

Comparison of Speculum and Non-speculum Vaginal Specimens in the Diagnosis of *Trichomonas vaginalis*

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Abstract: Trichomoniasis is the most prevalent non-viral sexually transmitted infection worldwide. Apart from being a key biologic indicator of sexual activity, it's been associated with the transmission of Human Immunodeficiency virus. The use of vaginal speculum is traditionally required for specimen collection. The limited application of this step in various primary health care facilities in resource-poor settings due to difficulties with sterilization of speculae among others, has grossly reduced the offer of testing for Trichomoniasis. Self-collected vaginal swab could be an acceptable alternative specimen for diagnosis of trichomoniasis in these settings. We compared the yield of *Trichomonas vaginalis* from speculum and non-speculum based specimens. Five hundred women between 18 – 45 years presenting with abnormal vaginal discharge in two health care facilities in Rivers State, Nigeria, were enrolled. Three specimens were collected from each woman; two non-speculum based /non-invasive and the third, speculum based. Microscopists were trained and the procedure standardized. Wet mount microscopy was performed on all specimens within thirty minutes of collection. Three Microscopists examined each specimen independently. All infected patients were managed according to existing guidelines. Ethical approval was obtained and data analyzed using SPSS version 21. Median age of participants was 31 years. Of the 500 women, 53 were infected giving an overall prevalence of 10.6%. Using the speculum-based specimens, 90.5% (48) of infected women were detected while 83.0% (44) and 30.1% (16) were detected using the self-collected swab and first void urine respectively. Interestingly, first void urine was able to detect five (5) more cases that were not detected by the speculum-based method. In low income settings, use of self-collected swabs in combination with first void urine is likely to increase detection rate of *Trichomonas vaginalis* when compared with the use of speculum-based specimen only.

Keywords: Trichomoniasis, Speculum, Diagnosis, *Trichomonas vaginalis*

1. Introduction

Trichomonas vaginalis (*T. vaginalis*) infection is the most prevalent non-viral sexually transmitted infection worldwide [1]. Estimated global incidence was about 156 million cases in 2016 [2]. In Nigeria prevalence varies across regions [3-8]. *T. vaginalis* has been recognized as a key biologic indicator of sexual activity as it is transmitted quite directly, primarily by sexual intercourse from human to human, being its only known host [9]. It has also been shown to be a risk factor for acquisition /infection and transmission of the Human

Immunodeficiency Virus (HIV) [10-12]. In Nigeria where HIV infection remains a public health burden [13, 14] it is essential to optimize efforts to control risk factors associated with the transmission of this infection.

Several studies have also shown that pregnant women infected with *Trichomonas vaginalis* are at increased risk of adverse birth outcomes such as pre term delivery and low birth weight babies [15, 16]. This high index of neonatal mortality has to be addressed using all available preventive approach. It is desirable that health care facilities are in a position to promptly diagnose and treat this curable protozoan infection and in this way reduce the perinatal

impact of the infection as well.

Culture is considered the gold standard for its diagnosis [17, 18]. It is generally accepted in developing countries that the diagnosis of trichomoniasis can be based on microscopic visualization of the motile trichomonads. Acceptability of this approach is due to the low technical demand, rapidity, achievable cost and acceptable sensitivity and specificity of the process [19]. This can be achieved through direct saline wet mount of vaginal secretion. Some authors argue that in few instances, the absence of trichomonads in saline mount microscopy can be a false negative result and may require further processing with a nucleic acid amplification technique, the Polymerase Chain Reaction [18, 20].

Speculum insertion into the vagina is traditionally a required step for specimen collection. The limited application of this step in various primary health care facilities has grossly reduced the offer of testing for *T. vaginalis*. The reasons for this limitation include; technical difficulties associated with sterilization of the speculum, correct use of speculum by health care workers, perceived pain reported by clients and cultural inhibitions as well as religious inclinations in the African setting. In some settings adolescent clients totally refused speculum insertion [21].

The use of urine as an alternative non-invasive specimen is being practiced in some settings, however; it has been documented that with this, the sensitivity of the wet microscopy as a diagnostic technique is significantly reduced [17, 18]. We propose that self-collected vaginal swab, a situation where patients collect their own specimens, could be an acceptable alternative sample for the diagnosis of trichomoniasis in patients who report copious abnormal vaginal discharge. This procedure is already acceptable for techniques that require DNA amplification for diagnosis of other sexually transmitted diseases such as genital chlamydia and gonorrhoea [22].

In this study we compared the yield of *T. vaginalis* from wet preparation using various specimen collection techniques namely; High vaginal swab (HVS), self-collected vaginal swab and first void urine (FVU) from a female population in a resource limited setting.

2. Method

Five hundred women aged between 18-45 years presenting with abnormal vaginal discharge in two healthcare facilities in Rivers State were enrolled into the study. Three specimens were collected from each woman; a Physician collected High vaginal swab, self-collected Swab and first void urine. To do this, Women were required to insert a sterile swab stick deep (about 3cm) into their vagina and keeping a hold on the handle of the stick, rotate clock wise and then anticlockwise and re-insert into the tube then subsequently provide 20-

30mls of first void urine in a sterile pre-labeled container. High vaginal swab (HVS) was thereafter taken in the course of pelvic examination by a Clinician. Vaginal Swabs were transported to the bacteriology bench of the laboratory immediately, placed in a test tube containing a few drops of sterile normal saline solution and wet mounts of the fluid examined microscopically for motile Trichomonads.

Urine specimens were transported to the urine bench of the laboratory, centrifuged at 2000rpm for 5 minutes, supernatant discarded and the sediment re-suspended in the drop of supernatant remaining in the tube. This was examined under the microscope.

Microscopists were trained and procedures standardized. Wet mount was carried out for all specimens within 30minutes of collection. Each Microscopist attached to a specimen was blind to the result of the other specimen types. A Positive Trichomonad result was only recorded once for each patient irrespective of the mode of collection of the specimen. All patients with infection were managed according to existing guidelines. Ethical approval and informed consent was obtained for this study. Data was analyzed using SPSS Version 21.

3. Results

The study participants' ages ranged from 18-45 years with a median age of 31 years. A total of 53 out of the 500 women investigated, were infected giving an overall prevalence of 10.6% (Figure 1).

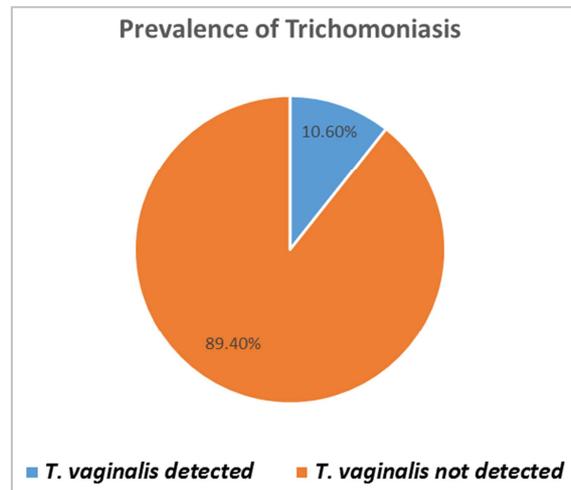


Figure 1. Prevalence of *T. vaginalis*.

From the speculum-based High vaginal swab, 90.5% (48/53) of infected women were detected while 83.0% (44/53) and 30.1% (16/53) were detected using the self-collected swab and first void urine respectively (Table 1).

Table 1. Comparison of specimen collection methods in detecting *Trichomonas vaginalis*.

Method of collection	Speculum Based	Non Speculum Based		
		Urine	Self-Collected (SC)	Urine plus SC
Number Detected N (%)	48 (90.5%)	16 (30.1%)	44 (83.0%)	49 (92.4%)

Four (4) of the infections which were diagnosed from the speculum-based specimens were missed using the self-collected swab, however; using the first void urine specimen, we detected 5 more infected women that were missed using the speculum-based specimen. These were probably women who had predominantly urethritis and so were missed by a carefully collected high vaginal swab using a speculum. On combination of self-collected swabs and first void urine for diagnosis, we were able to detect 49 out of 53 (92.4%) infected women.

4. Discussion

Many studies have shown that the prevalence of *Trichomonas* infection is highest among women 40 to 49 years old [23]. This is slightly higher than was observed in our study even though both age groups fall within the sexually active age group. Prevalence rates of trichomoniasis ranging from less than 1% to about 51.8% have been reported across Nigeria [24] some being much less and others quite similar to that in our study. In other parts of Africa and globally, prevalence rates ranging from 1.7% to even as high as 54% have been reported [22, 25, 26].

Self-collection of vaginal swab for diagnosis of sexually transmitted diseases is being advocated globally especially when pelvic examination will not add value to the management of the patient [27]. In Nigeria, Halpern *et al.* [13] opted for self-collected swabs to assay for gonococcal and chlamydial infections using genotypic techniques.

In an earlier study, Brabin *et al.* [3] showed that in southern Nigeria, the prevalence of sexually transmitted diseases was higher in the 17-19 years age group when compared to the higher age groups and this group of teenagers tend not to accept invasive vaginal procedures. Smith *et al.* [28] assessed the acceptability of self-collection of vaginal swabs amongst an adolescent population; all their clients opted for this approach. Holland-Hall *et al.* [21] also argued that the lack of the self-collection option could lead to missing over 70% of sexually transmitted diseases amongst adolescent girls in a juvenile facility.

Forney *et al.* [29] validated the use of self-collected swabs for longitudinal study of the vaginal flora.

Also important to note is that there was no difference in the vaginal microbiota when comparing self-collected and physician-collected vaginal swabs from these women. The primary focus on self-collected swabs had been to utilize this approach for procedures with amplification steps, however; our result shows that this innovative approach can also be validated for conventional diagnostic procedures in management of sexually transmitted diseases.

First void urine has also been shown to be of added value in diagnosis of trichomoniasis [20] as females may also have urethritis in addition to vaginitis. The usefulness of first void urine also applies to male partners who are infected with *Trichomonas vaginalis*. The combination of self-collected swabs and first void urine was noted to have a higher detection rate as it was able to detect 92.4% of infections, which is more than detected from either method alone. This is very exciting as we believe, it will

enable healthcare facilities equipped for microscopy but who lack technical expertise and other requirements to sustain aseptic speculum use, to carry out etiologic diagnosis of trichomoniasis. This is similar to reports and recommendations by Ogale *et al.* [30] and Shafer *et al.* [22] among others.

Other studies have shown a similar pattern to our findings with reports of comparable sensitivity, specificity and predictive values between self-collected and physician (speculum-based) collected swabs [31, 32] for diagnosis of *T. vaginalis* using various diagnostic methods.

5. Conclusion

We are of the opinion that self-collected swabs will contribute significantly in assisting health care providers practicing in resource poor settings to reduce missed cases in management of sexually transmitted diseases. The use of self-collected vaginal swabs in combination with first void urine is likely to increase the detection rate of *Trichomonas vaginalis* in low income settings, when compared with the use of speculum-based specimen only.

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