INTRODUCTION

Trichomoniasis is considered the most prevalent non-viral sexually transmitted infection worldwide with an estimated 276 million new cases annually ***(WHO, 2012).*** In Egypt a prevalence rate of 8.7 % among married women from Upper Egypt was recorded ***(Sullam et al., 2001).*** In another study a prevalence rate of 36% was encountered among Egyptian symptomatic women in the child bearing age, 20-45 years ***(Aboulghar et al., 2009).*** The factor which may lead to underestimation of prevalence rates of trichomoniasis is that about 50% of women and generally most infected males are asymptomatic ***(Lan et al., 2008).*** Besides, there are no guidelines for *T. vaginalis* screening of women, and clinicians often rely upon insensitive diagnostic methods ***(Aboulghar et al., 2009).***

Current knowledge of *Trichomonas vaginalis* (*T. vaginalis),* the causative agent of trichomoniasis, population genetics has been limited by a lack of appropriate tools. Crude genotyping markers such as random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) indicated genetic variation among *T. vaginalis* isolates and inconclusively detected evidence of population structure ***(Vanacova et al., 1997; Hampl et al., 2001; Snipes et al., 2000; Stiles et al., 2000; Rojas et al., 2004; Meade et al., 2009).*** These methods, however, are highly sensitive to

contaminating DNA or to slight variation in conditions, which may influence the interpretation of data collected with these techniques. Moreover, these studies have yielded discordant results for a number of different phenotypes including metronidazole resistance, geographical distribution, the presence of a linear double-stranded RNA virus known as *T. vaginalis* virus and clinical manifestation of infection in patients ***(Conrad et al., 2012).***

Multilocus sequence typing (MLST) of bacteria and eukaryotic pathogens has been used successfully to describe population diversity, delimit species, identify genetic components of important clinical phenotypes and track the spread of epidemics ***(Barker, 2002; Maiden, 2006; Tibayrenc, 2009; Conrad et al., 2011).*** Additional advantages of using MLST as a genotyping method are that the DNA sequences can be determined using automated technology and require minimal subjective interpretation of data ***(Maiden et al., 1998).*** Moreover, the portability of MLST data allows results from different laboratories to be compared ***(Maiden et al., 1998; Maiden, 2006).***

In Egypt, several studies were done to investigate the diversity of *T. vaginalis*, including isoenzyme patterns ***(Salem et al., 1992),*** serotyping ***(Azab et al., 1992),*** immunoblotting ***(Khalifa et al., 2004; El-Okbi et al., 2004, 2005),*** biological variability ***(Hussein et al., 2004),*** and HSP70- RFLP ***(Hussein et al., 2005).*** These studies concluded that the different clinical

isolates have different and common patterns at the levels of antigens, immunogens, pathogenicity and metronidazole resistance. However, multilocus genotyping of Egyptian *T. vaginalis* isolates need to be investigated. Hence, the aim of the present study is an attempt to elucidate the genetic diversity of Egyptian isolates of *T. vaginalis* by using multilocus genotyping. This would help in better understanding of the molecular epidemiology, phylogenetic relationship and population genetics of the Egyptian isolates of *T. vaginalis.*

REVIEW OF LITERATURE

Trichomonas vaginalis

I. Historical background

The protozoan parasite *Trichomonas vaginalis (T. vaginalis)* was first identified by the Parisian physician Alexandre Donne in 1836. Its 'undulating motion' and 'whip-like tail' was described in the purulent, frothy leucorrhea of women presenting with vaginal discharge and genital irritation. In 1837, he named it *T. vaginalis*, thereby creating the genus ***(Jamali et al., 2006)***. The name 'Trico-Monas' was given to these organisms because of similarities in morphology to two other protozoa known at that time, 'tricodes' and 'monas' ***(Roberts and Janovy, 2009).***

II. Taxonomy

According to ***Dyer (1990)*** *T. vaginalis* is classified as follows:

- **Phylum:** Zoomastigina—possess flagella
- **Class:** Parabasalia—presence of a parabasal body: Golgi associated with kinetosomes; axostyle (bundled microtubules); undulating membrane, an extension of the plasma membrane, enveloping the recurrent flagellum; occur in association with animals.

- **Order:** Trichomonadida (Kirby, 1947 emend. Honigberg, 1974)—four to six flagella, free or attached to an undulating membrane; no true cysts.

- **Family:** Trichomonadidae (Wenyon, 1926)—presence of a cytostome, three to five free flagella (one flagellum on the margin of the undulating membrane); axostyle protruding through the posterior of the cell.

- **Genus**: *Trichomonas*—four free flagella; one recurrent, along the outer margin of the undulating membrane; a costa at the base of the undulating membrane, and an axostyle extending through the cell.

- **Species**: *Trichomonas vaginalis* (Donne´, 1836).

III. Morphology

T. vaginalis is the most broadly studied parasite of all the trichomonads. The parasite does not form a cyst and the only existing form is the trophozoite ***(Marquardt et al., 2000).*** Light microscopy reveals various shapes of *T. vaginalis* including pyriform, amoeboid, ellipsoidal, ovoidal and spherical. External environmental conditions such as pH, temperature, oxygen tension, and ionic strength affect the shape of the trophozoites ***(Honigberg, 1989; Leitsch, 2016).*** Size of trophozoites range from 7 μm to 32 μm length by 5 μm to 12 μm width. Each has four anterior flagellae and a fifth recurrent one that runs toward the posterior end attached to the cell through an undulating

membrane. The membrane is supported on the cytosolic side by the costa that is a prominent striated fiber ***(Benchimol, 2004; Roberts and Janovy, 2009).*** By electron microscope, the costa is a periodic structure with alternating electron-dense and electron-lucent bands that display three distinct regions named head, neck and body ***(de Andrade Rosa et al., 2017).***

Inside the organism, a round nucleus located at its anterior portion (Figure 1) contains chromatin granules and a small karyosome. It is surrounded by a porous nuclear envelope. The trophozoite contains an axostyle which is a slender hyaline, rod-like structure that runs from the anterior towards the posterior end of the cell. It forms the capitulum in the anterior portion and a narrow tube in the posterior part ***(Lee et al., 2009)*** that protrudes through the posterior end of the parasite and terminates in a sharp point ***(Adegbaju and Morenikeji, 2008).*** It is thought to have a supportive function and a role in cell division ***(Benchimol, 2004).*** Also it may be used for attachment to surfaces and may induce tissue damage noted in trichomoniasis ***(Schwebke and Burgess, 2004).*** The trophozoite has a V-shaped parabasal apparatus that lies dorsal to the nucleus consisting of a parabasal body and two parabasal filaments ***(Adegbaju and Morenikeji, 2008).*** It lacks mitochondria and peroxisomes while having energy organelles involved in carbohydrate metabolism called the hydrogenosomes, arranged in association with the costa and the axostyle. The organelles are aligned in three rows parallel to the axostyle.

Hydrogenosomes lack detectable DNA, cytochromes or cristae, and the citric acid or Krebs cycle present in the mitochondria. Instead, they possess enzymes found in anaerobic bacteria which can produce molecular hydrogen; from which they derived their names ***(Bui et al., 1996; Garcia and Alderete 2007; Leitsch, 2016).*** However, the phylogenetic and biochemical analysis of hydrogenosomes indicate a common origin with mitochondria ***(Schneider et al., 2011; Leitsch, 2016).***

There were reports that *T. vaginalis* can round up and internalize the flagella under unfavorable growth conditions ***(Petrin et al., 1998).*** It was believed that these forms are pseudocysts, because they have not been reported to give rise to normal motile forms ***(Adegbaju and Morenikeji, 2008).*** The ability of intra-vaginally inoculated *T. vaginalis* pseudocysts to induce trichomoniasis in infected mice was evaluated. Pseudocysts formation was induced by using thermal-freezing cycle method. The infectivity of the pseudocysts was proved by the presence of *T. vaginalis* parasite in vaginal washes of mice inoculated *in vitro.* The parasites were maintained in the vaginal tissue for 72 hours post infection without any morphological changes. Accordingly *T. vaginalis* pseudocysts were considered an active form that can induce trichomoniasis ***(Hussein and Atwa, 2008).***

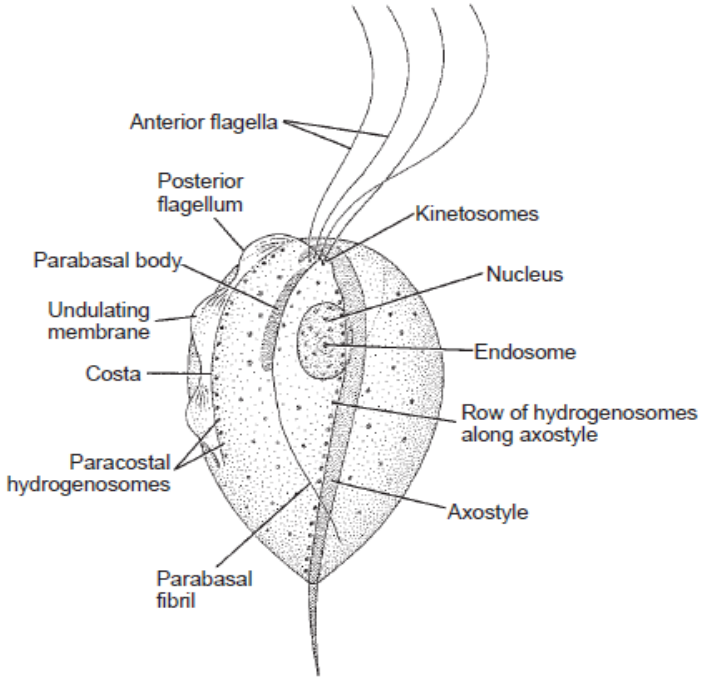

Figure (1): Morphology of *Trichomonas vaginalis* trophozoite from ***(Roberts and Janovy, 2009).***

VI. Life Cycle.

The life cycle of *T. vaginalis* is simple (Figure 2) occurring in one human host who is also its only natural host. After acquiring infection, the parasite resides in the lower urogenital tract. In females, these sites include vagina, cervix, urethra, bladder, Bartholin's glands, and Skene's glands. While in males, the organism has been isolated from the anterior urethra, epididymis, prostate and seminal vesicle ***(Krieger and Alderete, 1999; Sood and Kapil, 2008; Gatti et al., 2017).***

The trophozoites live in these sites replicating by binary fission until they are passed on to their next human host, where the whole process starts over again ***(Satterwhite, 2013).***

It is transmitted mainly through sexual intercourse. Usually, the organism dies outside of the human body unless it is protected from drying, so non-sexual transmission is rare. Infection may occur via sharing of soiled clothing, toilet seats, and in bath water ***(Krieger and Alderete, 1999).*** Transmission via perinatal route which occurs in about 5% of female children of infected mothers is usually self-limited ***(Schwandt et al., 2008; Swygard et al., 2004).***

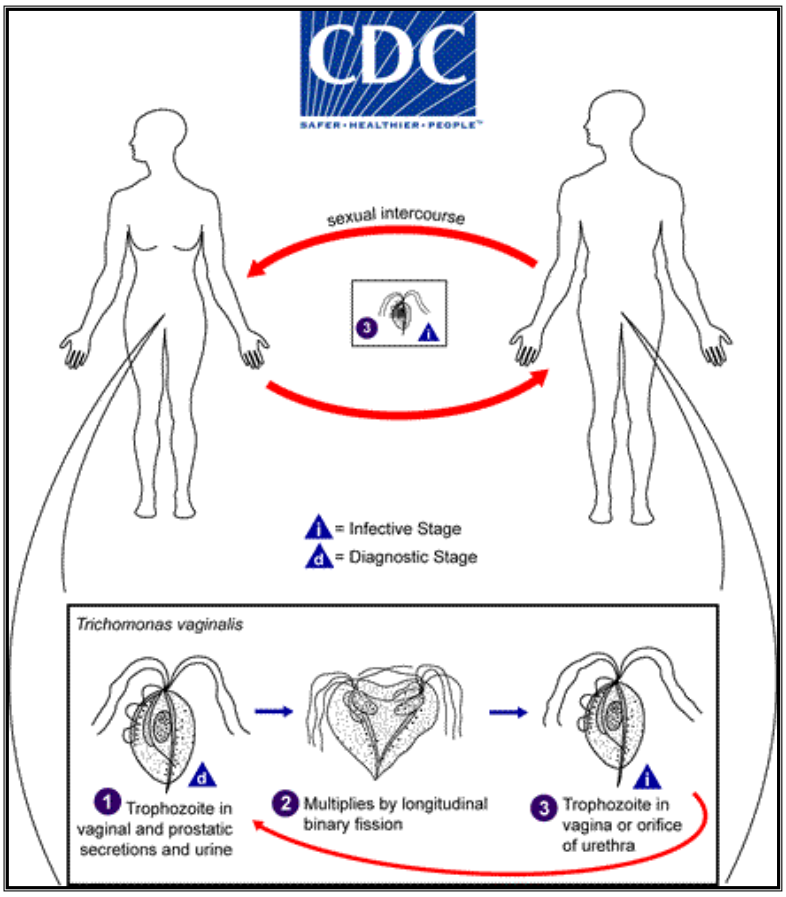

Figure (2): Life cycle of *Trichomonas vaginalis* from **"www.dpd.cdc.gov"** accessed online on (11/ 05/ 2017).

V. Reproduction

As with other protozoans, *T. vaginalis* divides by longitudinal binary fission. It undergoes a closed type of mitosis in which the nuclear envelope remains intact. The extra-nuclear spindle of microtubules develops from the attractrophores and begins the process of division. The attractrophores are two rod-shaped organelles attached to the base of the basal bodies which play the role of centrosomes ***(Brugerolle, 1975).*** The paradesmose is an extranuclear spindle extended between the attractophores. This spindle elongates, and then the daughter cells separate. Each subsequent daughter cell, in turn, produces any missing organelles. Consequently, one cell becomes two and after another period of time, these divide to become four and so on ***(Honigberg, 1989; Noel et al., 2003).***This type of doubling growth is called exponential growth. The organisms will double in number during a particular length of time called the doubling time ***(Petrin et al., 1998; Leitsch, 2016).***

During division, different forms of *T. vaginalis* may appear; those without flagella, those with flagella and having a dividing nucleus, and those with flagella and having multiple nuclei. They are considered to be developmental stages prior to the appearance of mononuclear flagellates. These shapes are associated with specific forms of cell division: the round shape is associated with amitotic cell division and the ovoid shape

with simple mitotic cell division ***(Abonyi, 1995; Gatti et al., 2017).***

VI. In vitro growth of T. vaginalis

Several media have been developed for *T. vaginalis* cultivation. These include the Cysteine-Peptone-Liver-Maltose medium ***(Johnson, 1947),*** the Basal Trypticase medium ***(Sprince and Kupferberg, 1947),*** the Simplified Trypticase Serum medium ***(Kupferberg et al., 1948).***

In 1957, the Diamond's Trypticase-Yeast-Maltose (TYM) medium was originally developed. Its main ingredients were a trypticase digest, yeast extract, cysteine, maltose, ascorbic acid, agar and sheep serum ***(Diamond, 1957)***. Several modifications of this medium have been made. Diamond later developed some modifications which were called modified Diamond's. In 1980, Klaas, made changes to the original Diamond formulation in the form of horse serum instead of sheep serum; elimination of agar; increased concentrations of maltose, cysteine, and ascorbic acid; and antibiotics were added to suppress the growth of bacteria ***(Fouts and Kraus, 1980).***

Diamond's medium is the most commonly used for cultivating the parasite. It is commercially available, with affordable price, in the form of a ready medium supplied in glass tubes, stored at 4°C before use, warmed to room temperature before inoculation and then incubated immediately at 37°C ***(Gelbart et al., 1990; Hobbs and Sena, 2013).***

Moreover, another form of culture medium is the InPouch TV® culture system. The InPouch TV® is a self-contained culture pouch that serves as the specimen's transport container, the growth chamber during incubation, and the slide during microscopy ***(Soba et al., 2015)***. It contains liquid medium in a clear pouch. Although it has been used successfully with both clinician-obtained and self-obtained specimens, it is costly than Diamond's media ***(Draper et al., 1993; Schwebke et al., 1997).*** The InPouch TV has the advantage of becoming quite useful in situations where the pelvic examination is not possible or desirable as in adolescents screening, or patients in developing countries. Another delayed-inoculation technique is available, allowing for initial reading of the wet preparation and then inoculation of the culture pouch if the wet preparation is negative ***(Schwebke and Burgess, 2004).*** Because it is made of optically clear plastic, once inoculated it requires no opening for microscopic examination. In contrast to Diamond's medium, InPouch TV® can be stored at room temperature ***(Hobbs and Sena, 2013).*** Furthermore, swab specimens may sit for up to 30 minutes at room temperature prior to pouch inoculation ***(Schwebke and Burgess, 2004).*** Once inoculated, it can remain at room temperature for up to 48 hours before incubation at 37 °C. Culture is to be examined microscopically each day for up to 5 days until proven negative ***(Hobbs and Sena, 2013).***

After inoculation, cultures from women with trichomoniasis usually appear positive within the first 3 days. However, cultures from men must be examined daily for 5 or more days before being considered negative. The possible cause is that extended incubation times are required to permit growth of detectable numbers of organisms from male specimens ***(Hobbs et al., 2006)***.

Trichomoniasis

I. Epidemiology and prevalence

Trichomoniasis is the most common, curable, non-viral sexually transmitted infection worldwide. It has been associated with vaginitis, cervicitis, urethritis, and pelvic inflammatory disease (PID). Trichomoniasis is a co-factor in human immunodeficiency virus (HIV) transmission and acquisition. Moreover, it also can affect birth outcomes ***(Sorvillo et al., 2001).***

The true prevalence and incidence of *T. vaginalis* infection are difficult to assess because it is not a reportable disease as in gonorrhea; moreover, there are no screening guidelines and most testing is limited to symptomatic individuals. Furthermore, the use of different diagnostic tools with varied sensitivity and specificity may cause errors in the number of infections reported ***(Huppert, 2009).***

In 1997, prevalence of vaginal trichomoniasis in the African populations, was between 11 and 25% ***(Klouman et al.,***

1997). In 2001, the global estimates of *T. vaginalis* incidence and prevalence among adults aged 15–49 years old reported an overall incidence of 54 cases per 1000 persons/year ***(WHO, 2001).*** Around the world, infection rates have been reported to be as high as 67% in Mongolia, 60% in Africa, and 40% in indigenous Australian women ***(WHO, 2005).***

According to WHO estimates, 276.4 million new infections occurred in 2008 with 11.5 % increase over the 2005 incidence rate ***(WHO, 2012).*** It was estimated that women account for 89% of prevalent *T. vaginalis* cases among the world, while over half of the new *T. vaginalis* infection cases each year occur in men ***(Kissinger, 2015).***

It is considered that rates of infection with *T. vaginalis* vary with geographic location, age, race, and community ***(Poole and McClelland, 2013).*** In the United States, two population-based studies that used PCR testing found rates of 2.3 % among adolescents and 3.1 % among women aged 14–49 years old ***(Miller et al., 2005; Sutton et al., 2007).***

Population-based studies in Africa show distinctly higher rates. In Zimbabwe, the rate was 9.5 % among both genders using antibody testing ***(Gregson et al., 2001).*** Using nucleic acid amplification techniques, the prevalence rate in Tanzania was 11 % ***(Klinger et al., 2006).*** Infections in Papau New Guinea also appearred to be 21 % in pregnant women, 1 % in

rural Vietnam, 0.37 % in Belgiumand 2.9 % in China ***(Depuydt et al., 2010; Wangnapi et al., 2015).***

In Egypt, in an early report, *T. vaginalis* was recorded in 17.38% of the female population in the child-bearing period ***(Salem et al., 1981).*** Later on, *T. vaginalis* was found in 8.7% of married women from rural and urban areas of Upper Egypt ***(Sullam et al., 2001)***. In another study, a prevalence rate of 36% was encountered among Egyptian symptomatic women in the child-bearing age; 20-45 years ***(Aboulghar et al., 2009).*** More recently, a cross-sectional study carried out in the Obstetrics and Gynecology Department at Kasr Al-Aini Cairo University Hospitals, 50 cases out of 1000 female patients in the child-bearing period were positive for trichomoniasis ***(Mahmoud et al., 2015).***

II. Clinical presentation and complications

The incubation period for the establishment of infection ranges from 4 to 28 days ***(Catterall, 1972).*** *T. vaginalis* primarily targets the squamous epithelial cells of the urogenital tract. It is site specific for the genitourinary tract and has been isolated from virtually all genitourinary structures. Along with vaginal epithelial cells, trichomonads can also affect the bladder, urethra, and paraurethral glands and present as a urinary tract infection. Fifty-six percent of *T. vaginalis* infections are initially asymptomatic, with one-third becoming