Comparison of Sensitivity and specificity of Direct Microscopy and Culture Methods as Diagnostic tool for fordiagnosing Trichomoniasisamong females of reproductive age, Port Harcourt,Nigeria.

ABSTRACT

Trichomoniasis is a sexually transmitted parasitic diseasecaused by *Trichomonasvaginalis* and is distributed worldwide.This study was a comparison of direct microscopy and culture methods in diagnosis of trichomoniasis in Port Harcourt, Nigeria. A descriptive cross-sectional study was adopted involving the detection of *Trichomonasvaginalis* using culture and direct microscopy techniques among females attending Rivers State University Teaching Hospital (RSUTH), Port Harcourt after ethical approval was sought and obtained. Samples were analyzed using direct wet preparation and culture technique. Sociodemographic data were obtained using well-structured questionnaires. A total of 650 specimens were examined, 450 were urine samples and 200 were high vaginal swab (HVS) samples which were examined directly under the microscope and cultured in Trichomonas medium (Oxoid CM0161) at 37ºc for 7 days.Out of 450 urine specimens examined, 4 were positive using direct microscopy with a prevalence of 0.9%($P>0.05$), while 22were positive using culture technique with a prevalence of 4.9% ($P<0.05$). 5 out of200 HVS were positive using direct microscopy with a prevalence of 2.5% ($P>0.05$), while 18 were positive using culture technique with a prevalence of 9% ($P<0.05$). Direct microscopy and culture techniques recorded a moderate agreement ($\kappa=0.412$) and was statistically significant ($P<0.05$). Culture of HVS recorded the highest sensitivity of 94.44% with specificity of 99.45%, while direct microscopy of HVS recorded a sensitivity of 80% with specificity of 99.49%. Culture of urine recorded a sensitivity of 90.91% with specificity of 99.53%, while direct microscopy of urine recorded a sensitivity of 75% with specificity of 99.78%.

Conclusion: It is recommended that culture should be incorporated as an additional method for routine testing of *Trichomonasvaginalis* in our health care centres for improved monitoring and control of the disease.

Keywords: Trichomoniasis, Trichomonasvaginalis, direct microscopy, culture.

1. INTRODUCTION

Trichomoniasis is a sexually transmitted infection (STI) caused by an anaerobic flagellated protozoan parasite called *Trichomonasvaginalis* that infects the human urogenital tract. *Trichomonasvaginalis* was originally considered a commensal organism until the 1950s when the understanding of its role as a sexually transmitted infection (STI) began to evolve[1].It differs from other flagellates in that, it exists only in trophozoite stage, cystic stages are not seen.*Trichomonasvaginalis* infects the squamous epithelium of the genital tract. It resides in the female lower genital tract and the male urethra and prostate, where it replicates by binary fission. *T. vaginalis* is transmitted among humans, its only known host, primarily by sexual intercourse.Transmission of *T. vaginalis* to neonates during passage through an infected birth canal is also possible. In the fetus and the neonates, complications such as abnormalities of the major organ systems as well as infections in form of pneumonia and conjunctivitis may also occur [2].

Symptoms include vaginal discharge (which is often diffuse, malodourous, yellow-green), dysuria, itching, vulvar irritation and abdominal pain. The normal vaginal pH is 4.5, but with *Trichomonasvaginalis* infection this increase markedly, often to >5. *Coplitismacularis* or small punctate haemorrhagic spots called strawberry (mucosa) cervix is seen in about 5% of women, though with colposcopy this rises to nearly 50%[3]. Other complications include infection of the adnexa, endometrium, Skene and Bartholin glands. In men, infection is frequently asymptomatic; occasionally, epididymitis, prostatitis, urethritis and decreased sperm cell [4].

Wet mount microscopy has been used for many decades to diagnose *T. vaginalis*by detecting actively motile organisms. This is the most practical and rapid method of diagnosis (allowing immediate treatment), but is relatively insensitive, several specimens may have to be examined to detect the organisms. The test is inexpensive, low technology and is point of care, however, it is insensitive, particularly in men.{5] Sensitivities range from 40 and less than >80% depending on the expertise of the reader and should be read within 10-20mins of collection, or the organisms lose motility and may be very difficult to see. The specimen should be diluted with a drop of saline and examined under low power with reduced illumination for the presence of actively moving organisms, urine sediments can be examined in the same way. As the jerky motility of the trophozoite diminishes, it may be possible to see the movement of the undulating membrane, particularly under high dry power. Specimens should never be refrigerated.

Culture media for *Trichomonasvaginalis* therefore need to include all the essential macromolecules, vitamins and minerals. In particular, serum is essential for the growth of trichomonads, since it provides lipids, fatty acids, amino acids and trace metals. In vitro, it grows optimally at a pH of 6.0-6.3, although it can also grow through a wide range of pHs, especially in the changing environment of the vagina. Various methods have been published regarding newer medium formulations and comparisons, including the use of modified Columbia agar [5,6]. Although the results of this study were excellent, the specimens were processed at bedside, an approach that is often not practical for specimen collection. Other culture mediums are Oxoid*Trichomonas* medium, Diamond medium, Cysteine-peptone-liver-maltose (CPLM) medium and plastic envelope medium (PEM) are often used. Cell culture methods use a variety of cell lines to recover *T. vaginalis* from clinical specimens, and they include McCoy cells. This has been reported to detect the organism at a concentration as low as 3 organism/ml [6]. Other diagnostic techniques are antigen or antibody detection and molecular methods.[7]

Primary prevention of *Trichomonasvaginalis* infection often relies on health promotion interventions to improve diseases awareness and behavior change [8], but male circumcision represents an important means for the prevention of *T. vaginalis* transmission and several studies have shown that partners of circumcised men are less at risk of acquiring trichomoniasis[9].

MATERIALS AND METHODS

Study Area

This research study was conducted in Port Harcourt, precisely in Rivers State University Teaching Hospital, (RSUTH) Port Harcourt. Port Harcourt which is the capital city of Rivers State is the 5th largest city in Nigeria and the only major city in Rivers State has a population of 1,382,592 from the 5,185,400 total population of Rivers State. (Rivers State government website, 2010). Rivers State University Teaching Hospital (RSUTH), formerly Braithwaite Memorial Specialist Hospital (BMSH) is located within latitude 4º 78N and 7º 01E Old GRA, Rivers State. The Hospital is a Tertiary Health Faculty and receives referrals from both private and public hospitals in Port Harcourt and its environs.

Study Population

The study population was four hundred and fifty females between the reproductive age of 15 to 55 years among pregnant women who were attending routine antenatal clinic, HIV positive patients, out patients and healthy volunteers in Rivers State University Teaching Hospital (RSUTH), Port Harcourt.The minimum sample size needed was determined based on Leslie-Kish formula [10]. A precision of 0.05 at 95% confidence interval was obtained thus. $N=z^2 (1-P)/D^2N$ = Minimum sample size required, Z= Standard normal deviate corresponding to confidence interval level of 95% (Standard value of 1.96). P = 48.8% Prevalence of trichomoniasis in a given time [11] D = margin of error to be tolerated at 5% (0.05); therefore, the minimum sample size is 384.

Sample Collection

A total of 200 High vaginal swab (HVS) specimens were collected aseptically using sterile cotton swab sticks from consented participants across the study area by qualified female medical laboratory scientists. A total of 450 urine specimens were collected by the patients themselves using clean sterile sample bottles as directed. Sample collection was targeted between 8am to 12pm.

Analytical Methods

Culture and direct microscopy techniques were employed in the detection of *T. vaginalis* trophozoites in specimens.

Culture Procedure

The bijou bottles were labelled correctly with subject's laboratory identification number. About 1-2ml of well mixed urine was pipetted into the medium, mixed and carefully sealed and high vaginal swabs were inserted into the medium, with the other end carefully broken to allow proper sealing of the bottles. These bottles were transferred into the cannister, an anaerobic condition was provided by inserting a lit candle into the cannister and properly covered. This was incubated at 37ºc for 3-7 days. From Day 3 of culture, a Pasteur pipette was used to pick an aliquot medium from the bottom of the bottle without agitating and smeared on a clean grease-free glass slide with a cover slip above it. This was examined microscopically using x10 and x40 objective lens. This microscopic examination was repeated till the end of the 7-day incubation period [12]TheTrichomonas medium used in this study was a medium based on that of Feinberg and Whittington for the detection of *T. vaginalis* and *Candida* species [13].

Detection: *Trichomonasvaginalis* flagellated trophozoites were identified in the medium by their characteristic wobbling or jerky motion. Absence of trophozoites after 7 days indicated a negative result.

2.6.2 Direct Microscopy Procedure

The urine sample was transferred into a centrifuge tube and spun using a bucket centrifuge at 3000 rpm for 5 minutes. The supernatant was decanted and the sediments preserved. The urine sediments were dropped on a clean grease-free glass slide with a cover slip placed above it and examined microscopically. Normal saline (0.85%) was added to the high vaginal swab specimen in drops and the solution was properly mixed. A smear was made on a

clean glass slide with a cover slip placed above it. This was examined microscopically at x10 and x40 objective lens[10].

Detection: Motile trophozoites were identified by their characteristic wobbling or jerky movement, which gives a positive result. The presence of epithelial cells, pus cells, yeast cells, bacteria, casts and crystals in urine were recorded. Presence of epithelial cells, pus cells and yeast cells were recorded in high vaginal swabs.

2.7. Statistical Analysis

Correlation of the diagnostic parameters of direct microscopy and culture method as a gold standard was done using accuracy test, chi-square and Kappa's test [14].

3. RESULTS

Out of a total of 650 specimens examined from consenting female subjects, 450 were urine specimens and 200 were HVS specimens.Subjects between the age of 46-55 years recorded the highest prevalence using HVS 4 (30.7%) and subjects between the age group 36-45 years usingurine specimen 7 (6.9%). Based on educational status, subjects who only attained primary education recorded the highest prevalence 100%.Risk factors associated withtrichomoniasis were lack of knowledge of the infection (7%), wearing tight underwear (9.6%) repeating underwear regularly (17.4%), and engaging in unprotected sex (7.7%).

On the basis of diagnostic method, Table 1 shows the level of agreement between culture and direct microscopy techniques in the diagnosis of trichomoniasis using Kappa (κ). Diagnosis of *T. vaginalis* using HVS specimen recorded moderate agreement with a kappa value of 0.412 while urine specimen showed fair agreement with a kappa value of 0.297. This was statistically significant ($P<0.05$).

Table 1.Level of Agreement between Culture and Direct Microscopy Techniques.

	Culture		Direct Microscopy		κ		P-value	
	Urine	**HVS**	**Urine**	**HVS**	**Urine**	**HVS**	**Urine**	**HVS**
Number Positive	22	18	4	5				
Number Negative	428	182	446	195				
Number Examined	450	200	450	200	0.297	0.412	0.000^{SS}	0.000^{SS}

Note <0 = *No agreement/disagreement*
0.0-0.20 = *Slight/Poor agreement*
0.21-0.40 = *Fair agreement*
0.41-0.60 = *Moderate agreement*
0.61-0.80 = *Substantial/Good agreement*
0.81-1.00 = *Almost perfect/Very good agreement*

Table 2 shows a comparative analysis of direct microscopy and culture techniques by their level of accuracy and disease prevalence. Culture of HVS recorded the highest prevalence 18 (9%) and urine 22 (4.9%), while direct microscopy of HVS recorded a prevalence of 5 (2.5%) and urine 4 (0.9%).Culture of HVS and urine specimens recorded the highest sensitivity of 94.44% and 90.91% respectively with specificity of 99.45% and 99.53% respectively, while direct microscopy of HVS and urine specimen recorded a sensitivity of 80% and 75% respectively with specificity of 99.49% and 99.78% respectively.

Table 2.Level of Accuracy between Culture and Direct Microscopy Techniques.

Factors/Parameters	Culture				Direct Microscopy			
	Urine		HVS		Urine		HVS	
	Value (%)	95% CI	Value (%)	95% CI	Value (%)	95% CI	Value (%)	95% CI
Sensitivity	90.91	70.84 to 98.88	94.44	72.71 to 99.86	75	19.41 to 99.37	80	28.36 to 99.49
Specificity	99.53	98.32 to 99.94	99.45	96.98 to 99.99	99.78	98.76 to 99.99	99.49	97.18 to 99.99
Positive Likelihood Ratio	194.55	48.51 to 780.28	171.89	24.26 to 1217.63	334.5	43.59 to 2567.16	156	21.04 to 1156.72
Negative Likelihood Ratio	0.09	0.02 to 0.34	0.06	0.01 to 0.38	0.25	0.05 to 1.37	0.2	0.03 to 1.16
Disease Prevalence	4.89	3.09 to 7.31	9	5.42 to 13.85	0.89	0.24 to 2.26	2.5	0.82 to 5.74
Positive Predictive Value	90.91	71.37 to 97.57	94.44	70.59 to 99.18	75	28.10 to 95.84	80	35.04 to 96.74
Negative Predictive Value	99.53	98.27 to 99.87	99.45	96.42 to 99.92	99.78	98.79 to 99.96	99.49	97.11 to 99.91
Accuracy	99.11	97.74 to 99.76	99	96.43 to 99.88	99.56	98.40 to 99.95	99	96.43 o 99.88

4. DISCUSSION

In the present study, direct microscopy and culture techniques were used in the detection of *Trichomonasvaginalis* in clinical specimens. There was no significant difference between both methods indicating that either of both methods can be used in diagnosing the parasite.

The findings of this study recorded a statistically significant moderate agreement between culture and direct microscopy techniques in the diagnosis of *T. vaginalis* for HVS (0.412) and a statistically significant fair agreement for urine (0.297). A direct wet preparation may be carried out for immediate diagnosis prior to results from culture which requires one-week incubation period before final decisions are made, hence, both techniques complement each other. This is similar to the observation made by Adjei*et al.,* [14] recording the agreement between various techniques including direct microscopy and culture.

Culture method recorded sensitivity of 94.44% (HVS), 90.91% (urine) and accuracy of 99% compared to direct microscopy with a sensitivity of 80% (HVS) and 75% (urine). The specificity of culture method was 99.53% (urine) and 99.45% (HVS) while that of direct microscopy was 99.78% (urine) and 99.49% (HVS). Culture is necessary for proper detection of *T. vaginalis* in clinical specimen because it is more sensitive, accurate and reliable. These findings are in harmony with other studies recording broth culture technique as the gold standard for detection of *T. vaginalis*[15]. Direct microscopy technique which is routinely practiced in many clinical laboratories in Nigeria is the reason for many false negative results gotten as trichomonads are usually missed because non-viable forms are usually mistaken as pus cells under the microscope. Direct microscopy cannot reveal low parasitaemia as non-viable forms cannot be detected and instances where increased speed of centrifugation affects motility of parasite rendering trophozoites immotile, whereas culture method aids in easy isolation of the parasite and gives room for undisturbed growth within 3-7days incubation under suitable anaerobic condition at 37^{o}c. Some scholars described the effect of chemical and temperature perturbations on trophozoites which results in their conversion to a cyst-like structure otherwise called pseudocyst, this form is poorly investigated. These pseudocysts are not identified by direct microscopy technique but when specimen is cultured, trichomonads may undergo excystation to its motile form (trophozoites) [16].

Trichomoniasis is an unpleasant parasitic infection that can go undiagnosed for years and can be transmitted by asymptomatic carriers. The prevalence of *T. vaginalis* infection, its co-infection with bacterial vaginosis and other sexually transmitted infections (HIV) causes adverse pregnancy outcome, pelvic inflammatory diseases, and tubal infertility, therefore trichomoniasis is an infection of public health concern [17,18].

5. CONCLUSION

In conclusion, this research is unique in terms of its contribution to patient treatment, being the first culture technique study carried out in Rivers State University Teaching Hospital(RSUTH), Port Harcourt to detect *Trichomonasvaginalis* in clinical specimens.Diagnosis of *T. vaginalis* infection is usually problematic due to highly sensitive nature of the parasite to drying effect and atmospheric oxygen. Hence, Culture at 37^{o}c under anaerobic conditions with a pH of 5.5-6.0 has better sensitivity and accuracy compared to direct wet preparation.It is recommended that culture technique be used in routine testing to improve diagnosis of *Trichomonasvaginalis*infection.

Ethical Consideration

Ethical approval for this research was sought and obtained from Rivers State Research and Ethics Committee()with number RSUTH/REC/2022146) for sample collection and analysis in the Rivers State University Teaching Hospital (RSUTH, laboratory.

Consent

As per international standards or university standards, patients' written consent has been collected and preserved by the author(s).

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