PREVALENCE OF TRICHOMONIASIS AMONG PATIENTS ATTENDING UMARU SANDA NDAYAKO GENERAL HOSPITAL BIDA, NIGER STATE, NIGERIA

BY

AHMAD, Nana Hauwau

M.TECH/SLS/2018/8761

DEPARTMENT OF MICROBIOLOGY

FEDERAL UNIVERSITY OF TECHNOLOGY MINNA

NOVEMBER, 2021.

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL FEDERAL UNIVERSITY OFTECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY IN MEDICAL MICROBIOLOGY

NOVEMBER, 2021

ABSTRACT

Trichomoniasis, which is caused by a parasitic protozoa called *Trichomonas vaginalis,* it is a common sexually transmitted parasitic disease (STPD) in Nigeria. It is one of the most common curable sexually transmitted diseases (STD) that infect the urogenital tract of sexually active men and women causing significant vaginal and cervical ulceration. This study was carried out to determine the prevalence and risk factors of trichomoniasis among patients attending Umaru Sanda Ndayako General Hospital Bida, Niger State, Nigeria. A total of five hundred (500) high vaginal swabs (HVS) and urine samples were aseptically collected with a sterile swab stick and universal sample bottles from 250 females and 250 males respectively. The samples were examined for *T. vaginalis* using standard microbiological techniques within one hour of collection. Out of the 500 samples examined, 47(9.4 %) were positive. Higher prevalence of infection was recorded among females (33.0 %) compared to males (14.0 %). The highest prevalence was recorded among individual aged 40-50years (18.2 %) while the least prevalence was among those aged 51 and above 0(0.0 %). The highest prevalence (14.5 %) was obtained among the married participants, followed by the single (11.1 %), and the least was obtained among divorced participants (2.4 %). For educational level, the highest prevalence was recorded among participant that had no formal education (11.21 %) while the least prevalence was recorded among those that had primary education (5.8 %). Based on occupation, highest *T. vaginalis* prevalence was obtained among the unclassified (12.4%) while the least was recorded among Students (3.3 %). Significant risk factors for *T. vaginalis* infection were gender (p=0.004) and marital status (p=0.003). There was no relationship between rate of infection, age group (p=0.237), occupation (p=0.348) and educational status (p=0.614) of individual. This study has recorded a significant prevalent rate of *T. vaginalis* infection in the study area. It is therefore necessary to expand the knowledge of individuals especially the women, about Trichomoniasis.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Trichomoniasis is a common sexually transmitted parasitic disease (STPD) in Nigeria, caused by *Trichomonas vaginalis*. It was reported by Krieger (2012) that Trichomoniasis is a "curable sexually transmitted disease (STD) that infects the urogenital tract of sexually active men and women inducing substantial vaginal and cervical ulceration".

Among the diseases that commonly cause vaginal symptoms, trichomoniasis infection comes after bacterial vaginosis and candidiasis (McClelland *et al.,* 2017). The causative organism known to as *Trichomonas vaginalis*, is located inside genitourinary structures (such as the urethra, ureter, bladder, genital organs, including the uterus). It has been reported that annually, an estimate of 2.5 to 3 million Americans get infection and about one billion people suffer trichomoniasis worldwide while in Nigeria approximately 25% of students and 20% of pregnant women are infected (Toth, 2013).

The infection is typically acquired via direct (sexual) and indirect (rarely through contact with formites or surfaces contaminated with fluids from infected individuals) contact (Nester *et al.,* 2011). Women particularly those with multiple sex partners contract trichomoniasis infection via sexual contact with infected men or via vulva to vulva contact also, through non venereal mode of transmission due to the open biological nature of women, while seated on lavatory seat (the seat containing infected urine that is passed within thirty to forty five minutes) while men contact the infection only when they involve themselves in a sexual contact with infected women. Men serve as vectors, they are infestors rather than been infected while the women as reservoirs.

It has been reported by Swygard *et al*. (2014) that more than 180 million women globally might be infected, with prevalence varying between studied populations and ranges from 5-29 % in men and 5-74 % in women. Prevalence is lesser in males when compared to females; this is due to the fact that the infection in men is mostly asymptomatic and they do not seek evaluation until their partners are confirmed. However, when symptoms do manifests they can include; irritation inside the penis, mild discharge and slight burning sensation following urination or ejaculation. The symptoms in women appear within 5-28days of exposure. The symptoms include greenish-yellow frothy discharge, painful urination, vaginal itching and discomfort during intercourse in 50 % of cases to pruritus or dysuria and pelvic inflammatory disease (PID) (Krieger, 2012).

Trichomoniasis causes genital inflammation which also increases a woman's risk of HIV infection if exposed to HIV / shedding HIV if the woman is already a carrier, increasing the chances of transmission. Most of the women that are asymptomatic and usually avoid diagnosis turn out to be positive of trichomoniasis infection. The observation is affirmed by a case study in Zimbabwe where 75% of women denied symptoms on direct questioning out of which 16% tested positive after screening (Rathore *et al.,* 2017). The infection of trichomoniasis is often confusing for example, in a case study where 200 Nigerian women with discharge about 74% of them were infected with *Trichomonas* while out of a hundred and forty-nine (149) Nigerian women with discharge, none were infected (Swygard *et al.,* 2014). As was reported by Rathore *et al*. (2017), trichomoniasis are often asymptomatic in both men and women at a ratio of 50-70 % which contribute majorly to the source of transmissions of the infection. Pregnancy in women can be affected due to the asymptomatic nature of most of the infections in females leading to preterm birth, low birth weight in infants that usually develop congenital health problems which degenerate later in life. Infants become infected as a

result of rape and possibly through exposure to other STDs. So also, it was reported that "the disease can be contracted congenitally during birth by the newborn babies" (Lawing *et al*., 2010).

The vaginal discharge is the common sample collected mostly for the diagnosis of trichomoniasis in women while urine is sample mostly used for diagnosis in men. The pH paper is used in order to differentiate *T. vaginalis* from yeast rapidly in women, it is also used for the evaluation of pH. As was reported by Gary and Garber (2015), that pH level of the vagina which normally is 4.5, rises to 6 and above with Trichomoniasis but is not altered during yeast infestation. This simply means that *T. vaginalis* infection raises the hydrogen ion concentration (pH) of the vagina.

A single oral dose of 2 g metronidazole or tinidazole can treat *T. vaginalis* infection. It is always better for the sex partners to be treated at the same time as re-infection occurs often, in every five people treated, one gets re-infected within 3 months of treatment and it is advisable for them to avoid intercourse until every symptom disappear (Anorlu *et al*., 2012). The investigation of the prevalence of trichomoniasis among patients attending Umaru Sanda Ndayako General Hospital in Bida is due to the lack of baseline data in many parts of the state especially in the study area and the asymptomatic nature of the infection.

1.2 Statement of the Research Problem

Trichomoniasis affects more than two million people annually (Centre for Disease Control (CDC), 2021) and prevalence of the infection is often associated with significant public health problems. Infection in the majority of the men and women are often asymptomatic thereby escaping diagnosis and treatment. As a result of this, frequent spread and transmissions of the disease (trichomoniasis). Trichomoniasis in

females adversely affects pregnancies resulting in premature birth of mostly low birth weight infants that usually develop congenital health problems later in life. Schwebke (2012) and Soper (2014) reported that *T. vaginalis* is a major cause of infertility and a common cause of non-gonococcal urethritis (NGU) in men.

1.3 Aim and Objectives of the Study

This study was aimed at determining the prevalence of Trichomoniasis among adults in Bida Local Government Area of Niger State.

The objectives of the study were to

i. isolate and identify *Trichomonas vaginalis* from infected individual

ii. determine the prevalence of *T. vaginalis* among patients attending Umaru Sanda General Hospital

iii. determine the socio-demographic factors (age group, sex, status) associated with prevalence of the infection.

1.4 Justification for the Study

Early diagnosis of the infection with trichomoniasis is important to avoid obvious adverse effects to the infected individual, their sex partners and the unborn babies. Knowing the prevalence of trichomoniasis infection will help health professionals and individuals to be conscious of the parasite and forestall its further spread. Also establishing the socio-demographic factors associated with this infection will also aid in the control measure.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Morphology and Classification of *Trichomonas vaginalis*

T. vaginalis is the most widely studied parasite of all the trichomonads. This urogenital pathogen varies in size and shape, with the average length and width being 10 and 7 mm, respectively (Mendoza-Lopez *et al*., 2020). Physiochemical conditions do alter the appearance of the parasite. In axenic culture, the shape of the protozoan tends to be more uniform, i.e., pear shaped or oval (Zhang *et al.*, 2012), but the parasite takes on a more amoeboid appearance when attached to vaginal epithelial cells (Zhang *et al.*, 2012).

T. vaginalis is a flagellated protozoan possessing five flagella, four of which are located at its anterior portion. The fifth flagellum is incorporated within the undulating membrane of the parasite (Zhang *et al.*, 2012), which is supported by a slender non-contractile costa. The flagella and the undulating membrane give this parasite a characteristic quivering motility (Mendoza-Lopez *et al*., 2020). Under unfavorable growth conditions, *T. vaginalis* can round up and internalize the flagella.

The cytoskeleton of *T. vaginalis* is composed of tubulin and actin fibers. Investigators have used monoclonal antibodies to the tubulin molecule and found that the axostylar tubulin reacted with both sheep and pig brain tubulin (Bastida-Corcuera *et al*., 2013). The nucleus in *T. vaginalis* is located at its anterior portion, and, as in other eukaryotes, it is surrounded by a porous nuclear envelope. A slender hyaline, rod-like structure, called an axostyle, commences at the nucleus and bisects the protozoan longitudinally. It protrudes through the posterior end of the parasite, terminating in a sharp point (Mendoza-Lopez *et al*., 2020). This structure (Figure 1.1, Table 2.1) is thought to anchor the parasite to vaginal epithelial cells.

Granules are commonly seen in living organisms under light microscopy. These organelles are catalase negative, indicating that they are not peroxisomes (Mendoza-Lopez *et al.*, 2020). Because they produce molecular hydrogen, they were named hydrogenosomes and have been found to be important in metabolism (Mallo *et al*., 2013). There are two sets of these granules: paracostal and paraxostylar. The latter set is arranged along the axostyle in three parallel rows, which is a distinguishing feature of *T. vaginalis*. Glycogen granules are also present in *T. vaginalis* and can be observed by transmission electron microscopy (Schwebke and Burgess, 2012). *T. vaginalis* demonstrates hydrolase activity and contains lysosome-like structures such as phagosomes (Beri *et al*., 2020).

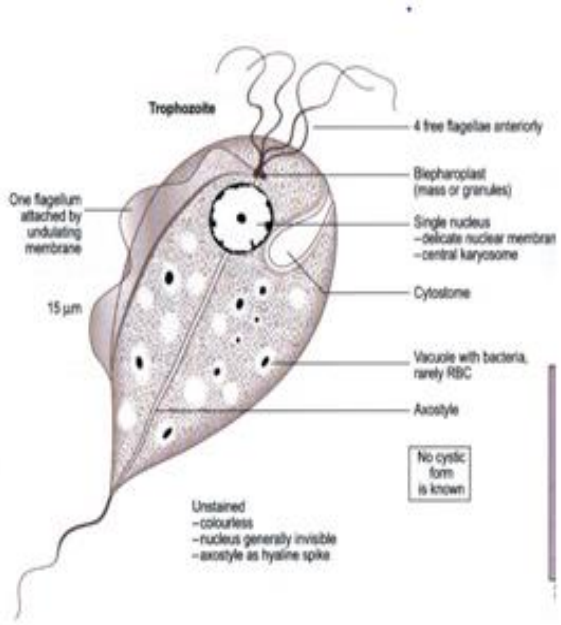

Figure 1.1: Structure of *Trichomonas vaginalis*

Table 2.1: The morphological characteristics of *T. vaginalis*

Morphology	Characteristics
Shape	Amoeboid or pyriform (pear shaped)
Size	~9–23x7micrometre (average 13mm)
Flagella	Anterior
	Recurrent one (originate from blepharoplast)
Internal organalles	Nucleus, Axostyle, Costa, Pelta
	Cytoskeleton (made up of actin, tubulin, kinesin and dynenin)
	Hydrogenosome without cytochrome, electron transport chain (ETC) enzymes and DNA

Source: (Palmieri *et al*., 2021)

2.1.1 Classification of *T. vaginalis*

Domain: Eukarya

Kingdom: Protista

Phylum: Metamonada

Class: Parabasilia

Order: Trichomonadida

Genus: *Trichomonas*

Species: *Trichomonas vaginalis*

2.2 Epidemiology and Modes of Classification

Trichomoniasis is a sexually transmissible infection that is capable of causing substantial morbidity. *T. vaginalis* is the only pathogenic species of the genus *Trichomonas* and has a worldwide distribution. The disease affects individuals in all racial groups and depending on the type of subpopulation studied prevalence rates vary between 0 and 65 %. In men, the organism can be found in the urethra, periurethral glands, prostate, epididymis, and in semen.

In 2001, World Health Organisation (WHO) estimated that 174 million cases occur worldwide annually, making *T. vaginalis* infection the most common non-viral Sexually Transmitted Infection (STI). Trichomonas infection has been encountered in every continent and climate and with no seasonal variability. It has a cosmopolitan distribution and has been identified in all racial groups and socioeconomic strata. The estimated incidence is more than 170 million cases worldwide Lewis *et al*. (2021), and at least 2 to 3 million symptomatic infections occur annually among sexually active women in the United States (Nolan *et al*., 2020). The incidence of trichomoniasis is as high as 56 % in patients attending Sexually Transmitted Disease (STD) clinics (Territo *et al*., 2021). This rate depends on many factors including age, sexual activity, number of sexual partners, other STDs, sexual customs, phase of the menstrual cycle, techniques of examination, specimen collection, and laboratory technique.

The prevalence of trichomoniasis at Sexually Transmitted Disease (STD) clinics varies from 8 to 31 %. In KwaZulu-Natal, trichomoniasis is one of the most commonly reported STIs in females Hodiwala and Narayankhedkar (2016). A prevalence rate of 28 % among STD clinic attendees was reported in 2004. It was found that the prevalence of trichomoniasis was lower in women attending antenatal clinics. Studies conducted amongst pregnant women in South Africa showed prevalence rates between 15 and

41.4%. The prevalence of trichomoniasis was 2.8 to 8.5 % in India. These figures underestimate the real prevalence, since up to one half of all infections are asymptomatic and never diagnosed. Reports from China indicate that it accounts for more than 30 % of gynaecologic inflammatory diseases causing female sterility. A Korean study done on patients complaining of vaginal symptoms found that 10.4% of these patients were infected by *T. vaginalis*.

Humans are the only natural host for *T. vaginalis*. The trophozoite is transmitted from one person to another, usually by sexual intercourse. Four points support the belief that *T. vaginalis* is transmitted sexually. The most important evidence is the high rate of infection of the urethra and/or prostate in the male partners of infected females. Recurrent trichomonal vaginitis was cured only after the parasites had been eradicated from the genital tract of the patients' consorts. *T. vaginalis* is observed more frequently in females attending STD clinics and also in prostitutes than in postmenopausal women and virgins. Finally, the flagellates die outside the human body unless they are protected from drying (Kirby, 2020).

Nonsexual transmission has been observed in cases such as by contaminated douche nozzles, specula, or toilet seats through which trichomonads may find their way into the vagina. However, such cases are rare. Live *T. vaginalis* has been found in urine and in semen after several hours of exposure to air and in swimming pool water. Newborn infants of mothers infected by *T. vaginalis* have on occasion acquired a *T. vaginalis* urinary tract or vaginal infection. The organisms were acquired by 2 to 17 % of female neonates of infected women. Because of the wide spectrum of clinical disease, control of this infection relies on the screening of women and their partners and the appropriate treatment of infected individuals to prevent the continued spread of the disease. Asymptomatic carriers can unknowingly transmit *T. vaginalis* via unprotected sexual

contact. Therefore, asymptomatic infections are epidemiologically important. Sexual intercourse is the most common route of transmission; however, non-venereal transmission is extremely rare Hodiwala and Narayankhedkar (2016).

2.3 Trichomoniasis in Nigeria

Prevalence and distribution of sexually transmitted parasitic diseases (STPDs) amongst the majority of endemic parasitic diseases in Nigeria, only trichomoniasis is sexually transmitted. There are other parasites that could be transmitted through oral-genital and oral-anal routes eliciting other forms of sexually transmitted diseases. Out of the 36 states in Nigeria, only 26 states including Abuja could access the studies for *T. vaginalis*. These studies traversed through different geo-political zones of the country. In spite of this, there is still little/no literature on *T. vaginalis* infection prevalence in some states. There was a resently on the study of *T. vaginalis* in Niger State at Federal University of Technology Minna as reported by Samuel *et al.* (2021) among the 500 level parasitology students, a prevelance of 40.17 % was recorded. The data available provide the prevalence of trichomoniasis across the country ranging from zero to fifty-one percent. Most of these studies were conducted on pregnant women, using vaginal swab as preferred samples (Asemota, 2018).

2.3.1 Prevalence of trichomoniasis across the six geopolitical zones and their states

Prevalence of trichomoniasis across the six Geopolitical Zones is as follows: The highest (36.6%) and lowest (1.3%) prevalence in South East was recorded in Ebonyi among the HIV/AIDS patients and Imo State among the males and Females respectively (Nweze and Mouneke, 2012). The highest (44.5%) and lowest (0%) prevalence in South South was recorded in Akwa Ibom among the HIV seropositive people and Edo State among the pregnant women respectively (Akinbo and Oronsaye, 2017). Unlike the prevalence in

South West which recorded the highest (74.5%) in Lagos among the Patients with vaginal Discharge and lowest (0%) in Ondo State among Seropositive and seronegative pregnant women (Akinbo and Oronsaye, 2017). But in North West, the lowest (1.3%) prevalence was recorded at Jigawa State among pregnant women (Abubakar *et al*., 2013) while the highest (19.2%) was in Kaduna among the Pregnant women (Anorlu *et al.,* 2012).

The highest (20.5%) and lowest (2.6%) prevalence in North East recorded in Borno among the Female IDPs and Adamawa State among Health men, women, antenatal females, and sick men and women with no trichomoniasis hereditary respectively (Mairiga *et al.,* 2012). The highest (51.8%) and lowest (3.73%) prevalence in North Central was recorded in Plateau among the HIV-1 infected women (Abdulazeez *et al.,* 2017) and FCT, Abuja Jigawa State among School pupils (Okojokwu *et al*., 2015) respectively.

The sex with higher prevalence of trichomoniasis infection is said to be the sexually active women (Jatau *et al*., 2016). *T. vaginalis* infection in male is generally asymptomatic. It is very important to treat male partners since asymptomatic carriers serve as vectors of the disease. *T. vaginalis* is found in the prostate and urethra of the males, also in the uterus, kidneys, bladder, ovaries, fallopian tubes and vagina of the female. It has been suggested from the Nigerian report that trichomoniasis could be lower in rural communities than in urban areas (Obiajuru, 2011; Njoku *et al*., 2012). This has been proved in a research carried out by Obiajuru and Ogbulie (2017) which shows that those residing in the rural areas record a lesser prevalence (39.16%) of trichomoniasis than those in the urban areas (57.70%).

Also, the sexually active aged group recorded the highest prevalence from the findings (that is ranging from 11 to 45 years old) (Amadi and Nwagbo, 2013). *T. vaginalis* is generally high amongst pregnant women (Ojurongbe, 2017). It was reported for example in a study from Zaria that the 16 -25years were positive for *T. vaginalis* with a prevalence of 53.57 %, while the prevalence of 1.8% for *T. vaginalis* was recorded in Lagos among the pregnant women aged 21-30 years old. Just like the findings in Abeokuta, South-West of Nigeria, the prevalence 21.3% was recorded for the pregnant female ranging between 20 and 30 years old.

Contrary to the record from the findings in Anambra, South-East of Nigeria, the pregnant women was said to have a lesser prevalence (16.7%) than the non- pregnant women (17.8%) (Iwueze *et al*., 2014). This is in consistent with the findings in Maiduguri that showed higher rate (20.8%) of infection among non-pregnant women (Hamafyelto and Ikeh, 2017). Report on marital status shows low prevalence 0.4% among the unmarried women as compared to the prevalence of 2.9% among the married women (Kojokwu *et al.,* 2015). It was reported by Amadi and Nwagbo the prevalence of 9.72% in single women in Abia, 21.6% and 11% among married women in Abeokuta and Maiduguri respectively (Etuketu *et al*., 2015; Hamafyelto and Ikeh, 2017).

Data have shown that HIV has increased the chances of infection with *T. vaginalis* (Kojokwu *et al.,* 2015). It was observed by Isiaka-Lawal *et al*. (2014) that the prevalence of *T. vaginalis* infection was lesser in HIV negative feminine gender as compared to those that are HIV positive in North-North Nigeria, contrary to that in South-West (Lagos to be precise), a prevalence of 35.8% was recorded among the HIV positive (Oyetunde and Chelsea, 2016).

The highest prevalence of *T. vaginalis* was observed among the patients that are involved in multiple sexual partner, low educational level, HIV positive, lack of personal hygiene and low socio-economic status. According to level of education, prevalence rate is higher with 22.3 % among the uneducated women and lower with 1.0% amongst their educated counterparts (Hamafyelto and Ikeh, 2017). It was also reported by Usanga *et al*. (2019) that the feminine gender with the prevalence 6.4% was recorded among those with secondary level of education. There are other risk factors associated with acquiring *T. vaginalis* infection as reported by Aboyeji and Nwabuisi (2013), Obiajuru, *et al.* (2014), Ulogu *et al*. (2017) and Usanga *et al.* (2019) include; increasing poverty, unemployment, violence against women and children.

2.4 Symptoms and Causes of Trichomoniasis

2.4.1 Symptoms of trichomoniasis

One may infect others before knowing it because a large number of infected people never have symptoms. In fact, men rarely show any sign of infection. When symptoms occur, they tend to appear within 5 to 28 days of exposure. The symptoms are thin white, yellow or greenish vaginal discharge that has a bad odour, white discharge from the penis, genital itching or irritation, burning or painful urination, burning after ejaculation, pain or discomfort during intercourse (CDC, 2021).

2.4.2 Causes of trichomoniasis

A parasite called *T. vaginalis* causes this sexually transmitted disease. Once an individual is infected, infection can be transferred to others through vaginal-penile or vaginal-vaginal intercourse, anal sex, oral sex, genital touching (CDC, 2021).

2.5 Pathogenesis of *T. vaginalis*

T. vaginalis is a flagellated parasitic protozoan, typically pyriform but occasionally amoeboid in shape, extracellular to genitourinary track epithelium with a primarily

anaerobic lifestyle (Harp and Chowdhury, 2017). The individual organism is 10–20 μm long and 2–14 μm wide. Four flagella project from the anterior portion of the cell and one flagellum extends backwards to the middle of the organism, forming an undulating membrane. An axostyle extends from the posterior aspect of the organism.

T. vaginalis has a large genome (strain G3, 176, 441, 227 bp) with approximately 60,000 protein coding genes organized into six chromosomes (Carlton *et al*., 2017). *T. vaginalis* is a highly predatory obligate parasite that phagocytoses bacteria, vaginal epithelial cells and erythrocytes and is itself ingested by macrophages. *T. vaginalis* uses carbohydrates as its main energy source via fermentative metabolism under aerobic and anaerobic conditions.

T. vaginalis primarily 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. Infection may persist for long periods, possibly months or even years, in women but generally persists less than 10 days in males.

The parasite does not appear to have a cyst form and does not survive well in the external environment, but can survive outside the human body in a wet environment for more than three hours. *T. vaginalis* (TV) pseudocyst have been found to be more virulent in animals and could have relevance for human, particularly in the case of neoplasia (Afzan and Suresh, 2012). Trophozoites of *T. vaginalis* are transmitted from person to person through sexual intercourse (Palmieri *et al*., 2021). Non-sexual transmission of *T. vaginalis* is rare. Cystic stages are unknown for *T. vaginalis*. The trophozoite attaches to mucosal surfaces of the lower urogenital tract and divides by longitudinal binary fission. Invitro studies suggest that trophozoites have a 4–28 day incubation period. *T. vaginalis*

survives long-term in the varying and adverse acidic environment of the vagina through various successful host parasitisms. Visceral epithelial cell (VEC) surface or extracellular matrix (ECM) proteins play different roles in binding diverse target molecules from the host or other microbes from the mucosal surfaces that are mediated by *T. vaginalis* surface proteins including LPG, cytokines and cytoskeletal protein a-actinin.

Although *T. vaginalis* is the most intensely studied trichomonad and is the world's most common cause of nonviral STDs, the exact mechanism of its pathogenesis has not been clearly elucidated. While searching for a virulence assay (Westrop *et al*., 2017), Honigberg and coworkers used microscopy and biochemical analysis to define trichomonad structure and function (Bhakta *et al*., 2020; de Souza and Attias, 2018) as well as many behavioral and cytochemical interactions between the parasite and the host cell (de Souza and Attias, 2018).

Modern research has focused on the initial events required to establish infection. Many mechanisms are thought to be involved and include cell-to-cell adhesion Ayona *et al*. (2020), hemolysis, and the excretion of soluble factors such as extracellular proteinases and CDF. The interaction of *T. vaginalis* with the members of the resident flora of the vagina may be an important factor as well (Tam *et al*., 2021), and, like many other protozoans, *T. vaginalis* has demonstrated many mechanisms which are used to evade the host immune system (Mercer and Johnson, 2018; Zimmann *et al*., 2021). The host-parasite relationship is very complex, and the broad range of clinical symptoms cannot likely be attributed to a single pathogenic mechanism. All clinical isolates of *T. vaginalis* appear to be capable of infection and disease production. The cell surface of the trichomonad plays a major role in adhesion, host-parasite interaction, and nutrient

acquisition, and the proteins and glycoproteins displayed on the surface have functions in this regard.

T. vaginalis has over 300 candidate surface proteins which belong to ten different protein families with at least one inferred transmembrane (TM) domain and share one or more features with other pathogen surface proteins. Three surface protein families are BspA-like proteins (TpLRR-containing proteins), GP63-like proteins and adhesins or others (Štáfková, 2018). The BspA-like proteins are the largest gene family encoding potential surface proteins and share a specific type of leucine-rich repeat (LRR), the TpLRR. The GP63-like proteins are the second largest gene family of candidate surface proteins encoding 77 paralogues, and 53 possess potential TM domains. GP63 are metalloproteinases (MMP) belonging to the metzincin class (EC 3.4.24.36), characterized by the motif HExxHxxGxxH (x represents any amino acid residues) forming an extended zinc-binding motif and a catalytic site.

Additional candidates of this family are 28 subtilisin-like serine proteases, nine different serine proteases and five calpain-like cysteine proteases. The calpain-like cysteine proteases possess 22 to 23 identifiable TM domains and function as surface proteases that transport important protein fragments or small peptides for energy generation from amino acids and redox balancing. The third surface protein family of *T. vaginalis* shares domains with other surface proteins related to mucosal pathogens. The members of this family are identified at different stages of infection during mucosal contact and help *T. vaginalis* to escape from the host adaptive immune response.

T. vaginalis is a highly predatory obligate parasite that phagocytoses bacteria, vaginal epithelial cells (VECs) and erythrocytes, and is itself ingested by macrophages (Núñez-Troconis, 2020; Palmieri *et al.*, 2021). *T. vaginalis* uses carbohydrates as its main energy

source via fermentative metabolism under aerobic and anaerobic conditions. *T. vaginalis* lacks the ability to synthesize many macromolecules de novo, particularly purine, pyrimidine, and many lipids, including cholesterol. These nutrients are acquired from the vaginal secretions or through phagocytosis of host and bacterial cells.

T. vaginalis has a broad range of transport capabilities that facilitate transport of complex carbohydrates and amino acids through the members of the cation-chloride co-transporter (CCC) family proteins, and help survival by reflecting osmotic changes in a mucosal environment. *T. vaginalis* has an unusual biosynthetic pathway for synthesis of non-protein lipid anchors (inositol phosphoceramide) of surface lipophosphoglycans (LPG) (Wang, 2021). With a microaerophilic (aerobic) lifestyle, *T. vaginalis* uses redox and antioxidant systems to counter the detrimental effects of oxygen and express a wide range of genes encoding for defense molecules including superoxide dismutases, thioredoxin reductases, peroxiredoxins, and rubrerythrins (Table 2.2).

Table 2.2 Pathogenicity of *T. vaginalis*

Pathogenesis	Characteristics
Surface proteins	3000, Grouped into three major categories (BspA-like proteins, GP63-like proteins, adhesins and other proteins)
Energy sources	Dihydrolase metabolism; enzyme amino-transferases and glutamate dehydrogenase synthesizes glutamate, aspartate, alanine, glutamine and glycine; enzyme cysteine synthase synthesizes cysteine; synthesize proline from arginine; synthesize phospholipids; metabolize threonine.

(Source: Palmieri *et al*., 2021)

2.5.1 Adhesion/adherence and adhesins

T. vaginalis possesses multiple mechanisms for colonization in the vaginal tract due to a dynamic hormonal influence from the menstrual cycle on the exfoliation of valued

environmental components (VECs) and a constantly changing environment. After cytoadherence, *T. vaginalis* transforms to an amoeboid structure with increasing cell-to-cell surface contact, forming cytoplasmic projections that interdigitate with target cells. The interactions of *T. vaginalis* with mucin, VECs and ECM molecules persist in a non-self-limiting fashion. *T. vaginalis* releases cysteine proteinases into the vaginal milieu resulting in desquamation of the vaginal and cervical epithelia. This facilitates efficient cytotoxicity toward host cells with complex interactions similar to the situation for other cell to cell contacts. The 71LPG is a major adherence factor in *T. vaginalis*, although studies of the molecular basis of adhesion of *T. vaginalis* to human cells have revealed that several other genes including adhesion proteins (AP), fibronectin (FN)-binding protein, laminin binding protein, a-actinin, enolase, phosphoglucomutase, and conserved GTP-binding protein (GTP-BP)] expressions are unregulated (Wang *et al*., 2021).

The adhesion of the parasite to the epithelial cell seems to be mediated by four adhesion proteins: AP65, AP51, AP33, and AP23 Ayona *et al*. (2020), which act in a specific receptor-ligand fashion (Ayona *et al*., 2020). This depends on time, temperature and pH, prompting *T. vaginalis* to become flat and laminate itself to the host cell (Wang *et al*., 2021). AP65 is encoded by at least three genes in a multiple-gene family which are curiously similar to the genes encoding malic enzyme. Much of the evidence for the role of AP in pathogenesis has come from co-culture experiments in which antibodies to AP are shown to reduce parasite adhesion and subsequent cytopathic effects (CE) on host cells.

The ap65-1 gene encodes a 65 kDa malic enzyme involved in cytoadherence. The transcription of this gene is critically regulated by the coordination of two similar but opposite oriented DNA regulatory regions, MRE-1/MRE-2r and MRE-2f; both of the regulatory proteins bind for multiple Myb-like proteins and binding varies with iron

concentrations (Wang *et al*., 2021). Myb1 proteins bind ap65-1 promoter at a proximal site in higher iron levels, whereas Myb2 protein binds in iron-depleted conditions (Wang *et al*., 2021).

Adhesion of trichomonads to the epithelial cells in the vaginal environment is a critical step in the pathogenesis of the parasite (Mercer and Johnson, 2018). Attachment to cells is time, temperature, and pH dependent. *T. vaginalis* appears more inclined to parasitize vaginal epithelial cell lines than other cell types in vitro. This is not surprising since epithelial cells are likely to be the principal cell type with which the parasite would interact in vivo. The surface of the trichomonad cell is a mosaic of adhesins, receptors to host extracellular matrix proteins, and carbohydrates, which provide the basis for ligand-receptor binding (Pekmezovic *et al*., 2019).

The adhesins are alternately expressed on the surface with a highly immunogenic glycoprotein, P270 (Mercer and Johnson, 2018). Gene expression of the four adhesins is coordinately upregulated at the transcriptional level by iron (Ayona *et al*., 2020). Contact-initiated ameboid transformation, which is marked by the production of pseudopodia followed by the upregulation of adhesin synthesis, suggests evidence for a sophisticated signal transduction system. Adhesion seems to also require the presence of cysteine proteinases (CPs) (Gould *et al*., 2017).

It has been observed that the side opposite the undulating membrane and the recurrent flagellum of the parasite attaches itself to the epithelial cells Mercer and Johnson (2018), and the microfilaments become concentrated in the parasite on the side that is in contact with the vaginal epithelium (Štáfková *et al*., 2018). While the adhesins are concentrated on the side opposite the undulating membrane, laminin-binding proteins are ubiquitous on the entire surface of *T. vaginalis* (Ayona *et al*., 2020). Laminin, a glycoprotein

localized in the basement membrane of the epithelium, promotes cell adhesion, differentiation, shape, and motility in normal cells, and it has been shown to have chemotactic properties (Ayona *et al*., 2020). *T. vaginalis* was observed adhering to laminin-coated plastic (Ayona *et al*., 2020) and endocytosing laminin-covered polystyrene particles. Similar laminin receptors have also been found on macrophages, bacteria, and cancer cells, but their role in *T. vaginalis* pathogenesis remains undefined.

Similarly, *T. vaginalis* has receptors for another extracellular matrix adhesion glycoprotein, fibronectin, which is secreted in both the basement membrane and serum. Although trichomonads can become coated with host fibronectin (and other serum proteins) Mercer and Johnson (2018), it is unclear whether fibronectin receptors function in nutrient acquisition Mercer and Johnson (2018), adherence Ayona *et al*.(2020) or a combination of the two (Ayona *et al.*, 2020). Lectin-binding carbohydrates on the surface of the trichomonad cell were characterized by the group of Westrop *et al*. (2017), after Ayona *et al*. (2020) reported their role in adherence to glass.

The presence of surface carbohydrates (D-lactose and *N*-acetyl-D-glucosamine) appears to be correlated with virulence (Westrop *et al*., 2017). Surface saccharides seem to be involved in *T. vaginalis* hemolysis of erythrocytes and phagocytosis of the target cells and may be associated with drug susceptibility. On the other hand, endogenous lectins on the surface of the trichomonad cell may be important in adhesion (Shi *et al*., 2018). Neuraminidase seems to be excreted both on the surface and in the culture media (Pekmezovic *et al*., 2019). Cleavage of Sisalic acid on the surface of host cells may be important for adhesion (Pekmezovic *et al*., 2019), but sialic acid present on the surface of *T. vaginalis* does not seem to be involved (Pekmezovic *et al.*, 2019).

2.5.2 Hemolysis

The vaginal mucosa may be a poor nutritional milieu for microbes. Since the ability to synthesize lipids is lacking in *T. vaginalis*, erythrocytes may be a prime source of fatty acids that are needed by the parasite. In addition to lipids, iron is an important nutrient for *T. vaginalis* and may also be acquired via the lysis of erythrocytes. Metabolically active parasites are necessary for lysis of erythrocytes. CP inhibitors greatly reduced erythrocyte lysis, which suggests that CPs may be a lytic factor involved in hemolysis. Hemolysis in vitro is greatest at the normal vaginal pH of 4.5, suggesting that this parasite characteristic occurs within the vaginal microenvironment (Pekmezovic *et al*., 2019). This lysis of the erythrocytes appears to be mediated by protein receptors on the surfaces of both erythrocytes and parasites (Pekmezovic *et al*., 2019), and empirical evidence suggests that perforin-like proteins may be involved. Five adhesin molecules have been identified, three of them identical to the ones that mediate adherence to epithelial cells (Ayona *et al*., 2020).

Hemolysis seems to occur in three steps. A specific ligand-receptor interaction allows the trichomonad to attach itself to the erythrocyte. This is followed by the release of perforin-like proteins (possibly CPs), which form pores in the erythrocyte membrane. Finally, *T. vaginalis* detaches itself from the cell and cell lysis occurs. Unlike its behavior with epithelial cells, *T. vaginalis* has been observed to phagocytes erythrocytes (Pekmezovic *et al*., 2019). Hemolytic activity appears to be correlated with virulence.

2.5.3 Proteinases

Characteristics of proteinases have been well summarized by North and extensive work has been done in isolating and purifying the proteinases of *T. vaginalis*. *T. vaginalis* has between 11 and 23 distinct CP activities, most of which are lysosomal (Zimmann *et al.*,

2021). The CPs of *T. vaginalis* is by far the most abundant of the parasitic protozoa. CPs has been implicated as probable lytic factors in the hemolysis of erythrocytes.

In addition, CP activity is required for the adherence of *T. vaginalis* to epithelial cells (Gould *et al*., 2017). Pretreating trichomonads with *N*a-*p*-tosyl-L-lysine chloromethyl ketone HCl (TLCK), a CP inhibitor, caused a marked decline in their ability to adhere to epithelial cells. When CP was added to TLCK-treated cells, their ability to attach themselves to the host was restored (Gould *et al*., 2017). This indicates that the action of proteinase on the surface of the parasite is a prerequisite for host attachment. *T. vaginalis* CPs also have the ability to degrade host immunoglobulins G and A (IgG and IgA), both of which are present in the vagina (Zimmann *et al*., 2021).

2.5.4 Contact-independent mechanisms of pathogenicity and cell-detaching factor

Although contact-dependent mechanisms play a significant role in the pathogenesis of *T. vaginalis*, contact-independent mechanisms are also involved. The first to report on contact-independent mechanisms was Hogue Mercer *et al*. (2018), who noted that cell-free filtrates had similar adverse effects on cell culture to those of the organism itself. The idea that some soluble cytotoxin may play a role in the pathogenic effects has also been proposed by others (Bhakta *et al.,* 2020). Hemolysis and cytotoxicity, for example, cannot be explained solely by the contact-dependent mechanisms, since these effects can be seen in the absence of cell-to-cell contact. While pH and acidic metabolites can be partly responsible for these effects the organism has been shown to produce other factors which cause cytopathic effect.

It has been shown that a cell-free product of *T. vaginalis*, Cell-Detaching Factor **(**CDF), causes cytopathic effects in cell culture (Warin, 2020). When the cell-free filtrate of a *T. vaginalis* culture is applied in vitro to a cell culture monolayer, the cells of the monolayer detach and clump together but remain viable. The detachment of the cell

monolayer in vitro is thought to be analogous to the sloughing of vaginal epithelial cells seen in the vaginal mucosa during acute infections (Warin, 2020). Cell-detaching factor (CDF) activity is probably a factor in pathogenesis, since *Pentatrichomonas hominis*, a nonpathogenic species, does not show CDF activity (Warin, 2020). Cell-detaching factor, which is thought to be an extracellular factor, was found to be a 200-kDa glycoprotein and it is heat and acid labile (Warin, 2020). The concentration of CDF in the filtrates varied with three factors: the duration of *T. vaginalis* growth prior to filtrate preparation, the initial inoculum size, and the pH of the filtrate prior to harvesting (Warin, 2020).

Activity was also found to be affected by pH as reported by Warin (2020). Also, the optimum pH of the cell-free filtrate was found to be around 7.2. This is of clinical relevance since the normal pH of the vagina is 4.5 but is greater than 5.0 during trichomoniasis. The rise in vaginal pH during trichomoniasis may therefore be crucial in the pathogenesis of the disease. Cell-detaching factor levels have been shown to correlate with the severity of the clinical symptoms of vaginitis. Increasing production of CDF was associated with increased severity of clinical disease. Cell-Detaching Factor is also immunogenic, and the detaching activity is inactivated by human sera reactive to *T. vaginalis* (Waring, 2020). Thus, local vaginal antibodies may decrease the effects of CDF. It is not certain whether the regulation of CDF production (its concentration), its activity, its immunogenicity, or a combination of the three plays a role in the severity of symptoms. Indeed, all of the pathogenic mechanisms (contact dependent, contact independent, and immune response) are probably important in the virulence of this disease.

Several investigators have been unable to demonstrate a cytopathic effect with cell-free filtrates of *T. vaginalis* culture (Štáfková *et al*., 2018). *Trichomonas vaginalis* is known

to excrete lactic and acetic acids in cell culture, and so unless the pH of the cell culture is maintained, the pH drops below 4.5 (Waring, 2020). The intolerance of CDF to acidic pH may explain why these investigators could not demonstrate its activity (Lee *et al*., 2017).

2.5.6 Interaction with the vaginal flora

The establishment of *T. vaginalis* in the vagina is puzzling indeed, since the normal pH of the vagina is a very acidic 4.5 while this organism thrives in a less acidic pH of .5. The relationship between protective lactobacilli and *T. vaginalis* is not completely understood. The rise in the pH of the vagina is also marked, with a concomitant reduction (or complete loss) of *Lactobacillus acidophilus* and an increase in the proportion of anaerobic bacteria. It appears that in vitro, with a controlled pH, lactobacilli do not affect the growth of *T. vaginalis*; however, the parasite seems to have a deleterious effect on *L. acidophilus* (Tam *et al*., 2021). Several mechanisms have been proposed: *T. vaginalis* has been observed to phagocytes bacteria (Dessì *et al.*, 2019) and this may occur with lactobacilli as well. Another hypothesis is that products, such as CDF or proteinases secreted by *T. vaginalis*, may destroy the lactobacilli (Nader-Macías *et al.,* 2021).

2.5.7 Immune system evasion

In a hostile changing environment, *T. vaginalis* can survive and flourish. Its ability to evade the host immune system is an important aspect of pathogenesis. Avoidance of complement is a strategic tactic which is used by *T. vaginalis* to overcome the human immune system. It has long been known that *T. vaginalis* activates the alternative pathway of complement, yet we are only just beginning to understand how the parasite escapes eradication. *T. vaginalis* has taken advantage of a niche in which little complement is present.

Like many other protozoan parasites, *T. vaginalis* displays phenotypic variation as a mechanism of immune evasion. Mercer and Johnson (2018) found out that two classes of markers are alternately expressed on the surface of the organism: the highly immunogenic glycoproteins (P270) and the adhesins (AP65, AP51, AP33, and AP23) (Mercer and Johnson, 2018). While all isolates (type I and type II) synthesize P270, only type II organisms can undergo phenotypic variation between cytoplasmic and cell surface expression of P270 (Núñez-Troconis, 2020). Thus, the phenotypes are described as A1 B2 (P270 positive) and A2 B1 (P270 negative) (Núñez-Troconis, 2020). The positivephenotype organisms lack adhesins and cannot cytoadhere or parasitize host cells (Núñez-Troconis, 2020). Only the organisms of the negative phenotype, which express the adhesins, have the ability to cytoadhere (Núñez-Troconis, 2020). After prolonged cultivation in vitro, the organisms shift toward the positive phenotype (Núñez-Troconis, 2020).

The P270 glycoprotein has been shown to have a single repetitive DREGRD epitope, which is important for antibody binding and it was found that some organisms bearing the P270 antigen on the cell surface are susceptible to antibody mediated, complement-independent lysis in vitro. The lack of P270 surface expression and the immune recessive nature of the adhesins permit the negative-phenotype organisms to survive antibody attack. The adhesin proteins appear to mimic the structure of malic enzyme, which may account for their poor immunogenicity (Ayona *et al*., 2020). This molecular mimicry is yet another example of how the trichomonad can escape detection by the immune system.

In addition to these mechanisms, *T. vaginalis* has numerous other ways of evading the immune system. Bhakta *et al*. (2020) have reported that numerous CPs secreted by *T. vaginalis* degrade IgG, IgM, and IgA, which allows the organism to survive the antibody

response. This parasite also secretes copious amounts of highly immunogenic soluble antigens. A continuous release of these antigens may neutralize antibody or cytotoxic T lymphocytes, thus short-circuiting specific anti-*T. vaginalis* defense mechanisms (Bhakta *et al*., 2020). As well, *T. vaginalis* can coat itself with host plasma proteins. This coating does not allow the hosts immune system to recognize the parasite as foreign (Mercer and Johnson, 2018). Thus, immune system mechanisms such as antigen presentation and complement-mediated lysis will not occur.

2.5.7 *T. vaginalis* RNA virus

Interestingly, only the P270-positive phenotype organisms have been found to harbor a double-stranded RNA virus Mercer and Johnson, (2018), termed *T. vaginalis* RNA virus (TVV). It seems that P270 surface expression is correlated with the presence of TVV Mercer and Johnson, (2018), since the loss of P270 surface expression was associated with the loss of TVV (Valenti *et al*., 2018). It has been suggested the presence of TVV may even upregulate P270 mRNA accumulation (Mercer and Johnson, 2018). This correlation, however, does not explain two independent paradoxical observations: during prolonged cultivation in vitro, a transition toward P270 surface expression is seen (Núñez-Troconis, 2020), yet TVV is associated only with fresh isolates and is lost after prolonged in vitro cultivation.

2.5.8 Hydrolases and cytotoxic molecules

A wide range of hydrolases (20–110 kDa) has been identified in *T. vaginalis* as cytoplasmic cysteine proteinases (Palmieri *et al*., 2021). These hydolases have trypsin-like activity and function as cell-detaching factors by degrading proteins (such as laminin, fibronectin, and other components) of the ECM and aid in the release of host cells from tissue and mucosal desquamation. *T. vaginalis* proteinases of 25, 27 and 34

kDa are specifically hydrolyzed synthetic substrates with arginine–arginine residues, whereas other proteinases have activity over a wide substrate range. Four different cysteine proteinase genes of *T. vaginalis* have 45% homology to cysteine proteinase genes of Dictyostelium discoideum, and are L-cathepsin and H-papain type proteinases (Wang, *et al*., 2021). These proteinases allow *T. vaginalis* to traverse the protective mucus barrier of host epithelium.

T. vaginalis produces several cytotoxic molecules and mediate cytotoxicity by damaging the target cell plasma membrane. Some of these molecules have perforin-like activity and create pores in erythrocyte membranes (Palmieri *et al*., 2021). *T. vaginalis* also secretes different lytic factors (LF) with phospholipase A2 activities to destroy nucleated cells, erythrocytes and specifically degrade phosphatidylcholine, underlying its unique pathogenesis.

2.5.9 Host response and innate immunity mechanism

T. vaginalis evades the immune system through complement mediated destruction, molecular mimicry and coating itself with host plasma proteins (Nemati *et al.,* 2018). Natural infection of *T. vaginalis* to humans seems to produce immunity that is only partially protective. *T. vaginalis* has unique abundant cell surface lipophosphoglycan (LPG) Nemati *et al.* (2018), a carbolipid molecule ($2–3x10^6$ copies/parasite) that similar to prokaryotic glycol conjugates. *T. vaginalis* produces immuno-suppressive cytokines (IL-10, TGFb) and causes caspase-mediated apoptosis in T-cells, macrophages and dendritic cells.

Moreover, recent comprehensive compositional and structural analysis of *T. vaginalis* revealed that LPG has specific LPG domains with pro inflammatory properties, and its outer branch saccharide and ceramide phospho-inositol glycan core (CPI-GC) activates NFkB, ERK1/2 and MEK1/2 (Nemati *et al.,* 2018). Furthermore, *T. vaginalis* induces

COX-2 expression, and up-regulates and activates toll-like receptors (TLR2, 4, and 9) via the p38 mitogen-activated protein kinase (MAPK) pathway. LPG CPI-GC contains terminal poly-Nacetylactosamine repeats that represent the ligand for the animal lectins called galectins (Nemati *et al*., 2018).

Cervical and VECs release galectin-1 and galectin-3 upon T. vaginalis infection and modulate the inflammatory responses in opposite fashion (galectin-1 suppressing and galectin-3 enhancing leucocyte response to inflammatory response). Also galectin-1 promotes viral attachment, whereas HIV-1 infected cells enhance viral replication, gelatin-3 and cytokine expression in VECs (Wang *et al.,* 2021). Thus, natural infection with *T. vaginalis* results in priming of acquired immune responses. Moreover, studies from the patients infected with *T. vaginalis* and HIV indicated that innate immunity involves chemotaxis and subsequent influx of neutrophils (Nemati *et al.,* 2018).

2.6 Clinical features of *T. vaginalis*

The majority of women and men with ***T. vaginalis*** are asymptomatic. One third of asymptomatic women become symptomatic within 6 months. Among those who do have symptoms, they include urethral discharge and dysuria. Among women, common sites of infection include the vagina, urethra and endocervix. Symptoms include vaginal discharge (which is often diffuse, malodorous, yellow-green), dysuria, itching, vulvar irritation and abdominal pain. The normal vaginal pH is 4.5, but with TV infection this increases markedly, often to >5. *Coplitis macularis* or strawberry cervix is seen in about 5 % of women, though with colposcopy, this rises to nearly 50% (Krieger, 2015). Other complications include infection of the adnexa, endometrium, and Skene and Bartholin glands. In men, it can cause epididymitis, prostatitis, and decreased sperm cell motility (Martinez-Garcia *et al*., 2016).

2.7 Sequelae of *T. vaginalis*

Reproductive outcomes studies show an association between *T. vaginalis* and vaginitis, cervicitis, urethritis, bacterial vaginosis, candidiasis, herpes simplex virus type-1 and type-2, Chlamydia, gonorrhea, and syphilis (Allsworth *et al*., 2019). *T. vaginalis* has also been associated with poor birth outcomes such as low birth weight, preterm delivery, pelvic inflammatory disease, and premature rupture of membranes (Silver *et al*., 2014). One study showed an association between maternal *T. vaginalis* infection and intellectual disability in children Mann *et al*. (2019). Although rare, *T. vaginalis* infection can be transmitted perinatally Schwandt *et al*. (2018) and cause vaginal and respiratory infections in neonates. HIV acquisition and transmission several cross-sectional and cohort studies that have indicated a higher risk for HIV acquisition among *T. vaginalis* positive compared to *T. vaginalis* negative women (Kissinger *et al.*, 2013). This greater susceptibility is biologically plausible for three reasons: inflammatory response to *T. vaginalis* infection results in the increased appearance of HIV target cells; *T. vaginalis* infection can impair the mechanical barrier to HIV via punctuate mucosal hemorrhages (Guenthner *et al*., 2015) and *T. vaginalis* infection may change the normal vaginal flora rendering it more permissive for bacterial vaginosis, which, in turn, can increase the risk of HIV acquisition (Moodley *et al*., 2012). These consequences facilitate HIV in *T. vaginalis* infected women.

Several studies have also demonstrated increased HIV expression among HIV positive / *T. vaginalis* positive women. A study estimates that in a community with a high prevalence of *T. vaginalis*, as much as 20% of HIV could be attributed to *T. vaginalis* infection (Chesson *et al*., 2014). It was also estimated that 6.2% of all HIV infections among US women may be attributed to *T. vaginalis* infection. Control of *T. vaginalis* therefore, may provide a cost-effective strategy for reducing HIV transmission especially

in settings where *T. vaginalis* is common (McClelland, 2018) or among subgroups that are at higher risk for *T. vaginalis* such as African Americans (Sorvillo *et al*., 2011).

Among HIV positive women, *T. vaginalis* has been associated with increased HIV vaginal shedding in several studies (Kissinger and Adamski, 2013). Fortunately, treatment for *T. vaginalis* has demonstrated reductions in HIV genital shedding in several studies. HIV positive men with urethritis in Malawi, with *T. vaginalis* diagnosed by NAAT, experienced a decrease in seminal HIV after metronidazole (MTZ) treatment (Prince *et al*., 2013). HIV vaginal shedding was decreased after treatment in one cohort of women, diagnosed by microscopy and culture in Kenya (Wang *et al*., 2011), and another, diagnosed by culture, in Louisiana, US (Kissinger *et al*., 2019). These data underscore the importance of screening and treatment among HIV positive persons.

Herpes Simplex virus (HSV-2) *T. vaginalis* appears to have a similar bi-directional association with HSV-2 as it does with HIV-1. Concomitant infection with *T. vaginalis* has been associated with HSV-2 shedding (Boselli *et al*., 2015) and women who have been found to have *T. vaginalis* have a higher incidence of HSV-2 (Gottilieb *et al*., 2014).

Neoplasia evidence that *T. vaginalis* is associated with HPV acquisition, thus there may be in indirect link between *T. vaginalis* and cervical neoplasia (Zhang and Begg, 2014). A meta-analysis found that *T. vaginalis* was associated with a 1.9 fold risk of cervical neoplasia. Studies of Finnish, Dutch, Belgian and Chinese women have all found elevated odds of cervical neoplasia among women who have *T. vaginalis* or vice versa (Roeters *et al*., 2011). An association was also found between *T. vaginalis* and prostate cancer in a study (Sutcliffe *et al*., 2019).

2.8 Diagnosis and Test

The diagnosis of *T. vaginalis* is becoming more precise and more tests have become available in the last decade. Wet mount microscopy has been used for many decades to diagnose *T. vaginalis*. The test is inexpensive, low technology and is point of care; however, it is insensitive, particularly in men. Sensitivities range from 50–70% depending on the expertise of the reader and should be read within 10 minutes of collection (Kingston *et al*., 2013).

Nucleic acid probe techniques are the most sensitive tests, moderately priced and fast, but require instrumentation. These tests are not considered point-of-care. The APTIMA *T. vaginalis* Assay (Hologic Gen-Probe, San Diego, CA) was United States Federal Drug Administration (FDA)-cleared in 2011 for use with urine, endocervical and vaginal swabs, and endocervical specimens collected in the Hologic Preserve Cyst solution (Thin-Prep) from females only. Sensitivity and specificity are both 95–100% (Nye *et al*., 2019).

There are two point-of-care (POC) tests that have been approved by the U.S FDA for diagnosis of *T. vaginalis* among women: OSOM Trichomonas Rapid Test (an immunochromatographic capillary flow dipstick technology (Huppert *et al*., 2017) and Affirm VP III (a nucleic acid probe test that evaluates for *T. vaginalis, G. vaginalis,* and *C. albicans* (Andrea and Chapin, (2011). Both tests are performed on vaginal secretions and have a sensitivity of more than 83% and a specificity of more than 97 %. Results of the OSOM test are available in about 10 mins, while results of the Affirm VP III test can be available within 45 mins.

It has been generally thought that only vaginal specimens should be collected for *T. vaginalis* testing among women. There is, however, some evidence that endocervical

specimens are suitable. Endocervical specimens have been found to be 88 % sensitive and 99% specific for TV by PCR compared to 90% and 99% for vaginal swab (Van Der Pol *et al*., 2016). Huppert showed that endocervical specimens were 100% sensitive and 98% specific by TMA compared to 100% sensitivity and specificity for vaginal specimen using latent class analysis. Nuclear acid amplification test (NAAT) testing too soon after treatment can result in detection of remnant trichomonad DNA, thus producing false positives. By 2–3 weeks post treatment most remnant DNA has cleared, however, one study found a 15% false positive rate at 3 weeks and further examination (Williams *et al*., 2014).

Therefore, about four diagnostic methods available for the detection of *Trichomonas vaginalis* which is known to be responsible for the sexually transmitted parasitic disease, trichomoniasis. These methods are microscopic wet mount preparation, staining methods, culture in laboratory medium, and molecular methods. Historically, detection of the parasite is made possible by examining urine and high vaginal swab (HVS) in a drop of saline or trichomonas diluents for the characteristic tombling and rotating motion of the parasite. In 2013, Amadi and Nwagbo reported that making use of only urine sample or vaginal swab is not sufficient for proper diagnosis of *T. vaginalis* infection and suggested that for better results both urine and vaginal swab should be used. As reported by Fouts and Kraus (2013) the result for the diagnosis of trichomoniasis using wet mount demonstration and staining of the parasite in the laboratory ranges from 20 to 80%. An observation was made by Arora and Arora (2005) that the combination of cultural method with microscopic wet mount demonstration is the most acceptable methods for effective diagnosis.

New molecular diagnostic tests with improved sensitivity have been developed in response to the increasing recognition by stakeholders of the importance of this wide-

spread STI. Thus, thediagnostics quality for trichomoniasis has significantly been improved by the detection of *T. vaginalis*, including rapid antigen detection and nucleic acid amplification tests, particularly in women (Hobbs and Sena, 2013). In America, Guillermo *et al*. (2014) reported 97% sensitivity for molecular amplification diagnostic methods, as against 70 and 36% sensitivities for culture and wet preparations respectively. Also, studies of genetic typing of the parasites have been reported to be more sensitive than other methods (Vanacova *et al.*, 2017; Van Der Schee *et al*., 2019). However, in Nigeria there is no report of the application molecular diagnosis tools on *T. vaginalis* infection, which may influence the prevalence data as presently reported from less reliable tests.

Different *T. vaginalis* diagnostic tests are given for female vaginal swab and urinary samples. For males, diagnostic tests are restricted. However, for males, urinary sediments, urethral swab culture, wet mount and PCR are used. These diagnostic tests for males are ~60 % sensitive and highly specific except PCR test, which is >90 % sensitive and >90 % specific as shown in Table 2.3 (Gaydos *et al.*, 2017)

Table 2.3: *T. vaginalis* diagnostic tests

	Characteristics	Sensitivity	Specificity
Old diagnostic tests (Microscopic evaluation)			
1. Papanicolaou (Pap) smear	Direct visualization of motile trichomonads in saline preparation. Perform within 10–20 min of sample collections	50 %	90 %
2. Staining techniques	Can detect 3-trichomonads/ml Most common dyes are acridine orange, leishman stain, periodic acid-schiff stain, Fontana dye	30-60 %	Less specific
3. Wet mount	A physiological saline preparation of vaginal secretions Trichomonads can be identified by size (similar to WBC), shape and quivering/twitching motility Require ~104 trichomonads/ml vaginal fluid	50-60 %	>90 %
4. The agar plate technique	A timesaving culture technique with microscopic examination of the whole clinical material obtained, require a turnaround time of 2–6 days Eliminates the need for slide preparations. Favorable for screening a low-risk population	>90 %	>90 %
New diagnostic tests			
1. Broth culture (Gold standard)	Require ~300–500 trichomonads/ml to culture at 37 8C for 2–7 days Culture media types (Diamond's media for culture in glass tube; InPouch TV media, a double pouched soft transparent plastic container, Biomed Diagnostics, USA; Trichosel media)	~85-95 %	>95-100 %
2. Odour test	Whiff test (amine odor test) Perform by mixing vaginal secretions with 10 % potassium hydroxide (KOH), gives strong fishy odor	-	Poor specificity due to similar results with bacterial

				vaginosis
3.	**XenoStrip-Tv technology**	Antigen specific color immunochromatographic ‘‘dipstick’’ test (mouse antibody bound to a nitrocellulose membrane) Results can be obtained within 10 min	~66 %	100 %
Newest diagnostic test				
1.	**Affirm VPIII test**	Unamplified RNA test (Becton Dickinson, Sparks, Maryland, USA) Rapid expensive test, require a complete laboratory, 30–60 min to perform	<90 % (false +ve from dead organisms)	99 %
2.	**Rapid antigen test**	Point-of-care lateral flow test strip device (OSOM TV, Genzyme Diagnostic, Cambridge, MA, USA) Detect T. vaginalis membrane proteins within 10 min	83-90 %	99-100 %
3.	**Nucleic acid amplification test (NAAT)**	Use of analyte-specific reagents to analyze transcription-mediated amplification (TMA)	96–98 % (vaginal swab), 88 % (urine specimen)	>98 %
4.	**TV Polymerase chain reaction (PCR)-based test**	Provides a good alternative to culture for vaginal specimens Limited availability	64–89 %	97–100 %

(Source: Gaydos *et al.*, 2017)

2.9 Management and Treatment

2.9.1 Treatment with 5-nitronimidazoles

For nearly four decades, metronidazole (MTZ) has been the treatment of choice for *T. vaginalis* (TV). MTZ belongs to the 5-nitronimidazole drug family and is reported to have about a 95 % success rate in curing TV along with its related compounds such as tinidazole (TNZ) and seconidazole (Epling, 2017). The World Health Organization (WHO) and the United States Centers for Disease Control and Prevention (CDC) guidelines for treatment of TV include: MTZ or TNZ 2 gm single dose as the recommended regimens, and MTZ 400–500 mg BID 7 day dose as the alternative treatment regimen. Abstinence from alcohol use should continue for 24 h after

completion of MTZ or 72 h after completion of TNZ. If a patient fails single dose MTZ therapy they can be given single dose TNZ or 7 day dose MTZ. If this fails, 2 g MTZ or TNZ for 5 days can be administered. If this fails and there is no history of sexual re-exposure, a consultation for medication resistance testing should be done (Kissinger, 2015).

2.9.2 Treatment among pregnant and lactating women

Metronidazole is a class B drug and several meta-analyses have found it to be safe in pregnant women in all stages of pregnancy. Tinidazole has not been evaluated in pregnant women and remains a class C drug. Treatment with 2 g MTZ is recommended by CDC at any time during pregnancy (Workowski and Berman, 2011) whereas WHO does not recommend treatment in the first trimester unless it is indicated for prevention of untoward birth outcomes. Both entities suggest 2 g dose. In lactating women who are administered MTZ, withholding breastfeeding during treatment and for 12–24 h after the last dose will reduce the exposure of the infant to metronidazole. For women treated with TNZ, interruption of breastfeeding is recommended during treatment and for 3 days after the last dose.

2.9.3 Treatment of recalcitrant *T. vaginalis* or allergies to metronidazole/ nitronimidazole

Persistent TV is usually treated with multi-dose MTZ or tinidazole (TNZ). The most common reactions reported from metronidazole are urticaria and facial edema, while other adverse reactions include flushing, fever and anaphylactic shock from immediate-type hypersensitivity have been reported. De-sensitization can be done, but only has about a 42 % cure rate. If TV remains persistent or the patient is allergic to these medications, other intravaginal treatments have been studied or are under investigation

TV including: Acetarsol, Boric acid Muzny *et al.* (2012), Furazolidone and Paromomycin (Nyirjesy *et al*., 2011). Nitrazoxanide was examined as an alternative oral agent for MTZ-resistant TV but was not found to be very effective. Several combination therapies including TNZ plus ampicillin and multi-dose nitazoxanide (NTZ) are also available. Some plant extracts have shown anti-TV activity, but these have not yet been tested in clinical trials (Vieira *et al*., 2015).

2.9.4 Treatment among HIV-infected women

In a randomized clinical trial (RCT) among HIV infected women with TV, multi-dose MTZ was found to be superior to single dose treatment (Kissinger and Hogben, 2011). Further analysis revealed that the superiority is only in the presence of bacterial vaginosis (BV) (Gatski *et al*., 2011). Studies have also found that antiretroviral therapy may interfere with the efficacy of MTZ among HIV-infected women (Adamski *et al*., 2014). It has been estimated that if CDC recommendation for *T. vaginalis* screening and treatment among HIV+ women is followed, that the lifetime cost of new HIV infections prevented would approximate US $159,264,000 via new HIV cases of secondary to female-to-male transmissions prevented (Lazenby *et al.*, 2014).

2.9.5 Repeat/ persistent infections

Repeat infections are common, ranging from 5–31 % Kissinger *et al*. (2018), and share similar sequelae to primary infections. While it is clear that the *T. vaginalis* repeat infection rate is unacceptably high, the source of these repeat infections is less clear. Possible sources of retest positives after treatment include; re-infection from an untreated/infected baseline partner, infection from a new partner, and treatment failure. Each of these sources of retest positives requires a different approach to prevent ongoing infection. For example, if the cause is reinfection, then assuring the original partners are

treated (i.e. expedited partner treatment or EPT) is needed. If the source is a new partner or treatment failure, then rescreening is needed.

There have only been a few randomized trials with good follow-up that have compared single dose MTZ to multi-dose. In these trials, cure rates for single vs. multidose MTZ have been shown to be similar (82–88 % vs. 92–94 %). Both studies found that the single dose had higher rates of side effects (notably nausea and vomiting) (Krashin *et al*., 2011; Kissinger *et al*., 2012).

Both studies found that the single dose had higher rates of side effects (notably nausea and vomiting). One study that examined the origins of repeat infectionfound treatment failure to be the most common cause (Kissinger *et al*., 2018). Potential causes of early repeat *T Vaginalis* infections include; drug resistance, non-adherence to treatment, clinical treatment failure, re-infection from an untreated partner. One study that examined the origins of repeat infection found treatment failure to be the most common cause. Potential causes of early repeat *T. vaginalis* infections include; drug resistance, non-adherence to treatment, clinical treatment failure, re-infection from an untreated partner.

Single dose therapy has removed adherence as an issue and *in vitro* resistance testing has consistently demonstrated low rates of resistance. Reported rates of MTZ resistance among mostly non-HIV infected women range from 2.2–9.6 % and were usually resolved with repeat MTZ treatment at the same or higher dosage. The most likely sources of repeat infections, therefore, are clinical treatment failure or re-infection from an untreated partner.

In one study of HIV+ and HIV- women, a large proportion of the repeat infections were attributed to treatment failure (i.e. no sexual exposure and no drug resistance). Resistance

appears to play only a minor role in explaining probable treatment failure. TV infected women who were given single dose MTZ and provided with medication to deliver to their sex partner(s), repeat infections rates were high (8 %) and nearly all (92 %) were attributed to clinical treatment failure(Kissinger *et al*., 2018). Repeat TV infections among HIV positive women are substantially higher with rates between 18.3 and 36.9 % Kissinger *et al*. (2018) and since these studies used culture, the true rate may be even higher. The molecular mechanism(s) of clinical resistance are poorly understood.

Sex partner treatment Sex partners of patients with TV should be treated. Commonly, patients are told by their providers to tell their partners to seek testing and treatment. This can be problematic because sensitive tests for men are not readily available. Providers may consider treating partners of positive patient presumptively. One method of presumptive partner treatment is called expedited partner therapy (EPT). EPT is the clinical practice of treating the sex partners of patients diagnosed with an STI by providing prescriptions or medications to the patient to take to his/her partner without the health care provider first examining the partner (Krieger, 2015).

One RCT demonstrated that partner treatment with 2 g TNZ resulted in a > 4 fold reduction in repeat infections among index women. Two other studies using 2 g MTZ for male partners of TV infected women found no effect of EPT (Kissinger *et al*., 2016) or a borderline effect(Schwebke and Desmond, 2014). While it is possible that the two studies that used MTZ were either underpowered or did not use the correct control arm, it is also possible, that TNZ is a better treatment for men.

2.10 Reproduction and Life Cycle

Although cell division has been extensively described through the use of microscopy, the life cycle of *T. vaginalis* is still poorly understood. Like many other protozoan parasites,

it is known to exist only as a trophozoite and lacks a cystic stage (Beri *et al*., 2020). Several oversized round forms of the trichomonads are known to exist in dividing, growth phase culture: those without flagella, those with flagella and a dividing nucleus, and those with flagella and multiple nuclei. It was thought that these forms are not stages in the life cycle but, rather, that they arise during certain unfavorable conditions (Beri *et al*., 2020). However, recent evidence suggests that they may be developmental stages preceding the appearance of mononuclear flagellates (Dias-Lopes *et al*., 2017). The round forms are morphologically different from the smaller, flagellated ovoid forms (Dias-Lopes *et al*., 2017). Furthermore, they appear to divide by amitotic budding rather than by the mitotic division of ovoid cells (Dias-Lopes *et al*., 2017). It is not certain how these round forms fit into the development of the organism (Dias-Lopes *et al*., 2017).

The small, ovoid flagellates generally reproduce by longitudinal binary fission, without the disappearance of the nuclear membrane (Coceres *et al*., 2021). According to Coceres *et al*. (2021), this event begins with the duplication of selected locomotor organelles, which is followed by the development of two attractophores flanking either side of the nucleus, which become the poles for division. From the attractophores develop chromosomal microtubules, which extend towards and into the nucleus, attaching to the centromeres of the chromosomes. Also extended between the attractophores is an extranuclear spindle, called the paradesmose. This extranuclear spindle elongates, and the daughter cells separate. Each daughter cell then produces any missing organelles.

2.11 Metabolism

T. vaginalis is a primitive eukaryotic organism. Although it is similar in many respects to other eukaryotes, it differs in its energy metabolism and shows remarkable similarity to primitive anaerobic bacteria. The hydrogenosomes Martin *et al*. (2020) are analogous to

the mitochondria of more advanced eukaryotes and carry out many of the same metabolic functions. The biochemistry and metabolism are well summarized by Muller (Westrop *et al*., 2017).

2.11.1 Carbohydrate and energy metabolism

Being one of the most ancient eukaryotes, *T. vaginalis* has features that are common to anaerobic bacteria as well as higher eukaryotic organisms in terms of its carbohydrate and energy metabolism. Carbohydrate metabolism is described as being fermentative under both anaerobic and aerobic conditions because glucose is incompletely oxidized (Hackstein *et al*., 2019). The metabolic products include acetate, lactate, malate, glycerol, CO_2, and, under anaerobic conditions, H_2 (Westrop *et al*., 2017). Carbohydrate metabolism occurs in two compartments: the cytoplasm and an organelle called the hydrogenosome, which is analogous to the mitochondria of higher eukaryotes Martin *et al*. (2020) and is found in a number of anaerobic parasitic protozoa. Within the cytoplasm, glucose is converted to phosphoenolpyruvate and subsequently to pyruvate via a classical Embden-Meyerhoff-Parnas pathway (Martin *et al*., 2020). Many of the enzymes in the pathway have been described, and several steps produce energy via substrate-level phosphorylation. Glycerol is produced from dihydroxyacetone phosphate by glycerol-3-phosphate dehydrogenase and glycerol-3-phosphatase (Westrop *et al*., 2017). Lactate is also produced in the cytosol via the reduction of pyruvate by lactate dehydrogenase (Hackstein *et al.*, 2019). Pyruvate, which is generated through glycolysis, is then metabolized further in the hydrogenosome (Westrop *et al*., 2017).

The hydrogenosome, like the mitochondrion, is 0.5 to 1.0 mm in diameter and is surrounded by a double membrane de Souza and Attias (2018). The hydrogenosomes are the site of fermentative oxidation of pyruvate Duarte and Huynen (2019), and they

produce ATP by substrate-level phosphorylation, produce hydrogen, process half of the carbohydrates of the cell, and contain homologous enzymes common to those found in both prokaryotes and eukaryotes (Westrop *et al*., 2017). Hydrogenosomes lack cristae and cytochromes, which are typically found in mitochondria. Furthermore, DNA is not present in the hydrogenosomes (Martin *et al*., 2020).

Biochemical studies of the hydrogenosome have revealed both similarities to and differences from mitochondria. Pyruvate: ferredoxin oxidoreductase, an enzyme not found in mitochondria, converts pyruvate to acetate Rada and Tachezy (2019). In this regard, *T. vaginalis* metabolism in the hydrogenosome is more closely related to that of the anaerobic bacteria Duarte and Huynen (2019). However, analysis of the ferredoxin protein in *T. vaginalis* shows it to be comparable to the [2Fe-2S] ferredoxins found in aerobic bacteria and in mitochondria (Braymer *et al.*, 2021) rather than in anaerobic bacteria. Another feature shared with mitochondria is the beta-succinyl coenzyme A synthetase enzyme, which catalyzes ATP production by substrate-level phosphorylation (Dolezal *et al*., 2019).

Using sequence alignments of the 18S-like rRNA of various eukaryotes, proposed that trichomonads branched off from the main line of the eukaryotic tree before true mitochondria arose. In support of this theory, Martin *et al*. (2020) have found a gene encoding a mitochondrial type of 70-kDa heat shock protein in *T. vaginalis* that has sequences which to date have been found only in mitochondrion HSP70 and proteobacterial DnaK. A third hypothesis suggests that hydrogenosomes originated through the endosymbiosis of an anaerobic bacterium and a primitive eukaryotic cell. The similarities between the hydrogenosome and the anaerobic bacterium, in terms of anaerobic metabolism, support this theory.

Johnson and coworkers have done extensive research on the hydrogenosome and have characterized some of the genes and their respective products associated with this organelle, which include ferredoxin (Braymer *et al*., 2021) and beta-succinyl coenzyme A synthetase (Dolezal *et al*., 2019). As in mitochondria, *T. vaginalis* hydrogenosomal proteins have highly conserved amino-terminal leader sequences, which are cleaved upon entering the hydrogenosome. In the genes studied to date, these short leader sequences between 8 and 11 amino acids show a high degree of similarity to the longer leader sequences (20 to 80 amino acids) found in mitochondria.

The leader sequences of the hydrogenosomal proteins are believed to be important in the targeting of specific proteins to the hydrogenosome (Dolezal *et al*., 2019). Although the hydrogenosomes are characteristic of *T. vaginalis*, their functions may not be essential for the parasite, since trichomonads without the hydrogenosomes can be cultured in vitro (Westrop *et al*., 2017). Prasad *et al*. (2020) reported that Ter Kuile and Muller have studied maltose utilization and transport. In most eubacteria and yeasts, maltose is transported into the cell and then hydrolyzed to glucose (Koller, 2019). These investigators have demonstrated that as in the intestinal epithelial cells of vertebrates, maltose is cleaved on the cell surface of the parasite to glucose via alpha glucosidase. Glucose is subsequently transported by the glucose transporter into the cytosol, where it is metabolized. This process, whereby the carbohydrate is metabolized on the membrane, does not seem to be advantageous for a unicellular organism, since glucose has been found to diffuse away from the parasite under culture conditions (Prasad *et al*., 2020).

It was reported by Macdonald (2021) chemostats have been used to study prokaryotes, but little work has been done with eukaryotic organisms. The use of chemostats has an advantage over the use of batch culture for growing organisms, since investigators can create conditions that closely approximate what occurs in vivo. Studies of *T. vaginalis*

carbohydrate metabolism show that although the parasite is not exceedingly energy efficient, it is able to adapt its metabolism according to available carbon sources (Macdonald, 2021). *T. vaginalis* has high maintenance energy, expending up to half its carbon flow on maintaining internal homeostasis (Macdonald, 2021). Maintaining homeostasis is crucial for *T. vaginalis* since the vaginal environment is constantly changing with regard to pH, hormones, menses, and the nutrient supply.

2.11.2 Lipid Metabolism

T. vaginalis contains cholesterol, phosphatidylethanolamine, phosphatidylcholine, and sphingomyelin as its major phospholipids (Wilson and Knoll, 2018; Panevska *et al*., 2019). Lipid precursors fail to incorporate themselves into phospholipids in *T. vaginalis*, showing that the parasite is unable to synthesize fatty acids and sterols (Wilson and Knoll, 2018). Although lacking in the metabolic pathways necessary for phospholipid and fatty acid synthesis, *T. vaginalis* hydrolyzes the fatty acyl groups of phospholipids, triacylglycerols, and cholesterols and use these groups in the acylation of phospholipids (Wilson and Knoll, 2018; Panevska *et al*., 2019). Glycerolphospholipids are incorporated into most phospholipids; further suggesting that turnover of lipids in the membrane of the cell does occur (Wilson and Knoll, 2018). Nevertheless, many enzymes involved in the biosynthesis of complex phospholipids are lacking in *T. vaginalis*, and therefore the parasite must rely on exogenous sources of lipid moieties to survive (Wilson and Knoll, 2018; Panevska *et al*., 2019).

It was postulated that de novo glycolipid and glycophosphingolipid synthesis occurs in trichomonads. Glycoconjugates found on the surface of the parasite are important in its survival within the host microenvironment (Ayona *et al*., 2020). A glycosphingolipid called TV1 has been identified and found to contain inositol phosphoceramide. This

appears to be a novel phospholipid since inositol phosphoceramide is normally substituted with ethanolamine. TV1 may be an intermediate in the generation of membrane anchor proteins in *T. vaginalis* (Martin *et al.*, 2017). Studies have shown that lipophosphoglycan-like glycoconjugates are also present on the cell surface. The role of these lipid moieties is not clear, although they may be involved in the survival of the parasite (Ayona *et al*., 2020). Further study is required to determine the exact role that the phospholipids play in the host-parasite interrelationship.

2.11.3 Amino Acid Metabolism

Investigators have used high-pressure liquid chromatography to determine the amino acids present in the trichomonad cell (Friedman *et al.,* 2021). Carbohydrates are the preferred source of nutrients for *T. vaginalis*; however, under conditions where carbohydrates are limiting, amino acids have been shown to sustain trichomonad growth and survival (Friedman *et al.,* 2021). When grown in the absence of maltose, *T. vaginalis* consumes greater amounts of amino acids, especially arginine, threonine, and leucine which are used in energy generation (Friedman *et al.,* 2021). Under normal culture conditions, *T. vaginalis* consumes large amounts of arginine and somewhat smaller amounts of methionine for use in energy production (Friedman *et al.,* 2021).

Alanine and more recently, glycine have been found to be end products in the metabolism of glucose (Vermathen *et al*., 2018; Friedman *et al.*, 2021). Alanine and leucine constitute the major amino acids within the trichomonad cell (Friedman *et al.,* 2021). Valine, glutamate, phenylalanine, glycine, and proline levels are also elevated under standard conditions (Vermathen *et al*., 2018). In *T. vaginalis*, the intracellular and extracellular concentrations of amino acids are quite similar, implying that some form of

equilibrium is being maintained between the free amino acids within the cell and its external environment (Vermathen *et al*., 2018).

Aminotransferases have not been extensively studied, but they have been shown to play a role in amino acid metabolism (Westrop *et al.*, 2017). Rowe and Lowe have purified an enzyme exhibiting dual activity: aspartate/aromatic amino acid: 2-oxoglutarate aminotransferase (Verdaguer *et al*., 2019). They have also found high activities of branched-chain and aromatic aminotransferases which explains why these amino acids are found predominantly with *T. vaginalis* (Verdaguer *et al*., 2019).

2.11.4 Nucleotide Metabolism

Trichomonas vaginalis lacks the ability to synthesize purines and pyrimidines and hence must resort to salvage pathways to generate nucleotides (Smith *et al*., 2017). Purine salvage is mediated by nucleoside phosphorylases and kinases (Smith *et al*., 2017), whereas phosphoribosyl transferases and nucleoside kinases are able to recover pyrimidines. *T. vaginalis* requires adenine and guanine or their nucleosides for growth, in addition to thymidine, cytidine, uracil, and/or uridine (Smith *et al*., 2017). It is known that in cultured and animal cells, nucleosides enter the cells by passive diffusion through a single carrier. However, other researchers have postulated that *T. vaginalis* may in fact contain two separate carriers for nucleotide transport. One carrier is believed to accommodate adenosine and pyrimidine nucleosides, while the second shows a high affinity for uridine and a separate site for guanosine transport. The presence of two nucleoside carriers has also been found in another parasitic protist, *Leishmania donovani* (Kelly *et al*., 2021).

2.12 Nutrition, Growth Requirements and Cultivation

Studies on the nutritional requirements of *T. vaginalis* came with the advent of the axenic culture technique. This organism evolved to survive in conjunction with host cells and various other members of the microbiological flora, and thus it was initially difficult to cultivate it independent of bacteria. Originally, an axenic culture was obtained by allowing the trichomonads to migrate down a tube, so that they swam free of other nonmotile organisms. Today, axenic culture is obtained through the use of antibiotics (Das *et al*., 2018). The contributions reported by Das *et al*. (2018) to the development of media for trichomonads has enabled studies of the nutritional requirements of *T. vaginalis*.

T. vaginalis is an obligate parasite in that it lacks the ability to synthesize many macromolecules de novo, particularly purines, pyrimidines, and many lipids. These nutrients are acquired from the vaginal secretions or through phagocytosis of host and bacterial cells (Lawrence, 2020). Culture media for *T. vaginalis* are thus required 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. Iron is also required, to maintain maximal levels of ferredoxin and pyruvate-ferredoxin oxidoreductase activity.

Diamond's TYM medium consists primarily of Trypticase, yeast, and maltose. The most popular medium for *T. vaginalis* currently is Diamond's TYI-S-33 medium, which is a nutrient broth of Trypticase, yeast extract, and iron, with the addition of fetal bovine serum and a vitamin 107-Tween 80 mixture. Semi-defined media and chemically defined media have been outlined by Linstead (Diamond, 2019). In 1981, Linstead reasoned that since *T. vaginalis* is so intimately associated with its host cells, a modified cell culture medium might be suitable. Linstead's semi-defined medium was developed from

modified CMRL 1066 (Gibco) containing Tween 80. Often, however, the use of serum is undesirable because the proteins within it can interfere with enzymatic studies and can bind directly to the trichomonad. Linstead (Diamond, 2019) therefore developed chemically defined media, DL7 and DL8, in which fetal calf serum was replaced with bovine serum albumin, cholesterol, and a mixture of fatty acids. Also supplied were 8 salts, various amino acids, and nucleic acid precursors, 7 carbohydrates, and 16 vitamins. also developed a serum-free cell culture system using a McCoy cell monolayer. The presence of eukaryotic cells is necessary for cultures without serum. In vitro, *T. vaginalis* grows optimally at a pH of 6.0 to 6.3 Das *et al*. (2018), although it does grow through a wide range of pH, especially in the changing environment of the vagina.

2.13 Disability Adjusted Life Years (DALYs) and Infection Burden

Evaluation of the disease burden is essential in determining the cost-effectiveness of control so as to ensure that control programs are focused appropriately. Disability adjusted life years (DALYs) is the trending metric measurement to assess disease burden (Brooker, 2010). Disability adjusted life year include years of life lost due to mortality (YLLs) and years lived with disability (YLDs). Most *T. vaginalis* survey is mainly on women, but reports of infection burden measured in DALYs are unavailable. Bartsch *et al.,* (2016) estimated the value of YLD based on available prevalence data and morbidity of the infection. It was reported that the YLD from *T. vaginalis* prevalence data in Nigeria ranged from 21 to 63; with the highest and least disease burden recorded in South-South and North-West respectively as shown in Table 2.4.

Table 2.4: *T. vaginalis* mean prevalence and estimated YLD according to geopolitical zones (1984-2017)

Region	Mean ± SD (%)	YLD /10 000
South-East	16.12 ± 9.9	44
South-South	23.3 ± 16.3	63
South-West	17.3 ± 20.2	47
North-West	7.6 ± 7.3	21
North-East	11.4 ± 9.0	31
North-Central	12.5 ± 12.4	34

Source: (Asemota, 2018)

2.14 Vaccination and Immunity to *T. vaginalis*

Recently, Abraham *et al*. was able to induce immunity to *T. vaginalis* in the mouse model, which may lead to the development of a vaccine. Immunity has been difficult to produce in vivo, since in humans, repeated infections with *T. vaginalis* do not confer immune protection (Volpedo *et al*., 2021). Despite this, antibodies can be found in the serum Nemati, *et al*. (2018) and vaginal secretions Nemati *et al*. (2018) of infected individuals and a cell-mediated immune response is also invoked.

To date, only one vaccine had been produced against *T. vaginalis*. The Solco-Trichovac vaccine was prepared from inactive lactobacilli and was thought to work by inducing antibodies to abnormal lactobacilli and *T. vaginalis* without adversely affecting the growth of normal lactobacilli in the vagina. However, a lack of antigenic similarity between this vaccine and *T. vaginalis* was shown, which makes this cross-reaction

hypothesis unlikely. Clinical trials of Solco-Trichovac have yielded inconclusive data (Woelber *et al*., 2020).

In the study as reported by Zhang and Begg (2014), whole, live trichomonads at different concentrations were injected subcutaneously into mice, first with Freund's complete adjuvant and then in a booster dose with the trichomonads and Freund's incomplete adjuvant. The mice were given estrogen and inoculated intravaginally with *Lactobacillus acidophilus* to simulate the conditions in the human vagina (Shazadi *et al*., 2021), they were then inoculated intravaginally with *T. vaginalis*. Immunized mice had significantly less intravaginal infection and had elevated antibody levels in the serum and vagina compared with the shaminoculated and naive control groups. Mice that had been infected vaginally, treated, and reinfected vaginally were not protected and did not mount an immune response. This suggests that antigen presentation may be crucial for developing protective immunity. This work provides new hope for the goal of vaccine development, and further studies are warranted.

2.15 Microbiome and *T. vaginalis*

There has been recent evidence that TV infection changes or is changed by the microbiome of women (Hirt and Sherrard, 2015) and TV treatment is altered by the microbiome (Kissinger *et al.,* 2011). One possible factor in the treatment failure of TV is vaginal flora disturbances. Bacterial vaginosis (BV) is a common vaginal condition in women of childbearing age. The prevalence of BV in the US varies from 29 % in a nationally representative sample (where the prevalence was 3.1 times greater for African-American women compared to whites), 44 % in a group of women at high-risk for HIV, and 56 % among injection drug users.

Like TV, BV can also increase a woman's susceptibility to HIV infection. Several studies have shown a strong association between TV and BV, meaning that the two frequently occur as co-infections among women. While these two vaginal infections have similar symptom and are treated with similar medication, the dosing is not the same. TV has been found to occur more often in the presence of women with a newly identified species of *Mycoplamsa* called Mnola or *Candidatus Mycoplasma girerdii* (Fettweis *et al.,* 2012). Brotman *et al.* (2012) found that TV was associated with vaginal microbiota consisting of low proportions of lactobacilli and high proportions of Mycoplasma, Parvimonas, Sneathia, and other anaerobes.

In a screening study of HIV-positive women, the prevalence of TV was higher among women who had altered vaginal flora and that the majority (61.0%) of HIV+/TV+ women also had BV. This high rate of BV that accompanies TV infection among HIV+ women has implications for treatment decisions since multi-dose MTZ is recommended for BV. Martin *et al*. found that TV prevalence was highest in the women with intermediate Nugent scores confirming the observations of Gatski *et al*., (2011). A heat map analysis of pyrosequencing data showed that the vaginal flora of 18/30 TV+ women had a similar unique microbiota characterized by high abundance of *Mycoplasma* sp. or *Ureaplasma* sp. and relatively low abundance of *Lactobaccilus sp*. and *Gardnerella* sp. Martin *et al.* (2013), reported that *T. vaginalis* directly influences microbial environment and confirms the potential importance of interactions between *T. vaginalis* and vaginal microbiota.

2.16 Prevention and Control of Trichomoniasis

2.16.1 Prevention of trichomoniasis

Trichomoniasis can be prevented through the following: use of condoms, get tested routinely for trichomoniasis, get treated if you have trichomoniasis or other STDs, tell

your sexual partner if you have trichomoniasis so they can get tested and treated and engage in monogamous relationship.

2.16.2 Control of trichomoniasis

Control and elimination may depend largely on extensive proper sex education, especially for the adolescent and youths (Amadi and Nwagbo, 2013; Hamafyelto and Ikeh, 2017). Also suggested are adequate treatment of both spouses, implementation of effective screening programmes and treatment at no cost (Hamafyelto and Ikeh, 2017). For improved health, adequate personal hygiene, avoidance of promiscuity, improved education of women on safe sex and the need to know partners' STI status are advocated (Iwueze *et al*., 2014; Oyetunde and Chelsea, 2016). It is recommended that routine STIs screening in sexually active patients especially among the young and singles should be incorporated into hospital care (Okonko *et al*., 2012). This is needed to prevent transmission of the parasite, because some infected women and most infected men show no signs of the disease like liquid discharge from the vagina or penis, irritation while urinating and genital itching. So this YLD information is vital to focusing efforts on getting women tested and treated. Based on the results from this study, trichomoniasis control in Nigeria should especially be of high priority in the South-South.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Bida is a Local Government Area as well as a city (the second largest) with a latitude and longitude coordinates of 9.083333, 6.016667 in Niger state. Bida which is densely populated with an estimate of 178,840, majority are the Nupe people and has a landmass of 51km square. Bida is located in the southern axis of Niger State. At the southwest of Minna (Capital of Niger State), is the location of Bida which is said to be an arid, dry town. Kutigi, Enagi, Badegi, Patigi, Lemu are few of the district in Bida. Some of the areas in Bida are Banma, G.R.A., Esso, Ifengi, Fogun and old Market to mention but a few. Few of the schools in Bida are Eyagi Day Secondary School, Government Day Secondary School, Edusoko University, Gibril Memorial International School and Federal Staff School. Local crafts have been produced in Bida for edges. The Durbar and Nupe Day Festivals are known in Bida. The head quarter of Nupe kingdom is known to be Bida, which is led by an emir the people address as Etsu Nupe.

3.2 Study Design

This was a cross sectional case-control study carried out among male and female adults visiting Umaru Sanda Ndayako General Hospital Bida, Niger State, Nigeria.

3.3 Study Population

The study population comprised five hundred (500) patients (250 males and 250 females) who attended Umaru Sanda Ndayako General Hospital during the course of the study after receiving informed consent from the patients. Only those that volunteered to take part in the study were included, patients aged 18 years and above were recruited for the study while those that refused to give their consent or aged below 18 years were

excluded. Questionnaire was administered to those who gave their consent. The designed questionaire is shown in Appendix A.

3.4 Ethical Clearance

Approval to carry out this research was obtained from the Research and Ethics Committee of the Umaru Sanda Ndayako General Hospital, Bida using the participants who volunteered.

3.5 Sample Size Determination

The under 18 years and those who just undergo MTZ/TNZ treatment for the past one week were excluded. The sample size was determined using equation 3.1.

$$N=Z^2P\ (1-P)/d^2 \qquad \text{(equation 3.1)}$$

where N=Sample size,

Z=Critical value at 95% confidence level,

P= an expected prevalence and

d=is precision or margin of error (5%).

Therefore, 500 sample sizes were collected for the study.

3.6 Sample Collection

The 250 samples of High Vaginal Swabs (HVS) were collected by the lab scientist using sterile cotton swaps sticks from the females while the 250 early morning urine samples were collected by the men (patients) themselves inside a sterile universal sample bottles. Samples were immediately transported to microbiology laboratory of Umaru Sanda Ndayako General Hospital Bida, for analysis within an hour of collection to avoid loss of jacking /tumbling motility of the organism and death that may follow due to the nature of the parasite.

3.7 Sample Transportation/Handling

The samples were immediately kept in a transport media (Stuarts) at 25^{o}C to prevent drying and loss of the parasite. The samples remain in the transport media for about 1 to 2 hours, this is because of the time it took for the samples to be collected and transported to the lab where the samples were processed immediately on getting to the laboratory.

3.8 Laboratory Analysis

3.8.1 Macroscopic identification

The macroscopic examination was done by checking for the yellowish-green colour, frothy odour and using paper strip to determine the pH level of the specimen.

3.8.2 Wet mount preparation

This was done by dropping a drop of 0.85% normal saline on a clean grease-free slide and the swab stick was rolled in the normal saline on the slide to make a smear. It was covered with a cover slip and then viewed under the microscope using x10 and x40 objective lens.

3.8.3 Microscopic identification of the parasite

The trophozoites of *T.vaginalis* which is about 8 to 15µm long appeared ovoid, round or pear like in shape. Rapid, jerky motility is accomplished with the aid of organism's four flagella, all of which originate from the anterior end. Only one extends posterrioly (Swygard *et al*., 2014).

3.9 Data Analysis

Percentages and Chi-square (χ^2) test were used in order to understand and interpret the test results perfectly. The percentages were used in order to compare the variables in a descriptive manner while the chi square (χ^2) test, for statistical comparism which is to

determine the significant relationship between variables at a significant P-value of less than or equal to 0.05(≤0.05). The SPSS software version 25.0 was used for data analysis and frequency Tables were generated.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Identification of *T. vaginalis*

Trichomonas vaginalis was identified from the infected individual based on the microscopic analysis of the samples (HVS and urine). The samples revealed the presence of trophozoites of *T. vaginalis* which is about 8 to 15 µm long appeared ovoid, round or pear like in shape. Rapid, jerky motility is accomplished with the aid of organism's four flagella, all of which originate from the anterior end. Only one extends posteriorly (Plate I).

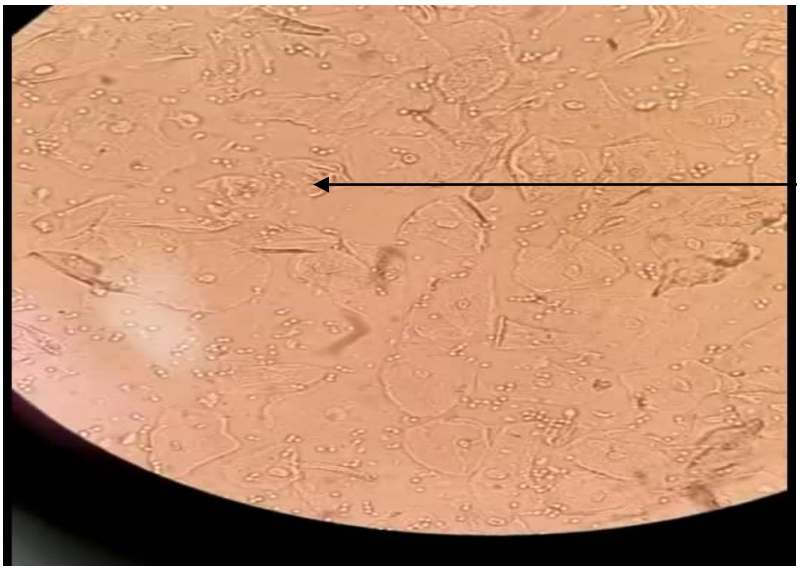

Plate I: ***Trichomonas vaginalis***

4.1.2 Prevalence of *T. vaginalis* among patients attending Umaru Sanda Ndayako General Hospital Bida

Out of 500 samples comprising 250 men and 250 women screened for *T. vaginalis* infection, 9.4% turned out to be infected. Females had a higher prevalence of 13.2% compared to males 5.6% as shown in the Table 4.1.

Table 4.1: Prevalence of *T. vaginalis* among patients attending the hospital

Gender	Number examined	Number infected (%)	Number uninfected (%)
Male	250	14(5.6)	236(94.4)
Female	250	33(13.2)	217(86.1)
Total	500	47(9.4)	453(90.6)

$\chi^2 = 8.478$, $p = 0.004$

4.1.3 Prevalence of *T. vaginalis* based on marital status

The highest prevalence 14.5% was obtained among the married participants, followed by single 11.1%, widowed 4.0% and the least was obtained among divorced participants 2.4% as shown in the Table 4.2.

Table 4.2: Prevalence of *T. vaginalis* based on Marital Status

Marital status	Number examined (%)	Number infected (%)	Number uninfected (%)
Single	144	16(11.1)	128 (88.9)
Married	173	25 (14.5)	148 (85.5)
Divorced	84	2 (2.4)	82 (97.6)
Widowed	99	4 (4.0)	95 (96.0)
Total	500	47 (9.4)	453 (90.6)

$\chi^2 = 13.876$, $p = 0.003$

4.1.4 Prevalence of *T. vaginalis* in accordance to age group

The highest prevalence of Trichomoniasis was recorded among patients aged 40-50 years with 18.2%, followed by 29-39 years 11.0%, then 18-28 years 7.5% while the least prevalence was recorded among patients aged 51-61 years 0.0% and above 61 years 0.0% as shown in Table 4.3.

Table 4.3: Distribution of *T. vaginalis* infection based on age group

Age range (Years)	Number Examined	Number Infected (%)	Number uninfected (%)
18-28	253	19 (7.5)	234 (92.5)
29-39	219	24 (11.0)	195 (89.0)
40-50	22	4 (18.2)	18 (81.8)
51-61	5	0 (0.0)	5 (100.0)
>61	1	0 (0.0)	1 (100.0)
Total	500	47 (9.4)	453(90.6)

$\chi^2 = 4.235$, p =0.237

4.1.5 Prevalence of *T. vaginalis* based on hydrogen ion concentration (pH)

Based on hydrogen ion concentration, the highest pH level (7.5) was recorded for the age group 40-50 years, followed by the age groups 18-28 and 29-39 (7.0) and the least was among the age groups 51-60 and above 61 as shown in Table 4.4

Table 4.4: Prevalence of *T. vaginalis* Based on Hydrogen Ion Concentration (pH)

Age Range (years)	Number examined	Number infected (%)	pH level
18-28	253	7.5	7.0
29-39	219	11.0	7.0
40-50	22	18.2	7.5
51-61	5	0.0	6.0
>61	1	0.0	6.0

4.1.6 Prevalence of *T. vaginalis* based on level of education of participants

The highest prevalence was recorded among participant that had no formal education 11.21%, followed by those that attended tertiary level of education 10.5%, those with secondary education 8.6% while the least prevalence was recorded among those that had primary education 5.8% as shown in the Table 4.5

Table 4.5: Prevalence of *T. vaginalis* based on level of education

Educational level	Number examined	Number infected (%)	Number uninfected (%)
None	107	12(11.2)	95(88.8)
Primary	69	4(5.8)	65(92.8)
Secondary	162	14 (8.6)	148 (91.4)
Tertiary	162	17(10.5)	145(89.5)
Total	500	47 (9.6)	453 (90.4)

$\chi^2 = 1.802$, $p = 0.614$

4.1.7 Prevalence of *T. vaginalis* based on occupation of participants

Based on occupation, highest *T. vaginalis* infection prevalence was obtained among the unclassified (not placed in the category of students, civil servant or private workers) 12.4%, followed by the public workers (civil servants) 11.3%, private workers 5.8% while the least was recorded among Students 3.3 % as shown in the Table 4.6.

Table 4.6: Prevalence of *T. vaginalis* based on occupation

Occupation	Number Examined	Number Infected (%)	Number uninfected (%)
Private workers	120	7(5.8)	113(94.2)
Civil servants	80	9 (11.3)	71(88.8)
Students	91	3 (3.3)	88 (96.7)
Unclassified	209	26 (12.4)	183(87.6)
Total	**500**	**47 (9.4%)**	**453(90.6)**

$\chi^2 = 4.456$, $p = 0.348$

The sociodemographic factor associated with *T.vaginalis* infection in the study area are gender (0.004) and marital status (0.003) however, age (0.237), occupation(0.348) and educational status(0.614) have no significant association with *T. vaginalis* infection.

4.2 Discussion

4.2.1 Overall prevalence of *T. vaginalis* among patients attending Umaru Sanda Ndayako General Hospital Bida

Prevalence of trichomoniasis changes, so also the nature of the organism and lack of the patients to respond to screening or admit to the presence of the symptoms may be due to one reason or the other. This singular attitude greatly promotes transmission of infection as many are undiagnosed (Sharma *et al*., 2014). Isolation and identification of *T. vaginalis* was done using single technique which was the microscopic analysis of both urine and high vaginal swabs collected from the patients which is contrary to the technique used by Van Gerwen and Muzny (2019) in Canada who used double technique

namely, microscopy and broth culture. This is due to the high sample population pop used, since the parasite was identified there was no need for additional technique, the HVS and urine samples used have a high specificity and sensitivity as compared to blood sample, The prevalence of *T. vaginalis* among patients attending Umaru Sanda Ndayako General Hospital during the study was 47(9.4%) out of 500 samples obtained. This is lower than the prevalence of 81(40.5%) reported by Ogomaka *et al*. (2018) in Oru-East LGA Imo State, 12% reported by Gberikon *et al*. (2015) in Benue, 15 % reported by Raimi and Ochayi, (2017) in FCT, Abuja and 15.71 reported by Alaku *et al*. (2014) in Nasarawa. However, other studies reported a lower prevalence of 1.3% in Imo state (Acholonu, 2018) and 2.6% in Adamawa (Abdulazeez *et al*., 2017). Variation in prevalence could be attributed to differences in study area, population type and diagnostic method. High prevalence of trichomoniasis reported in the present study could be attributed to sexual activities like multiple sex partners usually practiced in the study area and low level of awareness on the prevalence of the infection.

The prevalence of this study is contrary to that of Woken (2006) in some parts of Niger Delta Region of River state, as it is higher. The investigator stated that "the high prevalence rate of trichomoniasis might be due to little or lack of attention for trichomononiasis of public health importance". Also, even though trichomoniasis has been reported to be the most common sexually parasitic infection that is transmissible, it is yet to be given importance in the public health sector.

4.2.2 Prevalence of *T. vaginalis T. vaginalis* based on gender of patients

Gender is a significant factor ($p = 0.004$) that influences the distribution of trichomoniasis. Prevalence of infection among females 33(13.2 %) was higher than in males 14(5.6 %) and this is in consistent with the findings of Ogomaka *et al*. (2018) that

reported a higher prevalence of infection among females than in males. Both men and women can be infected with *T. vaginalis*, though trichomoniasis in men tends to be less prevalent and of shorter duration (Schwebke and Hook, 2013). Higher prevalence of infection recorded among females may be due to the fact that wet mount microscopy is usually attributed to low detection rate of *T. vaginalis* in men. The detection of the infection in male specimen could be affected due to high concentration of Zinc and anti-trichomonal substances in the prostate (Annang *et al*., 2016).

4.2.3 Prevalence of *T. vaginalis* based on marital status of patients

Prevalence of *T. vaginalis* infection is significantly associated with marital status of participants ($p = 0.004$). The highest prevalence of trichomoniasis was recorded among married participants while the least prevalence was recorded among divorced participants. This is in agreement with the study of Adeoye and Akande, (2017) but in contrast with the study of Tompkins *et al*. (2020) and Patel *et al*. (2018). This could be attributed to their rate of sexual activity. The prevalence of Trichomoniasis is usually higher in sexually active individuals (Opara *et al*., 2018). Married adults are known to be sexually active with some even having multiple partners especially those in polygamous marriage while the divorced participants are assumed to have no or reduced sexual activity.

4.2.4 Prevalence of *T. vaginalis* based on age of patients

The present study revealed that highest prevalence of infection was recorded among patients aged 40-50 years while the least prevalence was recorded among those aged above 50 years. Though, age is not a statistically significant ($p = 0.237$) factor in the distribution of trichomoniasis. This is similar with the findings of Ogomaka *et al*. (2018) that recorded a higher prevalence of infection among adults aged 29-39 years and the

least prevalence among adults aged 61-72years. Participants aged 40-50years were likely to have contacted the infection during sexual intercourse. It has been reported by that occurrence of the disease correlates with the rate of sexual activity among the group of individuals being studied. This proof that *T. vaginalis*is is sexually transmitted as the highest prevalence was recorded among patients with increased sexual activities and multiple sex partners.

4.2.5 Prevalence of *T. vaginalis* based on hydrogen ion concentration (pH) of specimen

The pH of the discharge and urine recorded among the individual aged above 50 years could be due to the low rate of infection among the age group. This could be justified since *Trichomonas* grows best in more alkaline environment (>6) than in the acidic and neutral environment (Gary and Garber, 2015). Consequently low pH of the vagina environment is highly unfavorable for *Trichomonas vaginalis* survival and this agrees with pH (7.5 and 7.0) level and infestation observed among adults aged 40-49, 18-28 and 29-39 years respectively.

4.5.6 Prevalence of *T. vaginalis* based on some socio-demographic factors

A major cause of sexually transmitted disease such as trichomoniasis in Nigeria has been associated with increasing poverty, unemployment (Woken, 2006) ignorance, poor personal hygiene and sexual recklessness among individuals. Though, there was no significant relationship between level of education ($p = 0.614$) and occupation ($p = 0.348$) of the participants in this present study. Highest prevalence of infection was recorded among unemployed patients while the least was recorded among students. This could be attributed to sexual recklessness, promiscuous life or in-ability of the patients to avoid contact with formites and the level of awareness among the participants as students

contributed to the low prevalence as they are more aware of sexually transmitted diseases such as trichomoniasis than other patients. The prevalence of infection was also very high in participants with no formal education whereas, those with primary level of education had the least prevalence. Similar study carried out in Sri Lanka reported a higher prevalence of *T. vaginalis* infection among women with low educational level than women with high educational level (Sumadhya *et al.*, 2012).

The high prevalence rate of *T. vaginalis* among patients attending Umaru Sanda General Hospital, Bida Nigeria might be due to poor sanitary condition of toilet facilities in the areas. Other reasons could be attributed to little or lack of personal hygiene such as the use of untidy under wears toilet rolls, sanitary pads, dirty towels, and clothes.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The parasite *T. vaginalis* from infected individual was isolated and identified using microscopic technique. Prevalence of trichomoniasis among patients attending Umaru Sanda Ndayako General Hospital Bida, Niger State, Nigeria recorded in this study was 47(9.4 %). Socio-demographic factors such as gender and marital status of participants had significant association with the prevalence of infection whereas, age, level of education and occupation of participants had no significant influence on the prevalence of trichomoniasis in the study area.

5.2 Recommendations

Based on the findings of this study, it is recommended that:

i. The Government should be screening of sexually transmitted infections (STIs) among the sexually active patients in health care centers so as to prevent further spread of the parasite as most of the patients especially the men have no signs and symptoms of been infected.
ii. There is a need for extensive sensitization of the public, especially the youths and married people on the implication of trichomoniasis by the health workers and students.
iii. Adequate treatment of spouses, implementation of effective screening programmes and treatment at no cost should be introduced.
iv. Individuals should avoid sexual recklessness especially those involved with multiple sexual partners.

v. Further studies should be carried out on the detection of *T. vaginalis*, employing molecular technique.

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LIST OF APPENDIX

APPENDIX A: Questionnaire

Code no……

Residential area:

Age:

Gender: Male ☐ Female ☐

Occupation:

Marital status: Single ☐ Married ☐ Divorced ☐ Others ☐

Type of marriage: Polygamous ☐ Monogamous ☐

Educational level: Uneducated ☐ Primary ☐ Secondary ☐ Tertiary ☐

Have you heard of Trichomoniasis? Yes ☐ No ☐

HIV status: Positive ☐ Negative ☐

MALE

Do you feel any irritation/itching inside the penis? Yes ☐ No ☐

Do you have a mild discharge? Yes ☐ No ☐

Do you have any burning sensation after urination/ejaculation? Yes ☐ No ☐

FEMALE

Are you pregnant? Yes ☐ No ☐

Any vaginal itching? Yes ☐ No ☐

Do you notice any vaginal discharge? Yes ☐ No ☐

Colour of vaginal discharge: White ☐ Green ☐ Yellow ☐ Pink ☐ Milkish ☐

Discomfort during urination/sexual intercourse? Yes ☐ No ☐