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## Molecular detection and risk factors associated with *Trichomonas vaginalis* infection among pregnant women in Osogbo and Ede, Southwest Nigeria

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### ABSTRACT

**Background:** Trichomoniasis is the most prevalent curable sexually transmitted disease globally. This study provides information on the prevalence and risk factors of Trichomoniasis among pregnant women in primary healthcare centres in Osogbo and Ede areas in Osun State, Nigeria.

**Methodology:** High vaginal swab samples were obtained from 145 consenting pregnant women using sterile swab sticks and analysed through microscopic examination and PCR diagnostic techniques. Questionnaires were administered to investigate the participants' socio-demographic characteristics, hygiene practices, and knowledge and practices (KAP) about trichomoniasis.

**Result:** The overall prevalence of trichomoniasis was 0%, as no trophozoite was detected in any sample. There was no statistical difference between the number of sexual partners, occupation, family type, and previous sexually transmitted infections. Many of the respondents (89.7%) were ignorant of this disease and its transmission mode.

**Conclusion:** The molecular technique employed in this study has further confirmed the zero prevalence of trichomoniasis in the study area and may be a promising diagnostic test.

**Keywords:** Trichomoniasis, laboratory diagnosis, Risk factors, Pregnant women, Osun State

## 1. INTRODUCTION

*Trichomonas vaginalis* is a highly predatory anaerobic pear-shaped obligatory protozoan parasite that lives outside the genitourinary tract epithelium. The parasite phagocytoses bacteria, vaginal epithelial cells, erythrocytes, and macrophages can ingest it. It causes trichomoniasis, a sexually transmitted parasitic infection in humans with significant public health implications [1]. Conception, menstruation, and concomitant anaerobic infections in the vaginal wall all diminish the acidity of the vaginal wall, from a semi-acidic pH (3.8–4.2) to a significantly lower acidic pH (5.0–6.0) which promotes the growth of *T. vaginalis* [2]. Trichomoniasis affects about 170-180 million people worldwide each year, with Africa accounting for 40% of the total [3]. According to Oyeyemi et al [4] and Simon et al [5], nearly half of women with trichomoniasis experience foul-smelling vaginal discharge, vulval irritation, inflammation, lower abdomen pain, and a strawberry cervix, which are more severe during pregnancy and menstruation. Trichomoniasis has been linked to early rupture of membranes, premature labor, low birth weight in infants, and in some situations, amplified HIV transmission in pregnant women, according to several studies by Uneke et al [6] and Simhan et al [7]. Despite this scourge, trichomoniasis receives little or no attention during antenatal care services, as the majority of routine ante-natal care treatments are focused on malaria and HIV [8]. *Trichomonas vaginalis* infection can be detected using a variety of diagnostic procedures. This study employed both microscopy and Polymerase Chain Reaction (PCR) to increase the detection of *T. vaginalis* among the participants. This study reports on the prevalence of trichomoniasis, perceptions of the infection, and risk factors related to this parasitic transmission among pregnant women in Primary Health Centres in Osun State, Nigeria.

## 2. METHODOLOGY

### 2.1 The Study Area

This study was carried out in six Primary Healthcare Centres (PHC): PHC Oke baale, PHC Oke Abesu, and PHC Isale Agbara in Osogbo and PHC Sekona, PHC Obada, and PHC Jagunjagun in Ede local government areas (LGAs), Osun State. Osogbo and Ede are located in the tropical vegetation zone in southwestern Nigeria. Osogbo has a landmass of approximately 47km<sup>2</sup> and a population of about 4,705,589, while Ede has a population of about 76,035 (National Bureau of Statistics,

2016). PHC Oke baale is located on latitude (7 45'N) and longitude (4 33'E), PHC Isale Agbara lies at latitude (7 44'N) and longitude (4 31'E). PHC Sekona lies approximately on latitude (7°46'N) and longitude (4 28'E), PHC Obada is located on latitude (7 43'N) and longitude (4 26'E), and PHC Jagunjagun lies at latitude (7 45'N) and longitude (4 29'E). The dominant ethnic group in both local governments is Yoruba. Islam and Christianity are common religions. The people in the study area are mostly civil servants and farmers.

### 2.2 Study Design

The cross-sectional descriptive study involved a survey of 145 pregnant women attending antenatal care who provided consent to participate. The participants selected for the study were aged 15-35. The sample size was computed with a 12.02% prevalence of *T. vaginalis* based on research conducted in Osun State by Hassan et al [9] using Fisher's formula for estimating sample size.

### 2.3 Ethics approval and consent to participate

Ethical approval was obtained from the Ministry of Health, Osogbo, Osun State, Nigeria. Written and verbal consent were obtained from the study participants before they were enrolled in the study. All the data collected from the participants were anonymized.

### 2.4 Sample Collection

A vaginal swab sample was collected from each study participant using a sterile disposable vaginal swab stick to determine the presence of trichomonal infection. Each sample was coded to match the unique survey number on the questionnaire.

### 2.5 Wet Mount Microscopy

Vaginal secretions were examined by rinsing the swab in a drop of 0.9% normal saline on a clean microscope slide, placing a coverslip, and viewing it under a microscope as described previously by Uneke et al [10] and Hobbs and Sena [11]. The microscopy was performed within 5 minutes of wet mount preparation; the slides were examined using x10 and x40 objectives for the presence of motile trichomonads.

### 2.6 DNA Extraction and PCR amplification

The genomic DNA was extracted using a NIMR BIOTECH Genomic DNA purification Kit. As described by the manufacturer's instructions.

#### 2.6.1 PCR method

Five microlitres (5 µl) of the extracted DNA were used

for PCR amplification. A reaction mix containing a total volume of 25 µl was formulated in a PCR tube. 12.5 µl of the One Taq master mix, primer concentration was optimised to a concentration of 10 µM, and 5.5 µl of nuclease-free water was also added to the mix. The DNA amplification reaction was performed using the BTUB 9; and BTUB 2 primers (FOWM Biotechnology limited) with product size 112 bp, targeting the *T. vaginalis* specific Betatubulin gene; 5 CAT TGA TAA CGA AGC TCT TTACGAT-3' - (positions 850 to 874); and 5 GCA TGT TGT GCC GGA CAT AAC CAT 3' (positions 961 to 938) respectively. The PCR reaction mix was placed in the thermal cycler, and amplification was carried out with the following cycling conditions: initial denaturation at 95°C for 15 min and 35 cycles; denaturation at 95°C for 30s- 35 cycles for annealing at 53°C for 60s-, extension at 72°C for 60s and final extension at 72°C for 5min. Amplification products were analysed by 1% agarose gel stained with ethidium bromide and visualized under ultra-violet light.

### 3. RESULTS

#### 3.1 The socio-demographic characteristics of participants

Most participants, 79.3%, were literate. Many of the participants, 46.2%, were aged 21-25 years. By Occupation, the majority were traders (81%), while More than half, 75.9%, practiced the Islamic religion (Table 1).

#### 3.2 Screening of vaginal swabs by microscopy and molecular method

The results of the wet mount microscopy showed that none of the samples was positive for *T. vaginalis*. The PCR amplification also showed that *T. vaginalis* was absent in any samples (Table 2).

#### 3.3 Knowledge about Trichomoniasis among patients receiving antenatal care in Osogbo and Ede, Southwest Nigeria

The questionnaire survey revealed the participants had little or no knowledge about the infection and its mode of transmission and are therefore more prone to infection (Table 3).

### 4. DISCUSSION

In our study, none of the 145 participants screened was infected with *T. vaginalis*, including the sexually active

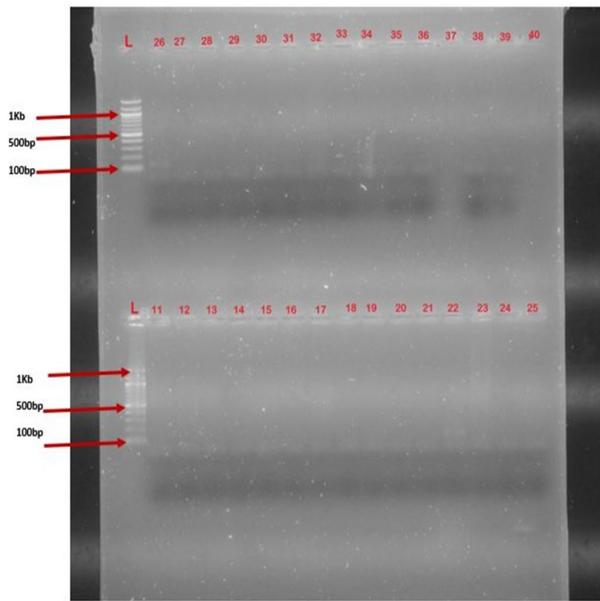
**Table 1:** Prevalence of *T. vaginalis* across the demographic groups

| PARAMETERS        | Number examined | Percentage Examined (%) |
|-------------------|-----------------|-------------------------|
| <b>AGE GROUP</b>  |                 |                         |
| 15-20             | 2               | 1.4                     |
| 21-25             | 67              | 46.2                    |
| 26-30             | 56              | 38.6                    |
| 31-35             | 20              | 13.8                    |
| Total             | 145             | 100.0%                  |
| <b>TRIMESTER</b>  |                 |                         |
| First             | 2               | 1.4                     |
| Second            | 30              | 20.6                    |
| Third             | 113             | 78.0                    |
| Total             | 145             | 100.0%                  |
| <b>EDUCATION</b>  |                 |                         |
| Illiterate        | 1               | 0.7                     |
| Primary           | 10              | 7.0                     |
| Secondary         | 115             | 79.3                    |
| Tertiary          | 19              | 13.0                    |
| Total             | 145             | 100.0%                  |
| <b>OCCUPATION</b> |                 |                         |
| Housewife         | 5               | 3.4                     |
| Civil servant     | 9               | 6.2                     |
| Nurse             | 2               | 1.4                     |
| Chemist           | 1               | 0.7                     |
| Artisan           | 31              | 21.4                    |
| Trader            | 87              | 60                      |
| Students          | 10              | 6.9                     |
| Total             | 145             | 100.0%                  |
| <b>RELIGION</b>   |                 |                         |
| Christianity      | 28              | 19.3                    |
| Islam             | 110             | 75.9                    |
| Others            | 7               | 4.8                     |
| Total             | 145             | 100.0%                  |

**Table 2:** Prevalence of *T.vaginalis* by microscopy and molecular Screening.

| Primary Healthcare- | No of persons examined | Prevalence by Microscopy | Prevalence by PCR (%) |
|---------------------|------------------------|--------------------------|-----------------------|
| Oke bale            | 12 (8.3%)              | 0                        | 0                     |
| Oke-Abesu           | 09 (6.1%)              | 0                        | 0                     |
| Isale-Agbara        | 57 (39.3%)             | 0                        | 0                     |
| Jagunjagun          | 26 (17.9%)             | 0                        | 0                     |
| Obada               | 21 (14.4%)             | 0                        | 0                     |
| Sekona              | 20 (14%)               | 0                        | 0                     |
| Total               | 145 (100%)             | 0(0%)                    | 0(0%)                 |

age groups, which is associated with low trichomoniasis prevalence in several findings in other parts of Southwest Nigeria [9,12-13]. This is further evidenced by a similar finding from our molecular screening of the samples by



**Figure 1:** Gel result showing no amplification of DNA to specific primers.

PCR. Previous studies have demonstrated a strong sensitivity (97-100%) and specificity (98-99%) of the Beta-tubulin primer set, BTUB 9/2 with a product size of 112bp, and other markers for detecting even low *T. vaginalis* infection in samples by PCR [14-16]. Although the specificity and sensitivity of both techniques could not be determined in this study due to the absence of a positive infection, these techniques have been proven to be highly efficient in detecting trichomonal infection.

Studies in various parts of Osun State have previously reported a low prevalence of the infection. Olaniran et al [3] reported 8.1% prevalence in Ilesha using Gram staining and direct microscopy diagnostic techniques among 310 non-pregnant female patients who had gynaecological complaints. Hassan et al [9] reported a prevalence of 12.02% in Olorunda local government area, Osun State using wet mount microscopy and Oxoid culture medium diagnostic techniques. Only 8 (0.9%) pregnant women were infected among the 882 sexually active women surveyed. Another study by Olowe et al [13] revealed a prevalence of 2% among 100 pregnant women receiving antenatal care at Ladoke Akintola University, Osogbo. Although no case of trichomoniasis was found in our study, this may indicate an extremely low prevalence of trichomoniasis in Osun State as was reported in previous investigations, which may be difficult to detect in a small sample of the population as in our study. The zero prevalence observed in this study compares to Ochei et al [17] and Okojokwu et al [18] in Edo and Plateau States respectively, where zero prevalence of *T. vaginalis* infec-

**Table 3:** Knowledge about trichomoniasis among patients receiving antenatal care in Osogbo and Ede Local Governments, Osun state, Nigeria

| Questions   | The number of people examined | Percentage of participants (%) |
|---|-------------------------------|--------------------------------|
| <b>Have you heard of Trichomoniasis before?</b>   |                               |                                |
| YES   | 15                            | 10.3                           |
| NO  | 130                           | 89.7                           |
| <b>How is the disease transmitted?</b>  |                               |                                |
| Sexual intercourse  | 6                             | 4.1                            |
| Toilet disease  | 5                             | 3.5                            |
| I don't know  | 134                           | 92.4                           |
| <b>Have you ever been treated for trichomoniasis before?</b>  |                               |                                |
| Yes   | 6                             | 4.1                            |
| No  | 139                           | 95.8                           |
| <b>How did you treat the infection (Trichomoniasis)?</b>  |                               |                                |
| Medical attention   | 8                             | 5.5                            |
| Self-medication/ drugs  | 3                             | 2.1                            |
| <b>When you have symptoms like vaginal itching, vulva irritation, etc, do you engage in sexual intercourse?</b> |                               |                                |
| YES   | 12                            | 8.2                            |
| NO  | 133                           | 91.7                           |
| <b>Have you had any other STD?</b>  |                               |                                |
| Yes   | 4                             | 2.8                            |
| No  | 141                           | 97.2                           |
| <b>Do you engage in unprotected sex with more than one partner?</b>   |                               |                                |
| Yes   | 5                             | 3.4                            |
| No  | 140                           | 96.6                           |
| <b>Number of sex partners</b>   |                               |                                |
| Yes   | 4                             | 2.8                            |
| No  | 141                           | 97.2                           |
| <b>Family Type</b>  |                               |                                |
| One   | 12                            | 85.5                           |
| More than One   | 09                            | 6.2                            |
| No answer   | 12                            | 8.3                            |
| <b>Family Type</b>  |                               |                                |
| Monogamous  | 134                           | 92.5                           |
| Polygamous  | 11                            | 7.6                            |

tion was attributed to improved healthy living and active control programs within the States. It is generally believed that the prevalence of *T. vaginalis* varies significantly based on the study population, settings, and different epidemiological factors. Unmarried individuals are believed to freely indulge in more sexual activities with multiple sexual partners [2, 18]. Therefore, a low prevalence of *T. vaginalis* infection in Osun State may be attributable to the attitude toward sexual activities and hygiene, affecting the infection risk. All the Pregnant women involved in this study were married so most had steady sexual partners and were also from religious

backgrounds, which promotes sexual values that reduce the risk of infection.

The knowledge, attitude, and practice of the study participants showed that the residents are not conversant with the parasite- *T. vaginalis*. The majority of the participants did not know about the disease or its transmission mode. The respondents' poor knowledge of the mode of transmission of this disease is worrisome as this may increase their risk of exposure.

The molecular technique employed in this study has helped to ascertain the low prevalence of trichomoniasis in these areas of Osun State. This could be because the majority of the participants were married and may not have multiple sexual partners compared to studies among sex workers and the HIV-positive population. Nonetheless, it is essential to maintain constant surveillance for this infection for prompt detection in the future and ensure adequate information about sexually transmitted infections is communicated to the women during an antenatal program.

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#### Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

#### Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request

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No external funding was received for conducting and completing this research work.

#### Authors' contributions

**OAS, MAA** and **SOO** designed the study; **SOO** collected and analysed human samples, **KF, OGD, NOS** and **MAR** assisted with data analysis and manuscript development. All authors approved the final copy of the manuscript.

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