# Diagnosis of *Trichotomies Vaginalis* in reproductive age group Libyan Ladies in Benghazi City using OSOM rapid test


**Abstract**

Trichomonas Vaginosis is a protozoal infestation considered among sexually transmitted diseases with a prevalence rate variable across societies and regions. Such infection is associated with some gynecological trouble and affects adversely the quality of life of women. The aim of the study is to study the prevalence of *Trichomonas vaginalis* infection using OSOM test ™. Two hundred women were investigated with history, speculum examination and OSOM test. The prevalence of Trichomonas infection was 4.0% with another 0.5% inconclusive result. The conservative nature and hygienic practice among the Libyan population may help in the lower prevalence of TV infection. But the prevalence is still high in comparison to some other countries in the Mediterranean region. History of irregular cycle and also Co-morbidities with hypertension, diabetes mellitus, polycystic ovarian syndrome and thyroid disorder as well as findings of whitish discharge and Odorous discharge have no statistically significant association with positive result. Frothy discharge was higher in positively testing with a statistically significant association and sensitivity as well as specificity and negative predictive value of the finding of frothy secretion were 100.0%, while the positive predictive value was 72.7%. The OSOM test should be available for use in maternity centers and women health clinics. A careful history and clinical findings should be obtained from the clinician. A further well designed study may be worthy to examine other characteristics might be related to the Trichomonas infection across different groups of women.

# List of Appendices

# List of Abbreviations

| Abbreviation | Meaning |
|---|---|
| AV | Aerobic vaginitis |
| AVF | Abnormal Vaginal Flora |
| BV | Bacterial Vaginosis |
| CI | Confidence Interval |
| DIV | Desquamative Inflammatory Vaginitis |
| HIV | Human Immunodeficiency Virus |
| I.V. | Intra Venous |
| L | Litre |
| Ml | Milliliter |
| MV | Mixed vaginitis |
| N / A | Not Applicable |
| NHANES | National Health and Nutrition Examination Survey |
| OR | Odds Ratio |
| RR | Risk Ratio |
| STD | Sexual Transmitted Disease |
| STI | Sexual Transmitted Infection |
| TV | Trichomonas Vaginosis |
| VVC | Vulvovaginal candidiosis |
| WHO | World Health Organization |

# 1. Introduction:

## 1.1 Introduction

Sexually transmitted infections (STIs) are group of infectious diseases caused by many varieties of organisms belong to different categories like bacteria, viruses, protozoal organisms and also fungi. Such infections are contagious and spread mainly through their habitation in genital tract and existence in genital secretions. Although STIs are a major public health concern around the world, there are certain challenges in developing and implementing STI prevention and control programmes due to clinical and laboratory diagnostic issues. Chlamydia trachomatis infections, trichomoniasis, and gonorrhoea are the most frequent STIs (Al-dunate et al., 2015). Vaginal infections and STIs account for a significant fraction of gynaecological outpatient visits, accounting for 10 to 20% of all consultations, and are common in women of reproductive age. The high risk of recurrence and a variety of major unfavourable pregnancy outcomes are also significant difficulties with these vaginal infections. Finally, they have a significant impact on the quality of life of these women, leaving them weary. Types of vaginal infections include three main well defined vaginal infections (Mendling *et al., 2016).*

1- bacterial vaginosis (BV),

2- vulvovaginal candidosis (VVC), and

3- Trichomoniasis/Trichomonas vaginitis (TV).

There are other more vaginal infections that have not yet been fully defined and identified. The 'intermediate flora' (based on Nugent score 4–6), as well as the less well defined'mixed flora' or 'abnormal vaginal flora' (AVF), may play a larger role in the risk of preterm birth than previously thought. Furthermore, aerobic bacteria are involved in vaginal infections, albeit their significance is unknown. Based on this information, a fourth, yet poorly understood type of vaginal infection has been identified: aerobic vaginitis (AV), which is likely the same as desquamative inflammatory vaginitis (DIV), but with more severe inflammation and complaints. Furthermore, mixed vaginitis (MV) and co-infection with BV, VVC, and TV are both conceivable, making diagnosis and treatment of vaginal infections difficult.

*Trichomonas vaginalis (TV) is a common motile protozoan parasite that is the world's most common non-viral sexually transmitted infection (STI). In 2008, the WHO estimated that TV caused 276.4 million new infections in people aged 15 to 49 years. Chlamydia trachomatis, Neisseria gonorrhoeae, and syphilis are all more common than Trichomonas vaginalis. TV viewing is expected to be 8.1 percent for women and 1.0 percent for males over the world.* In general, Africans or persons of African descent have higher rates of TV, as evidenced by higher rates in Sub- Saharan Africa.

The prevalence of the infection in Africa ranges from 5% to 74% in women and 5% to 29% in men, depending on the populations studied and the diagnostic methods used to detect the infection.

Among pregnant women, *T. vaginalis* infection has been associated with adverse birth outcomes such as preterm delivery, premature rupture of the membranes, and low birth weight. In addition to these reproductive health complications, mounting evidence suggests that *T. vaginalis* may be a cofactor for HIV-1 transmission and acquisition. *T. vaginalis* infection is generally given less emphasis than other STIs and is not a reportable infection. As a result, the infection is infrequently studied and little information exists about the risk factors for it; consequently, its public health importance remains poorly understood, and issues like partner management, antimicrobial resistance, associated conditions (eg, human immunodeficiency virus [HIV], pregnancy complications, and others), diagnostic methods, screening, reporting, and prevention of trichomoniasis take a considerable part of research in Western societies (Meites *et al.*,2015**)**.

Bacterial vaginosis is a common lower genital tract syndrome defined as a shift from normal hydrogen peroxide–producing lactobacilli to mixed anaerobes, such as Gardnerella species, Prevotella species, and Atopobium species (Yazısız H *et al,* 2020) and (Olson KM *et al*, 2018). Lactobacillus species comprise between 90 and 95 percent of the total bacteria count in the healthy vaginal flora and play a key role in maintaining balance and host defense against pathogens by producing several substances that inhibit the growth of deleterious microorganisms (Ling Z *et al*, 2010) and (Wood BA *et al*, 1975).

. Symptoms of bacterial vaginosis typically include off-white, thin, homogenous discharge or vaginal “fishy” odor, or both; however, many women with bacterial vaginosis are asymptomatic.

Worldwide bacterial vaginosis prevalence estimates range from 12% in Australian women and 29 percent in North American women to more than 50 percent in women from Eastern and Southern Africa (Tweats *et al*.,2012***)*** The prevalence of bacterial vaginosis in the United States is estimated to be 29.2 percent among all women ages 14 to 49 years (some of whom are pregnant), corresponding to 21 million women, according to National Health and Nutrition Examination Survey (NHANES) data from 2001 through 2004, the most recent years for which nationally representative estimates are available (Tarrant *et al***.,**2014). Prevalence varies most notably by race/ethnicity. The NHANES data from 2001 through 2004 showed significantly higher rates among African Americans (52.6%) and Mexican Americans (32%) than among non-Hispanic whites (23%) (Tarral A *et al*, 2014).

Among five studies published between 1995 and 2014, a higher prevalence of bacterial vaginosis (range, 25% to 50%) was observed among women who have sex with women (Bosserman *et al., 2011).* In the United States, the prevalence of bacterial vaginosis among pregnant women ranges from 5.8 to 19.3 percent and is influenced by the study population and the diagnostic criteria. The prevalence is higher in some races/ethnicities (Sobel *et al***.***, 2001).*

Studies conducted betweUpregnancy loss before 22 weeks (RR range, 3.1 [95% CI, 1.4 to 6.9]),(Obiero *et al.*,*2012*) pelvic inflammatory disease (magnitude not well defined), (Newman *et al.,2015*) postabortion sepsis (magnitude not well defined),(Ashshi *et al.,2015*) postpartum endometritis (odds ratio [OR], 5.8 [95% CI, 3.0 to 10.9]),(Joseph *et al.,2016*) and low birth weight (OR, 1.4 [95% CI, 1.1 to 1.8]).(Rowley *et al.,2019*).

**1.2. Importance of the study:**

Up to the best available knowledge, no published similar work in Libya. Use of OSM test

**1.3. Aims of the study:**

The aim of the study is to study the prevalence of *Trichomonas vaginalis* infection using OSOM test ™.

# 2.Review of literature:

**2.Review of literature:**

**2.1. Epidemiology of STI and vaginosis**

Analysis of data from 2009 to 2016, the estimated worldwide prevalence of chlamydia in 15–49-year-old women was 3.8 percent (95 percent UI: 3.3–4.5) and 2.7 percent (95 percent UI: 1.9–3.7) in men, with regional values ranging from 1.5 to 7.0 percent in women and 1.2 to 4.0 percent in males. The global estimate for gonorrhoea in women was 0.9 percent (95 percent UI: 0.7–1.1) and 0.7 percent (95 percent UI: 0.5–1.1) in men, with regional values ranging from 0.3 to 1.9 percent in women and 0.3 to 1.6 percent in males. In both men and women, the global report for syphilis was 0.5 percent (95 percent UI: 0.4–0.6), with regional estimates ranging from 0.1 to 1.6 percent. The WHO African Region had the highest prevalence for *chlamydia* in men, *gonorrhoea* in women and men, *trichomoniasis* in women and syphilis in men and women. The higher incidence of chlamydia in women and trichomoniasis in men was found in the WHO Region of the Americas.

**2.2. These incidence estimations correspond to totals of 124.3 million chlamydia cases, 30.6 million gonorrhoea cases, 110.4 million trichomoniasis cases, and 19.9 million syphilis cases (available from the data repository) (Rowley J et al, 2019). Epidemiology of trichomoniasis:**

The estimates for trichomoniasis were 5.3% (95% UI: 4.0–7.2) in women and 0.6% (95% UI: 0.4–0.9) in men, with regional values ranging from 1.6 to 11.7% in women and from 0.2 to 1.3% in men.

According to WHO's 2012 estimates were based upon literature reviews of prevalence data from 2005 through 2012 among general populations for genitourinary infection with ***chlamydia***, ***gonorrhoea***, and ***trichomoniasis***, and nationally reported data on syphilis seroprevalence among antenatal care attendees (Newman L *et al*, 2015).

Newman L, *et al* (2015) upon data during 2012, among women aged 15–49 years, the estimated global prevalence of chlamydia was 4.2% (95% CI(UI): 3.7–4.7%), gonorrhoea 0.8% (0.6–1.0%), trichomoniasis 5.0% (4.0–6.4%), and syphilis 0.5% (0.4–0.6%); among men, estimated chlamydia prevalence was 2.7% (2.0–3.6%), gonorrhoea 0.6% (0.4– 0.9%), trichomoniasis 0.6% (0.4–0.8%), and syphilis 0.48% (0.3–0.7%). These figures correspond to an estimated 131 million new cases of chlamydia (100–166 million), 78 million of gonorrhoea (53–110 million), 143 million of trichomoniasis (98–202 million), and 6 million of syphilis (4–8 million). Prevalence and incidence estimates varied by region and sex. According to Newman L, *et al* (2015) upon data during 2012, trichomoniasis global estimate was 5.0% (4.0–6.4%) and the regional values ranged from 1.0% to 11.5%. The lowest prevalence was reported in the European and South Asian regions. In eastern Mediterranean Region the prevalnec of trichomoniasis among women was around 6% while among men was 1% (Newman L *et al*, 2015).

In a case control study by Ashshi AM *et al* (2015) in Saudi Arabia including comparison of 7 STDs between normal and ectopic pregnancy, the prevalence of co-infections was significantly higher compared with single infection in the study participants and it was associated with 5 times greater risk of developing ectopic pregnancy including *Trichomonas vaginalis* (Ashshi AM *et al*, 2015).

*Trichomonas vaginalis* is considered as a curable STD despite the potential complications it may contribute to. A systematic review by Joseph Davey DL *et al* (2016) described studies performed in Kenya, Tanzania, Somalia, Ethiopia, Uganda, and Sudan for prevalence of *Trichomonas vaginalis* among pregnant women. The adjusted mean prevalence was similarly high for TV in 3 studies at 6.8% (95% CI, 4.6– 9.0) (Joseph Davey DL *et al*, 2016).

Rowley J *et al* (2019) published a meta-analysis including 76 data points regarding Prevalence and incidence of *Trichomonas vaginalis* along with some other sexually transmitted diseases. The global estimate for *Trichomonas vaginalis* infection among men was 0.6% (95% CI: 0.4 – 0.9). The highest prevalence among WHO regions was reported in African region; for women 11.7% (95% CI: 8.6 – 15.6) and for men 1.2% (95%CI: 0.7 – 1.8). While the Eastern Mediterranean Region (EMRO) which include Libya reported the lowest prevalence, for men0.2% (95%CI: 0.1 – 0.3) and for women 1.6% (95% CI: 1.1 – 2.3) (Rowley J *et al*, 2019).

Chemaitelly H *et al* (2019) demonstrated in a meta-analysis for studies including female sex workers in Middle East (Egypt, Iran and Pakistan) and found that the infection prevalence ranged from 0%-19.3%, with a median of 7.0%. The highest prevalence was in Egypt (Chemaitelly H *et al*, 2019).

T. vaginalis protozoa are the most common nonviral STI in the globe, according to (Crespillo-Andujar C et al, 2018), and their prevalence is rising. This parasite, which can cause urogenital tract infection, thrives in the genital tract of humans.

T. vaginalis has been found in seminal fluid and has been linked to sperm quality issues.

**2.3. Taxonomic classification of *Trichomonas vaginitis***

. Domain : Eukarya

. Kingdom : Protista

. Phylum : Metamonada

. Class : Parabasilia

. Family :Trichomonadida

. Genus : Trichomonas

. Species : *Trichomonas* vaginalis

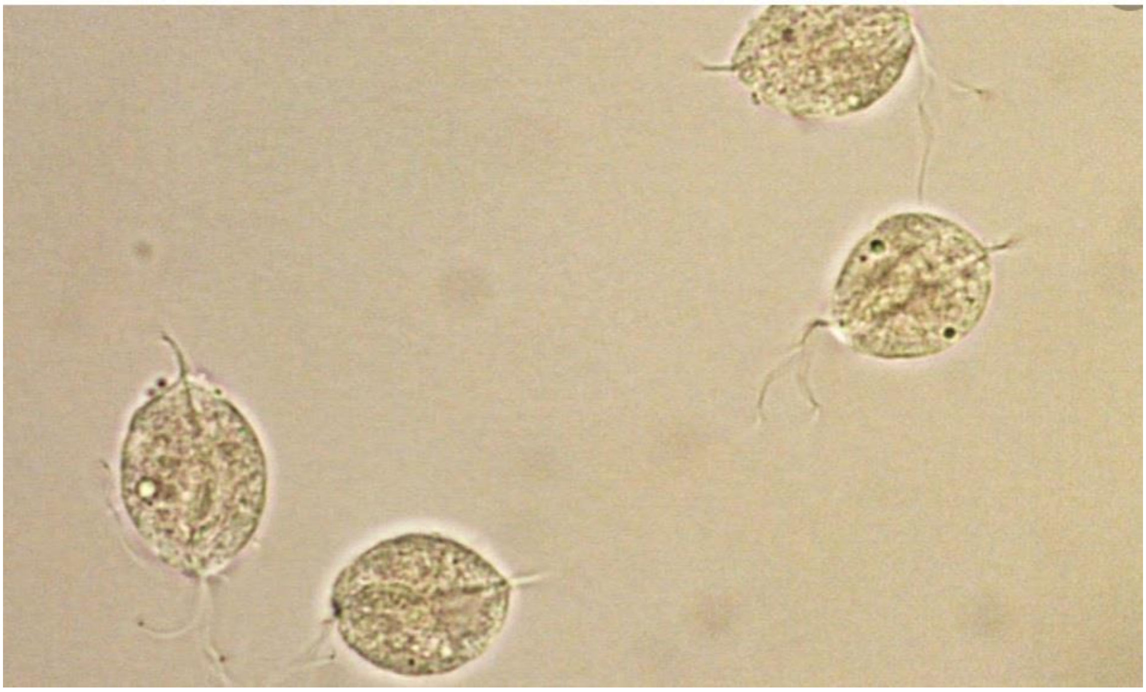

**Figure 1: Trichomonas virgin power 4$^{o}$**

**2.4. Pathogenesis of TV**

TV is a flagellated parasitic protozoan, typically pyriform (Harp *et al., 2011*) the individual organism is 10–20 µ m long and 2–14 µ m wide.

Four flagella project from the anterior portion of the cell (Carlton *et al*, .2007) TV is a highly predatory obligate parasite that phagocytoses bacteria, vaginal epithelial cells and erythrocytes. TV uses carbohydrates as its main energy source. Incubation period is generally between 4 and 28 days (Petrin *et al., 1998*). The squamous epithelium of the genital tract is largely infected by TV. TV replicates by binary fission in the female lower vaginal tract as well as the male urethra and prostate. TV is transmitted largely through sexual intercourse among humans, its only known host. In women, infection can last months or even years, whereas in men, it usually lasts less than 10 days (Krieger et al., 1995).TV has been linked to vaginitis, cervicitis, urethritis, bacterial vaginosis, candidiasis, and herpes simplex virus, according to studies (al., 2009). Low birth weight, preterm delivery, pelvic inflammatory illness, and premature rupture of membranes have all been linked to TV during pregnancy (Silver et al.,2014). One study found a link between maternal TV exposure and children's intellectual impairment (Mann et al., 2009).Rarely,TV infection can be transmitted perinatally and (Schwandt *et al.,*2008).cause vaginal and respiratory infections in neonates (Carter *et al.*,2008 & Temesvari *et al.*, 200).

## 2.5. HIV acquisition and transmission

Several cross-sectional and cohort studies have found that TV+ women had a higher risk of contracting HIV than TV- women (Kissinger *et al.,2013*).

This increased susceptibility is certainly plausible for three main reasons: the inflammatory response to TV infection causes the appearance of HIV target cells to enhance (Sardana et al., 1994). TV infection can impair the mechanical barrier to HIV via punctate mucosal hemorrhages (Guenthner *et al.*, 2005) and TV infection may change the normal vaginal flora rendering it more permissive for bacterial vaginosis (Moodley *et al., 2002*). Which, in turn, can increase the risk of HIV acquisition (Van *et al.*, 2009). These consequences facilitate HIV in TV-infected women. Several studies have also demonstrated increased HIV expression among HIV+/TV+ women. Some estimates that in a community with a high prevalence of TV, as much as 20 % of HIV could be attributed to TV infection (Sorvillo *et al.*, 1998 & Chesson *et al.*, 2004). Control of TV, therefore, may provide a cost-effective strategy for reducing HIV transmission especially in settings where TV is common (McClelland *et al.*, 2008 & Price *et al.*, 2006). Or among subgroups who are at higher risk for TV such as African Americans (Sorvillo *et al., 2001*).

Several studies have linked TV to increased HIV vaginal shedding in HIV+ women. Fortunately, several studies have shown that TV treatment reduces HIV genital shedding. MTZ treatment reduced seminal HIV in HIV+ men with urethritis in Malawi who had TV diagnosed by NAAT (Price et al., 2003).In one cohort of women diagnosed by microscopy and culture in Kenya, HIV vaginal shedding was reduced following treatment (Wang et al., 2001). And another, diagnosed by culture, in Louisiana, US (Kissinger *et al., 2009*). These data underscore the importance of screening and treatment among HIV positive persons.

**2.6. HSV-2:**

TV appears to have a bidirectional relationship with Herpes Simplex Virus II (HSV-2) in the same way as it does with HIV-1. HSV-2 shedding has been linked to concomitant TV infection (Boselli et al., 2005), and women who have TV have a higher HSV-2 infection rate (Gottlieb et al., 2004).

**2.7. Neoplasia:**

There is evidence that TV is linked to the acquisition of HPV, suggesting that there may be an indirect link between TV and cervical neoplasia. According to certain research, TV is linked to a 1.9-fold increased risk of cervical neoplasia (Zhang et al., 1994). Studies of Finnish, Dutch, Belgian and Chinese women have all found elevated odds (1.4–2.0) of cervical neoplasia among women who have TV or vice versa (Depuydt *et al., 2010 &*Viikki *et al.,* 2000 & Yap *et al., 1995*). Sutcliffe et atfound an association between TV and prostate cancer in one study but not in other studies (Sutcliffe *et al.*, 2009 & Sutcliff *et al.,* 2006).

The vagina, urethra, and endocervix are all common infection sites in women. Vaginal discharge (typically widespread, malodorous, and yellow-green), dysuria, itching, vulvar discomfort, and abdominal pain are all symptoms. The normal vaginal pH is 4.5, but when you have a TV infection, it rises dramatically, often to >5. (Petrin et al., 1998). Ther 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 *et al.,* 1996).

Trichomoniasis has been associated with poor reproductive health outcomes such as low birth weight (LBW) and premature birth (Cotch *et al.*, 1997 & Silver *et al.*, 2014) .TV infection is also associated with twofold to threefold increased risk of HIV acquisition and pelvic inflammatory disease (PID) among HIV-infected women (Van *et al.,* 2008 & Kissinger *et al.,*2013) .

Diagnostic testing for TV infection identification and treatment are advised for symptomatic women and men because TV infection is so frequent and can cause such serious side effects. Screening is only suggested for HIV-positive women and is only encouraged in places like sexually transmitted illness clinics and penal facilities for asymptomatic people (Workowski et al., 2015). Wet mount microscopy and culture techniques are the most common ways for detecting TV in vaginal swabs. Wet mount microscopy is the most common method for detection of TV, and although this technique is rapid and inexpensive, it is only about 36%–75% sensitive compared with culture even in the hands of trained microscopists NAATs (Nye *et al.,* 2009) may detect a prevalence of threefold to fivefold higher than wet preparation microscopy (Schwebke *et al.,2004*).

**2.7 Chemical and physical environment of female genital tract:**

The vagina and ectocervix and the lower female reproductive tract in general, comprise a good chemical and physical barrier to invading exogenous organisms, partially because of the structure of the stratified vaginal epithelium along with the excretion of cervicovaginal fluid (CVF). The CVF is eubiotic and viscoelastic serving as an effective lubricant to facilitate the trapping of exogenous organisms and also, it is an acidified medium in which there is many of antimicrobial molecules (antibodies, defensins etc.). The adhesion of those vaginal microbiota (Boris *et al.*, 1998) .enabled by the mucosal layer (mucus and layers of dead epithelial cells). In asymptomatic women, microbiota acidify the vagina through lactic acid-production whilst bacterial vaginosis-associated bacteria (BVAB) produce many short chain fatty acids (SCFAs) that contribute the development of a dysbiotic vaginal environment ( (Aroutcheva *et al*., 2001a,b; Valore *et al*., 2002; Yeoman *et al*., 2013)

**2.8. Diagnosis of TV**

1-Microscopic examination of a wet mount of vaginal (Schwebke *et al.,* 2004).

2-Fluid, looking for trichomonads sensitivity, ranges from 44–68% compared to culture (Hobbs *et al.,* 2013).

3-Culture was the gold standard for diagnosis of *T. vaginalis,* with a sensitivity of 81–94% (Garber *et al.,2005 &* Ohelmeyer *et al.,1998*). Diamond's medium is the traditional culture method used for the isolation of *T. vaginalis* (Garber *et al.*,1987). Itis time consuming and contamination with vaginal bacteria is common, making this technique difficult.

*4-T. vaginalis* NAATs for use in female urine, endocervical swab, as well as male urine. Diagnostic sensitivity and specificity for the NAAT range from 99.5–100% and 99.4–99.9% for female genital specimens and 97.2–99.9% for male urine specimens (Schwebke *et al.,* 2018).

5- The OSOM® Trichomonas Rapid Test (Sekisui, Framingham, MA), this trichomonas rapid antigen test is an immunochromatographic capillary-flow enzyme immunoassay based on trichomonas membrane proteins, which can detect trichomonas in 10 min.

Compared with wet preparation and culture, OSOM Trichomonas test has a good sensitivity, excellent specificity and compares favourability to NAAT assays with reported sensitivities of 83%– 90% (Nye *et al.*,2009 *&* Huppert *et al.*,2007 *&* Huppert *et al.*,2005).

The sensitivity and specificity of the OSOM test, wet mount, and culture conducted on vaginal swabs from 449 sexually active women were compared in an early study. According to the reference standards of a positive wet mount or culture test, the overall prevalence of TV was 23.4 percent. The OSOM test had a sensitivity and specificity of 83.3 percent and 98.8 percent, respectively, for vaginal swabs, and it outperformed wet preparation. The OSOM test is the only POC assay that is CLIA waived, which means it does not need to be done in a laboratory and does not require any special equipment. Its high sensitivity (83–90%) and specificity make it an appropriate test for resource-constrained environments. (Madhivanan *et al.,* 2013). It is currently recommended in Q women only.

**2.9 Diagnosis and clinical presentation of pathogens in vaginal infections:**

Anaerobic bacteria Gardnerella (facultative anaerobic), Atopobium, Prevotella, Mobiluncus, etc. Aerobic bacteria, Staphylococci, Streptococci, etc. Anaerobic and/or aerobic bacteria and/or *Candida*. C. *albicans* (80–90 %), C. *glabrata* (2–5 %), C. *krusei* (1-2 %) and *Trichomonas vaginalis* (Mendling W *et al*, 2016).

Symptoms and signs of vginosis include the following: Vaginal dysbiosis or infections are usually characterised by an abnormal vaginal discharge and other vaginal signs and symptoms. They can be differentiated into vaginosis, which does not show inflammation signs, and vaginitis (usually candidosis or aerobic infection), which leads to inflammation (Aldunate M *et al*, 2015).

Schmidt *et al*; 1994; Denmark; Nonpregnant, nonmenstruating women who did or did not complain of vaginal discharge and were gynecologically examined at a general practice; 31.6% complained of vaginal discharge.

Sonnex *et al*; 1995; United Kingdom Women attending three general practices or a hospital-based genitourinary clinic in the Cambridge area 135 (45.5) had vaginal discharge; 46 (15.5) had vaginal discharge and malodor from general practice; 54 (32.9) had vaginal discharge from genitourinary clinic.

Confirmed diagnosis with Gram stain Byun *et al*; 2016 South Korea; 34.9% (90) Cartwright *et al*; 2013; United States ; 64.6% (95) Schwebke *et al* (2018); 50.5% (99)

Criteria for diagnosis of vaginosis include: In clinical settings, at least three of four Amsel criteria are necessary for a diagnosis of BV:

- homogenous, thin, grayish-white vaginal discharge;
- vaginal pH >4.5, positive whiff-amine test; and clue cells present on a wet mount of vaginal fluid. In research settings, BV is defined by the Nugent score.
- Scores of 0–3 are graded as lactobacillus-predominate normal vaginal flora, 4–6 as intermediate flora with the emergence of *G. vaginalis/Bacteroides* morphotypes and 7–10 as BV flora with disappearance of lactobacillus species, numerous *G. Vaginalis / Bacteroides* and curved Gram-variable anaerobic rods. The appearance of curved Gram-variab le anaerobic rods results in Nugent scores of 9–10 (**Kristin *et al*.,2018).**

**Molecular diagnosis:**

Among molecular fingerprinting methods, PCR-DGGE represents a rapid and reliable technique to identify the predominant microbiota in various ecological niches.

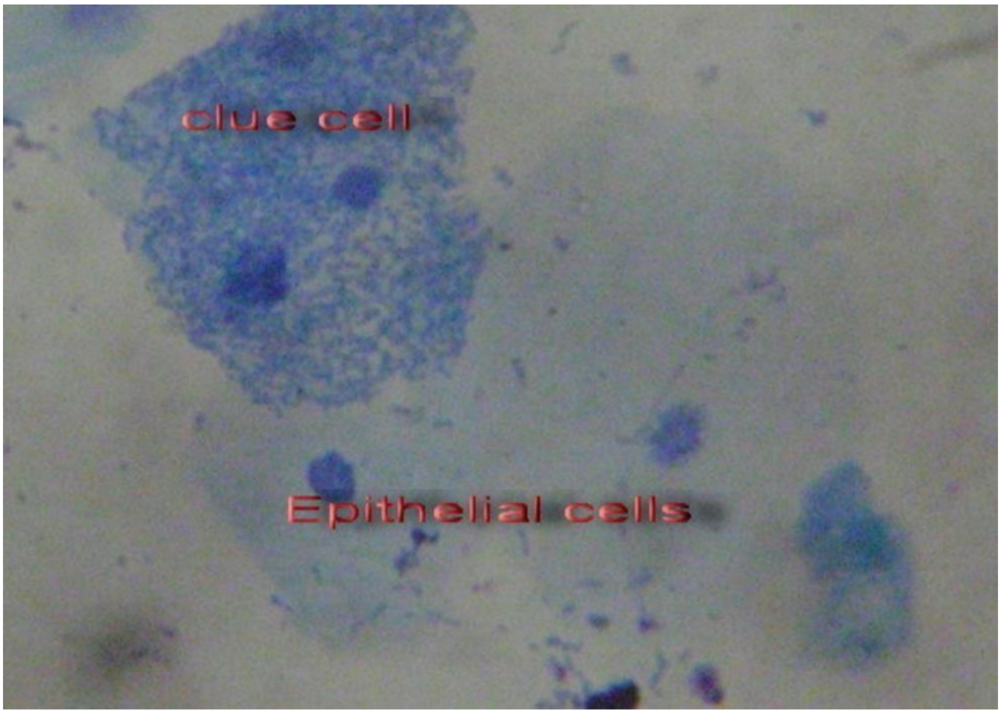


**Figure 2: Clue cell under light microscope**

Sequencing of 16S rRNA genes from different samples by constructing clone libraries, (typically at most a few thousand clones from a low number of individuals), has revolutionized our understanding of microbial systematics and diversity (Ling *et al*.,2010).

**Figure 3: Greenish Vaginal discharge in patient with TV**

## 2.10 Treatment of vaginosis:

The type of infection determines the anti-infective drug to use: 5-nitroimidazoles (e.g., metronidazole, tinidazole) are used to treat BV and TV, according to various criteria. VVC is treated with imidazoles or triazoles (e.g., clotrimazole, miconazole, and fluconazole), polyenes, or ciclopiroxolamine, which are given orally or locally. As a result, different antimicrobial drugs (antibiotics, antiseptics, antimycotics, and so on) are employed as first-line therapy depending on the pathogens involved, and controlled trials have demonstrated cure rates of 70–80 percent after 4 weeks of treatment. Anti-infectives with anti-anaerobe action are recommended for the treatment of BV, and routine therapy of sexual partners is usually not advised. Currently, metronidazole and clindamycin taken orally or applied vaginally are the mainstays of BV therapy. They have different spectra of antimicrobial activity but equivalent efficacy with regard to short-term and long-term cure rates (Meites E *et al*, 2015).

## 2.11 Treatment of trichomoniasis:

The US Food and Drug Administration (FDA) has approved metronidazole and tinidazole for the treatment of trichomoniasis since 1963. (since 2004). A single 2-g dose of metronidazole or tinidazole, administered orally or intravenously if necessary, is the standard treatment. The CDC also suggests taking metronidazole 500 mg twice a day for 7 days as an alternative. Tinidazole has a half-life of approximately 12.5 hours, compared with a half-life of 7.3 hours for metronidazole. Also they exhibit higher serum and genitourinary tract drug levels (Wood BA *et al*, 1975), (Viitanen J *et al,* 1985) and (Mannisto P *et al*, 1984).

. Tinidazole is approximately 10 times more expensive than metronidazole. Both metronidazole and tinidazole are 5-nitroimidazoles, which is currently the only class of antimicrobial medications approved for effective treatment of trichomoniasis and ***T. vaginalis*** infections. Other nitroimidazoles, such as secnidazole and ornidazole, have been used as antiparasitic agents in other countries but have not been approved for use within the United States. Another nitroimidazole called fexinidazole was favorably evaluated for toxicity and is undergoing research as a potential novel antiparasitic agent (Tweats D *et al*, 2012) and (Tarral A *et al*, 2014).

Persistent or recurrent infection due to antimicrobialresistant ***T. vaginalis*** or other causes should be distinguished from the possibility of reinfection from an untreated or insufficiently treated sex partner. The CDC's Division of STD Prevention and Division of Parasitic Diseases and Malaria have accumulated experience with testing and treatment of nitroimidazole-resistant ***T. vaginalis*** and can offer susceptibility testing and management recommendations upon request. Alternative treatment options are limited as no other FDA approved therapies are available. Combination regimens have not been systematically evaluated. The most anecdotal experience has been with intravaginal paromomycin in combination with high-dose tinidazole. Some studies demonstrated treatment success with agents including intravaginal paromomycin, intravaginal boric acid, nitazoxanide, and intravaginal metronidazole/miconazole (Bosserman EA *et al*, 2011), (Sobel JD *et al*, 2001), (Nyirjesy P *et al*, 2011),Tayal SC *et al*, 1998), (Muzny C *et al*, 2012), (Aggarwal A *et al*, 2008), (Dan M *et al*, 2007) and (Schwebke JR *et al*, 2013).

Toxicities with any of the topical regimens are not high, despite painful vulvar ulcers can occur uncommonly as a self-limited side effect of paromomycin. Other attempted treatments that have been reported with a < 50% success rate include intravaginal betadine (povidone-iodine), clotrimazole, acetic acid, furazolidone, gentian violet, nonoxynol-9, and potassium permanganate. To date, no topical microbicide has shown an effect on trichomoniasis (Obiero J *et al*, 2012).

## 3. Methods:

**Study design:**

Descriptive cross sectional.

**Patients and settings:**

Pregnant women during antenatal care in selected clinics.

**Patient selection criteria:**

All pregnant women attending antenatal care with symptoms of vaginal discharge or itching.

**Sample:**

Purposive sample.

**Techniques and materials:**

The patients were interviewed and clinically examined by certified physician. Then a swab was taken through speculum examination.

Microscopic examination of the swabs was undertaken with light microscope??? And OSOM™ trichomonas rapid test was also applied for vaginal washes.

OSOM™ trichomonas rapid test (by SEKISUI®) is a qualitative test for detection of Trichomonas vaginalis based on immunochromatographic, capillary flow, dipstick technology.

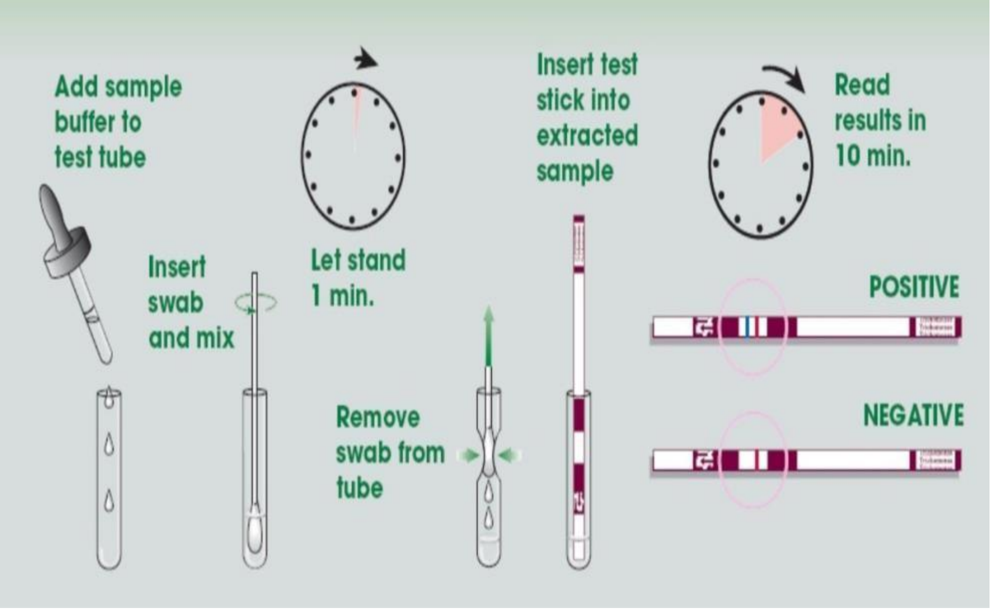


**Figure 4: Procedure of OSOM test**

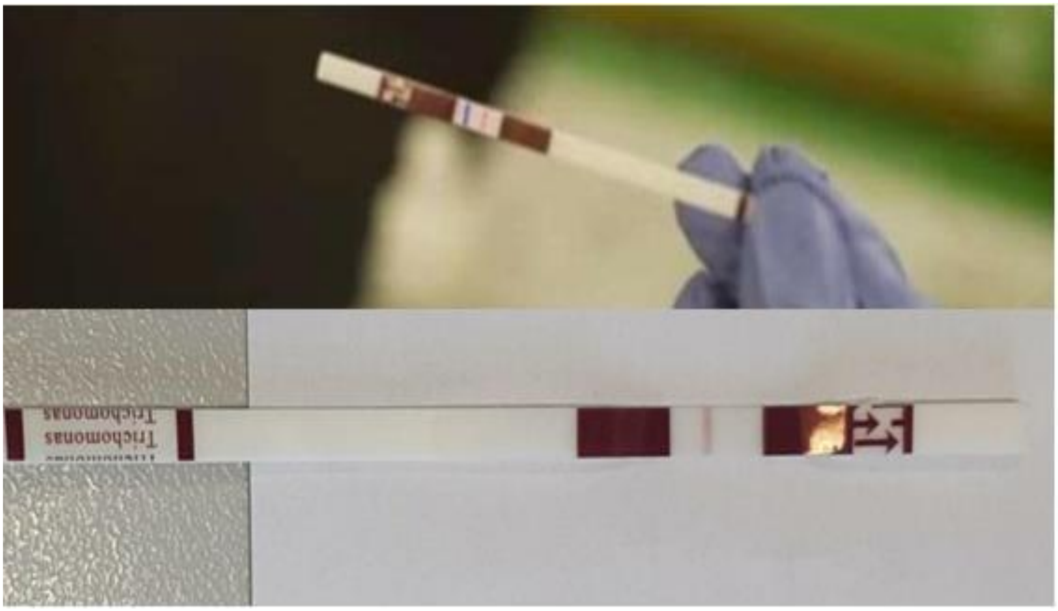


**Figure 5: Positive OSOM Test (Above) AND Negative OSOM test (Below)**

**Data synthesis:**

Data were registered on data collection form and transferred to Excel Microsoft® electronic register.

**Statistical methods:**

Statistical Package for Social Science (SPSS IBM v. 23) was used to analyze data. Descriptive statistics as frequency and percentage.

Inferential statistics were used when needed Chi-square ($X^2$) to find the difference in the distribution of the variables between the two groups, P-value were considered significant when $\leq 0.05$.

Data were presented in form of tables and figures, were the figures done by Microsoft Excel 2010.

# 4.Results

## 4.1. Health Characteristics of the Study Population:

Figure 6 shows the rates of different comorbid conditions among the population of the study. Most frequent conditions were PCOS and irregular cycles.

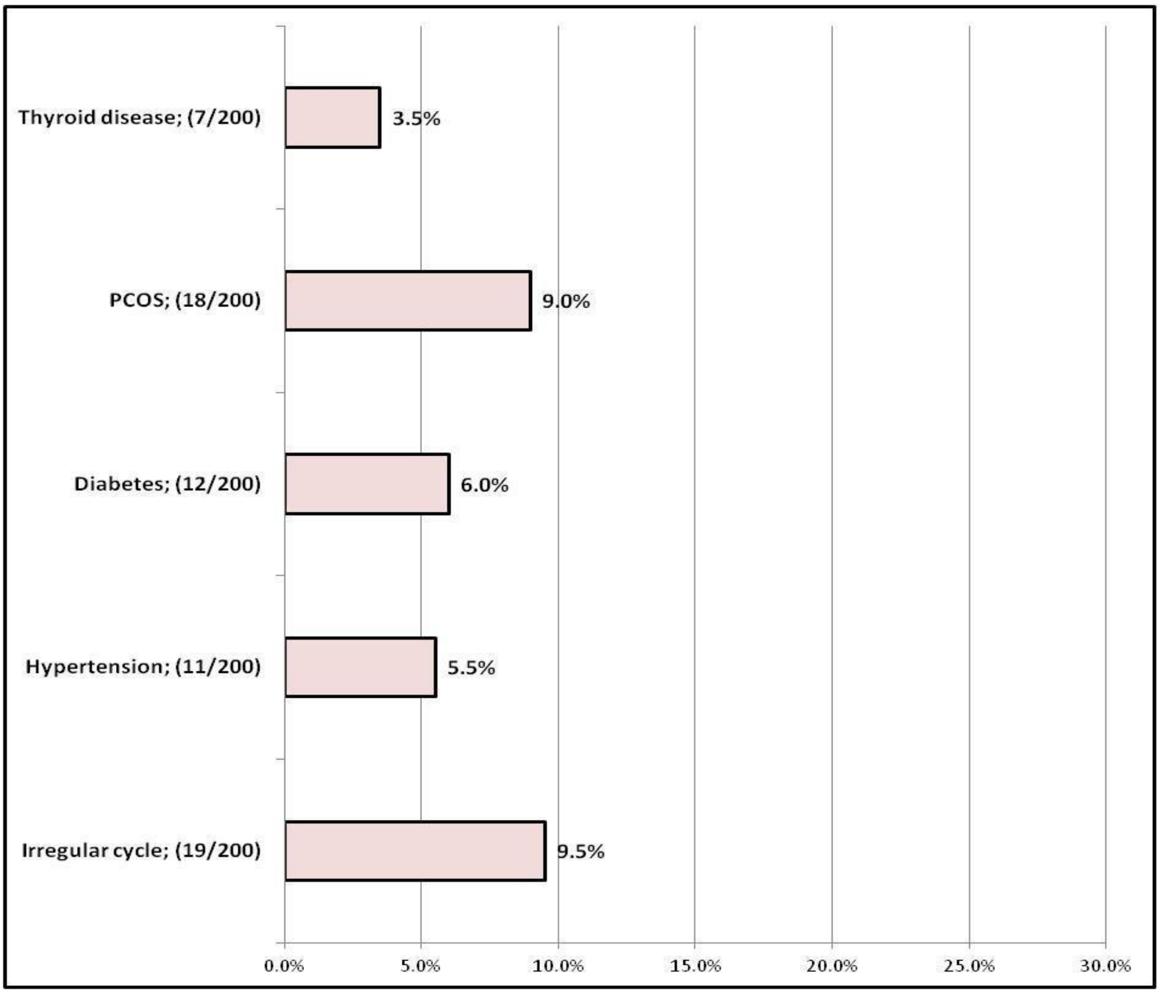


**Figure 6: Rates of co-morbid conditions among study population**

## 4.2. Symptoms distributions among the Study Population:

Figure 7 shows the distribution of different types of vaginal discharge according to the history. Sticky and whitish discharge were the most frequent.

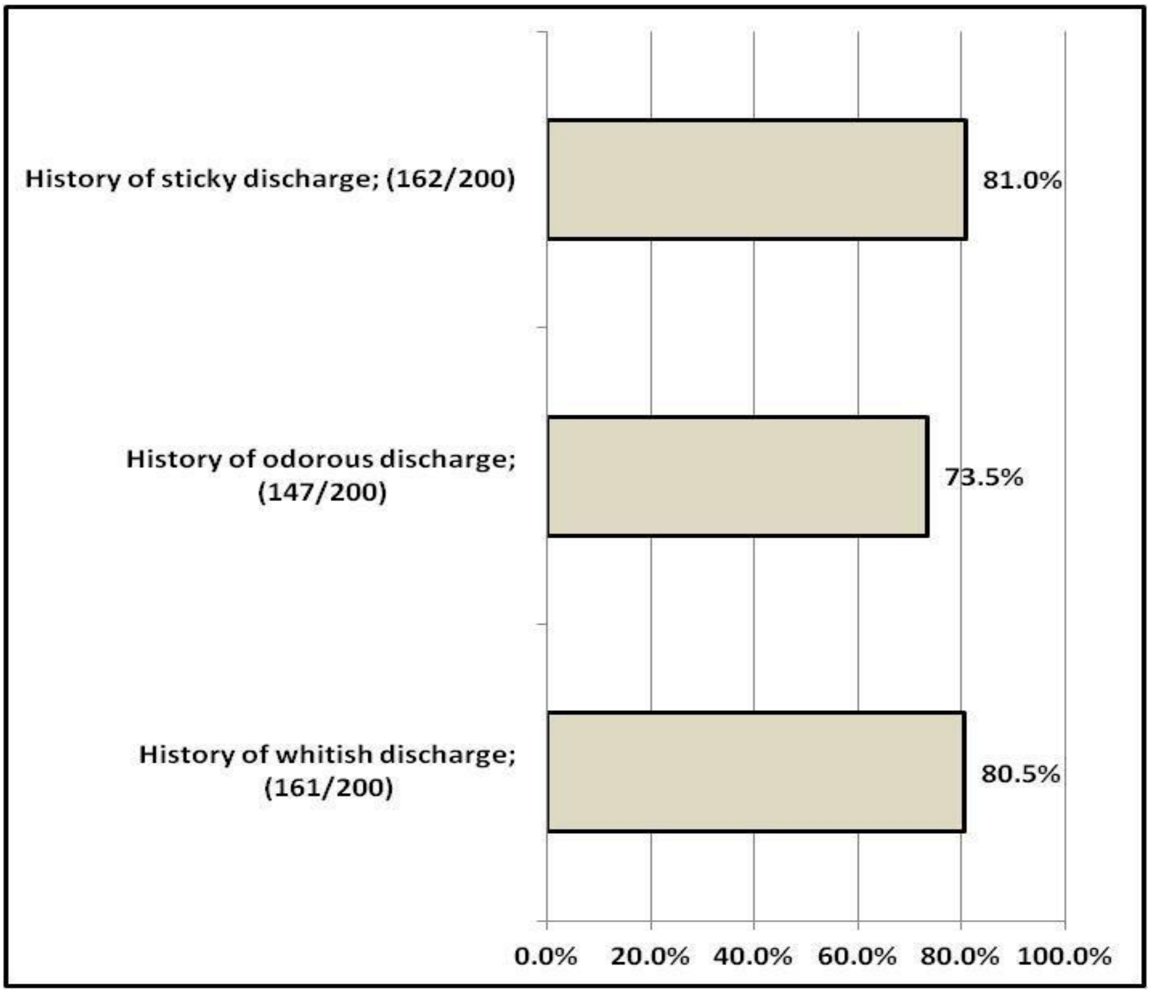


**Figure 7: Rates of different vaginal discharge characteristics among study population, history wise**

Figure 8 shows the rate of lower abdominal pain. Out of 200 cases 168 (84.0%) with lower abdominal pain , 32(16.0%) were without lower abdominal pain.

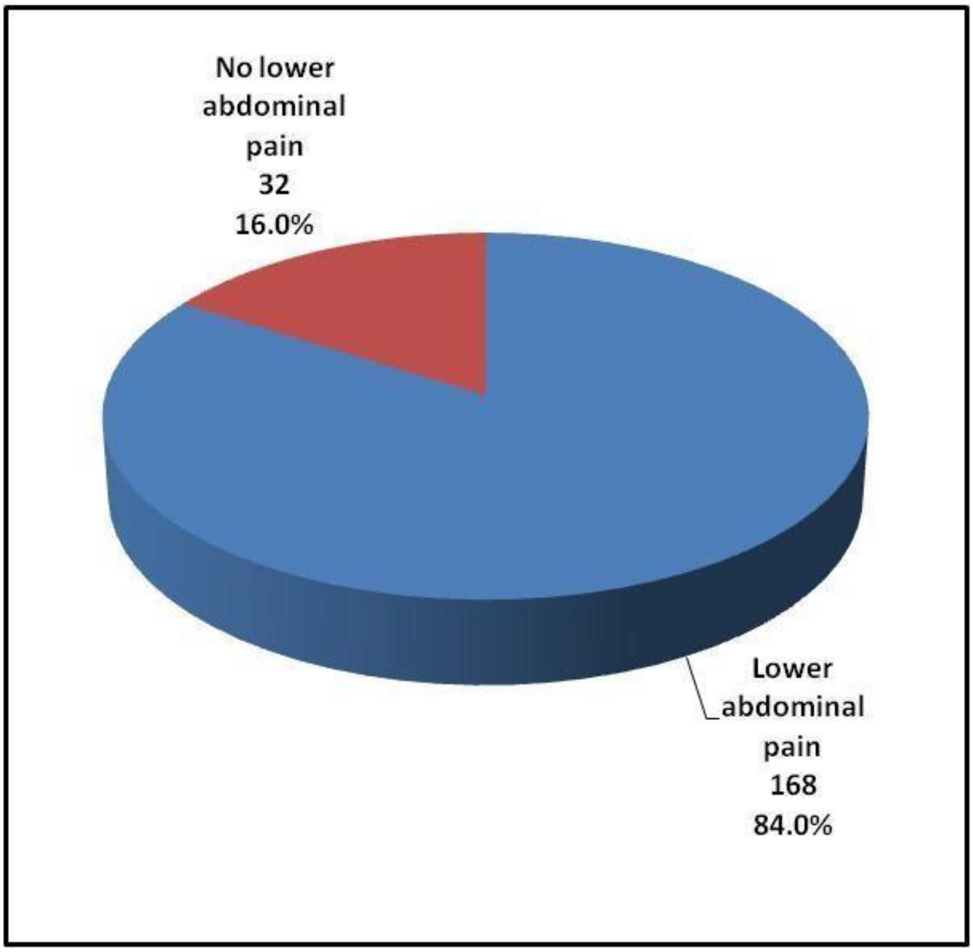


**Figure 8: Distribution of study population according to symptom of lower abdominal pain**

Figure 9 indicates that only 4.5% of the study population complains of per vaginal (PV) bleeding (9/200) and 191/200 (95.5%) without P.V bleeding.

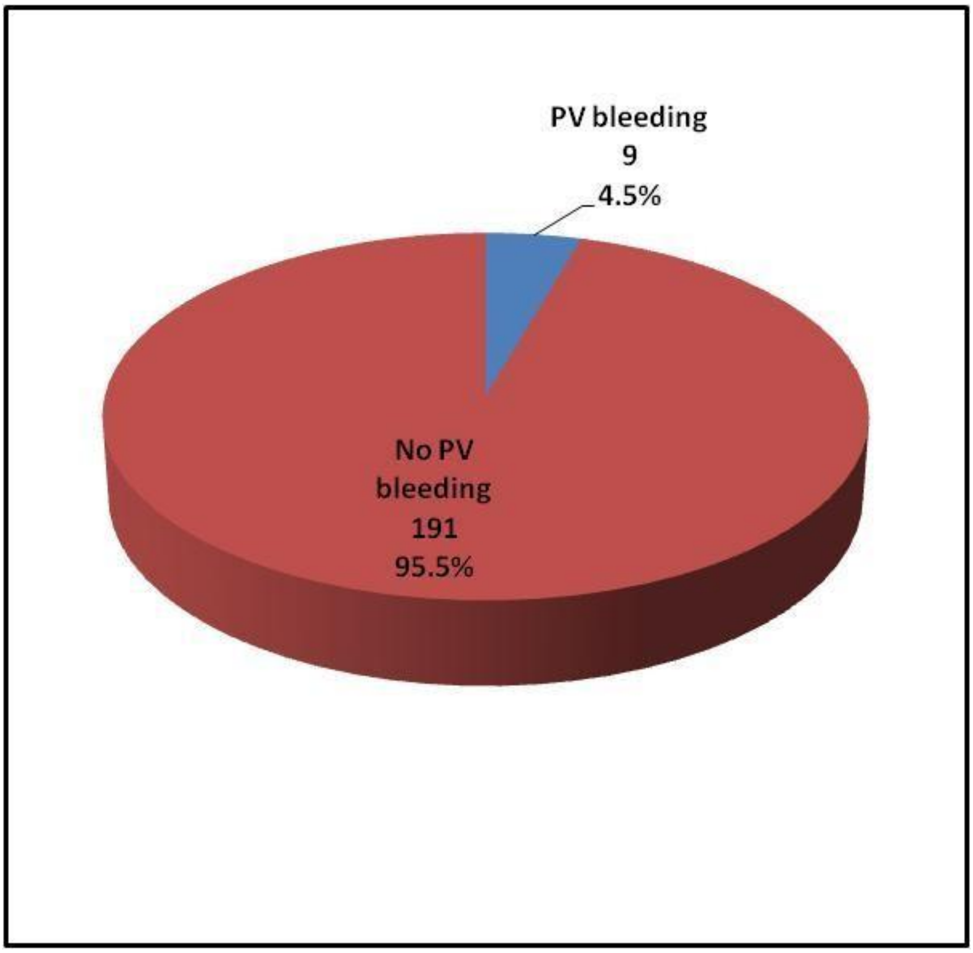


**Figure 9: Distribution of study population according to symptom of per vaginal bleeding**

Figure 10 shows that 187/200 cases (93.5%) had itching, 13/200 (6.5%) gave history\y of itching

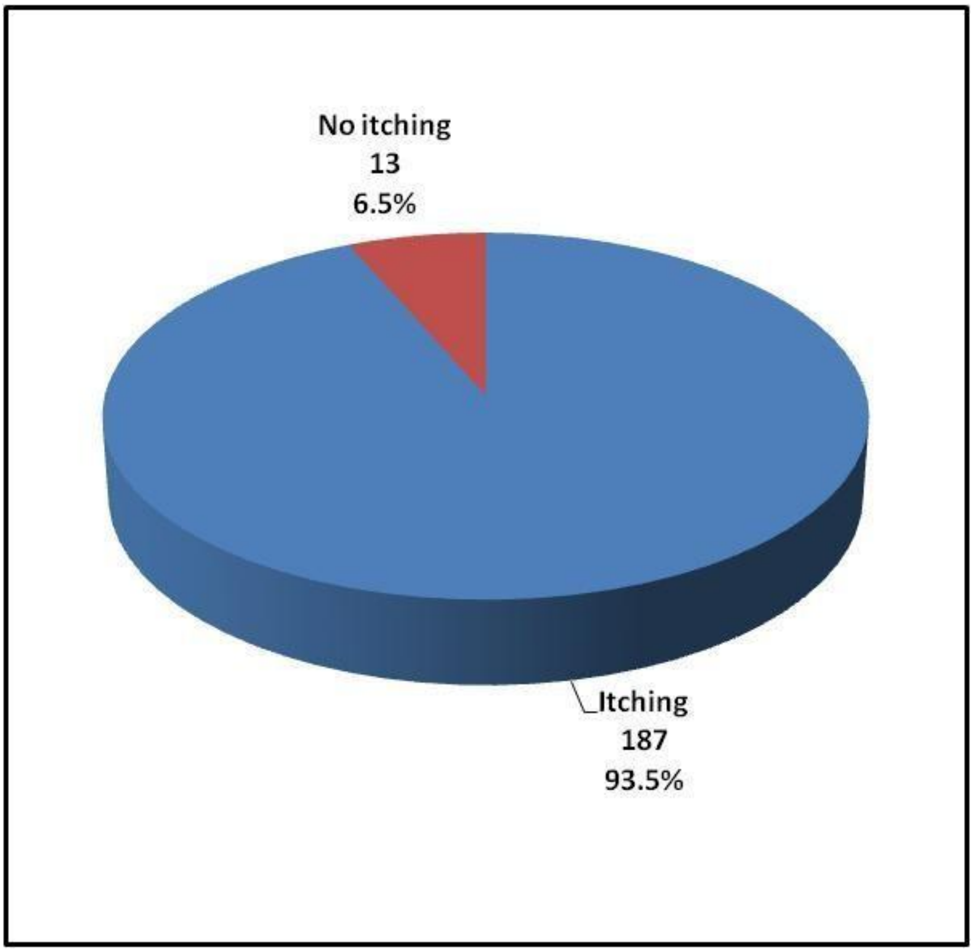


**Figure 10: Distribution of study population according to symptom of vulvar itching**

**187/200 cases (93.5%) had itching, 13/200 (6.5%) gave history\y of itching**

Figure 11 shows that Out of 200 87 (43.5%) had history of UTI, 113 (65.5%) had not UTI.

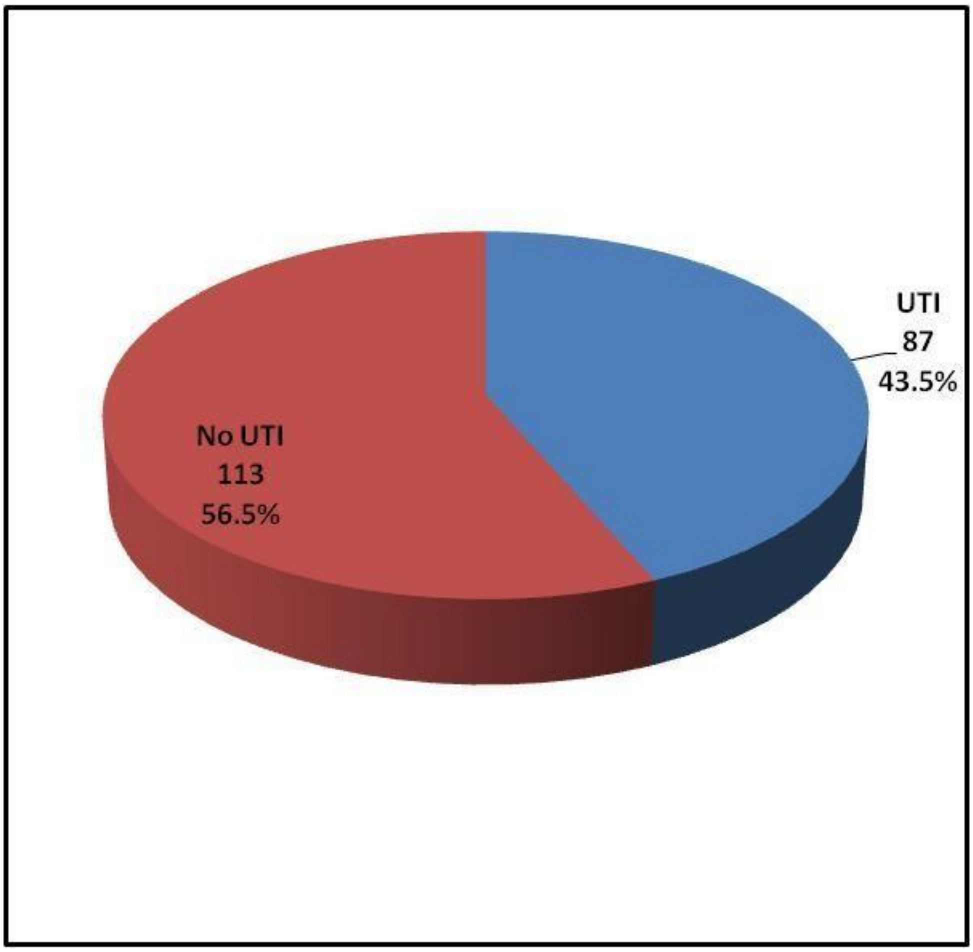


**Figure 11: Distribution of study population according to history of urinary tract infection**

## 4.3. Speculum findings distributions among the Study Population:

Figure 12 shows that 105/200 (52.5%) were with whitish vaginal discharge , 95/200 (47.5%) without whitish vaginal discharge

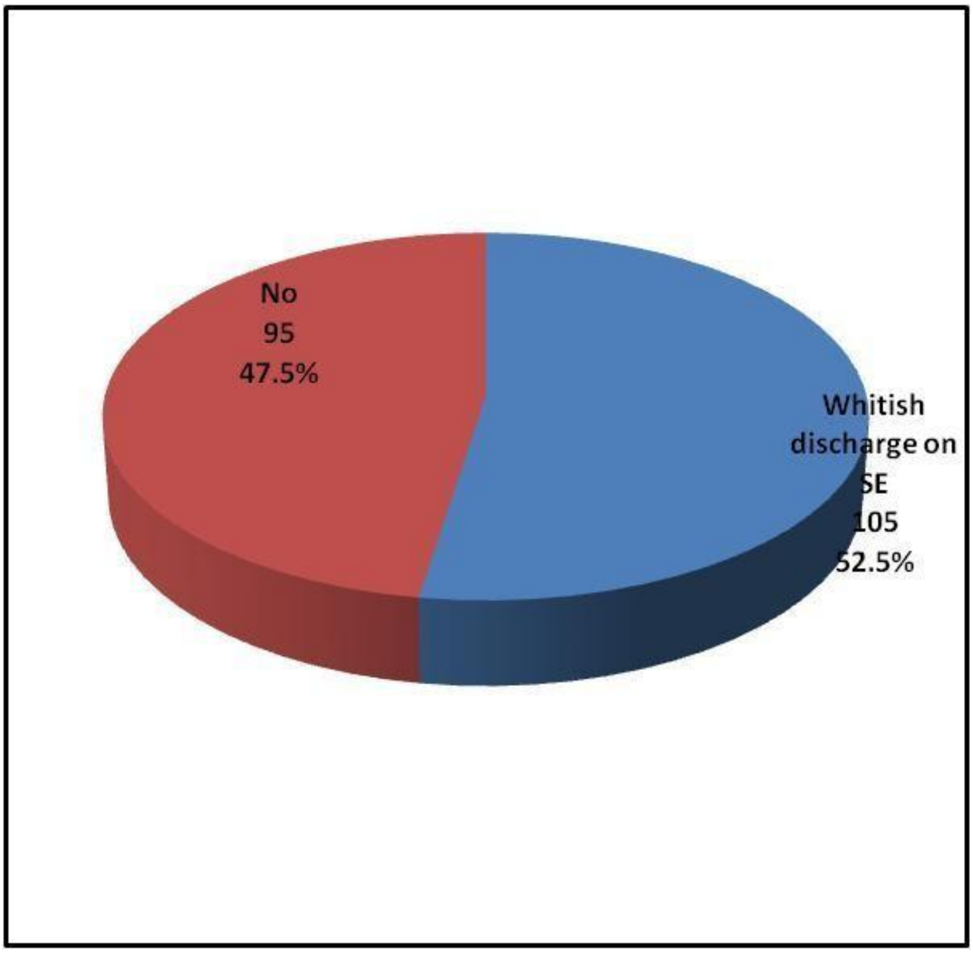


**Figure 12: Distribution of study population according to whitish vaginal discharge in speculum examination**

Figure 13 shows that out of 200 173 (86.5%) cases gave history of odorous vaginal discharge in speculum examination, 27 cases (13.5%) without.

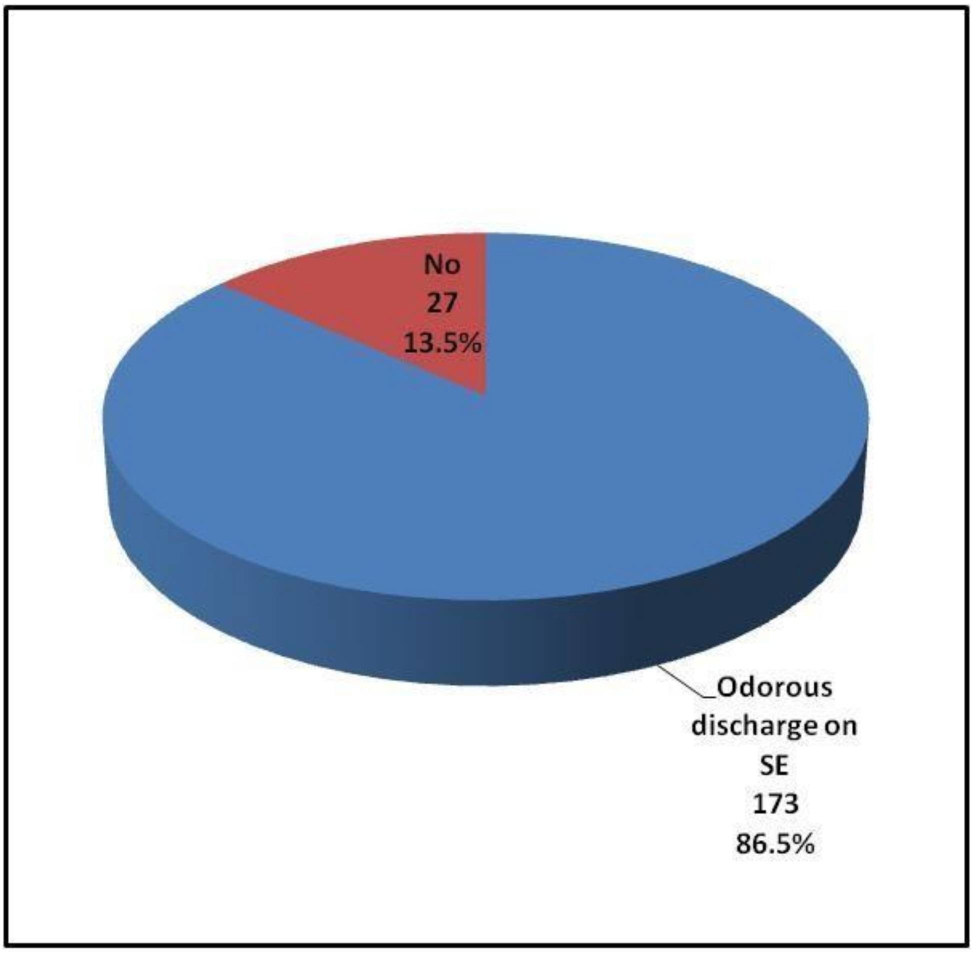


**Figure 13: Distribution of study population according to odorous vaginal discharge in speculum examination**

As in figure 14, frothy discharge and OSOM test result out of 200 cases 11(5.5%) case with frothy secretion thick discharge 72 cases (36.0%) and 117 cases (85.5%) with liquid discharge

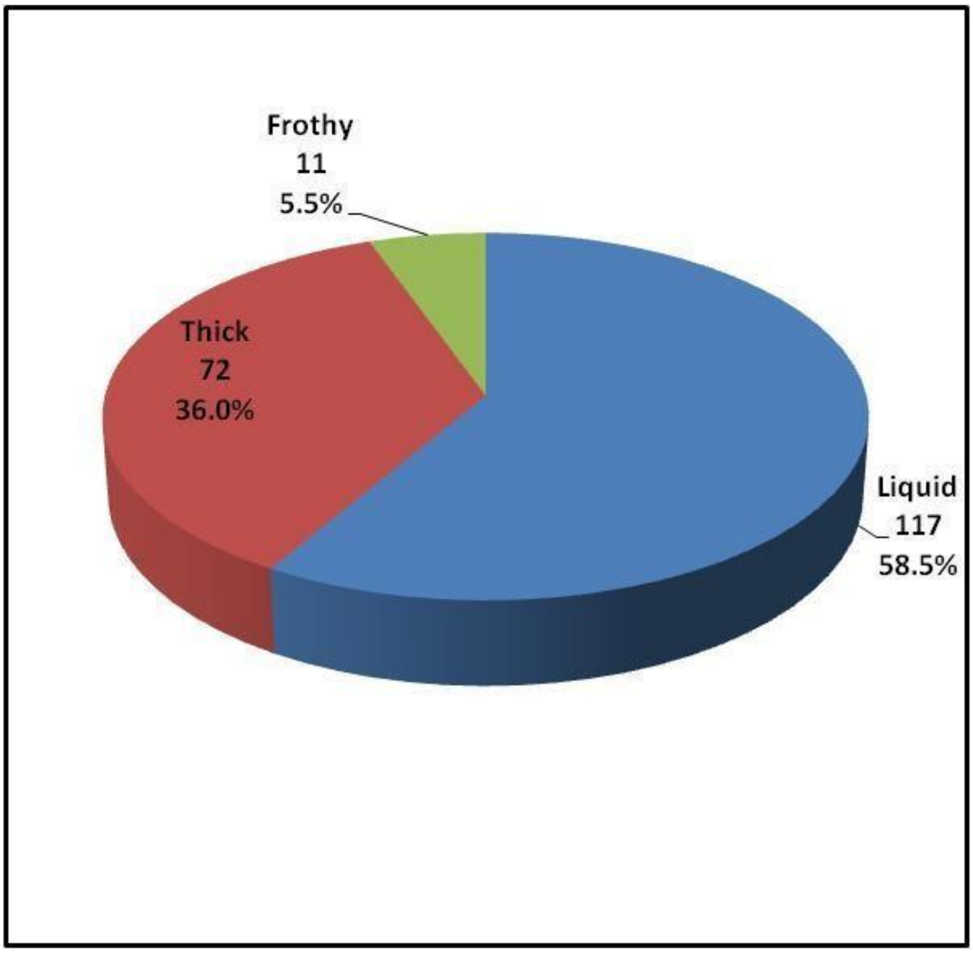


**Figure 14: Distribution of study population according to frothy vaginal discharge in speculum examination**

## 4.4. Analysis of Trichomonas findings the Study Population:

In this study out of 200 cases only 8cases (4.0%) were positive for OSOM test One case is invalid (0.5%), 191 (95.5%) were negative. See figure 15

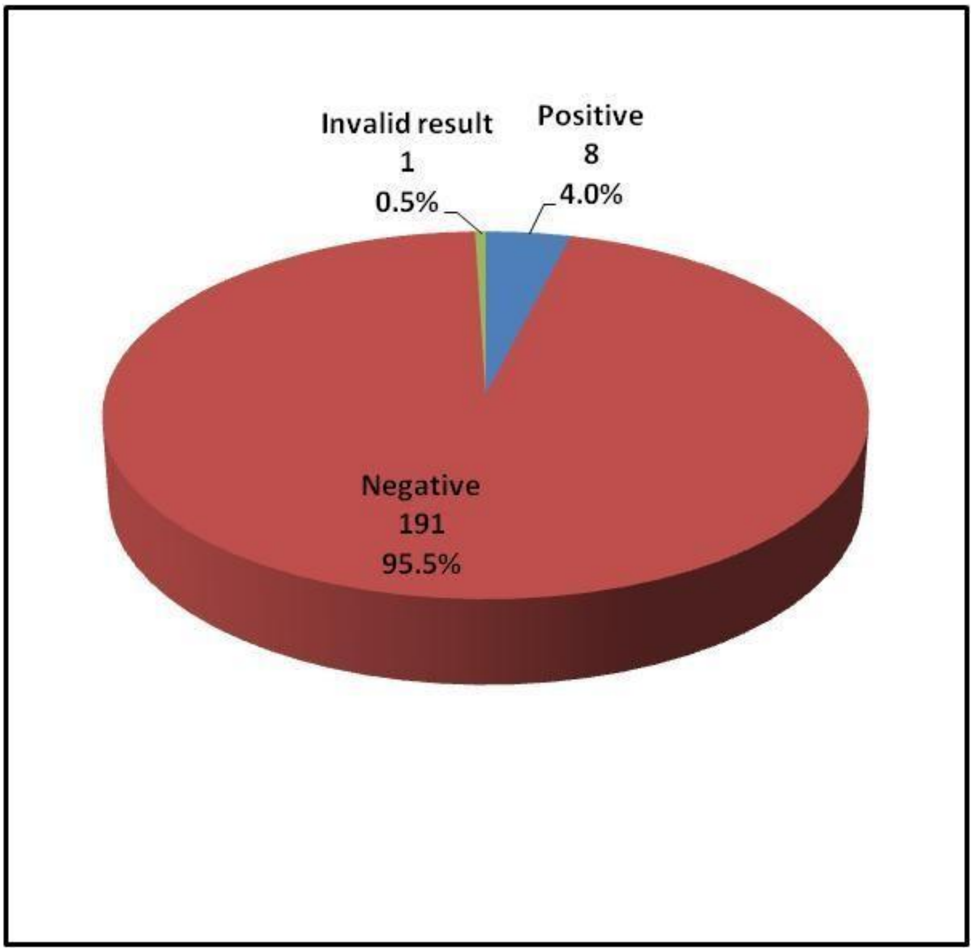


**Figure 15: Distribution of study population according to OSOM™ test result**

Out of 200 cases, 120 cases (60.0%) were Candida, 25 cases (12, 5%) were Bacteria, 47 cases (23, 5%) were mixed organisms and Trichomonas was in 8 cases (4.0%). See figure 16

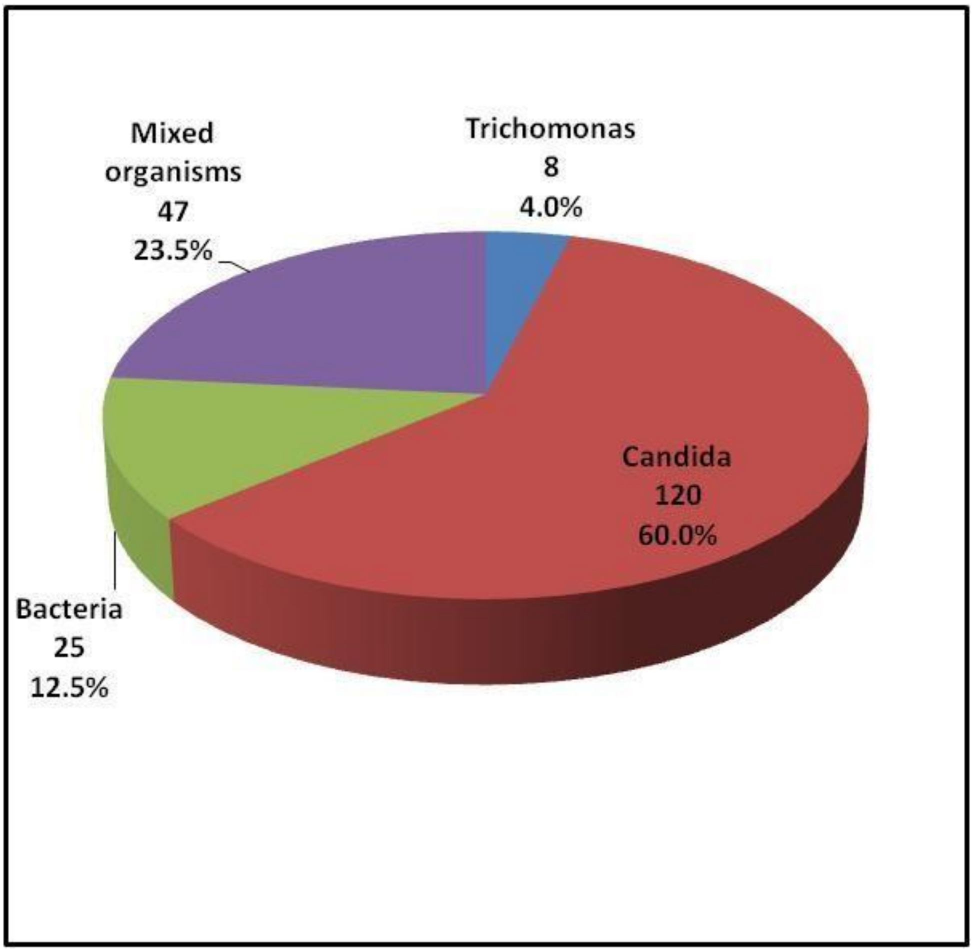


**Figure 16: Distribution of study population according to result of microscopic examination of vaginal discharge**

## 4.5. Analysis of OSOM test results among the Study Population:

Out of 200 cases 172 were from Benghazi 7/172 (4.1%) were positive, 165/172 (95.5) were negative. 28/200 (14.0%) cases were from areas out of Benghazi city. 1/28 (3.6%) were positive, 27/28 (96.4%) were negative. The difference was not statistically significant. See table 1

**Table 1: Residency and OSOM test result**

| Residency | OSOM test result | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Benghazi city | 7 | 165 | 172 |
| | 4.1% | 95.9% | 100.0% |
| Out of Benghazi city | 1 | 27 | 28 |
| | 3.6% | 96.4% | 100.0% |
| Total | 8 | 192 | 200 |
| | 4.0% | 96.0% | 100.0% |

Fisher's Exact Test, $P = 1.000$

Out of 200, 19 cases with irregular cycle, one case (5,3%) was positive OSOM; 18 cases (94.7%) were negative, 181 cases with normal cycle, 7 cases (3.9) were positive OSOM, 174 cases (96,1%) were negative OSOM. The difference was not statistically significant. See table 2

**Table 2: Irregular cycle and OSOM test result**

| Irregular cycle | OSOM test result | | Total |
|---|---|---|---|
| | **Positive** | **Negative** | |
| Yes | 1 | 18 | 19 |
| | 5.3% | 94.7% | 100.0% |
| No | 7 | 174 | 181 |
| | 3.9% | 96.1% | 100.0% |
| Total | 8 | 192 | 200 |
| | 4.0% | 96.0% | 100.0% |

Fisher's Exact Test, *P* = 0.557

Out of 200, 11 cases (100%) were hypertensive 189 cases with normal blood pressure. 8 cases (4.2%) were positive OSOM, 181 cases (95.8%) were negative. The difference was not statistically significant. See table 3

**Table 3: Hypertension and OSOM test result**

| Hypertension | OSOM test result | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Yes | 0 | 11 | 11 |
| | 0.0% | 100.0% | 100.0% |
| No | 8 | 181 | 189 |
| | 4.2% | 95.8% | 100.0% |
| Total | 8 | 192 | 200 |
| | 4.0% | 96.0% | 100.0% |

Fisher's Exact Test, *P* = 1.000

Out of 200, 12 cases (100%) were diabetic.  188 cases with normal blood sugar, 8 cases (4.3%) were positive OSOM. 180 cases (95.7%) were negative OSOM. The difference was not statistically significant. See table 4

**Table 4: Diabetes and OSOM test result**

| Diabetes | OSOM test result | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Yes | 0 | 12 | 12 |
| | 0.0% | 100.0% | 100.0% |
| No | 8 | 180 | 188 |
| | 4.3% | 95.7% | 100.0% |
| Total | 8 | 192 | 200 |
| | 4.0% | 96.0% | 100.0% |

Fisher's Exact Test, $P = 1.000$

Out of 200 18 cases were PCOS. One case (5.6%) was positive OSOM, 17 cases (94,4%) were negative, 182 cases without PCOS, 7 cases (3.8%) were positive OSOM and175cases (96.2%) were negative OSOM. The difference was not statistically significant. See table 5

**Table 5: PCOS and OSOM test result**

| PCOS | OSOM test result | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Yes | 1 | 17 | 18 |
| | 5.6% | 94.4% | 100.0% |
| No | 7 | 175 | 182 |
| | 3.8% | 96.2% | 100.0% |
| Total | 8 | 192 | 200 |
| | 4.0% | 96.0% | 100.0% |

Fisher's Exact Test, *P* = 0.536

Out of 200, 7 cases were thyroid disease. One of case (14.3%) was positive OSOM, 6 cases (85.7%) were negative, 193 cases were Euthyroid, 7 cases (3,6%) were positive and 186 cases (94.4%) were negative. The difference was not statistically significant. See table 6

**Table 6: Thyroid and OSOM test result**

| Thyroid | OSOM test result | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Yes | 1 | 6 | 7 |
| | 14.3% | 85.7% | 100.0% |
| No | 7 | 186 | 193 |
| | 3.6% | 96.4% | 100.0% |
| Total | 8 | 192 | 200 |
| | 4.0% | 96.0% | 100.0% |

Fisher's Exact Test, *P* = 0.252

No positive test result with patients with no symptoms of whitish vaginal discharge. This result was not statistically significant. See table 7

**Table 7: Symptom of Whitish discharge and OSOM test result**

| Whitish discharge | OSOM test result | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Yes | 8 | 153 | 161 |
| | 5.0% | 95.0% | 100.0% |
| No | 0 | 39 | 39 |
| | 0.0% | 100.0% | 100.0% |
| Total | 8 | 192 | 200 |
| | 4.0% | 96.0% | 100.0% |

Fisher's Exact Test, $P =$ 0.359

More proportion of positive test result with patients with odorous discharge. The result was not statistically significant. See table 8

**Table 8: Symptom of Odorous discharge and OSOM test result**

| Odorous discharge | OSOM test result | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Yes | 7 | 140 | 147 |
| | 4.8% | 95.2% | 100.0% |
| No | 1 | 51 | 52 |
| | 1.9% | 98.1% | 100.0% |
| Total | 8 | 191 | 199 |
| | 4.0% | 96.0% | 100.0% |

Fisher's Exact Test, $P =$ 0.683

Frothy discharge and OSOM test result out of 200 cases 11 case with frothy secretion, 8/11 (72.7%) were positive, 3/11 (27.3%) were negative. other discharge (liquid & thick) were in 189. 100% were negative by OSOM test. The result was statistically significant. See table 9

**Table 9: Symptom of Frothy discharge and OSOM test result**

| Frothy discharge | OSOM test result | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Frothy discharge | 8 | 3 | 11 |
| | 72.7% | 27.3% | 100.0% |
| Other types of discharge | 0 | 189 | 189 |
| | 0.0% | 100.0% | 100.0% |
| Total | 8 | 192 | 200 |
| | 4.0% | 96.0% | 100.0% |

Fisher's Exact Test, $P = 0.001$ (Significant)

Positive test results were found only with patients without whitish discharge on speculum examination. The result was statistically significant. See table 10

**Table 10: Whitish discharge on speculum examination and OSOM test result**

| SE Whitish discharge | OSOM test result | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Yes | 0 | 105 | 105 |
| | 0.0% | 100.0% | 100.0% |
| No | 8 | 87 | 95 |
| | 8.4% | 91.6% | 100.0% |
| Total | 8 | 192 | 200 |
| | 4.0% | 96.0% | 100.0% |

Fisher's Exact Test  $P$ = 0.002 (Significant)

Positive test results were found only with those with odorous discharge was found on speculum examination. The result was not statistically significant. See table 11

**Table 11: Odorous discharge on speculum examination and OSOM test result**

| SE Odorous discharge | OSOM test result | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Yes | 8 | 165 | 173 |
| | 4.6% | 95.4% | 100.0% |
| No | 0 | 27 | 27 |
| | 0.0% | 100.0% | 100.0% |
| Total | 8 | 192 | 200 |
| | 4.0% | 96.0% | 100.0% |

Fisher's Exact Test 0.601 (Non-significant)

Positive test results were found only with those with frothy discharge was found on speculum examination. The result was statistically significant. See table 12

**Table 12: Frothy discharge on speculum examination and OSOM test result**

| Frothy discharge | OSOM test result | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Frothy discharge | 8 | 3 | 11 |
| | 72.7% | 27.3% | 100.0% |
| Other types of discharge | 0 | 189 | 189 |
| | 0.0% | 100.0% | 100.0% |
| Total | 8 | 192 | 200 |
| | 4.0% | 96.0% | 100.0% |

Fisher's Exact Test $P < 0.001$ (Significant)

Patients with vaginal discharge with liquid or thick consistency on speculum examination show no positive test result. The result was statistically significant. See table 13

**Table 13: Consistency of discharge on speculum examination and OSOM test result**

| Consistency of discharge | OSOM test result | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Liquid | 0 | 117 | 117 |
| | 0.0% | 100.0% | 100.0% |
| Thick | 0 | 72 | 72 |
| | 0.0% | 100.0% | 100.0% |
| Frothy | 8 | 3 | 11 |
| | 72.7% | 27.3% | 100.0% |
| Total | 8 | 192 | 200 |
| | 4.0% | 96.0% | 100.0% |

Pearson Chi-Square 143.182 *P* <0.001

## 5. 1. Discussion:

The OSOM® Trichomonas Rapid Test (Sekisui, Framingham, MA) is a rapid antigen test which can detect trichomonas in 10 min. Compared with wet preparation and culture, OSOM Trichomonas test has a good sensitivity, excellent specificity and compares favourability to NAAT assays with reported sensitivities of 83%– 90% (Nye *et al.*,2009 *&* Huppert *et al.*,2007 *&* Huppert *et al.*,2005) .

In the present study, regarding OSOM test results, the prevalence of Trichomonas infection was 4.0% with another 0.5% inconclusive result. This result is comparable to findings of global estimates by Newman L, *et al* (2015) upon data during 2012 of 5.0% (4.0–6.4%) with regional variations from 1.0% to 11.5%. The eastern Mediterranean Region estimate of the prevalence of trichomoniasis among women was around 6% while among men was 1%.

The present study estimate compared to a systematic review by Joseph Davey DL *et al* (2016) described studies from Kenya, Tanzania, Somalia, Ethiopia, Uganda, and Sudan for prevalence of *Trichomonas vaginalis* among pregnant women. The adjusted mean prevalence was similarly high for TV in 3 studies at 6.8% (95% CI, 4.6– 9.0). The pregnancy condition of participants in those studies made comparison questionable as the pregnancy may affect the physiology of genital tract and increase susceptibility.

More recently, Rowley J *et al* (2019) published a meta-analysis including 76 data points investigating prevalence of TV infection and found that the highest prevalence among WHO regions was reported in African region; for women 11.7% (95% CI: 8.6 – 15.6). While the Eastern Mediterranean Region (EMRO) which include Libya reported the lowest prevalence for women 1.6% (95% CI: 1.1 – 2.3). The finding in the present study is still lower than the global and African estimate but higher than the EMRO one. Chemaitelly H *et al* (2019) demonstrated in a meta-analysis for studies including female sex workers in Middle East (Egypt, Iran and Pakistan) and found a high prevalence in Egypt approaching 19%.

The conservative nature and hygienic practice among the Libyan population may help in the lower prevalence of TV infection. But the prevalence is still high in comparison to some other countries in the Mediterranean region.

Among those 81.0% revealed a history of sticky vaginal discharge, 73.5% complained of an odorous discharge and 80.5% reported a whitish discharge. Also, 84.0

of participating women gave the history of lower abdominal pain and only 9/200 (4.5%) had vaginal bleeding. Urinary tract infection was a manifestation in the history of 43.5% of the study population.

Itching was reported by 93.5% of participating women in the study population.

History of irregular cycle and also Co-morbidities with hypertension, diabetes mellitus, polycystic ovarian syndrome and thyroid disorder has no statistically significant association with positive OSOM test result.

Findings of Whitish discharge ($P = 0.359$) and Odorous discharge ($P = 0.683$) had no significant association with OSOM test result.

Frothy discharge was found only in 11 cases and OSOM test result was poisitve among 8 of them (72.7%). Other types of discharge had no any OSOM test result. The difference indicates a statistically significant association ($P = 0.001$). Sensitivity as well as specificity and negative predictive value of the finding of frothy secretion were 100.0%, while the positive predictive value was 72.7%.

Compared to Hobbs *et al.,* 2013, looking for trichomonads sensitivity by vaginal discharge examination which was found ranged from 44–68% compared to culture; Garber *et al.,2005 &* Ohelmeyer *et al.,1998* using traditional culture method with a sensitivity of 81–94%. Anyhow, the finding of frothy secretion in the present study indicated much higher test performance. This may be due to the less prevalence of other sexually transmitted disease in our community.

The promising use of NAATs for *T. vaginalis* diagnosis proved diagnostic sensitivity and specificity range from 99.5–100% and 99.4–99.9% for female genital specimens and 97.2–99.9% for male urine specimens (Schwebke *et al.,* 2018). This is should be considered within the daily practice once economic value taken in account.

## 5.2. Conclusion and Recommendations:

Prevalence of Trichomonas Vaginosis in Libyan women estimate is 4.0% which is reasonable comparable to global and regional figures. OSOM test is a quick and helpful tool in screening and diagnosis of Trichomonas Vaginosis. The finding of frothy secretion can be used to predict the infection before performing the test.

The OSOM test should be available for use in maternity centers and women health clinics. A careful history and clinical findings should be obtained from the clinician. A further well designed study may be worthy to examine other characteristics might be related to the Trichomonas infection across different groups of women.

## Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

**Consent**

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

## References

Aggarwal A, Shier RM (2008). Recalcitrant Trichomonas vaginalis infections successfully treated with vaginal acidification. J Obstet Gynaecol Can; 30:55–8.

Allsworth JE, Ratner JA, Peipert JF (2009). Trichomoniasis and other sexuallytransmitted infections: results from the 2001–2004 National Health and Nutrition Examination Surveys. Sex Transm Dis.;36(12):738–44.

Aroutcheva A. A., Simoes J. A., Faro S. (2001b). Antimicrobial protein produced by vaginal Lactobacillus acidophilus that inhibits Gardnerella vaginalis. Infect. Dis. Obstet. Gynecol. 9, 33–39. 10.1155/S1064744901000060

Ashshi AM, Batwa SA, Kutbi SY, Malibary FA, Batwa M, Refaat B. Prevalence of 7 sexually transmitted organisms by multiplex real-time PCR in Fallopian tube specimens collected from Saudi women with and without ectopic pregnancy. *BMC Infect Dis*. 2015;15:569. Published 2015 Dec 15. doi:10.1186/s12879-015-1313-1

Boselli F, Chiossi G, Bortolamasi M, Gallinelli A. Prevalence and determinants of genital shedding of herpes simplex virus among women attending Italian colposcopy clinics. Eur J Obstet Gynecol Reprod Biol. 2005;118(1):86–90.

Bosserman EA, Helms DJ, Mosure DJ, Secor WE, Workowski KA. Utility of antimicrobial susceptibility testing in Trichomonas vaginalis-infected women with clinical treatment failure. Sex Transm Dis 2011; 38:983–7

Carlton JM, Hirt RP, Silva JC, Delcher AL, Schatz M, Zhao Q, *et al*. Draft genome sequence of the sexually transmitted pathogen Trichomonas vaginalis. Science. 2007;315(5809):207–12.

Carter JE, Whithaus KC. Neonatal respiratory tract involvement by *Trichomonas vaginalis*: a case report and review of the literature. Am J Trop Med Hyg. 2008;78(1):17–9.

Chemaitelly H, Weiss HA, Smolak A, Majed E, Abu-Raddad LJ. Epidemiology of *Treponema pallidum, Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis*, and herpes simplex virus type 2 among female sex workers in the Middle East and North Africa: systematic review and meta-analytics. *J Glob Health*. 2019;9(2):020408. doi:10.7189/jogh.09.020408

Chesson HW, Blandford JM, Pinkerton SD. Estimates of the annual number and cost of new HIV infections among women attributable to trichomoniasis in the United States. Sex Transm Dis. 2004;31(9):547–51.

Cotch MF, Pastorek JG, Nugent RP, *et al*. Trichomonas vaginalis associated with Low Birth Weight and Preterm delivery. *Sex Transm Dis.* 1997;24:353–60.

Crespillo-Andujar C, Díaz-Menéndez M, Mora-Rillo M. Evidence for Previously Unidentified Sexual Transmission of Protozoan Parasites. *Emerg Infect Dis*. 2018;24(3):602–603. doi:10.3201/eid2403.171838

Dan M, Sobel JD. Failure of nitazoxanide to cure trichomoniasis in three women. Sex Transm Dis 2007; 34:813–4.

Depuydt CE, Leuridan E, Van Damme P, Bogers J, Vereecken AJ, Donders GG. Epidemiology of Trichomonas vaginalis and human papillomavirus infection detected by real-time PCR in flanders. *Gynecol Obstet Invest.* 2010;**70**(4):273–280. doi: 10.1159/000314017.

Garber GE, Sibau L, Ma R, *et al*. : Cell culture compared with broth for detection of *Trichomonas vaginalis*. *J Clin Microbiol.* 1987;25(7):1275–9.

Gottlieb SL, Douglas Jr JM, Foster M, Schmid DS, Newman DR, Baron AE, *et al*. Incidence of herpes simplex virus type 2 infection in 5 sexually transmitted disease (STD) clinics and the effect of HIV/STD risk-reduction counseling. J Infect Dis. 2004;190(6):1059–67.

Guenthner PC, Secor WE, Dezzutti CS. *Trichomonas vaginalis*-induced epithelial monolayer disruption and human immunodeficiency virus type 1 (HIV-1) replication: implications for the sexual transmission of HIV-1. Infect Immun. 2005;73(7):4155–60.

Harp DF, Chowdhury I. Trichinosis’s: evaluation to execution. Eur J Obstet Gynecol Reprod Biol. 2011;157(1):3–9.

Huppert JS, Mortensen JE, Reed JL, *et al*. Rapid antigen testing compares favorably with transcription-mediated amplification assay for the detection of *Trichomonas vaginalis* in young women. Clin Infect Dis. 2007;45:194–8.

Huppert JS, Batteiger BE, Braslins P, *et al*. Use of an immunochromatographic assay for rapid detection of *Trichomonas vaginalis* in vaginal specimens. J Clin Microbiol. 2005;43:684–7.

Joseph Davey DL, Shull HI, Billings JD, Wang D, Adachi K, Klausner JD. Prevalence of Curable Sexually Transmitted Infections in Pregnant Women in Low- and Middle-Income Countries from 2010 to 2015: A Systematic Review. *Sex Transm Dis*. 2016;43(7):450–458. doi:10.1097/OLQ.0000000000000460

Kristin M . Olson, Louis J . Boohaker, Jane R . Schwebke, Stella Aslibekyan, and Christina A. Muzny. Comparisons of vaginal flora patterns among sexual behaviour groups of women: implications for the pathogenesis of bacterial vaginosis. Sex Health. 2018 Feb; 15(1): 61–67.

Kissinger P, Amedee A, Clark RA, Dumestre J, Theall KP, Myers L, *et al*. *Trichomonas vaginalis* treatment vaginal HIV-1 shedding. Sex Transm Dis. 2009;36(1):11–6.

Kissinger P, Adamski A. Trichomoniasis and HIV interactions: a review. Sex Transm Infect. 2013;89(6):426–33.

Krieger JN. Trichomoniasis in men: old issues and new data. Sex Transm Dis.1995;22(2):83–96.d.

Madhivanan P, Li T, Trammell S, *et al*. Performance of the OSOM Trichomonas rapid Test for diagnosis of Trichomonas vaginalis infection among women in Mysore, India. *Sex Health.* 2013;10:320–4.

Mannisto P, Karhunen M, Mattila J, *et al*. Concentrations of metronidazole and tinidazole in female reproductive organs after a single intravenous infusion and after repeated oral administration. Infection 1984; 12:197–201.

Mann JR, McDermott S, Barnes TL, Hardin J, Bao H, Zhou L. Trichomoniasis in pregnancy and mental retardation in children. Ann Epidemiol. 2009;19(12):891–9.

Martinez-Garcia F, Regadera J, Mayer R, Sanchez S, Nistal M. Protozoan infections in the male genital tract. J Urol. 1996;156(2 Pt 1):340–349. doi: 10.1097/00005392-199608000-00003.

McClelland RS. *Trichomonas vaginalis* infection: can we afford to do nothing? J Infect Dis. 2008;197(4):487–9.

Meites E, Gaydos CA, Hobbs MM, *et al*. A Review of Evidence-Based Care of Symptomatic Trichomoniasis and Asymptomatic Trichomonas vaginalis Infections. Clin Infect Dis. 2015;61 Suppl 8(Suppl 8):S837–S848. doi:10.1093/cid/civ738

Moodley P, Connolly C, Sturm AW. Interrelationships among human immunodeficiency virus type 1 infection, bacterial vaginosis, trichomoniasis, and the presence of yeasts. J Infect Dis. 2002;185(1):69–73.

Muriel Aldunate, Daniela Srbinovski, Anna C. Hearps, Catherine F. Latham, Paul A. Ramsland, Raffi Gugasyan, Richard A. Cone, and Gilda Tachedjian . Antimicrobial and immune modulatory effects of lactic acid and short chain fatty acids produced by vaginal microbiota associated with eubiosis and bacterial vaginosis. Front Physiol. 2015; 6: 164.

Muzny C, Barnes A, Mena L. Symptomatic Trichomonas vaginalis infection in the setting of severe nitroimidazole allergy: successful treatment with boric acid. Sex Health 2012; 9:389–91.

Newman L, Rowley J, Vander Hoorn S, *et al*. Global Estimates of the Prevalence and Incidence of Four Curable Sexually Transmitted Infections in 2012 Based on Systematic Review and Global Reporting. *PLoS One*. 2015;10(12):e0143304. Published 2015 Dec 8. doi:10.1371/journal.pone.0143304

Nyirjesy P, Gilbert J, Mulcahy LJ. Resistant trichomoniasis: successful treatment with combination therapy. Sex Transm Dis 2011; 38:962–3.

Nye MB, Schwebke JR, Body BA. Comparison of APTIMA Trichomonas vaginalis transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women. Am J Obstet Gynecol. 2009;200:188.e1–188.e7.

Nyirjesy P, Sobel JD, Weitz MV, Leaman DJ, Gelone SP. Difficult-totreat trichomoniasis: results with paromomycin cream. Clin Infect Dis 1998; 26:986–8.

Obiero J, Mwethera PG, Wiysonge CS. Topical microbicides for prevention of sexually transmitted infections. Cochrane Database Syst Rev 2012; 6:CD007961.

Ohlemeyer CL, Hornberger LL, Lynch DA, *et al*. : Diagnosis of *Trichomonas vaginalis* in adolescent females: InPouch TV culture versus wet-mount microscopy. *J Adolesc Health.* 1998;22(3):205–8. 10.1016/S1054-139X(97)00214-0

Petrin D, Delgaty K, Bhatt R, Garber G. Clinical and microbiological aspects of Trichomonas vaginalis. Clin Microbiol Rev. 1998;11(2):300–317.

Price MA, Stewart SR, Miller WC, Behets F, Dow WH, Martinson FE, *et al*. The cost-effectiveness of treating male trichomoniasis to avert HIV transmission in men seeking sexually transmitted disease care in Malawi. J Acquir Immune Defic Syndr. 2006;43(2):202–9.

Price MA, Zimba D, Hoffman IF, Kaydos-Daniels SC, Miller WC, Martinson F, *et al*. Addition of treatment for trichomoniasis to syndromic management of urethritis in Malawi: a randomized clinical trial. Sex Transm Dis. 2003;30(6):516–22.

Rowley J, Vander Hoorn S, Korenromp E, *et al*. Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. *Bull World Health Organ*. 2019;97(8):548–562P. doi:10.2471/BLT.18.228486

Nye MB, Schwebke JR, Body BA. Comparison of APTIMA Trichomonas vaginalis transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women. Am J Obstet Gynecol. 2009;200:188.e1–188.e7.

Schwebke JR, Burgess D. Trichomoniasis. Clin Microbiol Rev. 2004;17:794–803

Boris S., Suarez J. E., Vasquez F., Barbes C. (1998). Adherence of human vaginal lactobacilli to vaginal epithelial cells and interaction with uropathogens. Infect. Immun. 66, 1985–1989

Schwebke JR, Burgess D: Trichomoniasis. *Clin Microbiol Rev.* 2004;17(4):794–803, table of contents. 10.1128/CMR.17.4.794-803.2004

Schwebke JR, Gaydos CA, Davis T, *et al*. : Clinical Evaluation of the Cepheid Xpert TV Assay for Detection of *Trichomonas vaginalis* with Prospectively Collected Specimens from Men and Women. *J Clin Microbiol.* 2018;56(2): pii: e01091-17. 10.1128/JCM.01091-17

Sardana S, Sodhani P, Agarwal SS, Sehgal A, Roy M, Singh V, *et al*. Epidemiologic analysis of *Trichomonas vaginalis* infection in inflammatory smears. Acta Cytol. 1994;38(5):693–7.

Schwebke JR, Lensing SY, Sobel J. Intravaginal metronidazole/miconazole for the treatment of vaginal trichomoniasis. Sex Transm Dis 2013; 40:710–4.

Schwandt A, Williams C, Beigi RH. Perinatal transmission of *Trichomonas vaginalis:* a case report. J Reprod Med. 2008;53(1):59–61.

Silver BJ, Guy RJ, Kaldor JM, Jamil MS, Rumbold AR. Trichomonas vaginalis as a cause of perinatal morbidity: a systematic review and meta-analysis. Sex Transm Dis. 2014;41(6):369–76.

Silver BJ, Guy RJ, Kaldor JM, *et al*. Trichomonas vaginalis as a cause of perinatal morbidity: a systematic review and meta-analysis. *Sex Transm Dis.* 2014;41:369–76

Sobel JD, Nyirjesy P, Brown W. Tinidazole therapy for metronidazoleresistant vaginal trichomoniasis. Clin Infect Dis 2001; 33:1341–6.

Sorvillo F, Kerndt P. Trichomonas vaginalis and amplification of HIV-1 transmission. Lancet. 1998;351(9097):213–4.

Sorvillo F, Smith L, Kerndt P, Ash L. Trichomonas vaginalis, HIV, and African-Americans. Emerg Infect Dis. 2001;7(6):927–32.

Sutcliffe S, Alderete JF, Till C, Goodman PJ, Hsing AW, Zenilman JM, *et al*. Trichomonosis and subsequent risk of prostate cancer in the Prostate Cancer Prevention Trial. Int J Cancer. 2009;124(9):2082–7.

Sutcliffe S, Giovannucci E, Alderete JF, Chang TH, Gaydos CA, Zenilman JM, *et al*. Plasma antibodies against Trichomonas vaginalis and subsequent risk of prostate cancer. Cancer Epidemiol Biomarkers Prev. 2006;15(5):939–45.

Tarral A, Blesson S, Mordt OV, *et al*. Determination of an optimal dosing regimen for fexinidazole, a novel oral drug for the treatment of human African trypanosomiasis: first-in-human studies. Clin Pharmacokinet 2014; 53:565–80.

Tayal SC, Ochogwu SA, Bunce H. Paromomycin treatment of recalcitrant Trichomonas vaginalis. Int J STD AIDS 2010; 21:217–8.

Temesvari P, Kerekes A, Tege A, Szarka K. Demonstration of *Trichomonas vaginalis* in tracheal aspirates in infants with early respiratory failure. J Matern Fetal Neonatal Med. 2002;11(5):347–9.

Tweats D, Bourdin Trunz B, Torreele E. Genotoxicity profile of fexinidazole—a drug candidate in clinical development for human African trypanomiasis (sleeping sickness). Mutagenesis 2012; 27: 523–32.

Viitanen J, Haataja H, Mannisto PT. Concentrations of metronidazole and tinidazole in male genital tissues. Antimicrob Agents Chemother 1985; 28:812–4.

van de Wijgert JH, Morrison CS, Brown J, Kwok C, Van Der Pol B, Chipato T, *et al*. Disentangling contributions of reproductive tract infections to HIV acquisition in African Women. Sex Transm Dis. 2009;36(6):357–64.

Viikki M, Pukkala E, Nieminen P, Hakama M. Gynaecological infections as risk determinants of subsequent cervical neoplasia. *Acta Oncol.* 2000;**39**(1):71–75. doi: 10.1080/028418600431003.

Van Der Pol B, Kwok C, Pierre-Louis B, *et al*. Trichomonas vaginalis infection and human immunodeficiency virus acquisition in African women. J Infect Dis. 2008;197:548–54.

Valore E. V., Park C. H., Igreti S. L., Ganz T. (2002). Antimicrobial components of vaginal fluid. *Am. J. Obstet. Gynecol*. 187, 561–568. 10.1067/mob.2002.125280

Wang CC, McClelland RS, Reilly M, Overbaugh J, Emery SR, Mandaliya K, *et al*. The effect of treatment of vaginal infections on shedding of human immunodeficiency virus type 1. J Infect Dis. 2001;183(7):1017–22.

Werner Mendling, Ernst Rainer Weissenbacher, Stefan Gerber, Valdas Prasauskas, and Philipp Grob. Use of locally delivered dequalinium chloride in the treatment of vaginal infections: a review. Arch Gynecol Obstet. 2016; 293: 469–484

Wood BA, Monro AM. Pharmacokinetics of tinidazole and metronidazole in women after single large oral doses. Br J Vener Dis 1975; 51:51–3.

Workowski KA, Bolan GA Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep. 2015;64:1–137.

Yap EH, Ho TH, Chan YC, Thong TW, Ng GC, Ho LC, Singh M. Serum antibodies to Trichomonas vaginalis in invasive cervical cancer patients. *Genitourin Med.* 1995;**71**(6):402–404.

Yap EH, Ho TH, Chan YC, Thong TW, Ng GC, Ho LC, *et al*. Serum antibodies to *Trichomonas vaginalis* in invasive cervical cancer patients. Genitourin Med. 1995;71(6):402–4.

Yazısız H, Koyuncu Özyurt Ö, Öztürk Eryiğit F, Özhak B, Öngüt G, Özekinci M, Dönmez L, Çolak D, Gümüş S, Öğünç D. Evaluation of Microscopic Examination, Culture and Polymerase Chain Reaction Tests in the Diagnosis of Trichomonas vaginalis Infection. Mikrobiyol Bul. 2020 Jan;54(1):135- 143

Yeoman C. J., Thomas S. M., Miller M. E., Ulanov A. V., Torralba M., Lucas S., *et al*. . (2013). A multi-omic systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. *PLoS ONE* 8:e56111. 10.1371/journal.pone.0056111

Zhang ZF, Begg CB. Is Trichomonas vaginalis a cause of cervical neoplasia? Results f

Zongxin Ling, Jianming Kong, Fang Liu,1 Haibin Zhu, Xiaoyi Chen, Yuezhu Wang, Lanjuan Li, Karen E Nelson, Yaxian Xia, and Charlie Xiang. Molecular analysis of the diversity of vaginal microbiota associated with bacterial vaginosis. BMC Genomics. 2010; 11: 488. rom a combined analysis of 24 studies. Int J Epidemiol.1994;23(4):682–90