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

Zarine Khan[1,2], Aradhana Bhargava [1,2], Pratima Mittal[3], Rekha Bharti[3], Poonam Puri[4], Niti Khunger[4], Manju Bala [1,2*].

[1]Department of Microbiology, VMMC & Safdarjung Hospital, New Delhi, India, [2]Apex Regional STI Training, Research & Reference Laboratory, VMMC & Safdarjung Hospital, New Delhi, India, [3]Department of Obstetrics & Gynaecology, VMMC & Safdarjung Hospital, New Delhi, India, [4]Department of Dermatology & STD, VMMC & Safdarjung Hospital, New Delhi, India.

Correspondence:

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

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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

be effective in implementing the diagnostic approaches for STIs/RTIs in community based surveillance studies at national or regional level and therefore strengthening the National STI/RTI Control Programme.

ARTICLE SUMMARY

Strengths and limitations of this study

- This study determined that if diagnosis based on self-collected vaginal swabs is proven reliable, could contribute to early diagnosis and greatly increase the access to treatment.
- In this prospective study, specimens were obtained from 550 patients with vaginal discharge attending the two STI/RTI clinics after obtaining the written informed consent.
- Standard bedside tests, Gram staining, wet mount and culture (gold standard) were performed on both the self- and physician- collected samples for detecting bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis (TV).
- Corresponding findings were compared and analyzed using SPSS statistical analysis tool in terms of Cohen's Kappa values to detrmine the concordance between findings of self- and physician- collected samples.
- Limitation is that molecular tests were not performed, although gold standard test culture was performed which is more economical and less labour intensive also.

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

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

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

METHODS

The study was conducted in two National AIDS Control Organization (NACO) designated STI/RTI clinics in the Apex Regional STD Teaching, Training and Research Centre and Department of Obstetrics and Gynaecology, Vardhaman Mahavir Medical College and Safdarjung Hospital, New Delhi, India during January 2015 to May 2016. The thesis protocol was approved by the authors' Institute Ethics Committee (VMMC & SJH). Samples were collected from 550 sexually active females with vaginal discharge. Participants were between 18 and 45 years and written informed consent was obtained. Patients with history of antibiotic use or vaginal medication in the previous 14 days, pregnant patients, patients not willing to

participate were excluded from this study, patients with human immunodeficiency virus (HIV) infection and other serious illness or disability were excluded from this study.

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

The samples were examined by standard bedside tests, Gram staining, wet mount and culture (gold standard).[8] For each patient, the first of the three self-collected swabs was used for 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 criteria and Nugent's score, 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. 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".

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 computed for this is 0.890 with 95% confidence interval of 0.851 to 0.928, which indicates excellent agreement between self- and physician- collected samples.

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

Concordance for VVC:

The numbers of true negative and true positive cases were 406 and 144, respectively for VVC. There was no missed true positive result and only one case of false positive was found with self-collected samples. Very high concordance was observed with the kappa value at 0.994 with 95% confidence interval of 0.982 to 1.000. Table 2 also lists the cases of *Candida albicans* and non-albicans separately. For *C. albicans* infections, there was perfect concordance (kappa = 1.000) with 84 true positive and 466 true negative cases. For non-*albicans Candida* cases, there were 60

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

Concordance for TV:

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

Sensitivity, specificity, PPV and NPV of self-collected swabs in comparison to physician-collected swabs for diagnosis of BV, VVC and TV

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-obtained 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, 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]

Prevalence of BV among different age groups showed an increasing trend with age. The vaginal pH is dependent on the amount of lactic acid produced by the vaginal lactobacilli from glycogen. The glycogen production from the superficial cornified cells of the vaginal mucosa is dependent on estrogen stimulation, which is high during the reproductive age group and decreases with age. This increasing vaginal pH increases the susceptibility of aging women to BV.[19] Similar observation was made for VVC up to the age group of 30-40 years. However, the percentage dropped for the population above 40 years. This dip in prevalence can be attributed to normally decreasing sexual activities at this age group.[20] The above findings are in agreement with the findings of another study.[13]

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

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

A perfect match (kappa=1) between two methods was observed in this study for TV infections with a small number of positive cases (three positive cases out of 550).

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

(1) *Mature patients*: Almost 98% of the patients were of age 20 years or older with average age of 32 years. 96% of the population was married at some point. The maturity helped the patients follow the instructions and insert the swab properly in their genitalia for collecting the sample.

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

(3) *No delay in transportation of samples to the laboratory*: The samples were collected by both the methods inside the STI/RTI clinic and were transported to the STI laboratory immediately, which is located very near to the clinic. Specimens were processed immediately in the laboratory. Therefore, no transportation delay, sample labeling errors and sample contamination occurred leading to high concordance between the two methods.

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.

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.

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Competing interests None.

Patient consent Obtained.

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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

Age group	Number of patients with type of infection					
	BV N (%)	VVC N (%)			TV N (%)	Total N (%)
		C. albicans	Non-*albicans Candida* 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	Sensi-tivity (%)	Speci-ficity (%)	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															
C.albicans	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			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
Participants	**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
Test methods	**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
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	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
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Participants	**19**	Flow of participants, using a diagram	N/A
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Evaluation of reliability of self-collected vaginal swabs over physician-collected samples for diagnosis of bacterial vaginosis, candidiasis and trichomoniasis

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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.

Zarine Khan[1,2], Aradhana Bhargava [1,2], Pratima Mittal[3], Rekha Bharti[3], Poonam Puri[4], Niti Khunger[4], Manju Bala [1,2*].

[1]Department of Microbiology, VMMC & Safdarjung Hospital, New Delhi, India, [2]Apex Regional STI Training, Research & Reference Laboratory, VMMC & Safdarjung Hospital, New Delhi, India, [3]Department of Obstetrics & Gynaecology, VMMC & Safdarjung Hospital, New Delhi, India, [4]Department of Dermatology & STD, VMMC & Safdarjung Hospital, New Delhi, India.

Correspondence:

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

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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

implementing the diagnostic approaches for STIs/RTIs in community based surveillance studies at national or regional level and therefore strengthening the National STI/RTI Control Programme.

ARTICLE SUMMARY

Strengths and limitations of this study

- This study determined that if diagnosis based on self-collected vaginal swabs is proven reliable, it could contribute to early diagnosis and greatly increase the access to treatment.
- Specimens were obtained from 550 patients with vaginal discharge attending the two STI/RTI clinics after obtaining the written informed consent. Concordance of inexpensive standard bedside tests, such as Gram staining, wet mount and culture (gold standard), was evaluated on both the self- and physician- collected samples for detecting bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis (TV).
- Corresponding findings were compared and analyzed using SPSS statistical analysis tool in terms of Cohen's Kappa values to determine the concordance between findings of self- and physician- collected samples.
- Limitation is that molecular tests were not performed, although gold standard test culture was performed which is more economical and less labour intensive also.
- Another limitation was the low positivity of Trichomonas vaginalis in patients of vaginal discharge by culture.

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

pilot study from Goa, which suggested that self-collected swabs are an acceptable method of collection of vaginal specimens in women attending gynaecological clinics in India.[8] However, the main limitation of the study was a statistically small sample size. Moreover, the samples were examined only by Gram's stain for their sensitivity for the diagnosis of STIs and were not tested by other diagnostic techniques like wet mount and culture. Another study was performed in New Delhi,[9] which showed that self-collected swabs can reasonably approximate physician-collected swabs for BV only. However, this study also was limited by a statistically small sample size.

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

METHODS

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

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

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

The samples were examined by standard bedside tests, Gram staining, wet mount and culture (gold standard).[10] For each patient, the first of the three self-collected swabs was used for 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 criteria[11] and

Nugent's score[11], 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 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

prevalence of BV and VVC across different age groups of vaginal discharge patients was analyzed by paired t- test (two tailed), it was observed that the difference was not significant.

Concordance between results 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 Figure 2 and 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 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

computed for this is 0.890 with 95% confidence interval of 0.851 to 0.928, which indicates excellent agreement between self- and physician- collected samples. The weighted kappa value was 0.921 for this category.

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

Concordance for VVC:

The numbers of true negative and true positive cases were 406 and 144, respectively for VVC. There was no missed true positive result and only one case of false positive was found with self-collected samples. Very high concordance was observed with the kappa value at 0.994 with 95% confidence interval of 0.982 to 1.000. Table 2 also lists the cases of *Candida albicans* and non-albicans separately. For *C. albicans* infections, there was perfect concordance (kappa = 1.000) with 84 true positive and 466 true negative cases. For non-*albicans Candida* cases, there were 60 true positive, 489 true negative and 1 false positive cases using self-collected swabs making the kappa value to be 0.991 with 95% confidence interval of 0.973 to 1.000.

Concordance for TV:

Only three cases were found positive for TV and the results were identical for self- and physician- collected samples yielding perfect concordance (kappa value of 1.000).

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

other studies.[14,17] 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.[18] Clinical implications of these non-*albicans Candida* species is their documented decreased susceptibility to azoles, due to the indiscriminate use of azole group of antifungals, especially fluconazole.[18] 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,[15,16] but higher prevalence rates ranging from 5.6% to 16.1% were reported in other studies. [7,14,19] 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.[20]

Prevalence of BV among different age groups showed a statistically insignificant increasing trend with age. The vaginal pH is dependent on the amount of lactic acid produced by the vaginal lactobacilli from glycogen. The glycogen production from the superficial cornified cells of the vaginal mucosa is dependent on estrogen stimulation, which is high during the reproductive age group and decreases with age. This increasing vaginal pH increases the susceptibility of aging women to BV.[21] Similar observation was made for VVC up to the age group of 30-40 years. However, the percentage dropped for the population above 40 years. This statistically insignificant dip in prevalence can be attributed to normally decreasing sexual activities at this age group.[22] The above findings are in agreement with the findings of another study.[16]

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

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

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

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

(1) *Mature patients*: Almost 98% of the patients were of age 20 years or older with average age of 32 years. 96% of the population was married at some point, which helped the patients to insert the swab properly in their genitalia for collecting the sample.

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

(3) *No delay in transportation of samples to the laboratory*: The samples were collected by both the methods inside the STI/RTI clinic and were transported to the STI laboratory immediately, which is located very near to the clinic. Specimens were processed immediately in the laboratory. Therefore, no transportation delay, sample labeling errors and sample contamination occurred leading to high concordance between the two methods.

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 to patients who refrain from getting gynaecological examination either due to social or cultural misconceptions. An early and accurate diagnosis based on inexpensive standard 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 also.

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, prevention and timely management of curable STIs and RTIs is particularly important. 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.

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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 n/N (%)**	**VVC n/N (%)**			**TV n/N (%)**
		C. albicans	**Non-*albicans Candida* 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*)

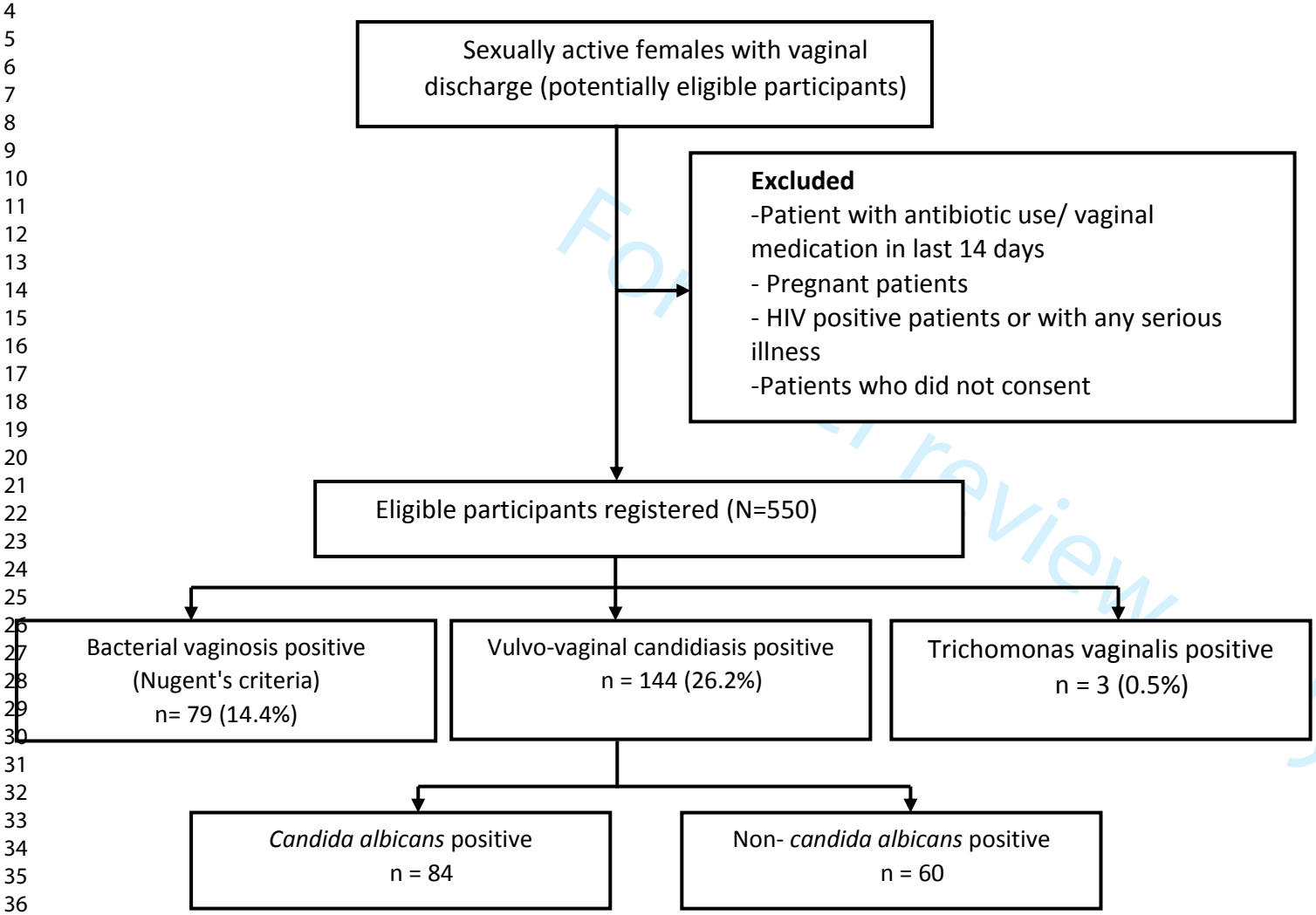
ABLE 2 Concordance between physician- and self- collected swabs for diagnosis of bacterial vaginosis, vulvovaginal candidiasis and trichomonas vaginitis for a sample size N=550. Outcomes are categorized with combination of "P" and "S" (representing Physician- and Self- collected, respectively) with "+", "-" 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.

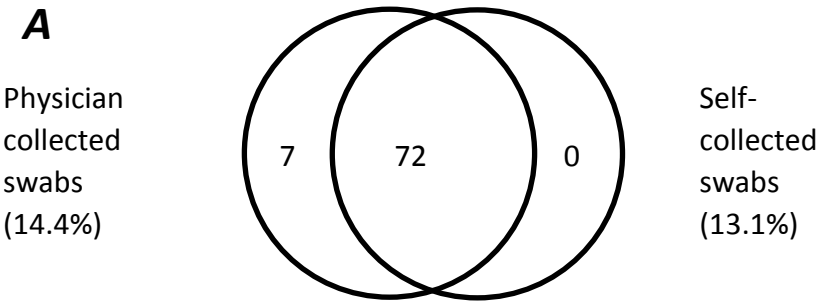
Criterion Used	Number of patients (physician-collected versus self-collected)									Kappa (95% CI)	Prevalence	Sensi-tivity (%)	Speci-ficity (%)	PPV (%)	NPV (%)
	P+/S+	P+/Si	P+/S-	Pi/S+	Pi/Si	Pi/S-	P-/S+	P-/Si	P-/S-						
Bacterial vaginosis															
Nugent – 3 category	72	7	0	0	82	13	0	9	367	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															
C.albicans	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.

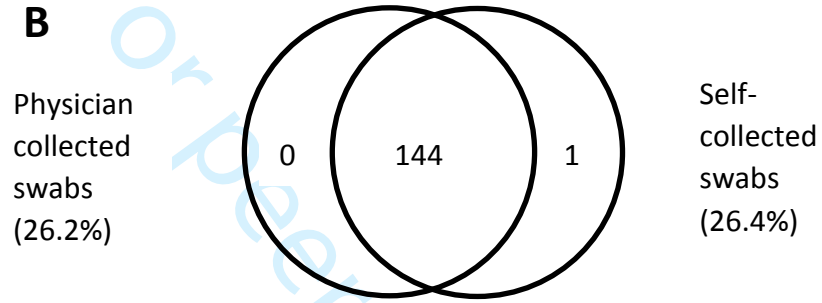
Figure 1: Prevalence of bacterial vaginosis (BV), vulvo-vaginal candidiasis (VVC) and Trichomonas *vaginalis* (TV) in 550 females presenting with vaginal discharge diagnosed by physician collected swabs.

Figure 2: A comparison of etiological diagnosis of vaginal discharge by physician and self-collected swabs (N=550). (A) A total of 79 bacterial vaginosis cases were identified by physician collected swabs. (B) A total of 144 vulvo-vaginal candidiasis cases were identified by physician collected swabs. (C) A total of 3 trichomonas vaginalis cases were identified by physician collected swabs.

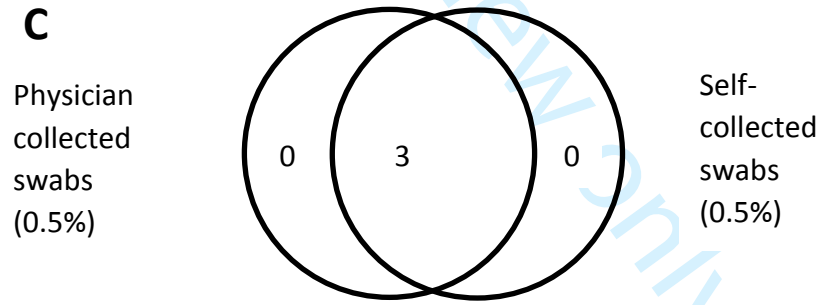




Bacterial vaginosis positive cases (n=79)



Vulvo-vaginal candidiasis positive cases (n=144)



Trichomonas vaginalis positive cases (n=3)

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			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
Participants	**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
Test methods	**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
Analysis	**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			
Participants	**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
Test results	**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

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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

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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

Zarine Khan[1,2], Aradhana Bhargava [1,2], Pratima Mittal[3], Rekha Bharti[3], Poonam Puri[4], Niti Khunger[4], Manju Bala [1,2*].

[1]Department of Microbiology, VMMC & Safdarjung Hospital, New Delhi, India, [2]Apex Regional STI Training, Research & Reference Laboratory, VMMC & Safdarjung Hospital, New Delhi, India, [3]Department of Obstetrics & Gynaecology, VMMC & Safdarjung Hospital, New Delhi, India, [4]Department of Dermatology & STD, VMMC & Safdarjung Hospital, New Delhi, India.

Correspondence:

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

Key words: Vaginal discharge; bacterial vaginosis; vulvovaginal candidiasis; trichomonas vaginitis, self-collected vaginal swabs; physician-collected vaginal swabs.

Word count: 2776

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

implementing the diagnostic approaches for STIs/RTIs in community based surveillance studies at national or regional level and therefore strengthening the National STI/RTI Control Programme.

ARTICLE SUMMARY

Strengths and limitations of this study

- This study determined that if diagnosis based on self-collected vaginal swabs is proven reliable, it could contribute to early diagnosis and greatly increase the access to treatment.
- Specimens were obtained from 550 patients with vaginal discharge attending the two STI/RTI clinics after obtaining the written informed consent. Concordance of inexpensive standard bedside tests, such as Gram staining, wet mount and culture (gold standard), was evaluated on both the self- and physician- collected samples for detecting bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis (TV).
- Corresponding findings were compared and analyzed using SPSS statistical analysis tool in terms of Cohen's Kappa values to determine the concordance between findings of self- and physician- collected samples.
- Limitation is that molecular tests were not performed, although gold standard test culture was performed which is more economical and less labour intensive also.
- Another limitation was the low positivity of Trichomonas vaginalis in patients of vaginal discharge by culture.

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

pilot study from Goa, which suggested that self-collected swabs are an acceptable method of collection of vaginal specimens in women attending gynaecological clinics in India.[8] However, the main limitation of the study was a statistically small sample size. Moreover, the samples were examined only by Gram's stain for their sensitivity for the diagnosis of STIs and were not tested by other diagnostic techniques like wet mount and culture. Another study was performed in New Delhi,[9] which showed that self-collected swabs can reasonably approximate physician-collected swabs for BV only. However, this study also was limited by a statistically small sample size.

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

METHODS

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

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

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

The samples were examined by standard bedside tests, Gram staining, wet mount and culture (gold standard).[10] For each patient, the first of the three self-collected swabs was used for 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 criteria[11] and

Nugent's score[11], 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 1analyses 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

prevalence of BV and VVC across different age groups of vaginal discharge patients was analyzed by paired t- test (two tailed), it was observed that the difference was not significant.

Concordance between results 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 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

computed for this is 0.890 with 95% confidence interval of 0.851 to 0.928, which indicates excellent agreement between self- and physician- collected samples. The weighted kappa value was 0.921 for this category.

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

Concordance for VVC:

The numbers of true negative and true positive cases were 406 and 144, respectively for VVC. There was no missed true positive result and only one case of false positive was found with self-collected samples. Very high concordance was observed with the kappa value at 0.994 with 95% confidence interval of 0.982 to 1.000. Table 2 also lists the cases of *Candida albicans* and non-albicans separately. For *C. albicans* infections, there was perfect concordance (kappa = 1.000) with 84 true positive and 466 true negative cases. For non-*albicans Candida* cases, there were 60 true positive, 489 true negative and 1 false positive cases using self-collected swabs making the kappa value to be 0.991 with 95% confidence interval of 0.973 to 1.000.

Concordance for TV:

Only three cases were found positive for TV and the results were identical for self- and physician- collected samples yielding perfect concordance (kappa value of 1.000).

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

other studies.[14,17] 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.[18] Clinical implications of these non-*albicans Candida* species is their documented decreased susceptibility to azoles, due to the indiscriminate use of azole group of antifungals, especially fluconazole.[18] 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,[15,16] but higher prevalence rates ranging from 5.6% to 16.1% were reported in other studies. [7,14,19] 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.[20]

Prevalence of BV among different age groups showed a statistically insignificant increasing trend with age. The vaginal pH is dependent on the amount of lactic acid produced by the vaginal lactobacilli from glycogen. The glycogen production from the superficial cornified cells of the vaginal mucosa is dependent on estrogen stimulation, which is high during the reproductive age group and decreases with age. This increasing vaginal pH increases the susceptibility of aging women to BV.[21] Similar observation was made for VVC up to the age group of 30-40 years. However, though statistically insignificant, the percentage dropped for the population above 40 years. The above findings are in agreement with the findings of another study.[16]

In the present study, in general the kappa values were remarkably higher than the values reported in similar studies. For diagnosis of BV, the Nugent score based method with two and

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

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

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

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

(1) *Mature patients*: Almost 98% of the patients were of age 20 years or older with average age of 32 years, which helped the patients to collect the sample properly.

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

(3) *No delay in transportation of samples to the laboratory*: The samples were collected by both the methods inside the STI/RTI clinic and were transported to the STI laboratory immediately, which is located very near to the clinic. Specimens were processed immediately in the laboratory. Therefore, no transportation delay, sample labeling errors and sample contamination occurred leading to high concordance between the two methods.

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, prevention and timely management of curable STIs and RTIs is particularly important. 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.

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Competing interests None declared.

Patient consent Obtained.

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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 n/N (%)**	**VVC n/N (%)**			**TV n/N (%)**
		C. albicans	**Non-*albicans***	**Total VVC**	

			***Candida* 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	

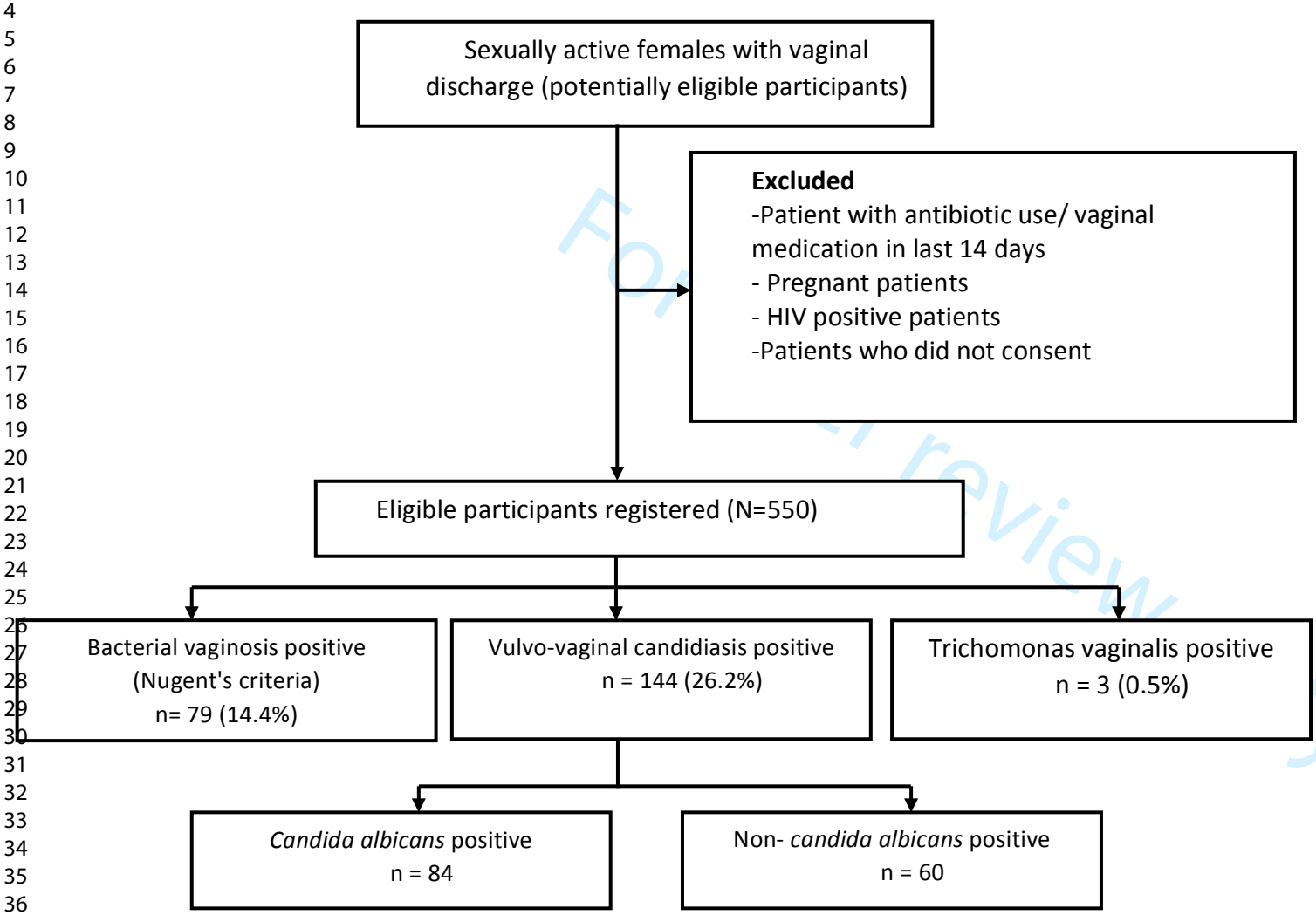
*Paired t-test has been used to evaluate prevalence in various age groups, BV (Bacterial vaginosis), VVC (Vulvo-vaginal candidiasis) and TV (Trichomonas *vaginalis*)

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 "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	Sensi-tivity (%)	Speci-ficity (%)	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															
C.albicans	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.

Figure 1: Prevalence of bacterial vaginosis (BV), vulvo-vaginal candidiasis (VVC) and Trichomonas *vaginalis* (TV) in 550 females presenting with vaginal discharge diagnosed by physician collected swabs.



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			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
Participants	**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
Test methods	**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
Analysis	**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			
Participants	**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
Test results	**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

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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

Zarine Khan[1,2], Aradhana Bhargava [1,2], Pratima Mittal[3], Rekha Bharti[3], Poonam Puri[4], Niti Khunger[4], Manju Bala [1,2*].

[1]Department of Microbiology, VMMC & Safdarjung Hospital, New Delhi, India, [2]Apex Regional STI Training, Research & Reference Laboratory, VMMC & Safdarjung Hospital, New Delhi, India, [3]Department of Obstetrics & Gynaecology, VMMC & Safdarjung Hospital, New Delhi, India, [4]Department of Dermatology & STD, VMMC & Safdarjung Hospital, New Delhi, India.

Correspondence:

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

Key words: Vaginal discharge; bacterial vaginosis; vulvovaginal candidiasis; trichomonas vaginitis, self-collected vaginal swabs; physician-collected vaginal swabs.

Word count: 2776

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

implementing the diagnostic approaches for STIs/RTIs in community based surveillance studies at national or regional level and therefore strengthening the National STI/RTI Control Programme.

ARTICLE SUMMARY

Strengths and limitations of this study

- This study determined that if diagnosis based on self-collected vaginal swabs is proven reliable, it could contribute to early diagnosis and greatly increase the access to treatment.
- Specimens were obtained from 550 patients with vaginal discharge attending the two STI/RTI clinics after obtaining the written informed consent. Concordance of inexpensive standard bedside tests, such as Gram staining, wet mount and culture (gold standard), was evaluated on both the self- and physician- collected samples for detecting bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis (TV).
- Corresponding findings were compared and analyzed using SPSS statistical analysis tool in terms of Cohen's Kappa values to determine the concordance between findings of self- and physician- collected samples.
- Limitation is that molecular tests were not performed, although gold standard test culture was performed which is more economical and less labour intensive also.
- Another limitation was the low positivity of Trichomonas vaginalis in patients of vaginal discharge by culture.

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

pilot study from Goa, which suggested that self-collected swabs are an acceptable method of collection of vaginal specimens in women attending gynaecological clinics in India.[8] However, the main limitation of the study was a statistically small sample size. Moreover, the samples were examined only by Gram's stain for their sensitivity for the diagnosis of STIs and were not tested by other diagnostic techniques like wet mount and culture. Another study was performed in New Delhi,[9] which showed that self-collected swabs can reasonably approximate physician-collected swabs for BV only. However, this study also was limited by a statistically small sample size.

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

METHODS

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

maximum value of 400 corresponding to the maximum heterogeneity condition of P=50%. Accordingly, a sample size larger than 400 was chosen and samples were collected from 550 sexually active females with vaginal discharge. A patient information sheet and consent form were presented and approved by the protocol review committee and ethical committee of the institution. After explaining the study details to the potentially eligible participants with the help of a pre-designed patient information sheet; a written informed consent was obtained on patient consent form. Both the patient information sheet and the consent form were designed in English and Hindi (local language). Patients with history of antibiotic use or vaginal medication in the previous 14 days, pregnant patients, patients unwilling/ unable to participate and patients with human immunodeficiency virus (HIV) infection were excluded from this study.

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

The samples were examined by standard bedside tests, Gram staining, wet mount and culture (gold standard).[10] For each patient, the first of the three self-collected swabs was used for

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 criteria[11] and Nugent's score[11], 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 1analyses 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 prevalence of BV and VVC across different age groups of vaginal discharge patients was analyzed by paired t- test (two tailed), it was observed that the difference was not significant.

Concordance between results 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 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 computed for this is 0.890 with 95% confidence interval of 0.851 to 0.928, which indicates excellent agreement between self- and physician- collected samples. The weighted kappa value was 0.921 for this category.

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

Concordance for VVC:

The numbers of true negative and true positive cases were 406 and 144, respectively for VVC. There was no missed true positive result and only one case of false positive was found with self-collected samples. Very high concordance was observed with the kappa value at 0.994 with 95% confidence interval of 0.982 to 1.000. Table 2 also lists the cases of *Candida albicans* and non-albicans separately. For *C. albicans* infections, there was perfect concordance (kappa = 1.000) with 84 true positive and 466 true negative cases. For non-*albicans Candida* cases, there were 60 true positive, 489 true negative and 1 false positive cases using self-collected swabs making the kappa value to be 0.991 with 95% confidence interval of 0.973 to 1.000.

Concordance for TV:

Only three cases were found positive for TV and the results were identical for self- and physician- collected samples yielding perfect concordance (kappa value of 1.000).

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 patients with history of antibiotic use or vaginal medication in the previous 14 days, pregnant patients, patients unwilling/ unable to participate and HIV patients. Even though oral antibiotics may potentially increase women's propensity towards vaginal candidiasis, but antibiotics such as metronidazole also alter the vaginal flora by killing Gardnerella vaginalis. Also, with the

availability of these antibiotics as over the counter drug, it was difficult to exactly know the exact antibiotic taken by the patient before visiting our tertiary reference clinic; hence this exclusion criteria. As it is not advisable to perform self-collection of vaginal swabs in pregnant woman they were not included in the study. Studies on HIV patient requires ethical clearance from national organization in addition to the institutional ethical clearance and similar studies in literature have not included HIV patients, hence the exclusion. These differences in the exclusion criteria, contribute to 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 other studies.[14,17] 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.[18] Clinical implications of these non-*albicans Candida* species is their documented decreased susceptibility to azoles, due to the indiscriminate use of azole group of antifungals, especially fluconazole.[18] 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,[15,16] but higher prevalence rates ranging from 5.6% to 16.1% were reported in other studies. [7,14,19] The higher prevalence rates were mostly observed in studies employing higher sensitive molecular techniques such as nucleic acid amplification techniques (NAAT) for detecting TV as compared to conventional culture technique used in this work. Additionally, variation in geographical locations can contribute to the difference.[20] Unlike developed countries,

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

Prevalence of BV among different age groups showed a statistically insignificant increasing trend with age. The vaginal pH is dependent on the amount of lactic acid produced by the vaginal lactobacilli from glycogen. The glycogen production from the superficial cornified cells of the vaginal mucosa is dependent on estrogen stimulation, which is high during the reproductive age group and decreases with age. This increasing vaginal pH increases the susceptibility of aging women to BV.[21] Similar observation was made for VVC up to the age group of 30-40 years. However, though statistically insignificant, the percentage dropped for the population above 40 years. The above findings are in agreement with the findings of another study.[16]

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

A near-perfect concordance was observed in cases of VVC, with a kappa value of 0.995. In VVC cases, self-collected samples produced one false positive result as compared to physician-collected sample. This can possibly be attributed to the contamination of the swab with

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

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

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

(1) *Mature patients*: Almost 98% of the patients were of age 20 years or older with average age of 32 years, which helped the patients to collect the sample properly.

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

(3) *No delay in transportation of samples to the laboratory*: The samples were collected by both the methods inside the STI/RTI clinic and were transported to the STI laboratory immediately, which is located very near to the clinic. Specimens were processed immediately in the laboratory. Therefore, no transportation delay, sample labeling errors and sample contamination occurred leading to high concordance between the two methods.

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,

prevention and timely management of curable STIs and RTIs is particularly important. 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.

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Competing interests None declared.

Patient consent Obtained.

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

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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				
	BV n/N (%)	**VVC n/N (%)**			**TV n/N (%)**
Age group		***C. albicans***	**Non-*albicans Candida* 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 (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	

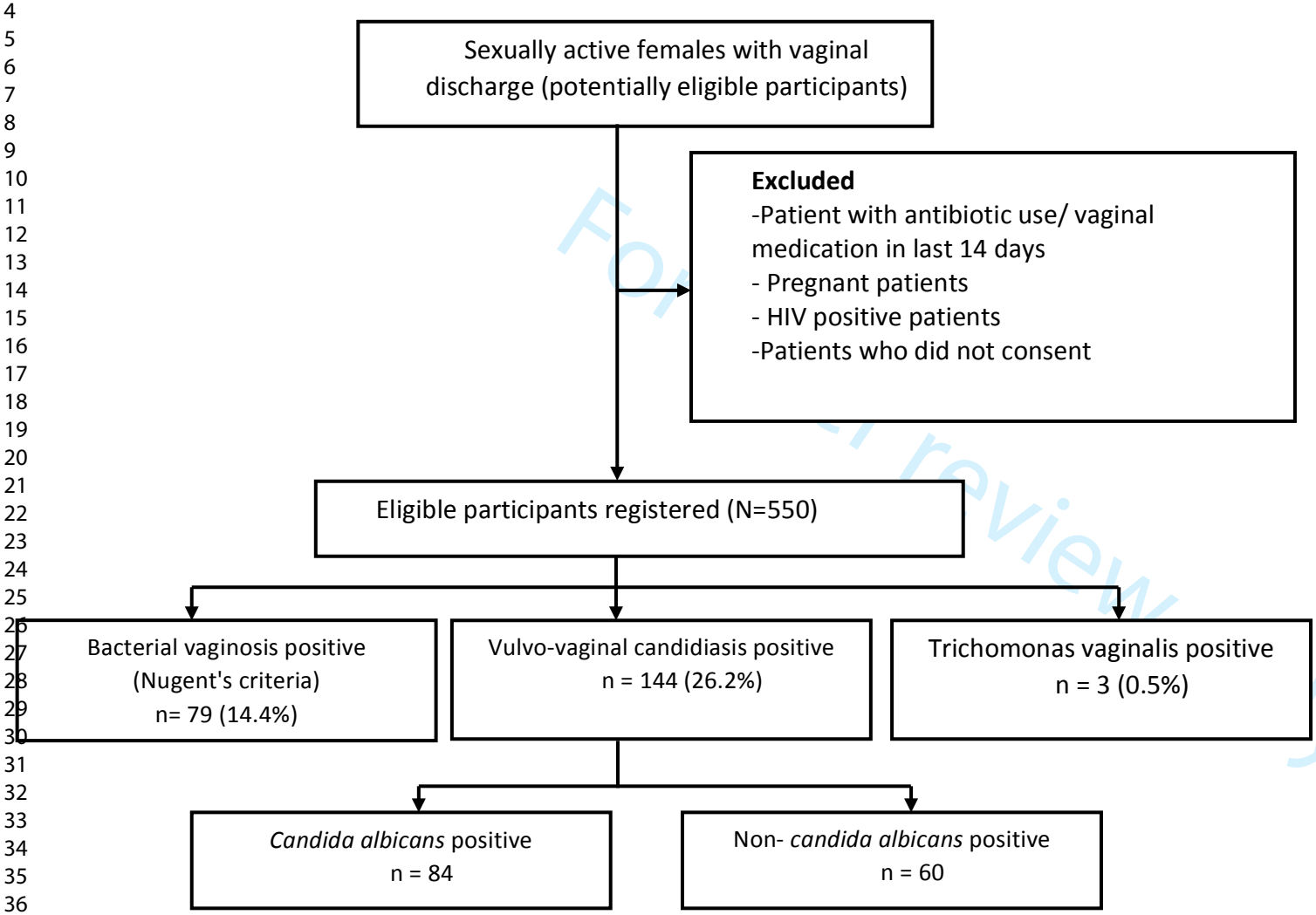
*Paired t-test has been used to evaluate prevalence in various age groups, BV (Bacterial vaginosis), VVC (Vulvo-vaginal candidiasis) and TV (Trichomonas *vaginalis*)

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 "Si" (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	Sensi-tivity (%)	Speci-ficity (%)	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															
C.albicans	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.

Figure 1: Prevalence of bacterial vaginosis (BV), vulvo-vaginal candidiasis (VVC) and Trichomonas *vaginalis* (TV) in 550 females presenting with vaginal discharge diagnosed by physician collected swabs.



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			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
Participants	**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
Test methods	**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
Analysis	**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			
Participants	**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
Test results	**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