collected
18 vaginal swabs are a viable and accurate method for diagnosing vaginal infections, which may
19 have adverse outcomes including preterm birth, low birth weight, post-operative infections and
20 increased risk of acquisition and transmission of STIs including the HIV infection. Hence,
21 prevention and timely management of curable STIs and RTIs is particularly important. The
22 findings of this study will help in planning and implementing the diagnostic approaches for
23 STIs/RTIs in community based surveillance studies at national or regional level and also in the
24 effective day-to-day STI/RTI diagnosis and management in the peripheral health settings.
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39

40 **Contributors**

41
42 MB, ZK, AB designed the study, coordinated the work and finalized the draft of the manuscript.
43
44 ZK participated in all the data collection, testing, carried out analysis of data and prepared first
45 draft of the manuscript. PM, RB, PP, NK helped in designing the study, sample collection and
46 collaborated in writing of the manuscript. Guarantor of the article: MB.
47
48
49
50

51 **Ethical approval**

52
53
54
55
56
57
58
59
60

The study was approved the Institute Ethics Committee of VMMC & Safdarjung hospital with approval number IEC/VMMC/SJH/Thesis/November-2014/429 and date 25 November 2014.

Acknowledgements

We thank the Medical Superintendent and Principal, VMMC & Safdarjung Hospital for permitting us to carry out this study. The authors are thankful to the Head, Department of Microbiology and Apex Regional STD Teaching, Training & Research Centre for granting permission to Dr. Zarine Khan to carry out her thesis work. We are grateful to Mrs. Ranjana Gupta and Ms. Hemlata Saxena for their technical assistance.

Competing interests None.

Patient consent Obtained.

Funding There was no funding for the study from any funding agency.

Data sharing statement: There is no additional data available.

REFERENCES

1. **Fule SR**, Fule RP, Tankhiwale NS. Clinical and laboratory evidence of *Trichomonas vaginalis* infection among women of reproductive age in rural area. *Indian J Med Microbiol* 2012;**30**:314.
2. **Singh RH**, Zenilman JM, Brown KM, *et al*. The role of physical examination in diagnosing common causes of vaginitis: a prospective study. *Sex Transm Infect* 2013;**89**:185–90.
3. **Forney LJ**, Gajer P, Williams CJ, *et al*. Comparison of self-collected and physician-collected vaginal swabs for microbiome analysis. *J Clin Microbiol* 2010;**48**:1741–8.
4. **Huppert JS**, Hesse EA, Bernard MC, *et al*. Accuracy and trust of self-testing for bacterial vaginosis. *J Adolesc Health* 2012;**51**:400–5.

- 1
2
3 5. **Nelson DB**, Bellamy S, Gray TS, et al. Self-collected versus provider-collected vaginal
4 swabs for the diagnosis of bacterial vaginosis: An assessment of validity and reliability. *J*
5
6 *Clin Epi* 2003 56(9):862-60.
7
8
- 9
10 6. **Garrow SC**, Smith DW, Harnett GB. The diagnosis of chlamydia, gonorrhoea, and
11
12 trichomonas infections by self-obtained low vaginal swabs, in remote northern Australian
13
14 clinical practice. *Sex Transm Infect* 2002;**78**:278–81.
15
16
- 17 7. **Knox J**, Tabrizi SN, Miller P, et al. Evaluation of self-collected samples in contrast to
18
19 practitioner-collected samples for detection of *Chlamydia trachomatis*, *Neisseria*
20
21 *gonorrhoeae*, and *Trichomonas vaginalis* by polymerase chain reaction among women living
22
23 in remote areas. *Sex Transm Dis* 2002;**29**:647–54.
24
25
- 26 8. **Tanksale VS**, Sahasrabhojane M, Patel V, et al. The reliability of a structured examination
27
28 protocol and self-collected vaginal swabs: a pilot study of gynaecological outpatients in Goa,
29
30 India. *Sex Transm Infect* 2003;**79**:251–3.
31
32
- 33 9. **Kashyap B**, Singh R, Bhalla P, et al. Reliability of self-collected versus provider-collected
34
35 vaginal swabs for the diagnosis of bacterial vaginosis. *Int J STD AIDS* 2008 19(8): 510-3.
36
37
- 38 10. **Department of AIDS control**, Ministry of Health and Family Welfare, Government of
39
40 India. Laboratory Manual for Diagnosis of Sexually Transmitted and Reproductive Tract
41
42 Infections. February 2014. Available from: URL: [http://www.indiahivinfo.naco.gov.in/sites/
43
44 default/files/media-gallery/STI_Report.pdf](http://www.indiahivinfo.naco.gov.in/sites/default/files/media-gallery/STI_Report.pdf).
45
46
- 47 11. Unemo, M. (2013). *Laboratory diagnosis of sexually transmitted infections, including human*
48
49 *immunodeficiency virus*. 1st ed. Geneva, Switzerland: WHO Document Production Services,
50
51 p.86. (ISBN 978 92 4 150584 0)
52
53
54
55
56
57
58
59
60

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
12. **Merrill RM.** Fundamentals of Epidemiology and Biostatistics. Jones & Bartlett Publishers, 2012. p 167-8.
 13. **Strauss RA,** Eucker B, Savitz DA, Thorp JM. Diagnosis of bacterial vaginosis from self-obtained vaginal swabs. *Infect Dis Obstet Gynecol* 2005;**13**:31–5.
 14. **Passos MR,** Varella RQ, Barreto NA, *et al.* Accuracy of a self-collection kit for the microbiological study of the vaginal content. *Braz J Infect Dis* 2007;**11**:249–53.
 15. **Puri, KJ,** Madan, A, Bajaj, K. Incidence of various causes of vaginal discharge among sexually active females in age group 20-40 years. *Indian J Dermatol Venereol Leprol* 2003;**69**:122.
 16. **Gandhi TN,** Patel MG, Jain MR. Prospective study of vaginal discharge and prevalence of Vulvovaginal candidiasis in a tertiary care hospital. *Int J Curr Res Rev* 2015;**7**:34-8.
 17. **Pereira DC,** Backes LT, Calil LN, *et al.* A six-year epidemiological survey of vulvovaginal candidiasis in cytopathology reports in the state of Rio Grande do Sul, Brazil. *Rev Patol Trop* 2012;**41**:163-8.
 18. **Tellapragada C,** Eshwara VK, Johar R, *et al.* Antifungal susceptibility patterns, in vitro production of virulence factors, and evaluation of diagnostic modalities for the speciation of pathogenic candida from blood stream infections and vulvovaginal candidiasis. *J Pathog* 2014;142864.
 19. **Holland-Hall CM,** Wiesenfeld HC, Murray PJ. Self-collected vaginal swabs for the detection of multiple sexually transmitted infections in adolescent girls. *J Pediatr Adolesc Gynecol* 2002;**15**:307–13.
 20. **Malla NA,** Gupta IN, Mahajan RC. Human trichomoniasis. *Indian J Med Microbiol* 2001;**19**:6-13.

- 1
2
3 21. **Padubidri VG**, Daftary SN. Shaw's Textbook of gynecology. 16th ed. New Delhi: Elsevier
4 Health Sciences; 2014:379-90.
5
6
7
8 22. **Jindal N**, Gill P, Aggarwal A. An epidemiological study of vulvovaginal candidiasis in
9 women of childbearing age. *Indian J Med Microbiol* 2007;**25**:175.

12 **Key messages**

- 15 • This study has shown that VVC was the most prevalent (26.2%) cause of vaginal
16 discharge, followed by BV (14.4%) and TV (0.5%).
 - 17 • High values of Cohen's Kappa were obtained for all three infections: 0.95 (BV), 0.99
18 (VVC) and 1.0 (TV).
 - 19 • High concordance of self-collected swabs with physician-collected swabs proves the
20 efficacy of self-collected swabs in diagnosing the major causes of vaginal discharge, with
21 high sensitivity and specificity.
 - 22 • This will also help in early diagnosis and management of patients in resource-constrained
23 and peripheral settings thereby strengthening National STI/RTI control programs
24 worldwide.
- 25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1 Prevalence of various types of infections in patients with vaginal discharge based on physician-collected samples among various age groups,

Age group	Number of patients with type of infection				
	BV n/N (%)	VVC n/N (%)		TV n/N (%)	
		<i>C. albicans</i>	Non- <i>albicans</i> <i>Candida</i> species	Total VVC	
<20	1/11 (9.1)	1/11 (9.1)	0/11 (0.0)	1/11 (9.1)	0/11 (0.0)
20-30	25/179 (14.0)	32/179 (17.9)	14/179 (7.8)	46/179 (25.7)	1/179 (0.6)
31-40	33/258 (12.8)	40/258 (15.5)	36/258 (14.0)	76/258 (29.5)	1/258 (0.4)
>40	20/102 (19.6)	11/102 (10.8)	10/102 (9.8)	21/102 (20.6)	1/102 (1.0)
Total	79/550 (14.4)	84/550 (15.3)	60/550 (10.9)	144/550 (26.2)	3/550 (0.5)
Sig (two tailed)*	0.069	0.073	0.062	0.116	

*Paired t-test has been used to evaluate prevalence in various age groups, BV (Bacterial vaginosis), VVC (Vulvo-vaginal candidiasis) and TV (*Trichomonas vaginalis*)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

ABLE 2 Concordance between physician- and self- collected swabs for diagnosis of bacterial vaginosis, vulvovaginal candidiasis and trichomonas vaginitis for a sample size N=550. Outcomes are categorized with combination of “P” and “i” (representing Physician- and Self- collected, respectively) with “+”, “-” and “i” for positive, negative and intermediate, respectively. Example: P+/Si represents the cases where the diagnosis was positive for physician-collected and intermediate for self-collected samples.

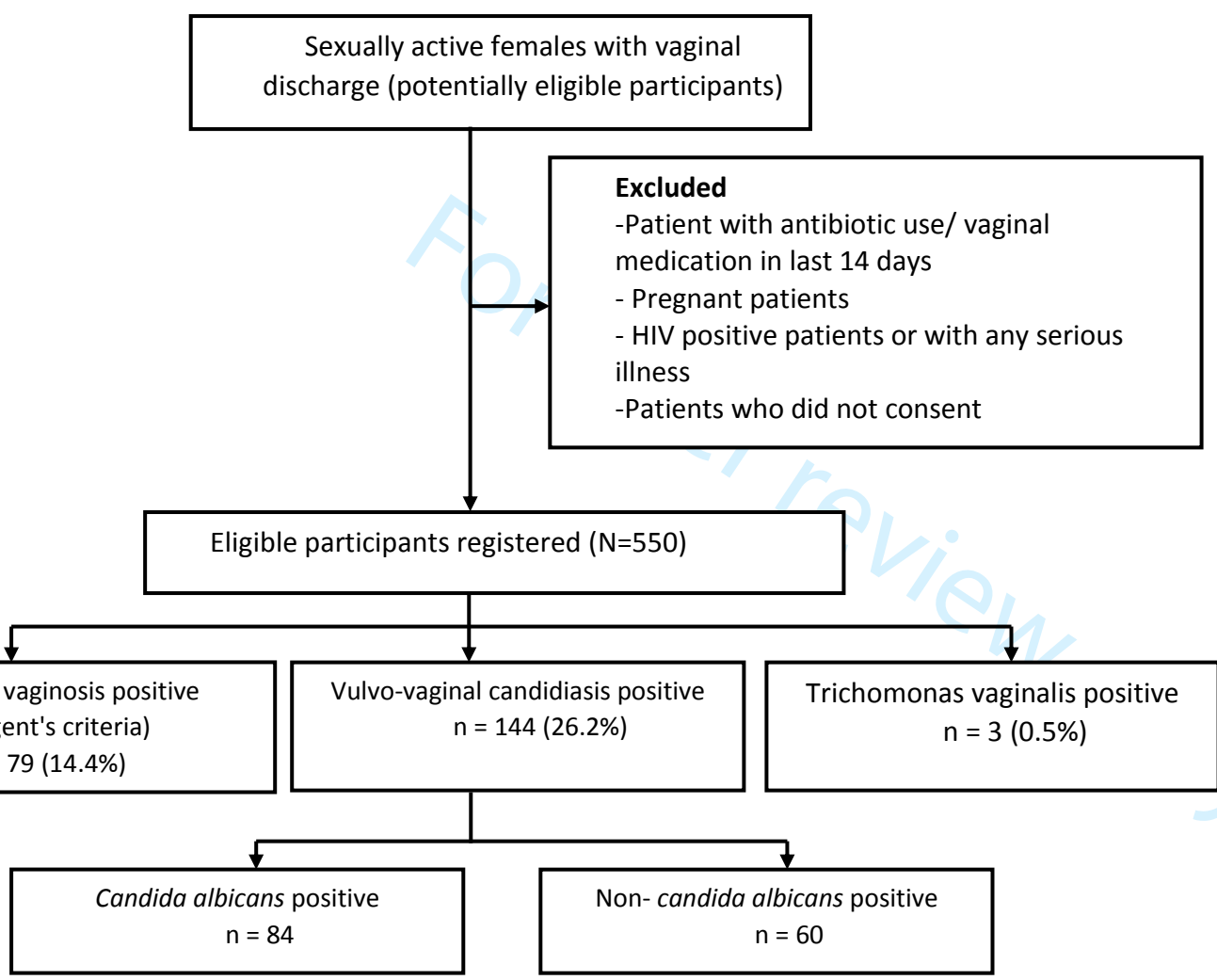
Criterion Used	Number of patients (physician-collected versus self-collected)									Kappa (95% CI)	Prevalence	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	P+/S+	P+/Si	P+/S-	Pi/S+	Pi/Si	Pi/S-	P-/S+	P-/Si	P-/S-						
Bacterial vaginosis															
Nugent – 3 category	72	7	0	0	82	13	0	9	367	0.890 (0.851 -0.928)	14.4	11.1	100	100	98.5
Nugent – 2 category	72	–	7	–	–	–	0	–	471	0.946 (0.907 -0.986)	14.4	11.1	100	100	98.5
Vulvovaginal candidiasis															
<i>C.albicans</i>	84	–	0	–	–	–	0	–	466	1.000 (1.000 -1.000)	15.3	100	100	100	100
Non-albicans	60	–	0	–	–	–	1	–	489	0.991 (0.973 -1.000)	10.9	100	99.8	98.4	100
All	144	–	0	–	–	–	1	–	405	0.995 (0.986 -1.000)	26.2	100	99.8	99.3	100
Trichomonas vaginitis															
TV culture	3	–	0	–	–	–	0	0	547	1.000 (1.000 -1.000)	0.5	100	100	100	100
PPV, Positive predictive value; NPV, Negative predictive value.															

bmjopen-2018-025013.g02 August 2019. Downloaded from http://bmjopen.bmj.com/ on April 19, 2024 by guest. Protected by copyright.

1
2
3
4
5
6 Figure 1: Prevalence of bacterial vaginosis (BV), vulvo-vaginal candidiasis (VVC) and
7
8 *Trichomonas vaginalis* (TV) in 550 females presenting with vaginal discharge diagnosed by
9
10 physician collected swabs.
11
12
13

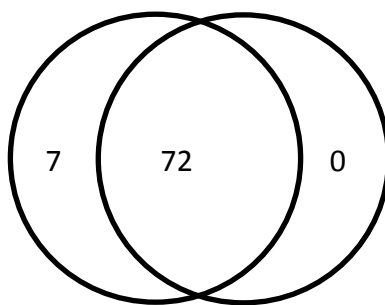
14
15 Figure 2: A comparison of etiological diagnosis of vaginal discharge by physician and self-
16
17 collected swabs (N=550). (A) A total of 79 bacterial vaginosis cases were identified by physician
18
19 collected swabs. (B) A total of 144 vulvo-vaginal candidiasis cases were identified by physician
20
21 collected swabs. (C) A total of 3 trichomonas vaginalis cases were identified by physician
22
23 collected swabs.
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46



A

Physician
collected
swabs
(14.4%)

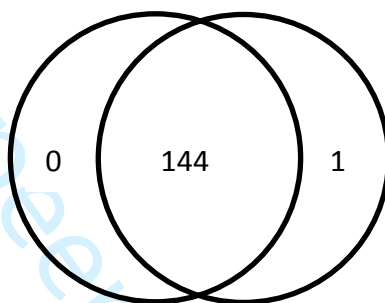


Self-
collected
swabs
(13.1%)

Bacterial vaginosis positive cases (n=79)

B

Physician
collected
swabs
(26.2%)

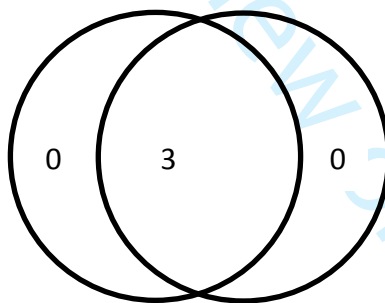


Self-
collected
swabs
(26.4%)

Vulvo-vaginal candidiasis positive cases (n=144)

C

Physician
collected
swabs
(0.5%)



Self-
collected
swabs
(0.5%)

Trichomonas vaginalis positive cases (n=3)

BMJ Open: first published as 10.1136/bmjopen-2018-025013 on 27 August 2019. Downloaded from <http://bmjopen.bmj.com/> on April 19, 2024 by guest. Protected by copyright.

Section & Topic	No	Item	Reported on page
TITLE OR ABSTRACT		STARD 2015 Research check-list	
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2-3
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4-5
	4	Study objectives and hypotheses	5
METHODS			
<i>Study design</i>	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
<i>Participants</i>	6	Eligibility criteria	5-6
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	5-6
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5-6
	9	Whether participants formed a consecutive, random or convenience series	5-6
<i>Test methods</i>	10a	Index test, in sufficient detail to allow replication	6
	10b	Reference standard, in sufficient detail to allow replication	6
	11	Rationale for choosing the reference standard (if alternatives exist)	6
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	6
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	6
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	6
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	6
<i>Analysis</i>	14	Methods for estimating or comparing measures of diagnostic accuracy	6-7
	15	How indeterminate index test or reference standard results were handled	N/A
	16	How missing data on the index test and reference standard were handled	N/A
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	6
	18	Intended sample size and how it was determined	5
RESULTS			
<i>Participants</i>	19	Flow of participants, using a diagram	20
	20	Baseline demographic and clinical characteristics of participants	8
	21a	Distribution of severity of disease in those with the target condition	NA
	21b	Distribution of alternative diagnoses in those without the target condition	7-8
	22	Time interval and any clinical interventions between index test and reference standard	7-9
<i>Test results</i>	23	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Table 1 & 2, 21-23
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	8-10
	25	Any adverse events from performing the index test or the reference standard	Nil
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	3,15
	27	Implications for practice, including the intended use and clinical role of the index test	14,15
OTHER INFORMATION			
	28	Registration number and name of registry	N/A
	29	Where the full study protocol can be accessed	6
	30	Sources of funding and other support; role of funders	15,16

BMJ Open

Evaluation of reliability of self-collected vaginal swabs over physician-collected samples for diagnosis of bacterial vaginosis, candidiasis and trichomoniasis in a resource limited setting: A cross sectional study in India

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-025013.R2
Article Type:	Research
Date Submitted by the Author:	21-Jun-2019
Complete List of Authors:	Khan, Zarine; VMMC & Safdarjung Hospital, Department of Microbiology Bhargava, Aradhana; VMMC and Safdarjung Hospital, Apex Regional STI Training, Research & Reference Laboratory Mittal, Pratima; VMMC & Safdarjung Hospital, Department of Obstetrics & Gynaecology Bharti, Rekha; VMMC & Safdarjung Hospital, Department of Obstetrics & Gynaecology Puri, Poonam; VMMC and Safdarjung Hospital, Department of Dermatology & STD Khunger, Niti; VMMC and Safdarjung Hospital, Department of Dermatology & STD Bala, Manju; VMMC and Safdarjung Hospital, Apex Regional STI Training, Research & Reference Laboratory
Primary Subject Heading:	Sexual health
Secondary Subject Heading:	Reproductive medicine, Epidemiology
Keywords:	Microbiology < BASIC SCIENCES, GENITOURINARY MEDICINE, Diagnostic microbiology < INFECTIOUS DISEASES

SCHOLARONE™
Manuscripts

1
2
3 **Evaluation of reliability of self-collected vaginal swabs over physician-collected samples for**
4 **diagnosis of bacterial vaginosis, candidiasis and trichomoniasis, in a resource limited**
5 **setting: A cross sectional study in India**
6
7
8
9

10
11
12 **Zarine Khan^{1,2}, Aradhana Bhargava ^{1,2}, Pratima Mittal³, Rekha Bharti³, Poonam Puri⁴,**
13 **Niti Khunger⁴, Manju Bala ^{1,2*}.**
14
15
16

17
18
19 ¹Department of Microbiology, VMMC & Safdarjung Hospital, New Delhi, India, ²Apex
20 Regional STI Training, Research & Reference Laboratory, VMMC & Safdarjung Hospital, New
21 Delhi, India, ³Department of Obstetrics & Gynaecology, VMMC & Safdarjung Hospital, New
22 Delhi, India, ⁴Department of Dermatology & STD, VMMC & Safdarjung Hospital, New Delhi,
23 India.
24
25
26
27
28
29
30
31
32

33 **Correspondence:**
34

35 Dr. Manju Bala, Consultant and Professor (Microbiology), Apex Regional STI Training,
36 Research & Reference Laboratory, Vardhman Mahavir Medical College & Safdarjung Hospital,
37 New Delhi, India. Telephone number: 91-11-26196740, Fax number: 91-11-26163072, Email:
38 manjubala_2@hotmail.com
39
40
41
42
43
44

45
46
47 **Key words:** Vaginal discharge; bacterial vaginosis; vulvovaginal candidiasis; trichomonas
48 vaginitis, self-collected vaginal swabs; physician-collected vaginal swabs.
49
50
51
52

53 **Word count: 2776**
54
55
56
57
58
59
60

ABSTRACT

Objectives Self-collected vaginal swabs can facilitate diagnosis of Vaginal discharge (VD) in resource-limited settings, provided reliability of the method is established. The aim of this study was to evaluate the concordance between self-collected and physician-collected vaginal swabs for etiological diagnosis of VD and to determine the prevalence of bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis (TV).

Methods A total of 550 females (median age: 32 years; range: 18-45 years) attending two STI/RTI clinics with VD from January 2015 to May 2016 were included in the study after obtaining written informed consent. Swabs were self-collected by patients after instructions and subsequently by a physician under speculum examination. Samples were processed for standard bedside tests, Gram staining, wet mount and culture (gold standard) according to national guidelines. Concordance between the two methods was determined by the Cohen's kappa value.

Results BV, VVC and TV were diagnosed in 79(14.4%), 144(26.2%) and 3(0.5%) patients respectively. VVC coexisted with BV in 58(10.5%) patients. There was no co-infection of TV with BV or VVC. *C. albicans* was isolated in 84(58.3%) VVC cases. Sensitivity, specificity, PPV and NPV of self-collected swabs for diagnosing BV was 91.1%, 100%, 100% and 98.5% respectively while for the *C. albicans* VVC and TV, sensitivity, specificity, PPV and NPV all were 100% as compared to physician-collected swabs. Highly concordant results were obtained between two methods by the Kappa values of 0.95 (BV), 0.99 (VVC) and 1.0 (TV).

Conclusion The comparative performance of self- and physician-collected vaginal swabs establishes self-collection of samples for BV, VVC and TV as a viable alternative tool in the management of sexually transmitted infections/ reproductive tract infections (STIs/RTIs) especially in peripheral and resource-constrained settings. This would be effective in

1
2
3 implementing the diagnostic approaches for STIs/RTIs in community based surveillance studies
4
5 at national or regional level and therefore strengthening the National STI/RTI Control
6
7 Programme.
8
9

10 11 12 **ARTICLE SUMMARY**

13 14 **Strengths and limitations of this study**

- 15
16
17 • This study determined that if diagnosis based on self-collected vaginal swabs is proven
18
19 reliable, it could contribute to early diagnosis and greatly increase the access to treatment.
20
21
- 22
23 • Specimens were obtained from 550 patients with vaginal discharge attending the two
24
25 STI/RTI clinics after obtaining the written informed consent. Concordance of
26
27 inexpensive standard bedside tests, such as Gram staining, wet mount and culture (gold
28
29 standard), was evaluated on both the self- and physician- collected samples for detecting
30
31 bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis
32
33 (TV).
34
- 35
36 • Corresponding findings were compared and analyzed using SPSS statistical analysis tool
37
38 in terms of Cohen's Kappa values to determine the concordance between findings of self-
39
40 and physician- collected samples.
41
- 42
43 • Limitation is that molecular tests were not performed, although gold standard test culture
44
45 was performed which is more economical and less labour intensive also.
46
- 47
48 • Another limitation was the low positivity of Trichomonas vaginalis in patients of vaginal
49
50 discharge by culture.
51
52
53
54
55
56
57
58
59
60

INTRODUCTION

Vaginal discharge is a frequently encountered complaint in women attending Sexually Transmitted Infections (STIs)/Reproductive Tract Infections (RTIs) clinics globally. It is mainly caused by curable bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis (TV).¹ Existing practice to diagnose vaginal discharge includes a speculum examination where the clinician inspects the external genitalia, vagina and cervix, assesses characteristics of the discharge, notes physical signs such as inflammation and lesions, and collects specimens for laboratory testing. The speculum examination requires a trained clinician and a proper setup. The discomfort of a pelvic examination may lead women to delay or avoid seeking care, which may result in lower enrollment rates and higher rates of loss to follow-up.^{2,3}

Even though high rates of STIs/RTIs are observed in developing countries due to various social and environmental factors such as sex ratio imbalances, urbanization and rural to urban migration, reliable detection of these infections are difficult due to poor infrastructure and lack of skilled workforce. Additionally, there is low female STI enrollment rate in developing countries due to women's reluctance to undergo gynecological examination, originating from cultural, religious and socio-economic factors.

Therefore, developing accurate approaches to diagnosing lower genital infections without a speculum examination would be advantageous to both clinicians and patients.² Self-collected vaginal swabs are the only practical and financially feasible method to use for sampling in field-based longitudinal cohort studies.³ As most of the studies comparing the reliability of self-collected vaginal swabs have been conducted in developed countries such as USA^{3,4,5} and Australia^{6,7} mostly using advanced molecular techniques such as nucleic acid amplification tests, limited literature on such studies is available from developing countries. In India, there was a

1
2
3 pilot study from Goa, which suggested that self-collected swabs are an acceptable method of
4 collection of vaginal specimens in women attending gynaecological clinics in India.⁸ However,
5 the main limitation of the study was a statistically small sample size. Moreover, the samples
6 were examined only by Gram's stain for their sensitivity for the diagnosis of STIs and were not
7 tested by other diagnostic techniques like wet mount and culture. Another study was performed
8 in New Delhi,⁹ which showed that self-collected swabs can reasonably approximate physician-
9 collected swabs for BV only. However, this study also was limited by a statistically small sample
10 size.
11
12
13
14
15
16
17
18
19
20
21

22 Hence, the current study was aimed at establishing reliability of self- collected vaginal
23 swabs against physician-collected swabs by determining its sensitivity, specificity, positive
24 predictive value (PPV) and negative predictive value (NPV) with significant number of samples
25 and quality reliable tests. Additionally, the fungal (*Candida albicans* or non-*albicans Candida*
26 species) bacterial (Bacterial vaginosis) and parasitic (*Trichomonas vaginalis*) etiology of vaginal
27 discharge and prevalence of various types of infections and co-infections were also studied.
28
29
30
31
32
33
34
35
36
37

38 METHODS

39
40 The study was conducted in two National AIDS Control Organization (NACO) designated
41 STI/RTI clinics in the Apex Regional STD Teaching, Training and Research Centre and
42 Department of Obstetrics and Gynaecology, Vardhaman Mahavir Medical College and
43 Safdarjung Hospital, New Delhi, India during January 2015 to May 2016. The thesis protocol
44 was approved by the authors' Institute Ethics Committee (VMMC & SJH). Sample size was
45 calculated according to the following formula: $S = \frac{4P(1-P)}{L^2}$ (where S = Sample size, P = Estimated
46 prevalence, L = Margin of error at 5%). Accordingly, samples were collected from 550 sexually
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 active females with vaginal discharge. After explaining the study details to the potentially
4 eligible participants with the help of a pre-designed patient information sheet; a written informed
5 consent was obtained on patient consent form. Both the patient information sheet and the consent
6 form were designed in English and Hindi (local language). Patients with history of antibiotic use
7 or vaginal medication in the previous 14 days, pregnant patients, patients unwilling/ unable to
8 participate and patients with human immunodeficiency virus (HIV) infection were excluded
9 from this study.

10
11 A total of six vaginal swabs, including three self-collected samples, were collected from
12 each participant. Participants were given instructions on appropriate specimen collection
13 technique before the speculum examination. They were instructed to insert the vaginal swab 1 to
14 2 inches into the vagina, twisting the swab to collect material on all sides of the tip, wipe in
15 several full circles on the vaginal wall, keep the swab in the vagina for 20 seconds, and then
16 carefully remove the swab and place it in a sterile tube. Subsequently, the female clinician
17 examined the participants and specimens were collected following the same procedure with
18 gloved hands under speculum examination. Proper indexing of the samples was performed and
19 the examining microbiologist was blind regarding the origin (physician- or self- collected) of the
20 swabs.

21
22 The samples were examined by standard bedside tests, Gram staining, wet mount and
23 culture (gold standard).¹⁰ For each patient, the first of the three self-collected swabs was used for
24 pH and whiff test, the second for wet mount and Gram staining, whereas the third swab was used
25 for Candida and Trichomonas culture. The same tests were repeated for the three physician
26 collected swabs. Bacterial vaginosis was diagnosed with the use of Amsel's criteria¹¹ and
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Nugent's score¹¹, whereas candidiasis and trichomoniasis were diagnosed based upon
4
5 microscopy and culture results.
6
7

8 9 **Patient and Public Involvement**

10
11 Patients were not involved in this study in developing the design, recruitment or conduction of
12
13 the study. Patients were not informed about the comparative results of the study but were given
14
15 the report of the diagnosis based on physician collected samples as per routine practice. The
16
17 result will be disseminated through this publication.
18
19

20 21 22 23 **Statistical analysis**

24
25 Data was analyzed using Microsoft excel and SPSS software version 21.0. Prevalence of
26
27 bacterial, fungal and parasitic causative agents of vaginal discharge was studied in patients
28
29 presenting with vaginal discharge. Also, their prevalence in various age groups was statistically
30
31 analyzed using t-test (two- tailed). Sensitivity, specificity, positive predictive value and negative
32
33 predictive value of self-collected specimens versus physician-collected specimens were
34
35 calculated. Concordance between results obtained from self-collected and physician collected
36
37 swabs was determined by calculating the Cohen's kappa value.
38
39

40
41
42 The value of Kappa is defined as
43

$$44 \quad \kappa = \frac{p_o - p_e}{1 - p_e},$$

45
46
47 where p_o is the observed level of agreement and p_e is the expected level of agreement. The value
48
49 of κ lies between -1 and 1. A value of 1 implies perfect agreement whereas -1 implies perfect
50
51 disagreement. When the two findings agree purely by chance, the value of kappa will be zero.¹²
52
53
54
55
56
57

RESULTS

Prevalence of BV, VVC and TV in patients with vaginal discharge using physician collected samples

The results obtained with physician-collected specimens were treated as the “standard results”. Prevalence of the three types of vaginal infections, namely BV, VVC and TV, as diagnosed by the Nugent score, candida culture and trichomonas culture respectively, are summarized in Figure 1. Out of the 550 patients presenting with vaginal discharge, 144 (26.2%) cases of VVC, 79 (14.4%) of BV, and 3 (0.5%) cases of TV were detected. Therefore, VVC was the most predominant infection in this population. A significant number of VVC infections were caused by non-albican species of *Candida* i.e. 60 out of 144 (41.7%) of the total VVC patients. BV and VVC coexisted in 21 (3.8%) patients. None of the TV infected patient had any co-infection with BV or VVC.

Table 1 analyses prevalence of various infections in different age groups. Maximum number of infections were found in the age group of 31-40 years (101/258) followed by 20-30 years (64/179) and >40 years (38/102). Only 2/11 participants less than 20 years of age were infected. In the age group 20 years or less, 9.1% (1/11) patients were found positive for BV and as well as for VVC. Among the 179 participants in the 20-30 years range, prevalence of both BV and VVC increased to 14% and 25.7%, respectively. In the age group 30-40 years (n=258), the prevalence of BV reduced slightly to 12.8% but the prevalence for VVC continued to increase (29.5%). For the remaining patients aged 40 years or more (n=102), the prevalence of BV sharply increased to 19.6% whereas the prevalence decreased to 20.6% for VVC. When the

1
2
3 prevalence of BV and VVC across different age groups of vaginal discharge patients was
4 analyzed by paired t- test (two tailed), it was observed that the difference was not significant.
5
6
7
8
9

10 **Concordance between results from self- and physician- collected samples**

11 Cohen's Kappa was used as the metric of agreement between the two collection methods. The
12 data related to the concordance of the two methods for diagnosis of BV, VVC and TV are
13 depicted in Table 2.
14
15
16
17
18

19 *Concordance for BV:*

20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
Diagnosis of BV was performed on the basis of Nugent's scoring. Of the total 550 participants,
376 (68.4%) showed a healthy vaginal flora (Nugent score 0-3), 95 (17.3%) were categorized as
"intermediate" (Nugent score 4-6) and the remaining 79 (14.4%) were diagnosed as BV (Nugent
score 7-10) based upon findings of physician collected swabs. For the Nugent score based
comparison; the outcome of a diagnosis could be classified in two different ways: (a) Three
categories: BV positive or BV intermediate or BV negative and (b) Two categories: BV positive
or BV non-positive by putting both intermediate and negative in the same bin of "non-positive".

For the three-category classification, both self- and physician- collection methods agreed
on 367 true negative cases, 82 intermediate cases and 72 true positive cases. Out of the BV
negative cases, self-collection method found nine cases as intermediate but no false positives.
For cases diagnosed as intermediate by physician-collected method, 13 were found negative by
self-collection method but, again, no false positives were detected. However, there were seven
cases of true positive diagnosis which were diagnosed as intermediate by self-collected samples,
but no true positive cases were diagnosed as negative by self-collected samples. The kappa value

1
2
3 computed for this is 0.890 with 95% confidence interval of 0.851 to 0.928, which indicates
4 excellent agreement between self- and physician- collected samples. The weighted kappa value
5 was 0.921 for this category.
6
7
8
9

10 For the two-category case, both methods diagnosed 471 non-positive cases and 72
11 positive cases. Self-collection method missed seven cases of BV positive results in this case, but
12 no false positive case was observed by the self-collection method. The kappa value computed for
13 this case is 0.946 with 95% confidence interval of 0.907 to 0.986 suggesting excellent agreement
14 between self- and physician- collected samples.
15
16
17
18
19
20
21
22
23

24 *Concordance for VVC:*

25
26 The numbers of true negative and true positive cases were 406 and 144, respectively for VVC.
27 There was no missed true positive result and only one case of false positive was found with self-
28 collected samples. Very high concordance was observed with the kappa value at 0.994 with 95%
29 confidence interval of 0.982 to 1.000. Table 2 also lists the cases of *Candida albicans* and non-
30 *albicans* separately. For *C. albicans* infections, there was perfect concordance (kappa = 1.000)
31 with 84 true positive and 466 true negative cases. For non-*albicans Candida* cases, there were 60
32 true positive, 489 true negative and 1 false positive cases using self-collected swabs making the
33 kappa value to be 0.991 with 95% confidence interval of 0.973 to 1.000.
34
35
36
37
38
39
40
41
42
43
44
45
46

47 *Concordance for TV:*

48
49 Only three cases were found positive for TV and the results were identical for self- and
50 physician- collected samples yielding perfect concordance (kappa value of 1.000).
51
52
53
54
55
56
57
58
59
60

Sensitivity, specificity, PPV and NPV of self-collected swabs in comparison to physician-collected swabs

Table 2 shows the sensitivity, specificity, PPV and NPV of diagnosis using self-collected swabs when compared to physician-collected swabs, for BV, VVC and TV. Self-collection method had acceptable sensitivity, specificity, PPV and NPV of 91.1%, 100%, 100% and 98.5% for diagnosing BV using Nugent score. For VVC, including both *C. albicans* and non-*albicans Candida* species, the self-collection method had high sensitivity of 100%, specificity of 99.8%, PPV of 99.3%, and NPV of 100%. The values for non-*albicans* VVC, were identical to the overall VVC cases except that the PPV (98.4%) was less. The sensitivity, specificity, PPV and NPV were all 100% for self-collected swabs as compared to physician-collected swabs for the *C. albicans* VVC and for TV.

DISCUSSION

The prevalence of BV in women presenting with vaginal discharge to STI/RTI clinic was 14.4%. In the literature the prevalence varies widely from 10.7% to 45%.^{4,13,14} In our study, we excluded all patients above 45 years of age, pregnant women and HIV patients. This difference in the exclusion criteria is one of the causes of the varying prevalence rates particularly because prevalence of BV increases with age and immune-deficient conditions. More importantly, geographical locations and cultural practices often have significant impact on the prevalence rates.

The prevalence for VVC was 26.2% in this study, which agrees well with the prevalence of other studies.^{15,16} However, much smaller prevalence rates of 2.8% to 8.5% were reported in

1
2
3 three categories had kappa values of 0.946 and 0.89, respectively, which are much higher than
4 kappa values of 0.71 and 0.72, correspondingly, reported in Strauss et al.¹³ In a similar study
5 performed by Huppert et al.,⁴ the reliability of self-collected vaginal swabs was established,
6 albeit with a value of kappa of 0.53. This can be attributed to the use of vaginal pH as the only
7 indicator of BV, instead of a more comprehensive Nugent score as used in this work.
8
9

10
11 A near-perfect concordance was observed in cases of VVC, with a kappa value of 0.995.
12 In VVC cases, self-collected samples produced one false positive result as compared to
13 physician-collected sample. This can possibly be attributed to the contamination of the swab with
14 skin commensal flora during self-collection or it may be because of scanty discharge; where after
15 self-collection not enough sample was left for collection by the physician. One of the limitation
16 of the study was that the self and physician collection was not alternated for every consecutive
17 patients. But the high agreement in both the methods negates the influence of this factor on the
18 results.
19
20
21
22
23
24
25
26
27
28
29
30
31
32

33 A perfect match (kappa=1) between two methods was observed in this study for TV
34 infections with a small number of positive cases (three positive cases out of 550). In order to
35 maintain the uniformity in data presentation the kappa value was calculated despite the low
36 positivity.
37
38
39
40
41

42 Thus, the current study findings highlight that near-perfect match was observed between self-
43 collected and physician collected swabs for diagnosis of BV, VVC and TV and it was proven by
44 the high values of kappa (all greater than 0.9). The high concordance can partly be attributed to
45 the following reasons:
46
47
48
49

50
51 (1) *Mature patients*: Almost 98% of the patients were of age 20 years or older with average age
52 of 32 years, which helped the patients to collect the sample properly.
53
54
55
56
57
58
59
60

1
2
3 (2) *Sample collection under supervision*: In the clinic, the patients were under supervision and
4
5 were provided with clarifications whenever in doubt. However, most of the patients were
6
7 able to collect the sample without further help post-verbal instructions.
8
9

10 (3) *No delay in transportation of samples to the laboratory*: The samples were collected by both
11
12 the methods inside the STI/RTI clinic and were transported to the STI laboratory
13
14 immediately, which is located very near to the clinic. Specimens were processed immediately
15
16 in the laboratory. Therefore, no transportation delay, sample labeling errors and sample
17
18 contamination occurred leading to high concordance between the two methods.
19
20
21
22
23

24 Even when used in resource constrained peripheral areas, self-collected swabs can perform
25
26 really well if mislabeling and transport error can be minimized and online or other forms of
27
28 assistance can be provided in case of doubts. The proliferation of mobile phones even in remote
29
30 corners of India can facilitate such online assistance. From this study, it was established beyond
31
32 doubt that self-collected swabs yield diagnostic results as accurate as physician-collected swabs
33
34 for almost all practical purposes.
35
36
37
38
39

40 When replicated in peripheral resource constrained settings, self-collected swabs would
41
42 provide alternative method of sample collection for patients who refrain from getting
43
44 gynaecological examination either due to social or cultural misconceptions. An early and
45
46 accurate diagnosis based on this inexpensive method would make testing more approachable,
47
48 economical and would improve the treatment outcomes. When integrated with proper quality
49
50 assurance, self-collected swabs may form an important diagnostic tool in community based
51
52
53
54
55
56
57
58
59
60

1
2
3 studies. A limitation of this study is that molecular tests were not performed, although gold
4 standard test culture was performed which is more economical and less labour intensive.
5
6
7
8
9

10 **CONCLUSION**

11
12 It was demonstrated that with specific instructions and guidance, self-collected swabs can
13 approximate physician-collected swabs with a high degree of reliability. Therefore, self-collected
14 vaginal swabs are a viable and accurate method for diagnosing vaginal infections, which may
15 have adverse outcomes including preterm birth, low birth weight, post-operative infections and
16 increased risk of acquisition and transmission of STIs including the HIV infection. Hence,
17 prevention and timely management of curable STIs and RTIs is particularly important. The
18 findings of this study will help in planning and implementing the diagnostic approaches for
19 STIs/RTIs in community based surveillance studies at national or regional level and also in the
20 effective day-to-day STI/RTI diagnosis and management in the peripheral health settings.
21
22
23
24
25
26
27
28
29
30
31
32
33
34

35 **Contributors**

36 MB, ZK, AB designed the study, coordinated the work and finalized the draft of the manuscript.
37
38 ZK participated in all the data collection, testing, carried out analysis of data and prepared first
39 draft of the manuscript. PM, RB, PP, NK helped in designing the study, sample collection and
40 collaborated in writing of the manuscript. Guarantor of the article: MB.
41
42
43
44
45
46

47 **Ethical approval**

48
49 The study was approved the Institute Ethics Committee of VMMC & Safdarjung hospital with
50 approval number IEC/VMMC/SJH/Thesis/November-2014/429 and date 25 November 2014.
51
52
53

54 **Acknowledgements**

We thank the Medical Superintendent and Principal, VMMC & Safdarjung Hospital for permitting us to carry out this study. The authors are thankful to the Head, Department of Microbiology and Apex Regional STD Teaching, Training & Research Centre for granting permission to Dr. Zarine Khan to carry out her thesis work. We are grateful to Mrs. Ranjana Gupta and Ms. Hemlata Saxena for their technical assistance.

Competing interests None declared.

Patient consent Obtained.

Funding None declared.

Data sharing statement: Data are available upon reasonable request.

REFERENCES

1. **Fule SR**, Fule RP, Tankhiwale NS. Clinical and laboratory evidence of *Trichomonas vaginalis* infection among women of reproductive age in rural area. *Indian J Med Microbiol* 2012;**30**:314.
2. **Singh RH**, Zenilman JM, Brown KM, *et al*. The role of physical examination in diagnosing common causes of vaginitis: a prospective study. *Sex Transm Infect* 2013;**89**:185–90.
3. **Forney LJ**, Gajer P, Williams CJ, *et al*. Comparison of self-collected and physician-collected vaginal swabs for microbiome analysis. *J Clin Microbiol* 2010;**48**:1741–8.
4. **Huppert JS**, Hesse EA, Bernard MC, *et al*. Accuracy and trust of self-testing for bacterial vaginosis. *J Adolesc Health* 2012;**51**:400–5.
5. **Nelson DB**, Bellamy S, Gray TS, *et al*. Self-collected versus provider-collected vaginal swabs for the diagnosis of bacterial vaginosis: An assessment of validity and reliability. *J Clin Epi* 2003 **56**(9):862-60.

- 1
2
3 6. **Garrow SC**, Smith DW, Harnett GB. The diagnosis of chlamydia, gonorrhoea, and
4 trichomonas infections by self-obtained low vaginal swabs, in remote northern Australian
5 clinical practice. *Sex Transm Infect* 2002;**78**:278–81.
6
7
- 8
9
10 7. **Knox J**, Tabrizi SN, Miller P, *et al*. Evaluation of self-collected samples in contrast to
11 practitioner-collected samples for detection of *Chlamydia trachomatis*, *Neisseria*
12 *gonorrhoeae*, and *Trichomonas vaginalis* by polymerase chain reaction among women living
13 in remote areas. *Sex Transm Dis* 2002;**29**:647–54.
14
15
- 16
17 8. **Tanksale VS**, Sahasrabhojane M, Patel V, *et al*. The reliability of a structured examination
18 protocol and self-collected vaginal swabs: a pilot study of gynaecological outpatients in Goa,
19 India. *Sex Transm Infect* 2003;**79**:251–3.
20
21
- 22
23 9. **Kashyap B**, Singh R, Bhalla P, *et al*. Reliability of self-collected versus provider-collected
24 vaginal swabs for the diagnosis of bacterial vaginosis. *Int J STD AIDS* 2008 19(8): 510-3.
25
26
- 27
28 10. **Department of AIDS control**, Ministry of Health and Family Welfare, Government of
29 India. Laboratory Manual for Diagnosis of Sexually Transmitted and Reproductive Tract
30 Infections. February 2014. Available from: URL: [http://www.indiahivinfo.naco.gov.in/sites/
31 default/files/media-gallery/STI_Report.pdf](http://www.indiahivinfo.naco.gov.in/sites/default/files/media-gallery/STI_Report.pdf).
32
33
- 34
35 11. Unemo, M. (2013). *Laboratory diagnosis of sexually transmitted infections, including human*
36 *immunodeficiency virus*. 1st ed. Geneva, Switzerland: WHO Document Production Services,
37 p.86. (ISBN 978 92 4 150584 0)
38
39
- 40
41 12. **Merrill RM**. Fundamentals of Epidemiology and Biostatistics. Jones & Bartlett Publishers,
42 2012. p 167-8.
43
44
- 45
46 13. **Strauss RA**, Eucker B, Savitz DA, Thorp JM. Diagnosis of bacterial vaginosis from self-
47 obtained vaginal swabs. *Infect Dis Obstet Gynecol* 2005;**13**:31–5.
48
49
50
51
52
53
54
55
56
57
58
59

14. **Passos MR**, Varella RQ, Barreto NA, *et al.* Accuracy of a self-collection kit for the microbiological study of the vaginal content. *Braz J Infect Dis* 2007;**11**:249–53.
15. **Puri, KJ**, Madan, A, Bajaj, K. Incidence of various causes of vaginal discharge among sexually active females in age group 20-40 years. *Indian J Dermatol Venereol Leprol* 2003;**69**:122.
16. **Gandhi TN**, Patel MG, Jain MR. Prospective study of vaginal discharge and prevalence of Vulvovaginal candidiasis in a tertiary care hospital. *Int J Curr Res Rev* 2015;**7**:34-8.
17. **Pereira DC**, Backes LT, Calil LN, *et al.* A six-year epidemiological survey of vulvovaginal candidiasis in cytopathology reports in the state of Rio Grande do Sul, Brazil. *Rev Patol Trop* 2012;**41**:163-8.
18. **Tellapragada C**, Eshwara VK, Johar R, *et al.* Antifungal susceptibility patterns, in vitro production of virulence factors, and evaluation of diagnostic modalities for the speciation of pathogenic candida from blood stream infections and vulvovaginal candidiasis. *J Pathog* 2014;142864.
19. **Holland-Hall CM**, Wiesenfeld HC, Murray PJ. Self-collected vaginal swabs for the detection of multiple sexually transmitted infections in adolescent girls. *J Pediatr Adolesc Gynecol* 2002;**15**:307–13.
20. **Malla NA**, Gupta IN, Mahajan RC. Human trichomoniasis. *Indian J Med Microbiol* 2001;**19**:6-13.
21. **Padubidri VG**, Daftary SN. Shaw's Textbook of gynecology. 16th ed. New Delhi: Elsevier Health Sciences; 2014:379-90.

Key messages

- This study has shown that VVC was the most prevalent (26.2%) cause of vaginal discharge, followed by BV (14.4%) and TV (0.5%).
- High values of Cohen's Kappa were obtained for all three infections: 0.95 (BV), 0.99 (VVC) and 1.0 (TV).
- High concordance of self-collected swabs with physician-collected swabs proves the efficacy of self-collected swabs in diagnosing the major causes of vaginal discharge, with high sensitivity and specificity.
- This will also help in early diagnosis and management of patients in resource-constrained and peripheral settings thereby strengthening National STI/RTI control programs worldwide.

Table 1 Prevalence of various types of infections in patients with vaginal discharge based on physician-collected samples among various age groups,

Number of patients with type of infection				
Age group	BV	VVC		TV
	n/N (%)	<i>C. albicans</i>	Non- <i>albicans</i>	n/N (%)
			Total VVC	

	<i>Candida</i> species				
<20	1/11 (9.1)	1/11 (9.1)	0/11 (0.0)	1/11 (9.1)	0/11 (0.0)
20-30	25/179 (14.0)	32/179 (17.9)	14/179 (7.8)	46/179 (25.7)	1/179 (0.6)
31-40	33/258 (12.8)	40/258 (15.5)	36/258 (14.0)	76/258 (29.5)	1/258 (0.4)
>40	20/102 (19.6)	11/102 (10.8)	10/102 (9.8)	21/102 (20.6)	1/102 (1.0)
Total	79/550 (14.4)	84/550 (15.3)	60/550 (10.9)	144/550 (26.2)	3/550 (0.5)
Sig (two tailed)*	0.069	0.073	0.062	0.116	

*Paired t-test has been used to evaluate prevalence in various age groups, BV (Bacterial vaginosis), VVC (Vulvo-vaginal candidiasis) and TV (*Trichomonas vaginalis*)

bmjopen-2018-025013.g022 August 2019. Downloaded from http://bmjopen.bmj.com/ on April 19, 2024 by guest. Protected by copyright.

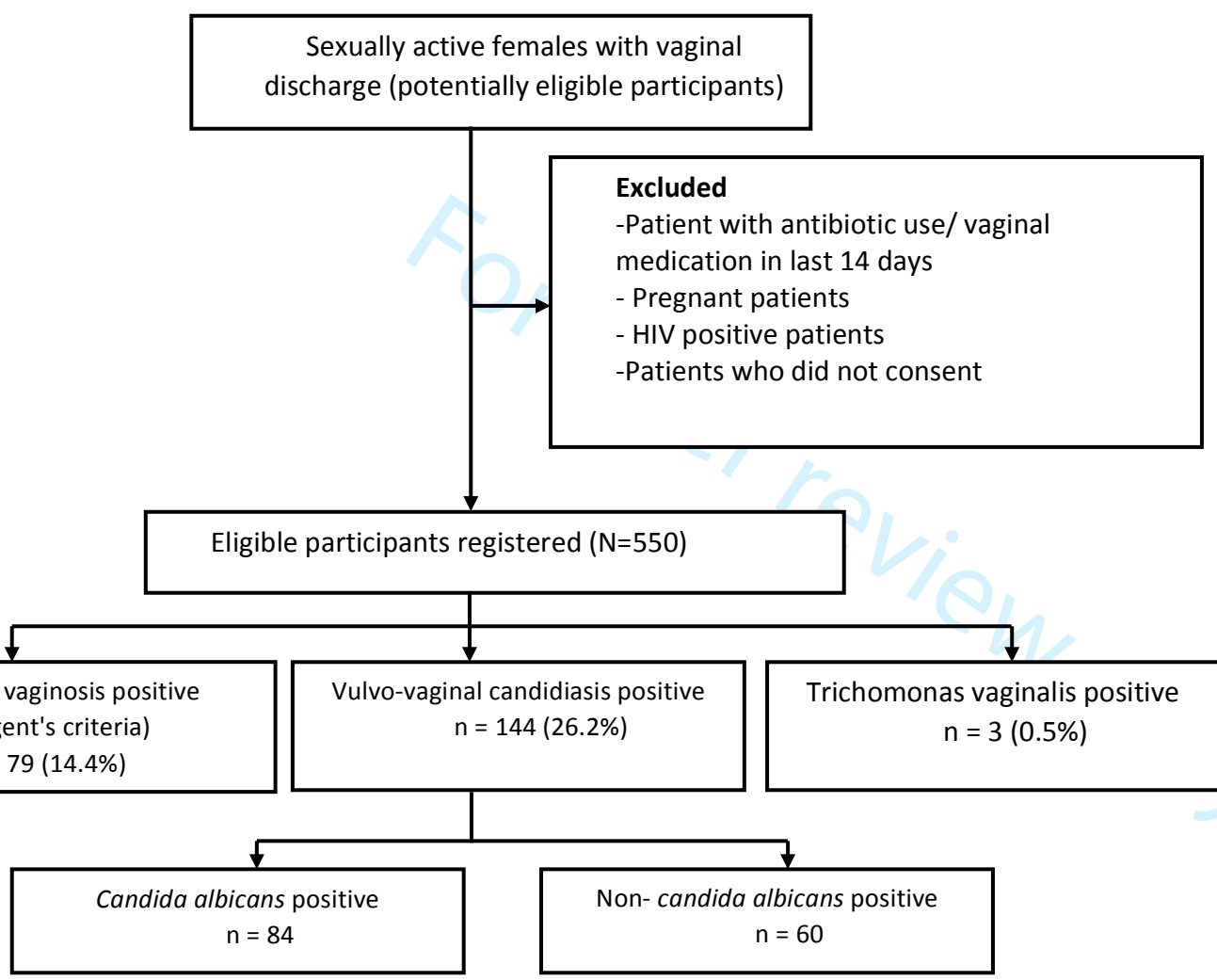
ABLE 2 Concordance between physician- and self- collected swabs for diagnosis of bacterial vaginosis, vulvovaginal candidiasis and trichomonas vaginitis for a sample size N=550. Outcomes are categorized with combination of “P” and “i” (representing Physician- and Self- collected, respectively) with “+”, “-” and “i” for positive, negative and intermediate, respectively. Example: P+/Si represents the cases where the diagnosis was positive for physician-collected and intermediate for self-collected samples.

Criterion Used	Number of patients (physician-collected versus self-collected)									Kappa (95% CI)	Prevalence	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	P+/S+	P+/Si	P+/S-	Pi/S+	Pi/Si	Pi/S-	P-/S+	P-/Si	P-/S-						
Bacterial vaginosis															
Nugent – 3 category	72	7	0	0	82	13	0	9	367	0.890 (0.851 -0.928)	14.4	11.1	100	100	98.5
Nugent – 2 category	72	–	7	–	–	–	0	–	471	0.946 (0.907 -0.986)	14.4	11.1	100	100	98.5
Vulvovaginal candidiasis															
<i>C.albicans</i>	84	–	0	–	–	–	0	–	466	1.000 (1.000 -1.000)	15.3	100	100	100	100
Non-albicans	60	–	0	–	–	–	1	–	489	0.991 (0.973 -1.000)	10.9	100	99.8	98.4	100
All	144	–	0	–	–	–	1	–	405	0.995 (0.986 -1.000)	26.2	100	99.8	99.3	100
Trichomonas vaginitis															
TV culture	3	–	0	–	–	–	0	0	547	1.000 (1.000 -1.000)	0.5	100	100	100	100
PPV, Positive predictive value; NPV, Negative predictive value.															

1
2
3
4
5
6 Figure 1: Prevalence of bacterial vaginosis (BV), vulvo-vaginal candidiasis (VVC) and
7
8 *Trichomonas vaginalis* (TV) in 550 females presenting with vaginal discharge diagnosed by
9
10 physician collected swabs.
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46



Section & Topic	No	Item	Reported on page
TITLE OR ABSTRACT		STARD 2015 Research check-list	
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2-3
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4-5
	4	Study objectives and hypotheses	5
METHODS			
<i>Study design</i>	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
<i>Participants</i>	6	Eligibility criteria	5-6
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	5-6
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5-6
	9	Whether participants formed a consecutive, random or convenience series	5-6
<i>Test methods</i>	10a	Index test, in sufficient detail to allow replication	6
	10b	Reference standard, in sufficient detail to allow replication	6
	11	Rationale for choosing the reference standard (if alternatives exist)	6
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	6
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	6
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	6
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	6
<i>Analysis</i>	14	Methods for estimating or comparing measures of diagnostic accuracy	6-7
	15	How indeterminate index test or reference standard results were handled	N/A
	16	How missing data on the index test and reference standard were handled	N/A
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	6
	18	Intended sample size and how it was determined	5
RESULTS			
<i>Participants</i>	19	Flow of participants, using a diagram	20
	20	Baseline demographic and clinical characteristics of participants	8
	21a	Distribution of severity of disease in those with the target condition	NA
	21b	Distribution of alternative diagnoses in those without the target condition	7-8
	22	Time interval and any clinical interventions between index test and reference standard	7-9
<i>Test results</i>	23	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Table 1 & 2, 21-23
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	8-10
	25	Any adverse events from performing the index test or the reference standard	Nil
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	3,15
	27	Implications for practice, including the intended use and clinical role of the index test	14,15
OTHER INFORMATION			
	28	Registration number and name of registry	N/A
	29	Where the full study protocol can be accessed	6
	30	Sources of funding and other support; role of funders	15,16

BMJ Open

Evaluation of reliability of self-collected vaginal swabs over physician-collected samples for diagnosis of bacterial vaginosis, candidiasis and trichomoniasis, in a resource limited setting: A cross sectional study in India

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-025013.R3
Article Type:	Research
Date Submitted by the Author:	20-Jul-2019
Complete List of Authors:	Khan, Zarine; VMMC & Safdarjung Hospital, Department of Microbiology Bhargava, Aradhana; VMMC and Safdarjung Hospital, Apex Regional STI Training, Research & Reference Laboratory Mittal, Pratima; VMMC & Safdarjung Hospital, Department of Obstetrics & Gynaecology Bharti, Rekha; VMMC & Safdarjung Hospital, Department of Obstetrics & Gynaecology Puri, Poonam; VMMC and Safdarjung Hospital, Department of Dermatology & STD Khunger, Niti; VMMC and Safdarjung Hospital, Department of Dermatology & STD Bala, Manju; VMMC and Safdarjung Hospital, Apex Regional STI Training, Research & Reference Laboratory
Primary Subject Heading:	Sexual health
Secondary Subject Heading:	Reproductive medicine, Epidemiology
Keywords:	Microbiology < BASIC SCIENCES, GENITOURINARY MEDICINE, Diagnostic microbiology < INFECTIOUS DISEASES

SCHOLARONE™
Manuscripts

1
2
3 **Evaluation of reliability of self-collected vaginal swabs over physician-collected samples for**
4 **diagnosis of bacterial vaginosis, candidiasis and trichomoniasis, in a resource limited**
5 **setting: A cross sectional study in India**
6
7
8
9

10
11
12 **Zarine Khan^{1,2}, Aradhana Bhargava ^{1,2}, Pratima Mittal³, Rekha Bharti³, Poonam Puri⁴,**
13 **Niti Khunger⁴, Manju Bala ^{1,2*}.**
14
15
16

17
18
19 ¹Department of Microbiology, VMMC & Safdarjung Hospital, New Delhi, India, ²Apex
20 Regional STI Training, Research & Reference Laboratory, VMMC & Safdarjung Hospital, New
21 Delhi, India, ³Department of Obstetrics & Gynaecology, VMMC & Safdarjung Hospital, New
22 Delhi, India, ⁴Department of Dermatology & STD, VMMC & Safdarjung Hospital, New Delhi,
23 India.
24
25
26
27
28
29
30
31
32

33 **Correspondence:**
34

35 Dr. Manju Bala, Consultant and Professor (Microbiology), Apex Regional STI Training,
36 Research & Reference Laboratory, Vardhman Mahavir Medical College & Safdarjung Hospital,
37 New Delhi, India. Telephone number: 91-11-26196740, Fax number: 91-11-26163072, Email:
38 manjubala_2@hotmail.com
39
40
41
42
43
44
45
46

47 **Key words:** Vaginal discharge; bacterial vaginosis; vulvovaginal candidiasis; trichomonas
48 vaginitis, self-collected vaginal swabs; physician-collected vaginal swabs.
49
50
51
52
53

54 **Word count: 2776**
55
56
57
58
59
60

ABSTRACT

Objectives Self-collected vaginal swabs can facilitate diagnosis of Vaginal discharge (VD) in resource-limited settings, provided reliability of the method is established. The aim of this study was to evaluate the concordance between self-collected and physician-collected vaginal swabs for etiological diagnosis of VD and to determine the prevalence of bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis (TV).

Methods A total of 550 females (median age: 32 years; range: 18-45 years) attending two STI/RTI clinics with VD from January 2015 to May 2016 were included in the study after obtaining written informed consent. Swabs were self-collected by patients after instructions and subsequently by a physician under speculum examination. Samples were processed for standard bedside tests, Gram staining, wet mount and culture (gold standard) according to national guidelines. Concordance between the two methods was determined by the Cohen's kappa value.

Results BV, VVC and TV were diagnosed in 79(14.4%), 144(26.2%) and 3(0.5%) patients respectively. VVC coexisted with BV in 58(10.5%) patients. There was no co-infection of TV with BV or VVC. *C. albicans* was isolated in 84(58.3%) VVC cases. Sensitivity, specificity, PPV and NPV of self-collected swabs for diagnosing BV was 91.1%, 100%, 100% and 98.5% respectively while for the *C. albicans* VVC and TV, sensitivity, specificity, PPV and NPV all were 100% as compared to physician-collected swabs. Highly concordant results were obtained between two methods by the Kappa values of 0.95 (BV), 0.99 (VVC) and 1.0 (TV).

Conclusion The comparative performance of self- and physician-collected vaginal swabs establishes self-collection of samples for BV, VVC and TV as a viable alternative tool in the management of sexually transmitted infections/ reproductive tract infections (STIs/RTIs) especially in peripheral and resource-constrained settings. This would be effective in

1
2
3 implementing the diagnostic approaches for STIs/RTIs in community based surveillance studies
4
5 at national or regional level and therefore strengthening the National STI/RTI Control
6
7 Programme.
8
9

11 12 **ARTICLE SUMMARY**

13 14 **Strengths and limitations of this study**

- 15
16
17 • This study determined that if diagnosis based on self-collected vaginal swabs is proven
18
19 reliable, it could contribute to early diagnosis and greatly increase the access to treatment.
20
21
- 22
23 • Specimens were obtained from 550 patients with vaginal discharge attending the two
24
25 STI/RTI clinics after obtaining the written informed consent. Concordance of
26
27 inexpensive standard bedside tests, such as Gram staining, wet mount and culture (gold
28
29 standard), was evaluated on both the self- and physician- collected samples for detecting
30
31 bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis
32
33 (TV).
34
- 35
36 • Corresponding findings were compared and analyzed using SPSS statistical analysis tool
37
38 in terms of Cohen's Kappa values to determine the concordance between findings of self-
39
40 and physician- collected samples.
41
- 42
43 • Limitation is that molecular tests were not performed, although gold standard test culture
44
45 was performed which is more economical and less labour intensive also.
46
- 47
48 • Another limitation was the low positivity of *Trichomonas vaginalis* in patients of vaginal
49
50 discharge by culture.
51
52
53
54
55
56
57
58
59
60

INTRODUCTION

Vaginal discharge is a frequently encountered complaint in women attending Sexually Transmitted Infections (STIs)/Reproductive Tract Infections (RTIs) clinics globally. It is mainly caused by curable bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis (TV).¹ Existing practice to diagnose vaginal discharge includes a speculum examination where the clinician inspects the external genitalia, vagina and cervix, assesses characteristics of the discharge, notes physical signs such as inflammation and lesions, and collects specimens for laboratory testing. The speculum examination requires a trained clinician and a proper setup. The discomfort of a pelvic examination may lead women to delay or avoid seeking care, which may result in lower enrollment rates and higher rates of loss to follow-up.^{2,3}

Even though high rates of STIs/RTIs are observed in developing countries due to various social and environmental factors such as sex ratio imbalances, urbanization and rural to urban migration, reliable detection of these infections are difficult due to poor infrastructure and lack of skilled workforce. Additionally, there is low female STI enrollment rate in developing countries due to women's reluctance to undergo gynecological examination, originating from cultural, religious and socio-economic factors.

Therefore, developing accurate approaches to diagnosing lower genital infections without a speculum examination would be advantageous to both clinicians and patients.² Self-collected vaginal swabs are the only practical and financially feasible method to use for sampling in field-based longitudinal cohort studies.³ As most of the studies comparing the reliability of self-collected vaginal swabs have been conducted in developed countries such as USA^{3,4,5} and Australia^{6,7} mostly using advanced molecular techniques such as nucleic acid amplification tests, limited literature on such studies is available from developing countries. In India, there was a

1
2
3 pilot study from Goa, which suggested that self-collected swabs are an acceptable method of
4 collection of vaginal specimens in women attending gynaecological clinics in India.⁸ However,
5 the main limitation of the study was a statistically small sample size. Moreover, the samples
6 were examined only by Gram's stain for their sensitivity for the diagnosis of STIs and were not
7 tested by other diagnostic techniques like wet mount and culture. Another study was performed
8 in New Delhi,⁹ which showed that self-collected swabs can reasonably approximate physician-
9 collected swabs for BV only. However, this study also was limited by a statistically small sample
10 size.
11
12
13
14
15
16
17
18
19
20
21

22 Hence, the current study was aimed at establishing reliability of self- collected vaginal
23 swabs against physician-collected swabs by determining its sensitivity, specificity, positive
24 predictive value (PPV) and negative predictive value (NPV) with significant number of samples
25 and quality reliable tests. Additionally, the fungal (*Candida albicans* or non-*albicans Candida*
26 species) bacterial (Bacterial vaginosis) and parasitic (*Trichomonas vaginalis*) etiology of vaginal
27 discharge and prevalence of various types of infections and co-infections were also studied.
28
29
30
31
32
33
34
35
36
37

38 METHODS

39
40 The study was conducted in two National AIDS Control Organization (NACO) designated
41 STI/RTI clinics in the Apex Regional STD Teaching, Training and Research Centre and
42 Department of Obstetrics and Gynaecology, Vardhaman Mahavir Medical College and
43 Safdarjung Hospital, New Delhi, India during January 2015 to May 2016. The thesis protocol
44 was approved by the authors' Institute Ethics Committee (VMMC & SJH). Sample size was
45 calculated according to the following formula: $S = \frac{4P(1-P)}{L^2}$ (where S = Sample size, P = Estimated
46 prevalence, L = Margin of error at 5%). For 5% margin of error, required sample size gets the
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 maximum value of 400 corresponding to the maximum heterogeneity condition of $P=50\%$.
4 Accordingly, a sample size larger than 400 was chosen and samples were collected from 550
5 sexually active females with vaginal discharge. A patient information sheet and consent form
6 were presented and approved by the protocol review committee and ethical committee of the
7 institution. After explaining the study details to the potentially eligible participants with the help
8 of a pre-designed patient information sheet; a written informed consent was obtained on patient
9 consent form. Both the patient information sheet and the consent form were designed in English
10 and Hindi (local language). Patients with history of antibiotic use or vaginal medication in the
11 previous 14 days, pregnant patients, patients unwilling/ unable to participate and patients with
12 human immunodeficiency virus (HIV) infection were excluded from this study.
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27

28 A total of six vaginal swabs, including three self-collected samples, were collected from
29 each participant. Participants were given instructions on appropriate specimen collection
30 technique before the speculum examination. They were instructed to insert the vaginal swab 1 to
31 2 inches into the vagina, twisting the swab to collect material on all sides of the tip, wipe in
32 several full circles on the vaginal wall, keep the swab in the vagina for 20 seconds, and then
33 carefully remove the swab and place it in a sterile tube. Subsequently, the female clinician
34 examined the participants and specimens were collected following the same procedure with
35 gloved hands under speculum examination. Proper indexing of the samples was performed and
36 the examining microbiologist was blind regarding the origin (physician- or self- collected) of the
37 swabs.
38
39
40
41
42
43
44
45
46
47
48
49
50

51 The samples were examined by standard bedside tests, Gram staining, wet mount and
52 culture (gold standard).¹⁰ For each patient, the first of the three self-collected swabs was used for
53
54
55
56
57
58
59
60

pH and whiff test, the second for wet mount and Gram staining, whereas the third swab was used for Candida and Trichomonas culture. The same tests were repeated for the three physician collected swabs. Bacterial vaginosis was diagnosed with the use of Amsel's criteria¹¹ and Nugent's score¹¹, whereas candidiasis and trichomoniasis were diagnosed based upon microscopy and culture results.

Patient and Public Involvement

Patients were not involved in this study in developing the design, recruitment or conduction of the study. Patients were not informed about the comparative results of the study but were given the report of the diagnosis based on physician collected samples as per routine practice. The result will be disseminated through this publication.

Statistical analysis

Data was analyzed using Microsoft excel and SPSS software version 21.0. Prevalence of bacterial, fungal and parasitic causative agents of vaginal discharge was studied in patients presenting with vaginal discharge. Also, their prevalence in various age groups was statistically analyzed using t-test (two- tailed). Sensitivity, specificity, positive predictive value and negative predictive value of self-collected specimens versus physician-collected specimens were calculated. Concordance between results obtained from self-collected and physician collected swabs was determined by calculating the Cohen's kappa value.

The value of Kappa is defined as

$$\kappa = \frac{p_o - p_e}{1 - p_e},$$

where p_o is the observed level of agreement and p_e is the expected level of agreement. The value of κ lies between -1 and 1 . A value of 1 implies perfect agreement whereas -1 implies perfect disagreement. When the two findings agree purely by chance, the value of kappa will be zero.¹²

RESULTS

Prevalence of BV, VVC and TV in patients with vaginal discharge using physician collected samples

The results obtained with physician-collected specimens were treated as the “standard results”. Prevalence of the three types of vaginal infections, namely BV, VVC and TV, as diagnosed by the Nugent score, candida culture and trichomonas culture respectively, are summarized in Figure 1. Out of the 550 patients presenting with vaginal discharge, 144 (26.2%) cases of VVC, 79 (14.4%) of BV, and 3 (0.5%) cases of TV were detected. Therefore, VVC was the most predominant infection in this population. A significant number of VVC infections were caused by non-albican species of *Candida* i.e. 60 out of 144 (41.7%) of the total VVC patients. BV and VVC coexisted in 21 (3.8%) patients. None of the TV infected patient had any co-infection with BV or VVC.

Table 1 analyses prevalence of various infections in different age groups. Maximum number of infections were found in the age group of 31-40 years (101/258) followed by 20-30 years (64/179) and >40 years (38/102). Only 2/11 participants less than 20 years of age were infected. In the age group 20 years or less, 9.1% (1/11) patients were found positive for BV and as well as for VVC. Among the 179 participants in the 20-30 years range, prevalence of both BV and VVC increased to 14% and 25.7%, respectively. In the age group 30-40 years (n=258), the

1
2
3 prevalence of BV reduced slightly to 12.8% but the prevalence for VVC continued to increase
4 (29.5%). For the remaining patients aged 40 years or more (n=102), the prevalence of BV
5 sharply increased to 19.6% whereas the prevalence decreased to 20.6% for VVC. When the
6 prevalence of BV and VVC across different age groups of vaginal discharge patients was
7 analyzed by paired t- test (two tailed), it was observed that the difference was not significant.
8
9
10
11
12
13

14 15 16 17 **Concordance between results from self- and physician- collected samples**

18
19 Cohen's Kappa was used as the metric of agreement between the two collection methods. The
20 data related to the concordance of the two methods for diagnosis of BV, VVC and TV are
21 depicted in Table 2.
22
23
24
25

26 27 28 *Concordance for BV:*

29
30
31 Diagnosis of BV was performed on the basis of Nugent's scoring. Of the total 550 participants,
32 376 (68.4%) showed a healthy vaginal flora (Nugent score 0-3), 95 (17.3%) were categorized as
33 "intermediate" (Nugent score 4-6) and the remaining 79 (14.4%) were diagnosed as BV (Nugent
34 score 7-10) based upon findings of physician collected swabs. For the Nugent score based
35 comparison; the outcome of a diagnosis could be classified in two different ways: (a) Three
36 categories: BV positive or BV intermediate or BV negative and (b) Two categories: BV positive
37 or BV non-positive by putting both intermediate and negative in the same bin of "non-positive".
38
39
40
41
42
43
44
45
46

47 For the three-category classification, both self- and physician- collection methods agreed
48 on 367 true negative cases, 82 intermediate cases and 72 true positive cases. Out of the BV
49 negative cases, self-collection method found nine cases as intermediate but no false positives.
50 For cases diagnosed as intermediate by physician-collected method, 13 were found negative by
51
52
53
54
55
56
57
58
59
60

1
2
3 self-collection method but, again, no false positives were detected. However, there were seven
4 cases of true positive diagnosis which were diagnosed as intermediate by self-collected samples,
5 but no true positive cases were diagnosed as negative by self-collected samples. The kappa value
6 computed for this is 0.890 with 95% confidence interval of 0.851 to 0.928, which indicates
7 excellent agreement between self- and physician- collected samples. The weighted kappa value
8 was 0.921 for this category.
9

10
11 For the two-category case, both methods diagnosed 471 non-positive cases and 72
12 positive cases. Self-collection method missed seven cases of BV positive results in this case, but
13 no false positive case was observed by the self-collection method. The kappa value computed for
14 this case is 0.946 with 95% confidence interval of 0.907 to 0.986 suggesting excellent agreement
15 between self- and physician- collected samples.
16

17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 *Concordance for VVC:*

32
33 The numbers of true negative and true positive cases were 406 and 144, respectively for VVC.
34 There was no missed true positive result and only one case of false positive was found with self-
35 collected samples. Very high concordance was observed with the kappa value at 0.994 with 95%
36 confidence interval of 0.982 to 1.000. Table 2 also lists the cases of *Candida albicans* and non-
37 *albicans* separately. For *C. albicans* infections, there was perfect concordance (kappa = 1.000)
38 with 84 true positive and 466 true negative cases. For non-*albicans Candida* cases, there were 60
39 true positive, 489 true negative and 1 false positive cases using self-collected swabs making the
40 kappa value to be 0.991 with 95% confidence interval of 0.973 to 1.000.
41
42
43
44
45
46
47
48
49
50
51

52 53 54 *Concordance for TV:*

1
2
3 Only three cases were found positive for TV and the results were identical for self- and
4
5 physician- collected samples yielding perfect concordance (kappa value of 1.000).
6
7

8 9 10 **Sensitivity, specificity, PPV and NPV of self-collected swabs in comparison to physician-** 11 **collected swabs** 12 13

14 Table 2 shows the sensitivity, specificity, PPV and NPV of diagnosis using self-collected swabs
15 when compared to physician-collected swabs, for BV, VVC and TV. Self-collection method had
16 acceptable sensitivity, specificity, PPV and NPV of 91.1%, 100%, 100% and 98.5% for
17 diagnosing BV using Nugent score. For VVC, including both *C. albicans* and non-*albicans*
18 *Candida* species, the self-collection method had high sensitivity of 100%, specificity of 99.8%,
19 PPV of 99.3%, and NPV of 100%. The values for non-*albicans* VVC, were identical to the
20 overall VVC cases except that the PPV (98.4%) was less. The sensitivity, specificity, PPV and
21 NPV were all 100% for self-collected swabs as compared to physician-collected swabs for the *C.*
22 *albicans* VVC and for TV.
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37

38 **DISCUSSION**

39
40
41
42 The prevalence of BV in women presenting with vaginal discharge to STI/RTI clinic was 14.4%.
43 In the literature the prevalence varies widely from 10.7% to 45%.^{4,13,14} In our study, we excluded
44 patients with history of antibiotic use or vaginal medication in the previous 14 days, pregnant
45 patients, patients unwilling/ unable to participate and HIV patients. Even though oral antibiotics
46 may potentially increase women's propensity towards vaginal candidiasis, but antibiotics such as
47 metronidazole also alter the vaginal flora by killing *Gardnerella vaginalis*. Also, with the
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 availability of these antibiotics as over the counter drug, it was difficult to exactly know the
4 exact antibiotic taken by the patient before visiting our tertiary reference clinic; hence this
5 exclusion criteria. As it is not advisable to perform self-collection of vaginal swabs in pregnant
6 woman they were not included in the study. Studies on HIV patient requires ethical clearance
7 from national organization in addition to the institutional ethical clearance and similar studies in
8 literature have not included HIV patients, hence the exclusion. These differences in the exclusion
9 criteria, contribute to the varying prevalence rates particularly because prevalence of BV
10 increases with age and immune-deficient conditions. More importantly, geographical locations
11 and cultural practices often have significant impact on the prevalence rates.
12
13
14
15
16
17
18
19
20
21
22
23

24 The prevalence for VVC was 26.2% in this study, which agrees well with the prevalence
25 of other studies.^{15,16} However, much smaller prevalence rates of 2.8% to 8.5% were reported in
26 other studies.^{14,17} Out of the 144 VVC positive cases, a significant number of isolates (60 or
27 41.7%) were due to non-*albican Candida* species. This conforms to the increasing trend of non-
28 *albicans* infections observed in the recent studies.¹⁸ Clinical implications of these non-*albicans*
29 *Candida* species is their documented decreased susceptibility to azoles, due to the indiscriminate
30 use of azole group of antifungals, especially fluconazole.¹⁸ Thus, early and accurate species
31 identification would be useful for the therapeutic management.
32
33
34
35
36
37
38
39
40
41

42 The prevalence of TV was quite low at 0.5% and was close to the values reported in a
43 few studies,^{15,16} but higher prevalence rates ranging from 5.6% to 16.1% were reported in other
44 studies.^{7,14,19} The higher prevalence rates were mostly observed in studies employing higher
45 sensitive molecular techniques such as nucleic acid amplification techniques (NAAT) for
46 detecting TV as compared to conventional culture technique used in this work. Additionally,
47 variation in geographical locations can contribute to the difference.²⁰ Unlike developed countries,
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 India being a large but resource limited country, newer advanced NAAT techniques are still used
4 only in tertiary care or research settings. The advanced tests would not be available in the rural
5
6 settings in the near future and were hence not used in this study.
7
8
9

10
11
12 Prevalence of BV among different age groups showed a statistically insignificant
13 increasing trend with age. The vaginal pH is dependent on the amount of lactic acid produced by
14 the vaginal lactobacilli from glycogen. The glycogen production from the superficial cornified
15 cells of the vaginal mucosa is dependent on estrogen stimulation, which is high during the
16 reproductive age group and decreases with age. This increasing vaginal pH increases the
17 susceptibility of aging women to BV.²¹ Similar observation was made for VVC up to the age
18 group of 30-40 years. However, though statistically insignificant, the percentage dropped for the
19 population above 40 years. The above findings are in agreement with the findings of another
20 study.¹⁶
21
22
23
24
25
26
27
28
29
30
31
32

33 In the present study, in general the kappa values were remarkably higher than the values
34 reported in similar studies. For diagnosis of BV, the Nugent score based method with two and
35 three categories had kappa values of 0.946 and 0.89, respectively, which are much higher than
36 kappa values of 0.71 and 0.72, correspondingly, reported in Strauss et al.¹³ In a similar study
37 performed by Huppert et al.,⁴ the reliability of self-collected vaginal swabs was established,
38 albeit with a value of kappa of 0.53. This can be attributed to the use of vaginal pH as the only
39 indicator of BV, instead of a more comprehensive Nugent score as used in this work.
40
41
42
43
44
45
46
47
48

49 A near-perfect concordance was observed in cases of VVC, with a kappa value of 0.995.
50 In VVC cases, self-collected samples produced one false positive result as compared to
51 physician-collected sample. This can possibly be attributed to the contamination of the swab with
52
53
54
55
56
57
58
59
60

1
2
3 skin commensal flora during self-collection or it may be because of scanty discharge; where after
4 self-collection not enough sample was left for collection by the physician. One of the limitation
5 of the study was that the self and physician collection was not alternated for every consecutive
6 patients. But the high agreement in both the methods negates the influence of this factor on the
7 results.
8
9

10
11
12 A perfect match ($\kappa=1$) between two methods was observed in this study for TV
13 infections with a small number of positive cases (three positive cases out of 550). In order to
14 maintain the uniformity in data presentation the kappa value was calculated despite the low
15 positivity.
16
17

18
19 Thus, the current study findings highlight that near-perfect match was observed between self-
20 collected and physician collected swabs for diagnosis of BV, VVC and TV and it was proven by
21 the high values of kappa (all greater than 0.9). The high concordance can partly be attributed to
22 the following reasons:
23

- 24
25
26
27
28
29
30
31
32
33 (1) *Mature patients*: Almost 98% of the patients were of age 20 years or older with average age
34 of 32 years, which helped the patients to collect the sample properly.
35
36
37 (2) *Sample collection under supervision*: In the clinic, the patients were under supervision and
38 were provided with clarifications whenever in doubt. However, most of the patients were
39 able to collect the sample without further help post-verbal instructions.
40
41
42
43
44 (3) *No delay in transportation of samples to the laboratory*: The samples were collected by both
45 the methods inside the STI/RTI clinic and were transported to the STI laboratory
46 immediately, which is located very near to the clinic. Specimens were processed immediately
47 in the laboratory. Therefore, no transportation delay, sample labeling errors and sample
48 contamination occurred leading to high concordance between the two methods.
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Even when used in resource constrained peripheral areas, self-collected swabs can perform really well if mislabeling and transport error can be minimized and online or other forms of assistance can be provided in case of doubts. The proliferation of mobile phones even in remote corners of India can facilitate such online assistance. From this study, it was established beyond doubt that self-collected swabs yield diagnostic results as accurate as physician-collected swabs for almost all practical purposes.

When replicated in peripheral resource constrained settings, self-collected swabs would provide alternative method of sample collection for patients who refrain from getting gynaecological examination either due to social or cultural misconceptions. An early and accurate diagnosis based on this inexpensive method would make testing more approachable, economical and would improve the treatment outcomes. When integrated with proper quality assurance, self-collected swabs may form an important diagnostic tool in community based studies. A limitation of this study is that molecular tests were not performed, although gold standard test culture was performed which is more economical and less labour intensive.

CONCLUSION

It was demonstrated that with specific instructions and guidance, self-collected swabs can approximate physician-collected swabs with a high degree of reliability. Therefore, self-collected vaginal swabs are a viable and accurate method for diagnosing vaginal infections, which may have adverse outcomes including preterm birth, low birth weight, post-operative infections and increased risk of acquisition and transmission of STIs including the HIV infection. Hence,

1
2
3 prevention and timely management of curable STIs and RTIs is particularly important. The
4 findings of this study will help in planning and implementing the diagnostic approaches for
5 STIs/RTIs in community based surveillance studies at national or regional level and also in the
6 effective day-to-day STI/RTI diagnosis and management in the peripheral health settings.
7
8
9
10
11
12
13

14 **Contributors**

15 MB, ZK, AB designed the study, coordinated the work and finalized the draft of the manuscript.
16
17 ZK participated in all the data collection, testing, carried out analysis of data and prepared first
18 draft of the manuscript. PM, RB, PP, NK helped in designing the study, sample collection and
19 collaborated in writing of the manuscript. Guarantor of the article: MB.
20
21
22
23
24
25

26 **Ethical approval**

27 The study was approved the Institute Ethics Committee of VMMC & Safdarjung hospital with
28 approval number IEC/VMMC/SJH/Thesis/November-2014/429 and date 25 November 2014.
29
30
31
32

33 **Acknowledgements**

34 We thank the Medical Superintendent and Principal, VMMC & Safdarjung Hospital for
35 permitting us to carry out this study. The authors are thankful to the Head, Department of
36 Microbiology and Apex Regional STD Teaching, Training & Research Centre for granting
37 permission to Dr. Zarine Khan to carry out her thesis work. We are grateful to Mrs. Ranjana
38 Gupta and Ms. Hemlata Saxena for their technical assistance.
39
40
41
42
43
44
45
46

47 **Competing interests** None declared.

48 **Patient consent** Obtained.

49 **Funding** None declared.
50
51
52
53
54
55
56
57
58
59
60

Data sharing statement: The deidentified participant data may be available upon request for a period of 1 year at the corresponding author mail ID.

REFERENCES

1. **Fule SR**, Fule RP, Tankhiwale NS. Clinical and laboratory evidence of *Trichomonas vaginalis* infection among women of reproductive age in rural area. *Indian J Med Microbiol* 2012;**30**:314.
2. **Singh RH**, Zenilman JM, Brown KM, *et al*. The role of physical examination in diagnosing common causes of vaginitis: a prospective study. *Sex Transm Infect* 2013;**89**:185–90.
3. **Forney LJ**, Gajer P, Williams CJ, *et al*. Comparison of self-collected and physician-collected vaginal swabs for microbiome analysis. *J Clin Microbiol* 2010;**48**:1741–8.
4. **Huppert JS**, Hesse EA, Bernard MC, *et al*. Accuracy and trust of self-testing for bacterial vaginosis. *J Adolesc Health* 2012;**51**:400–5.
5. **Nelson DB**, Bellamy S, Gray TS, *et al*. Self-collected versus provider-collected vaginal swabs for the diagnosis of bacterial vaginosis: An assessment of validity and reliability. *J Clin Epi* 2003 56(9):862–60.
6. **Garrow SC**, Smith DW, Harnett GB. The diagnosis of chlamydia, gonorrhoea, and trichomonas infections by self-obtained low vaginal swabs, in remote northern Australian clinical practice. *Sex Transm Infect* 2002;**78**:278–81.
7. **Knox J**, Tabrizi SN, Miller P, *et al*. Evaluation of self-collected samples in contrast to practitioner-collected samples for detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* by polymerase chain reaction among women living in remote areas. *Sex Transm Dis* 2002;**29**:647–54.

- 1
2
3 8. **Tanksale VS**, Sahasrabhojane M, Patel V, *et al.* The reliability of a structured examination
4 protocol and self-collected vaginal swabs: a pilot study of gynaecological outpatients in Goa,
5 India. *Sex Transm Infect* 2003;**79**:251–3.
6
7
- 8 9. **Kashyap B**, Singh R, Bhalla P, *et al.* Reliability of self-collected versus provider-collected
9 vaginal swabs for the diagnosis of bacterial vaginosis. *Int J STD AIDS* 2008 19(8): 510-3.
10
11
- 12 10. **Department of AIDS control**, Ministry of Health and Family Welfare, Government of
13 India. Laboratory Manual for Diagnosis of Sexually Transmitted and Reproductive Tract
14 Infections. February 2014. Available from: URL: [http://www.indiahivinfo.naco.gov.in/sites/
15 default/files/media-gallery/STI_Report.pdf](http://www.indiahivinfo.naco.gov.in/sites/default/files/media-gallery/STI_Report.pdf).
16
17
- 18 11. Unemo, M. (2013). *Laboratory diagnosis of sexually transmitted infections, including human*
19 *immunodeficiency virus*. 1st ed. Geneva, Switzerland: WHO Document Production Services,
20 p.86. (ISBN 978 92 4 150584 0)
21
22
- 23 12. **Merrill RM**. Fundamentals of Epidemiology and Biostatistics. Jones & Bartlett Publishers,
24 2012. p 167-8.
25
26
- 27 13. **Strauss RA**, Eucker B, Savitz DA, Thorp JM. Diagnosis of bacterial vaginosis from self-
28 obtained vaginal swabs. *Infect Dis Obstet Gynecol* 2005;**13**:31–5.
29
30
- 31 14. **Passos MR**, Varella RQ, Barreto NA, *et al.* Accuracy of a self-collection kit for the
32 microbiological study of the vaginal content. *Braz J Infect Dis* 2007;**11**:249–53.
33
34
- 35 15. **Puri, KJ**, Madan, A, Bajaj, K. Incidence of various causes of vaginal discharge among
36 sexually active females in age group 20-40 years. *Indian J Dermatol Venereol Leprol*
37 2003;**69**:122.
38
39
- 40 16. **Gandhi TN**, Patel MG, Jain MR. Prospective study of vaginal discharge and prevalence of
41 Vulvovaginal candidiasis in a tertiary care hospital. *Int J Curr Res Rev* 2015;**7**:34-8.
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 17. **Pereira DC**, Backes LT, Calil LN, *et al.* A six-year epidemiological survey of
4 vulvovaginal candidiasis in cytopathology reports in the state of Rio Grande do Sul, Brazil.
5
6 *Rev Patol Trop* 2012;**41**:163-8.
7
8
9
10 18. **Tellapragada C**, Eshwara VK, Johar R, *et al.* Antifungal susceptibility patterns, in vitro
11 production of virulence factors, and evaluation of diagnostic modalities for the speciation of
12 pathogenic candida from blood stream infections and vulvovaginal candidiasis. *J Pathog*
13 2014;142864.
14
15
16
17
18 19. **Holland-Hall CM**, Wiesenfeld HC, Murray PJ. Self-collected vaginal swabs for the
19 detection of multiple sexually transmitted infections in adolescent girls. *J Pediatr Adolesc*
20 *Gynecol* 2002;**15**:307–13.
21
22
23
24
25 20. **Malla NA**, Gupta IN, Mahajan RC. Human trichomoniasis. *Indian J Med Microbiol*
26 2001;**19**:6-13.
27
28
29
30 21. **Padubidri VG**, Daftary SN. Shaw's Textbook of gynecology. 16th ed. New Delhi: Elsevier
31 Health Sciences; 2014:379-90.
32
33
34
35
36
37

38 **Key messages**

- 39 • This study has shown that VVC was the most prevalent (26.2%) cause of vaginal
40 discharge, followed by BV (14.4%) and TV (0.5%).
41
42
- 43 • High values of Cohen's Kappa were obtained for all three infections: 0.95 (BV), 0.99
44 (VVC) and 1.0 (TV).
45
46
- 47 • High concordance of self-collected swabs with physician-collected swabs proves the
48 efficacy of self-collected swabs in diagnosing the major causes of vaginal discharge, with
49 high sensitivity and specificity.
50
51
52
53
54
55
56
57
58
59
60

- This will also help in early diagnosis and management of patients in resource-constrained and peripheral settings thereby strengthening National STI/RTI control programs worldwide.

Table 1 Prevalence of various types of infections in patients with vaginal discharge based on physician-collected samples among various age groups,

Age group	Number of patients with type of infection				
	BV n/N (%)	VVC n/N (%)			TV n/N (%)
		<i>C. albicans</i>	Non- <i>albicans</i> <i>Candida</i> species	Total VVC	
<20	1/11 (9.1)	1/11 (9.1)	0/11 (0.0)	1/11 (9.1)	0/11 (0.0)
20-30	25/179 (14.0)	32/179 (17.9)	14/179 (7.8)	46/179 (25.7)	1/179 (0.6)
31-40	33/258 (12.8)	40/258 (15.5)	36/258 (14.0)	76/258 (29.5)	1/258 (0.4)
>40	20/102	11/102	10/102 (9.8)	21/102	1/102

	(19.6)	(10.8)		(20.6)	(1.0)
Total	79/550	84/550	60/550	144/550	3/550
	(14.4)	(15.3)	(10.9)	(26.2)	(0.5)
Sig (two tailed)*	0.069	0.073	0.062	0.116	

*Paired t-test has been used to evaluate prevalence in various age groups, BV (Bacterial vaginosis), VVC (Vulvo-vaginal candidiasis) and TV (*Trichomonas vaginalis*)

TABLE 2 Concordance between physician- and self- collected swabs for diagnosis of bacterial vaginosis, vulvovaginal candidiasis and trichomonas vaginitis for a sample size N=550. Outcomes are categorized with combination of “P” and “i” (representing Physician- and Self- collected, respectively) with “+”, “-” and “i” for positive, negative and intermediate, respectively. Example: P+/Si represents the cases where the diagnosis was positive for physician-collected and intermediate for self-collected samples.

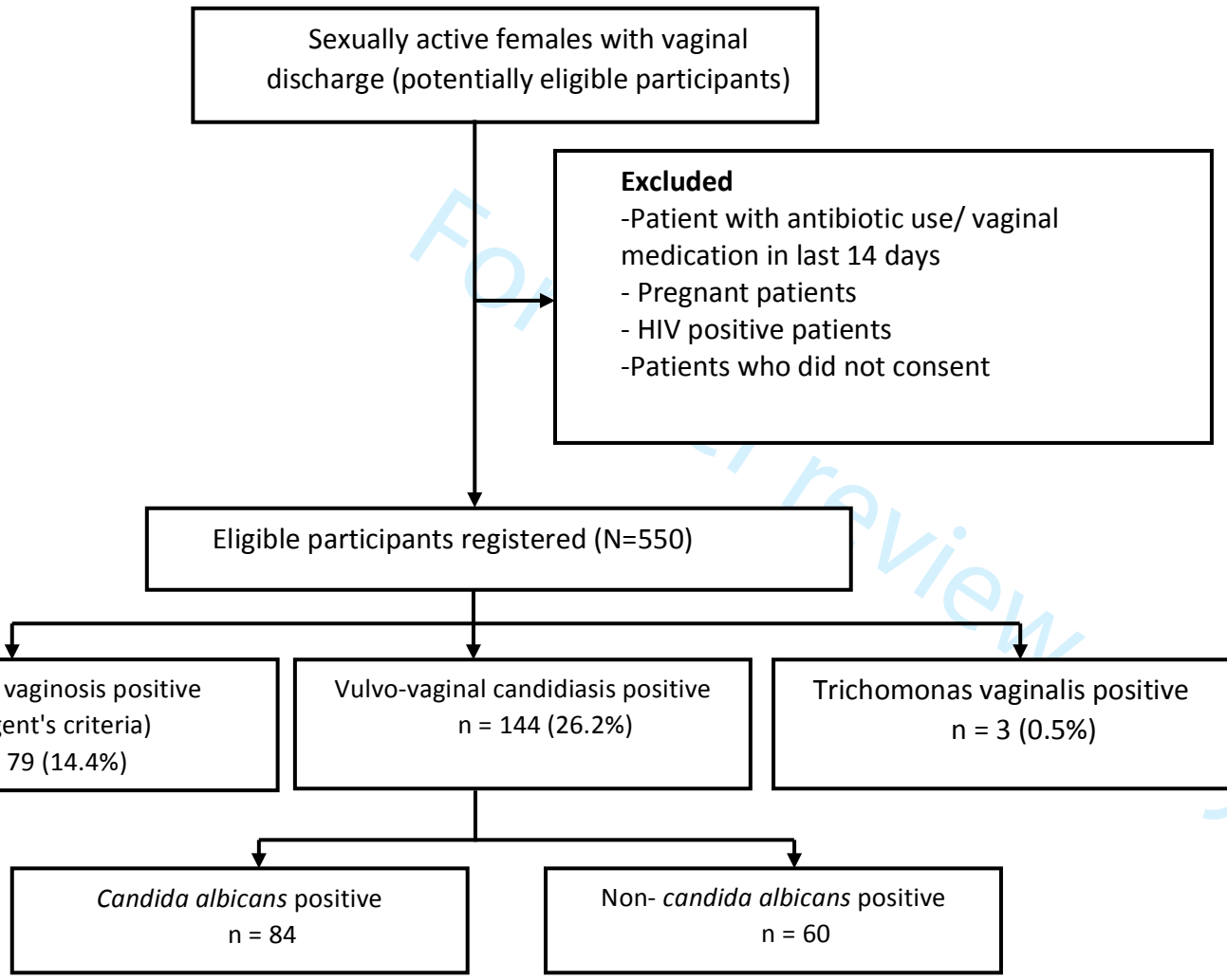
Criterion Used	Number of patients (physician-collected versus self-collected)									Kappa (95% CI)	Prevalence	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	
	P+/S+	P+/Si	P+/S-	Pi/S+	Pi/Si	Pi/S-	P-/S+	P-/Si	P-/S-							
Bacterial vaginosis																
Nugent – 3 category	72	7	0	0	82	13	0	9	367	0.890 (0.851 -0.928)	14.4	11.1	100	100	98.5	
Nugent – 2 category	72	–	7	–	–	–	0	–	471	0.946 (0.907 -0.986)	14.4	11.1	100	100	98.5	
Vulvovaginal candidiasis																
<i>C.albicans</i>	84	–	0	–	–	–	0	–	466	1.000 (1.000 -1.000)	15.3	100	100	100	100	
Non-albicans	60	–	0	–	–	–	1	–	489	0.991 (0.973 -1.000)	10.9	100	99.8	98.4	100	
All	144	–	0	–	–	–	1	–	405	0.995 (0.986 -1.000)	26.2	100	99.8	99.3	100	
Trichomonas vaginitis																
TV culture	3	–	0	–	–	–	0	0	547	1.000 (1.000 -1.000)	0.5	100	100	100	100	
PPV, Positive predictive value; NPV, Negative predictive value.																

bmjopen-2018-025013-2019-08-27 August 2019. Downloaded from http://bmjopen.bmj.com/ on April 19, 2024 by guest. Protected by copyright.

1
2
3
4
5 Figure 1: Prevalence of bacterial vaginosis (BV), vulvo-vaginal candidiasis (VVC) and
6 Trichomonas vaginalis (TV) in 550 females presenting with vaginal discharge diagnosed by
7
8 physician collected swabs.
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46



Section & Topic	No	Item	Reported on page
TITLE OR ABSTRACT		STARD 2015 Research check-list	
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2-3
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4-5
	4	Study objectives and hypotheses	5
METHODS			
<i>Study design</i>	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
<i>Participants</i>	6	Eligibility criteria	5-6
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	5-6
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5-6
	9	Whether participants formed a consecutive, random or convenience series	5-6
<i>Test methods</i>	10a	Index test, in sufficient detail to allow replication	6
	10b	Reference standard, in sufficient detail to allow replication	6
	11	Rationale for choosing the reference standard (if alternatives exist)	6
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	6
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	6
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	6
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	6
<i>Analysis</i>	14	Methods for estimating or comparing measures of diagnostic accuracy	6-7
	15	How indeterminate index test or reference standard results were handled	N/A
	16	How missing data on the index test and reference standard were handled	N/A
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	6
	18	Intended sample size and how it was determined	5
RESULTS			
<i>Participants</i>	19	Flow of participants, using a diagram	20
	20	Baseline demographic and clinical characteristics of participants	8
	21a	Distribution of severity of disease in those with the target condition	NA
	21b	Distribution of alternative diagnoses in those without the target condition	7-8
	22	Time interval and any clinical interventions between index test and reference standard	7-9
<i>Test results</i>	23	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Table 1 & 2, 21-23
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	8-10
	25	Any adverse events from performing the index test or the reference standard	Nil
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	3,15
	27	Implications for practice, including the intended use and clinical role of the index test	14,15
OTHER INFORMATION			
	28	Registration number and name of registry	N/A
	29	Where the full study protocol can be accessed	6
	30	Sources of funding and other support; role of funders	15,16