Sahel Journal of Life Sciences FUDMA (SAJOLS)
March 2025 Vol. 3(1): 107-114
ISSN: 3027-0456 (Print)
ISSN: 1595-5915(Online)
DOI: *https://doi.org/10.33003/sajols-2025-0301-14*

Research Article

Prevalence and Susceptibility of Trichomoniasis among Pregnant Women Attending Selected Primary Health Care Centers within Chikun Local Government Area, Kaduna State

***Idris, Z.[1], Musa, F. M.[1] and Nmadu, A. G.[2]**

[1]Department of Microbiology, Faculty of Pure and Applied Sciences, Kaduna State University, Nigeria
[2]Department of Community Medicine, College of Medicine, Kaduna State University, Nigeria
****Corresponding Author's email*: zubairuidris28@gmail.com; *Phone*: +234 8061569447**

ABSTRACT

Trichomoniasis is a disease caused by a protozoan parasite called *Trichomonas vaginalis* which is transmitted through sexual contact. Complications of *T. vaginalis* include cystitis, cervicitis, urethritis, infertility, low birth weight, neonatal morbidity, and mortality. This study, therefore, was carried out to determine the occurrence of Trichomoniasis among pregnant women attending ante-natal care services in some Primary Health Care Centers (PHC) within the Chikun Local Government Area of Kaduna State. A sample size of 360 was used in the study. High vaginal swab (HVS) samples were collected from each pregnant woman using the septic method. Wet mount preparation and culture method were used to identify *T. vaginalis* isolates, and the isolates were further confirmed using molecular techniques and a phylogenetic tree. The susceptibility pattern of the drug metronidazole against the isolates was determined using a standard method. The results obtained in this study revealed that 24 (6.7%) samples were positive for *T. vaginalis* in both wet mount and culture methods. A single 312bp product of the region was amplified in all 24 samples. Partial nucleotide sequences of *T. vaginalis* isolates using BLAST analysis with reference sequences in the GenBank database showed that twenty-four isolates belonged to *T. vaginalis.* All isolates were susceptible to the varied Minimum Inhibitory Concentration (MIC) after 24 hours of treatment with metronidazole (0.4, 0.8, 1.6, and 3.2 µg/ml) except the isolates treated with 0.1 µg/ml of metronidazole.

Keywords: Prevalence; Trichomoniasis; Pregnant women; Primary Health Cares; Susceptibility

Citation: Idris, Z., Musa, F.M. & Nmadu, A.G. (2025). Prevalence and Susceptibility of Trichomoniasis among Pregnant Women Attending Selected Primary Health Care Centers within Chikun Local Government Area, Kaduna State. *Sahel Journal of Life Sciences FUDMA*, 3(1): 107-114. DOI: *https://doi.org/10.33003/sajols-2025-0301-14*

INTRODUCTION

Trichomoniasis is a disease caused by a protozoan parasite called *Trichomonas vaginalis* which is transmitted through sexual contact. Approximately 180 million women are infected worldwide (Ojurongbe *et al.,* 2010), and it is estimated that 2-50 % of the infected persons are in Africa (Stary *et al*., 2018). *Trichomonas vaginalis* is the most common Sexually Transmission Parasitic Disease (STPD) in Nigeria and studies on STPDs remain relatively scanty in Nigeria (Amadi and Ngwabo 2018). In addition, control programs for *T. vaginalis* and trichomoniasis are lacking and this identified gap may have impacted the burden of infection on pregnant women in Nigeria (Hamafyelto *et al*., 2017).

According to Kissinger (2015), nearly more than 60% of women who are infected with *T. vaginalis* are asymptomatic and approximately 1/3 of asymptomatic women are to become symptomatic within 6 months after infection. The Infection in women is characterized by vaginitis, arthritis, and cervicitis, which can lead to serious health complications, such as infertility, miscarriage, low-birth-weight infants, susceptibility to viral infections such as herpes simplex virus, human

papillomavirus infection, and cervical cancer (Kissinger, 2015). Mother-to-child transmission of *Trichomonas vaginalis* can be acquired during delivery through an infected birth canal. It is estimated that 5 to 20% of female babies can acquire trichomoniasis infection through direct vulvovaginal contamination Fernado *et al.* (2012). In Nigeria, there are porosity of reported studies on the prevalence of Trichomoniasis which shows that the infection is still endemic across the regions Hassan *et al*. (2021). *Trichomonas vaginalis* has also been implicated as a cofactor in the transmission of HIV Lawing *et al*. (2020). It has also been reported that *Trichomonas vaginalis* causes psychosocial distress in infected patients. It has been reported that Trichomoniasis is one of the major causes of pathology in obstetrics and gynecology in women Conrad *et al.* (2012).

However, *T vaginalis* as an extracellular parasite colonizes the mucosal surfaces of the human genitourinary tract and causes infection that leads to damage of the mucosal membrane through an apparent variety of mechanisms, which are dependent on an epithelial cell such as adherence-based cytotoxicity and others of which are not released from soluble factors such as proteinases Fernando *et al.,* (2020) Moreover, many cases, *T. vaginalis* infection can result in an excessive host immune response that leads to inflammation and further damage of the mucosal (Sommer, 2015).

Trichomonas vaginalis can be found in vaginal prostatic or urethral secretions, semen, and urine of infected individuals. However, little effort has been made to improve the diagnosis of *T. vaginalis* in Primary Health Care settings and there are no existing guidelines for the screening of this parasite among pregnant women Fernando *et al*. (2020). Currently, the commonly used technique in the estimation of *T. vaginalis* in most health care clinics and other health facilities in Nigeria is wet mount microscopy which detects motile trichomonads in vaginal secretions, urine, and urethral fluids of the infected person by visual examination of the specimens under a microscope. This method has been reported as insensitive compared to molecular DNA amplification techniques and the chances of missing the parasites are high (Stary *et al*., 2018). Furthermore, treatment of patients infected with Trichomoniasis in most Primary Health Care (PHC) settings in Nigeria is based on signs and symptoms regardless of the asymptomatic nature of this infection. Indeed, the prevalence of *T. vaginalis* by wet mount microscopy has been underestimated and the true prevalence is not known (Akafyi *et al*., 2016). This infection causes adverse reproductive health problems and pregnancy outcomes in pregnant women and has also been recognized to play a critical role in the acquisition and transmission of HIV (Fernando *et al*., 2012). Other complications of *T. vaginalis* include cystitis, cervicitis, urethritis, infertility, low birth weight, neonatal morbidity, and mortality (Hamafyelto *et al.,* 2017). There is porosity of publications on the prevalence of trichomoniasis among pregnant women. This study is therefore was carried out to determine the occurrence of Trichomoniasis among pregnant women attending ante-natal care services in some Primary Health Care Centers (PHC) within the Chikun Local Government Area Kaduna State. Given the burden of trichomoniasis experienced by women of child bearing age, the impact of this infection in pregnancy, particularly with regard to adverse birth outcomes (ABOs), is important to consider

MATERIALS AND METHODS

Study Area

The study area was Chikun Local Government Area of Kaduna State. The Local Government has an area of about 445,659 km^2 with a population of 368,250 people as of 2006. The study covered Primary Health Care (PHC) Kujama, PHC Kakau, PHC Nasarawa, PHC Sabon Tasha, PHC Narayi, PHC Sabon Garin Nassarawa, PHC Unguwan Romi and PHC Maraban Rido in Chikun Local Government Area, Kaduna State. Chikun Local Government Area lies within latitude 1000'0" N -10050'0"N and within longitudes 6040'0"E - 7040'0"E.

Study Population

The target group in this study included pregnant women who were attending antenatal health care services in Primary Health Care Centers in Chikun local Government Kaduna State.

Sample Size Determination

The method described by Richard *et al.* (2018) was adopted for determining the sample size with a slight modification. This was done using the formula below.

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA)*; **101**: 11030-11035.

$$n = \frac{z^2 \times p(1-p)}{d^2}$$

Where;

n = number of samples

d = margin of error= 0.05

p = percentage of existing prevalence= 3.08% (Richard *et al.*, 2018)

z = t-value at 95% Confidence Interval (CI) =1.96

Therefore,

n= $(1.96)^2$ x 0.308 x (1- 0.308)/ $(0.05)^2$= 3.84 x 0.308 x 0.692/0025 =327.5

10% non-response rate = 32.75

Therefore, a sample size of 360 was used in the study.

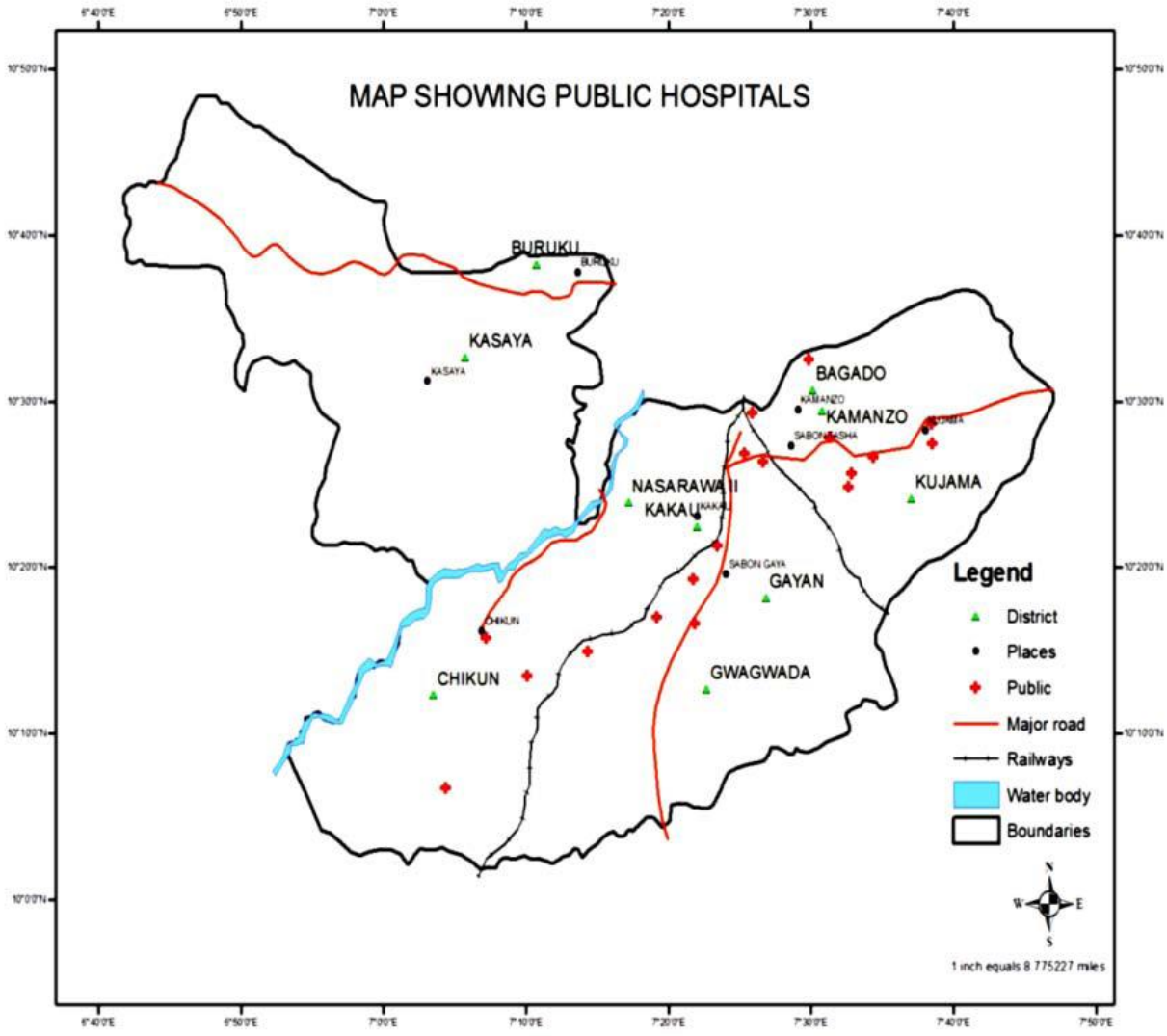

Figure 1. Map of Chikun Local Government Area Showing Sampled Public Health Centre

Ethical Considerations

Ethical clearance was obtained from Kaduna State Ministry of Health Ethical Committee with reference number MOH/744/VOL.1/1002. A consent form was signed by each pregnant woman who agreed to participate in this study. The data obtained from the participants was strictly kept confidential and the data generated was also coded so that it could not be directly connected to the participants. Participation in this research was entirely voluntary and any participants whose result turned out positive were referred to be treated immediately by a nurse.

Specimen Collection and Examination

A high vaginal swab (HVS) sample was collected from each pregnant woman through the help of health professionals working in the facility. Two sterile swabs aided with speculum were used to collect a vaginal exudate for wet mount preparations and culture.

Wet mount Preparation

High Vaginal Swab sample was used to make a wet preparation using a drop of normal saline on a clean glass slide which was covered with a cover slip and examined immediately under 10X and 40X objective lenses. A thorough microscopic observation of motile trophozoites of *Trichomonas vaginalis* was identified using ATLAS 'Microscopic examination of *Trichomonas vaginalis'* (Jatau *et al*., 2006).

Culture Method

The culture was performed using a Diamond culture medium as prepared according to the procedures provided by the Manufacturer. The vaginal swabs were placed into the medium and left to incubate

at 37°C for 5 days. The cultures were examined microscopically on days 1, 2, 3, 4, and 5 days after inoculation (Oladeide *et al*., 2016).

Molecular Method

DNA extraction was performed using a DNG-plus™ kit (Cinna Gene, Iran). DNA yield was quantified using NanoDrop (Thermo Scientific, USA) and then subjected to a PCR, using species-specific primers; Forward: 5'-GTTAATGGCAGAATCTTTGCAG-3'; Reverse: 5'-CTC GCAGTCCTATTGATCCTAAC-3' Tamura *et al*., 2004). Polymerase Chain Reaction Amplification was carried out using 18S *r*RNA and amplicons were visualized after electrophoresis in 1.5% agarose gel containing SYBR Safe (Invitrogen, USA).

Phylogenetic Reconstruction and Species Assignation

Amplicons were purified using a BigDye XTerminator purification kit (Thermo Fisher, USA) and sequenced in both directions, with primers used for the primary PCR. Sequences were trimmed and compared with homologous sequences in GenBank using the Basic Local Alignment Search Tool (BLAST). Based on ≤ 99% identity with the sequence deposited in GenBank, the strains were identified at the species level. To evaluate the intraspecific variability, and phylogenetic relationships within *Trichomonas* species, the sequences of our specimens were aligned against the GenBank sequences using BioEdit v7.0.0 software (Hall, 1999).

Drug Susceptibility Test

In vitro, drug susceptibility testing was carried out by the Meingassner method modified by the CDC (Sommer, 2015). Metronidazole Tablet was dissolved in distilled water sterilized through filtration (0.22 µm pore size) and stored at 4 ˚C. Serial twofold drug dilutions, ranging from 400 to 0.1µg/ml, were prepared using medium culture. Recommended trichomonad cells for aerobic and anaerobic susceptibility assays were 1 × 105 and 5 × 103 trophozoites per well, respectively (Ulogu *et al*., 2017). Both the minimum inhibitory concentration (MIC) and MLC were determined according to the method described by Rivera *et al.* (2019).

RESULTS

The Prevalence of *T. vaginalis* in high vaginal swab (HVS) samples of pregnant women attending Primary Health Care Centres in Chikun LGA

A total of 360 HVS samples from pregnant women were examined in the study, out of which 24 (6.7%) samples were positive for *T. vaginalis* in both wet mount and culture methods. Four (8.9%) positive samples each were identified from Primary Health Care (PHC) Kakau and PHC Nasarawa. At PHC MBR, PHC NRY, PHC SRS and PHC ROM 3 positive samples were identified each with a prevalence of 6.7%. Two positive samples were identified each from PHC KJM and PHC SGN each with a relative prevalence of 4.4% respectively Table 1.

Molecular Confirmation of *Trichomonas vaginalis* from High Vaginal Swabs (HVS) of Pregnant Women from the different Hospitals

Trichomonas vaginalis was successfully detected in 24 positive vaginal swab samples using PCR technique. A single 312bp product of the region was amplified in all 24 samples. Partial nucleotide sequences of *T. vaginalis* isolates using BLAST analysis with reference sequences in GenBank database showed that twenty four isolates belonged to *T. vaginalis* (Plate I).

The phylogenetic tree of *T. vaginalis* sequenced isolates was demonstrated in Figure 2. The phylogenic reconstruction demonstrates that all samples collected in the MBR01, KJM12, KAK10, NRY06, NSR03, STA14, SGR29 and ROM04 fall within the *T. vaginalis* clade, genetically close to *T. vaginalis* sequences from other reference sequences in GenBank.

Susceptibility of *T. vaginalis* Isolates to Metronidazole

All isolates were susceptible to the varied Minimum Inhibitory Concentration (MIC) after 24 hours of treatment with metronidazole (0.4, 0.8. 1.6 and 3.2 µg/ml) with the exception of the isolates treated with 0.1 µg/ml of metronidazole (Table 2).

Table 1: Prevalence of *T. vaginalis* in HVS of pregnant women attending PHCs in Chikun LGA (N= 360)

PHC Code	Number Examined	Number Positive	Percentage Positive (%)
MBR	45	3	6.7
KJM	45	2	4.4
KAK	45	4	8.9
NRY	45	3	6.7
NSR	45	4	8.9
SGR	45	2	4.4
STA	45	3	6.7
ROM	45	3	6.7

Keys*:* LGA: Local Government Area; PHCs: Primary Healthcare Centres; MBR: Mararaban Rido; KJM: Kujama; KAK" Kakau; NRY: Narayi; NSR: Nassarawa; SGR: Sabon-Gari; STA: Sabon-Tasha; ROM: Romi

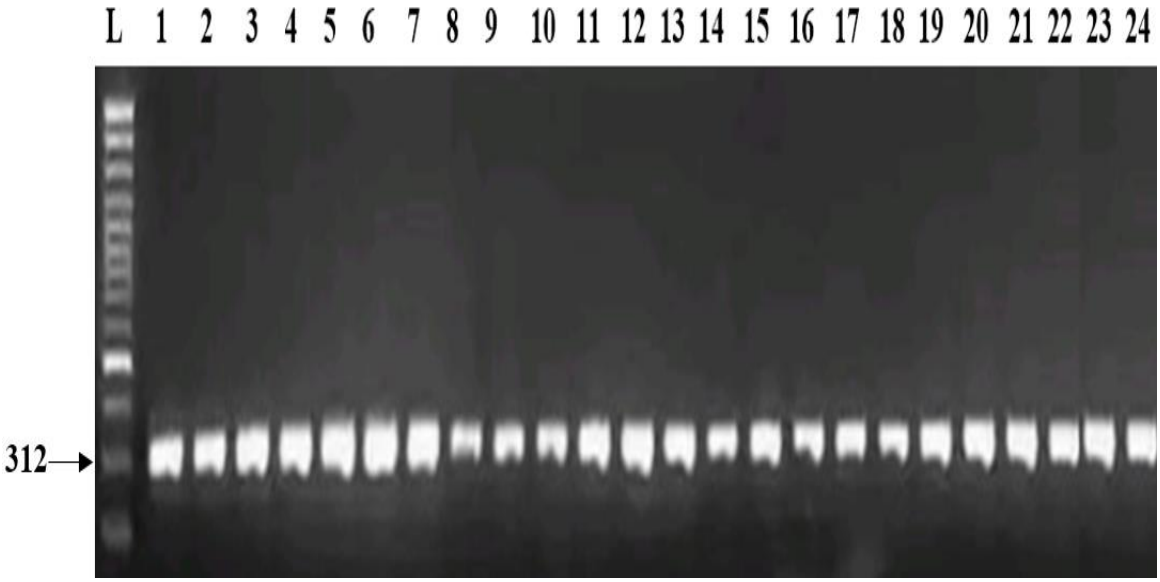

Plate I: Gel Image of Positive Samples Showing the PCR Amplicons of the 16S rRNA Gene of Trichomonad species

Key: M= Molecular Ladder, 1-24 = Sample of *T. vaginalis* Isolates

1 MBR 01	9 KAK 40	17 STA 14
2 MBR O6	10 NRY 06	18 STA 35
3 MBR17	11 NRY 25	19 STA 43
4 KJM 06	12 NRY 33	20 SGR 06
5 KJM 12	13 NSR 03	21 SGR 29
6 KAK 10	14 NSR 18	22 ROM 04
7 KAK 20	15 NSR 25	23 ROM 16
8 KAK 29	16 NSR 34	24 ROM 28

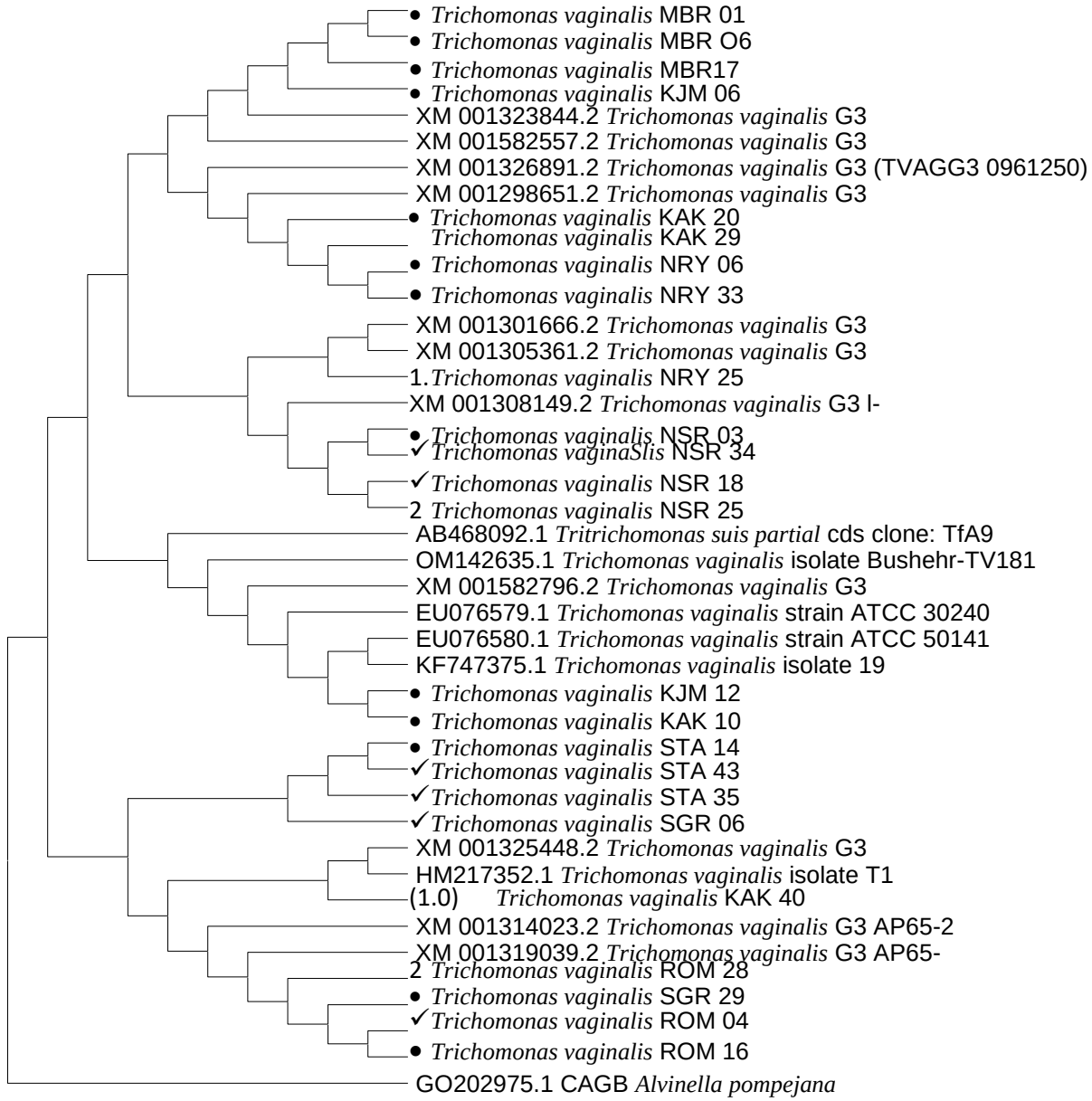

Figure 2: Phylogenetic Tree of Sequences obtained from *Trichomonas* sp. studied in this study and Comparison with the Sequences recorded in the gene bank.

Table 2: Susceptibility of *T. vaginitis* against Metronidazole

Conc (Ug/ml	No. of Sensitive Isolate	Percentage (%)
0.1	0	0
0.2	3	12.5
0.4	4	16.7
0.8	4	16.7
1.6	6	25
3.2	7	29.1

DISCUSSION

The present prevalence of *T. vaginalis* among pregnant women attending Primary Health care within Chikun LGA in this study was observed to be higher compared to the findings of Auta *et al.,* (2020) who reported the prevalence of *T. vaginalis* to be (4.49%) in General Hospital Sabon Tasha Chikun LGA in Kaduna State and lower to the findings of Mairiga *et al.* (2011) who reported (10.99%) in Maiduguri, North East Nigeria in their

study respectively. In contrast, other researchers such as Ojuronbge *et al.* 2010; Inusa *et al.,* (2018) found a relatively much higher prevalence of *T. vaginalis* infection among pregnant women in Abeokuta, to be 20.0% and 23.0% in Bauchi, Nigeria respectively. This disparity between studies could be attributed to differences in level of awareness about the mode of transmission of infections, increased in social lifestyle such as sex, among other factors.

Sequences of twenty-four isolates of *T. vaginalis* were obtained in this study. In an investigation in the Philippines, all the sequences clustered in a single clade, demonstrated low genetic polymorphism (Rivera *et al*., 2019). Ibáñez-Escribano *et al*. (2014) reported 99.7% nucleotide sequence identity among the vaginal specimens isolated from women. The 18s RNA sequences of the isolates showed high percentage sequence for identities to the existing sequences in the NCBI database. Using the same band fragment, Matini *et al*. (2012) reported two distinct reproducible banding patterns among the patients originating from different hospitals. In Chikun Local Government Area of Kaduna State, an earlier study demonstrated the presence of eight positive *T. vaginalis* within amplified fragments of 450bp in eight isolates (Alikhani *et al*., 2021). Nevertheless it was not able to obtain deep insights from the study area due to limited number of processed specimens. Based on phylogenetic analysis, *T. vaginalis* isolates demonstrated a high genetic homogeneity which clustered in a well-differentiated clade, supported by a bootstrap value of 100%, indicating no hybrid or intraspecific taxa among processed isolates. Consequently, a low genetic diversity was observed among *T. vaginalis* isolates of Chikun Local Government Area of Kaduna State.

The high inhibitory action of metronidazole implies that *T. vaginalis* is highly susceptible to the drug metronidazole. The result contradicts a similar study conducted by Josef and Josefine, (2011) on the susceptibility of *T. vaginalis* to selected antiparasites. This could be due some differences on the doses of minimal lethal concentrations differed 32-fold. It was further reported that there was no difference in the susceptibilities of *T. vaginalis* (Josef and Josefine, 2011).

CONCLUSION

The prevalence rate of *T. vaginalis* among pregnant women attending some Primary Health Care Centres in Chikun Local Government in this study was determined. Isolates of *T. vaginalis* were further confirmed using molecular technique and were identified. It was also found that *T. vaginalis* was susceptible to antiparasitic metronidazole. The study recommends that the parasite *T. vaginalis* should be studied using samples sources other than HVS for comprehensive analysis; also identification of the virulence gene present in the parasite using a molecular approach should be encouraged.

REFERENCES

Akafyi, D. E., Oko, J. O., Umar, M., Obafemi, A. and Michael, R. (2016). Prevalence of Bacterial *Trichomonas* and *Candidal vaginosis* among Females in Angwan-Fulani, Palladan in Zaria, Nigeria. *Journal of Applied Life Science International*, 5: 1-6.

Alikhani, M., Saberi, R., Hosseini, S. A., Rezaei, F., Pagheh, A. S. and Mirzaei, A. (2021). Identification of *Trichomonas vaginalis* genotypes using by actin gene and molecular based methods in southwest of Iran. *Rep Biochemical and Molecular Biology,* 10:135–143.

Amadi, A. N. C. and Nwagbo, A.K. (2018). *Trichomonas vaginalis* infection among women in Ikwuano, Abia State, Nigeria. *Journal of Applied Science Environmental Management*; 17: 389-393

Auta, I.K., Ibrahim, B. and Henry, D. (2020). Prevalence of *Trichomonas vaginalis* among ePregnant Women attending Antenatal Clinic in Two Health Facilities within Kaduna metropolis Kaduna Nigeria. *Science World Journal,* 15: 1: 98.

Conrad, M. D., Gorman, A. W. and Schillinger, J. A. (2012). Extensive genetic diversity, unique population structure and evidence of genetic exchange in the sexually transmitted parasite *Trichomonas vaginalis*. *PLoS Neglected Tropical Diseases* 6:e1573.

Fernado, S.D., Herath, S., Rodrigo, C. and Rajapakse, L. (2012). Clinical Features and Sociodemographic Factors Affecting *Trichomonas vaginalis* Infection in Women Attending a Central Sexually Transmitted Diseases Clinic in Sri Lanka. *Indian Journal of Sexually Transmitted Disease,* 33**:** 25-31.

Hall, T. (1999). ABioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98.

Hamafyelto, H.S. and Ikeh, I.E. (2017). Prevalence of *Trichomonas vaginalis* infection among female internally displaced persons in Maiduguri, Nigeria. *International Journal Tropical Disease Health*, 27: 1-7.

Hassan, A.O., Amoo, A.O., Adebimpe, W.O. and Akindele, A.A. (2021). Survey of Trichomoniasis in Osogbo, Southwestern Nigeria. *International Journal of BioMedical Research*, 2:607-9.

Ibáñez-Escribano, A., Nogal-Ruiz, J. J. and Arán, V. J. (2014). Determination of internal transcribed spacer regions (ITS) in *Trichomonas vaginalis*

isolates and differentiation among *Trichomonas* species. *Parasitological Inter* 63:427–431.

Inusa, S.D., Udechukwu, C.U., Panda, S.M. and Garba, F.G. (2018). Prevalence of Trichomoniasis among Pregnant Women Attending Ante-natal Clinic in Bauchi State, Nigeria. *GSC Biological and Pharmaceutical Sciences* 2(3): 010-016

Jatau, E.D., Olonitola, O.S. and Olayinka, A.T. (2006). Prevalence of Trichomonas infections among women attending antenatal clinics in Zaria, Nigeria. *Annals of African Medicine*, 5:178-181.

Josef G.M. and Josefine, T. (2011). Strain of Trichomonas vaginalis Resistant to Metronidazole and Other 5-Nitroimidazoles*. Journal of Antimicrobial Agents and Chemotherapy*, 15(2): 254-257

Kissinger, P. (2015). *Trichomonas vaginalis*: A review of epidemiologic, clinical and treatment issues. *Journal of Biomedical Centre of Infectious Diseases*, 15:307

Lawing, L.F., Hedges, S.R. and Schwebke, J.R. (2020). Detection of Trichomoniasis in Vaginal and Urine Specimens from Women by Culture and PCR. *Journal of Clinical Microbiology*, 38: 3585-3588

Mairiga, A.G., Balla, H.J. and Ahmad, M.I. (2011). Prevalence of *Trichomonas vaginalis* infections among antenatal clients in Maiduguri Nigeria. *International Journal Biomedical Research.* 2(4): 998–1002.

Matini, M., Rezaeian, M. amd Mohebali, M. (2012). Genotyping of *Trichomonas vaginalis* isolates in Iran by using single stranded conformational polymorphism-PCR technique and internal transcribed spacer regions. *Tropical Biomedical Journal,* 29:605–612

Ojurongbe, O., Taiwo, B.O., Dina, B.O., Sina-Agbaje, O.R., Bolaji, O.S. and Adeyeba, O.A. (2010). Prevalence of *T. vaginalis* Among Pregnant Women in Abeokuta Nigeria. *Sierra Leone Journal of BioMedical Research* 2(2): 82-86

Oladeinde, B.H., Oladeinde, O.B., Imade, O.S. and Onifade, A. A. (2016). *Trichomonas vaginalis* infection among women of reproductive age in a rural community in Nigeria. *Niger Journal Experimental Clinical Bioscience,* 4:1-5.

Richard, H.A., Agyeman, R.O., Nkrumah, N.O., Harriet, B.G., Awuah, G.M., Momodou, CL and Patrick, A.F. (2018). *Trichomonas vaginalis* infection and the diagnostic significance of detection tests among Ghanaian outpatients *Biomedical Journal of Science*, 18: 206.

Rivera, W. L., Ong, V. A. and Masalunga, M. C. (2019). Molecular characterization of *Trichomonas vaginalis* isolates from the Philippines. *Parasitological Resource Journal,* 106:105–110.

Sommer, U. (2015). Identification of *Trichomonas vaginalis* Cysteine Proteases that Induce Apoptosis in Human Vaginal Epithelial Cells. *Journal of Biology Chemistry*, 280:23853–23860.

Stary, A., Kuchinka-Koch, A. and Teodorowicz, L. (2018). Detection of *Trichomonas vaginalis* on Modified Columbia Agar in the Routine Laboratory. *Journal of Clinical Microbiology*, 40:3277-3286

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA)*; **101**: 11030-11035.

Ulogu, I.O., Obiajuru, I.O.C. and Ekejindu, I.M. (2017). Prevalence of Trichomoniasis amongst Women in Nnewi, Anambra State, Nigeria. *Nigerian Journal of Parasitology*, 28(1); 6-10.