Improving Detection of *Trichomonas vaginalis* infection among adult females in a Resource-Poor setting in Ogoni, Niger Delta, Nigeria

ABSTRACT

Aim: *Trichomonas vaginalis* infection is a neglected tropical parasitic infection affecting mostly women of child bearing age, especially those of low socio-economic status and those that have poor hygiene lifestyle. Unfortunately, the traditional wet mount microscopy routinely used in the rural health care settings, for the detection of this parasite from clinical specimen, hardly detect this parasite (false negative), thus making the diagnosis of trichomoniasis a huge challenge in the rural settings. The aim of this study was to determine the prevalence rate of *Trichomonas vaginalis* among adult females in Bori, a rural resource-poor setting in Ogoni, Niger Delta, Nigeria using modified Parasite Concentration-Giemsa staining technique.

Study Design: This was a randomized study of adult females attending Government Health Centers in the Ogoni areas of Rivers State, Nigeria between April and August, 2018.

Methodology: A total of five hundred (500) women, grouped into married (128) and unmarried (372), were enrolled in this study. A structured questionnaire was also administered to the participants for demographic data. Using sterile specula, high vaginal swab samples were collected and analyzed using the wet mount preparation and 10% Giemsa staining of centrifuged specimens.

Results: Results obtained showed that the prevalence rate was 1.8% (9 out of 500 subjects). The sensitivity of wet mount and Giemsa stain methods were 56.2% and 100% respectively while their specificity were 100% and 98.2% respectively. While wet mount detected 0.4%, Giemsa staining method detected 1.8%. Two (1.6%) out of 128 married women examined had *T. vaginalis* while 7 (1.9%) out of 372 unmarried women examined had *T. vaginalis.*

Conclusion: Although study recorded a low prevalence, *Trichomonas vaginalis* infections, exists in rural areas of Rivers State and therefore a source of concern because of its attendant morbidities. Early detection using improved diagnostic methods and in this case, the relatively inexpensive Parasite Concentration-Giemsa staining method, will improve management within the rural setting.. A meta screening of more rural communities is advocated.

Keywords: Trichomoniasis, Giemsa Staining, Wet mount, Prevalence

Introduction

Trichomonas vaginalis infection is a neglected tropical parasitic infection affecting mostly women of child bearing age, especially those of low socio-economic status and those that have poor hygiene lifestyle. The infection is therefore a major Public Health concern, being commonly transmitted in developing countries as well as in some developed regions such as the United States of America [1, 2]. The infection is generally acquired through sexual contact and sometimes through non-sexual contact, including fomites, with such clinical manifestations as vulva pruritis (itching) and burning, inflamed genitourinary tract surfaces, with a frothy yellow

or cream-coloured discharge. Some infected men have symptoms of urethritis, epididymitis or prostatitis. However, most infected persons (70 – 85%) have minimal or no symptoms and untreated infections might last for months [3]. *Trichomonas vaginalis* infection is a risk marker for other infections such as bacterial vaginosis, *Chlamydia trachomatis*, *Neisseria gonorhoeae* [4, 5]. It has also been reported to be associated with adverse pregnancy outcome, increase chance of sexual transmission of HIV [6], reversible infertility, neonatal morbidity and mortality, reproductive tract complications such as sepsis that accompanies abortion and caesarean section [5].

The prevalence rate of *Trichomonas vaginalis* infection varies from one location to another, with associated factors such as environment, educational status, and level of personal hygiene [2]. In the year 2011, WHO estimated that 160 million cases of infection are acquired globally [7]. In United States, Black American women were reported to have prevalence rate of 13% unlike white women of Latin origin which have very low prevalence rate of 1.8% [8].

In Nigeria, several workers from different parts of the country have reported different prevalence rates, ranging from 0.63% in Rivers State [9], 3.3% among adult women in Lagos [10] to 40.5% in Imo State [11]. There are different techniques used in detecting this parasite including- Culture, Microscopy (wet mount and stained), ELISA, and recently, polymerase chain reaction. However, most studies have dwelt on microscopic examination of motile trichomonad in normal saline preparation of vaginal fluid. Unfortunately, this traditional wet mount microscopy routinely used in the rural health care settings, for the detection of this parasite from clinical specimen, hardly detect this parasite (false negative), thus making the diagnosis of trichomoniasis a huge challenge in the rural settings. The aim of this study was to determine the prevalence of *Trichomonas vaginalis* among adult females in Bori, a rural resource-poor setting in Ogoni, Nigeria using modified Giemsa staining technique.

Materials and Methods

Study Area/Population: This comprised of 500 consenting adult females attending government health centers in the communities situated in and around Bori, a sub-urban setting in Ogoni, Rivers State of Nigeria.

Sample Collection and Processing: From April to August, 2018 and following ethical clearance obtained from Rivers State Hospital Management Board, High vaginal swabs (HVS) were collected from 500 consenting adult females. A sterile speculum was inserted into the posterior fornix of the vagina while the individual was in the lithotomic position. Then, sterile cotton wool swabs were aseptically inserted and the high area of the vagina of the patients was swabbed. The specimens collected were analyzed in two ways, which were wet mount examination and Giemsa stained analysis.

Wet Mount Examination: A wet smear (wet mount) was made of each HVS, immediately after collection, in a drop of physiological saline on a clean glass slide covered with a cover slip and examined microscopically under the low power (10x) and high power (40x) magnifications for presence of motile quick jerky motion of the protozoa. Trichomonads are Pear-shaped, motile flagellates with characteristic spasmodic, wobbling and rotating motions.

Giemsa Stain Examination: The specimens in the swab sticks were transferred into clean test tubes. 2mls of sterile normal saline was added to the tubes and vigorously shaken to dislodge all parasites into the saline solution. The swabs were then removed and the test tubes were centrifuged for 30 minutes at 1000 rpm. Using a Pasteur pipette, the supernatant in each tube was discarded, leaving the deposit. Following this, 5ml of absolute methanol (100% AnalaR methanol) was added to the deposit and gently but properly shaken. The mixture was centrifuged at 1000 rpm for another 30 minutes. The supernatant was removed leaving a thin layer of methanol as a liquid state which was then mixed properly. A drop of this mixture from each tube was placed on a clean grease-free microscopic glass slide. The slide was air-dried and incubated at 37^{o}C for 10 hrs. The slides were stained with Giemsa stain diluted with phosphate buffer solution pH 7.2 (1ml stock Giemsa solution into 9ml buffer) for 10 minutes after which they were washed, air-dried and examined under the microscope with oil immersion (X100) magnification for the presence of trichomonads.

Statistical Analysis: Microsoft Office Excel and Computer SPSS were used to analyze the data generated. Simple percentage was also used and prevalence was calculated to reflect the relative frequency of *Trichonomas vaginalis* infection with corresponding 95% confidence intervals.

RESULT

A total of 500 women, aged between 18-60 years, were enrolled in this study. Out of the 500 female, 9 (1.8%) were detected positive for *Trichomonas vaginalis* using 10% Giemsa stain technique, while only 2 (0.4%) *Trichomonas vaginalis* were detected using the conventional wet mount (Table 1)

Table 1: Detection of *Trichomonas vaginalis* using the Two Techniques

Method	Positive cases (%)	Negative case (%)
Giemsa staining (n=500)	9 (1.8)	491 (98.2)
Wet Mount (n=500)	2 (0.4)	498 (99.6)
p-value < 0.05		

The women used in the study were also grouped into two: married (n=128) and unmarried (n=372). Among the married women, *T. vaginalis* was detected in 2(1.56%) while the trophozoite of the organism was detected in 7(1.88%) unmarried women (Table 2).

Table 2: Distribution of *T. vaginalis* among Married and Unmarried Women

Marital Status	Positive %	Negative %

Married women (N=128)	2 (1.56)	126 (98.43)
Unmarried Women (N=372)	7 (1.88)	365 (98.12)
p-value >0.05		

The techniques used were compared in terms of sensitivity and specificity. The wet mount technique recorded a sensitivity of 56.25% (Table 3).

Table 3: Comparison of Techniques for Sensitivity and Specificity

Technique Used	**Sensitivity (%)**	**Specificity (%)**
Giemsa Stain	100	98.2
Wet Mount Examination	56.25	100

Discussion

Some factors, such as low social status and poor hygiene life style, have been reported as promoters of Trichomoniasis especially among women [8], hence the reason for carrying out this study in a resource-poor rural setting. Study recorded a prevalence rate of 1.8%. In Nigeria, several workers from different parts of the country have reported different prevalence rates, ranging from 0.63% in Port Harcourt, Rivers State [9], 3.3% among adult women in Lagos [10] to 40.5% in Imo State [11].

The present study in a rural setting has a higher prevalence rate in contrast to the work of Abah, [9] which was in an urban setting, all within the same State. In Sudan, a study compared prevalence rate of *Trichomonas vaginalis* infection between an urban and a rural area. The urban area recorded 4.8% against 15.6% prevalence rate recorded in the rural area [2]. The significant differences reported suggest varying level of awareness between rural and urban communities. The women in this present study were recruited from rural area with low, socio-economic status and poor hygiene.

Further to this, the technique used also accounted for improved detection rate. This study combined the use of wet preparation and Parasite Concentration-Giemsa stain techniques. While Giemsa stain detected 9 (1.8%) cases of *Trichomonas vaginalis,* wet mount detected 2 (0.4%), without any discordance. This observation is in concordance with work done by Paliwal *et al.*, [12] and further confirms that Giemsa stain is a more sensitive technique than wet mount preparation. Wet preparation for microscopy is currently the most common method for diagnosis of *Trichomonas vaginalis* infection *in the rural settings* because of its low cost.

The techniques used in this study were also compared in terms of sensitivity and specificity. The wet mount technique recorded a sensitivity of 56.25%, while the Giemsa staining technique recorded a specificity of 98.2% (Table 3). Before staining, the samples were centrifuged at low speed, confering the added advantage of concentrating the parasites. Although not compared against uncentrifuged samples in this study, it calls to reason that modifications to the preparation of samples before staining, improved its efficiency as the samples were concentrated and morphology of the parasite is preserved. A number of studies have reported different range of sensitivity of Giemsa-stained smears for diagnosis of *Tricohomoniasis.* Paliwal *et al.,* [12] reported a zero detection using wet mount as against 3.33% for Giemsa stained technique. Similarly, Sumadhya *et al.,* [13] also reported a lower sensitivity of wet mount compared to the Giemsa-stained technique. They adduced several factors that affect wet mount preparation, such as sample size, method of sample collection, variable absorption rate of cotton swab used, time of examination. It was noted that short time delays between collection and examination also decreased its sensitivity. In facilities lacking immediate microscopic protocols, Giemsa staining is very useful where duly prepared and fixed smears can be transported to the laboratory for diagnosis. Unlike wet mount examination, delay in transport may have no significant impact on its reliability for diagnosis of *Trichomoniasis.* According to Paliwal *et al.,* [12], the major challenge in using Giemsa-stained smear is that it is time consuming and requires technical expertise.

Moreover, the population in this present study was grouped into two viz; married and unmarried. Married women have lower prevalence (1.56%) than unmarried women (1.88%). This could be attributed to the fact that married women maintain single sex partner unlike unmarried women. The same observation was made by Abah [9], who reported lower prevalence of 0.4% in

married, than unmarried women. In rural areas, most individuals resort to quacks and also, over-the-counter prescriptions are very common and hence, the continuing spread of *Trichomonas vaginalis.*

Conclusion

The prevalence of *Trichomonas vaginalis* infection is low in the study communities. This is suggestive of increased health awareness, improved personal hygiene, practice of safe sex and other preventive practices in the study setting. As the Parasite Concentration-Giemsa staining had a higher diagnostic yield in comparison to wet mount examination, and can easily be carried out as a routine procedure, it should be used as an adjunct to wet mount microscopy in our routine laboratories.

ETHICAL APPROVAL

Ethical clearance was obtained from Rivers State Hospital Management Board, Port Harcourt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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