



## RESEARCH PAPER

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## Etiological agents of Vulvovaginitis amongst women complaining of genital tract infection in Soran City, Kurdistan, Iraq

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### Abstract

Vulvovaginitis is one of the commonest reproductive tract infections in women worldwide. There are three types of infectious vaginitis including, bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis. The present study aimed to characterize the causative agents of vulvovaginitis among women attending Soran Obstetrics and Gynecology hospital. Host-related and some behavioral risk factors which proposed as predisposing factors for this disease were also investigated. A pair of high vaginal swabs was collected from 97 vulvovaginitis women aged 18-52 years. Trichomoniasis was detected by wet and stained smears. Candidiasis was identified by direct examination and culturing on both Sabouraud dextrose agar and Chromagar medium. Bacterial infection was confirmed by Nugent scoring method after Gram staining. The present results showed that 85 (87.6%) cases were positive for etiologic agents and 12(12.3%) with nonpathogenic agents. Among the positive patients, the commonest organisms was bacteria 45%, followed by *Candida* species 15%, *Trichomoniasis* as 7%, and mixed infection with these three agents in 33%. Moreover, infection with non-*albicans Candida* like *C. galabrata*, *C. krusei*, and *C. dubliensis* were also detected. Pregnant women showed high percentage of infection with trichomoniasis and candidiasis. Women that used IUD as contraceptive methods, as well as those of non-contraceptive users were more susceptible to bacterial infection than the other. High percentage of women was infected with the etiologic agents that responsible for vulvovaginitis particularly with bacteria. Routine culturing of vaginal discharge must be performed for these patients; also antibiotics susceptibility of bacterial isolates should be determined. In addition, comprehensive healthcare education plan is needed to manage the disease.

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## Introduction

Vulvovaginitis includes infectious and noninfectious conditions involving vulva and vagina (Mc Cormack and Augenbraun, 2015). It is a common disease that affects women and girls of all ages specifically the sexually active women. It is considered as one of the most important public health issues in both developed and developing countries. Vaginal complaints are the most common reason for gynecological consultation and account for approximately 10 million office visits annually (Anderson *et al.*, 2004). Recently, Loveless and Myant (2017) summarized that this disease is the most prevalent gynecological concern presenting in pediatric and adolescent girls.

Bacterial vaginosis (BV), vulvovaginal candidiasis (VC), and trichomoniasis are diagnosed in 70% of patients, including 40% to 50% with BV, 20% to 25% have VC, and 15% to 20% infected with trichomoniasis. The remaining 30% are undiagnosed that can have physiologic discharge (leucorrhea), atrophic vaginitis, and vulvar dermatologic abnormalities, or vulvodynia. (Mulley, 2000; Vaginitis, 2006). The main characteristics of vaginitis are vaginal discharge, vulvar itching /irritation and malodor, which consider as important reason for women to seek help of a health care providers (Mills, 2017; Narayankhedhar *et al.*, 2015).

Normal vaginal ecosystem is a physiological important biomass, in which glycogen rich epithelial cells are always shed. As the cells autolysis, glycogen depolymerizes to glucose, which serves as an energy source for the commonly inhabit vagina *Lactobacilli* spp. These bacteria metabolize glucose to lactic acid, which lead to a normal vaginal pH of about 4.0 that inhibit the growth of pathogenic bacteria (Ravel *et al.*, 2011). In addition to producing lactic acid, lactobacilli may also produce hydrogen peroxide, which is bactericidal alone and highly effective when integrate with physiologic amounts of myeloperoxidase and chloride. (Klebanoff *et al.*, 1991). Several researchers pointed that human vaginal microbiota seem to play an important role in preventing many urogenital diseases, like BV, VC, sexually transmitted infections,

urinary tract inflammations (Gupta *et al.*, 1998; Martin *et al.*, 1999; Wiesenfeld *et al.*, 2003) and Human immunodeficiency virus type 1 infection. (Lai *et al.*, 2009; Taha *et al.*, 1998). *Trichomonas vaginalis* infection is the most prevalent non-viral sexually transmitted infection worldwide. It caused by unicellular, aerotolerant, flagellated protozoan parasite that restricted to human. Globally, an estimated of 170 million cases of trichomoniasis were reported. The majority of these cases were in developing countries (Gerbase *et al.*, 1998). In United States, approximately 3.7 million people are infected with *T. vaginalis*, more than chlamydia and gonorrhea collectively (Meites, 2013; Satterwhite *et al.*, 2013). Although this infection is common worldwide, it has been considered a “neglected” parasitic infection, because of a lack of public awareness and due to restricted knowledge of its sequelae and associated costs. (Meites, 2013; Secor *et al.*, 2014).

Furthermore, Alcaide and colleagues (2015) highlighted the high rates of reinfection or treatment failures in trichomoniasis women and emphasized the need for rescreening them after treatment by using sensitive and specific test. The center of disease control recommends the use of more sensitive method to detect *T. vaginalis* of both symptomatic and asymptomatic infections. Nucleic acid amplification tests (NAATs) are more sensitive than wet preparation in about 3-5 times, and both of vaginal secretions or urine can be tested by NAAT. (Workowski and Bolan, 2015).

Moreover, trichomoniasis increases the risk of complications in pregnancy. The percentage of preterm birth was increased by 42% among infected women. As well as the risks of delivery of a small for gestational age infant also increased. (Malone, 2017). There is strong evidence suggests the correlation between human immunodeficiency virus (HIV) and *T. vaginalis*. This parasitic infection in women infected with (HIV) enhances HIV transmission by increasing genital shedding of the virus (Hirt and Sherrard, 2015; Schwebke and Burgess, 2004). In addition, trichomoniasis has been interacted with incidence of cervical cancer (Boyle & Smith, 1999; Gram *et al.*, 1992).

VC is an infection caused by *Candida* species that affects millions of women yearly and considered as significant public health problem (Goncalves *et al.*, 2016). Some researchers have estimated that about 70-75% of adult women are inflected with at least one episode of VC during their lives .I t is considered as the second most common cause of vaginitis after BV (Sobel, 2007). Inhibition of normal microbial flora by antibiotics encourages the growth of yeasts, although VC sometimes happens after treatment of trichomoniasis or BV (Agnew and Hillier, 1995).

*Candida spp* perhaps become pathogenic and lead to candidiasis under some circumstances, such as immune-suppressed specifically due to steroid use and prolonged broad spectrum antibiotics treatment, pregnancy, used of oral contraceptive, malnutrition, diabetes, and obesity, (Nyrjesy 2014; Okungbowa *et al.*, 2003).

*C. albicans* is the most common cause (80%-90%), whereas other yeasts account for up to 20% of cases. *C. tropicalis* is isolated from 1% to 5% and may be associated with a higher rate of recurrence after standard treatments. (Horowitz *et al.*, 1985; Spinillo *et al.*, 1997). *C. glabrata* accounts for about 10% of vaginal yeast isolates (Geiger *et al.*, 1995; Spinillo *et al.*, 1997). The pathogenicity of *Candida* species is mediated by several virulence factors like, adhesion, hyphal and biofilm formation, extracellular hydrolytic enzyme production, and phenotypic switching (Goncalves *et al.*, 2016; Tamura *et al.*, 2007).

BV is considered as the main cause of symptomatic vaginitis with vaginal discharge and malodor with prevalence about 15% to 50%. It is the most common reason for lower genital tract disorder among women of child- bearing ages (Allsworth and Peipert, 2007). It reflects alteration in vaginal microbiota from H<sub>2</sub>O<sub>2</sub>-producing lactobacilli to many pathogenic bacteria (Hillier 1993). The disease is related to the disruption of vaginal environment rather than to the occurrence of these bacteria (Mills, 2017). Histologically, there is an absence of inflammation in biopsies of the vagina, therefor the term *vaginosis* rather than *vaginitis* was used. More recently, Yudin and Money (2017) recommended that

diagnosis and treatment of BV is important for symptom resolution in pregnant women. In another current study ,they concluded that BV significantly associate with preterm delivery ( Tellapragada *et al.*, 2017). Also this finding beside other adverse pregnancy outcomes were previously documented by several researchers (Carey and Klebanoff, 2005).

The goal of this study was to highlight the etiological agents of Vulvovaginitis among women complaining of genital tract infection in Soran city. Also the association between this disease and various behavioral risk factors was explained.

## Materials and methods

### *Samples collection*

High –vaginal swabs were collected from 97 vulvovaginitis women aged 18-52 years during October 2016 to March 2017. Pair of vaginal swab was obtained from each participant after speculum examination and clinical presentation which was confirmed by specialized gynecologist in Soran obstetrics and gynecology hospital and private clinics in Soran city/Erbil-Iraq. The first swab was used for culturing, and the second was used for direct wet smear examination, 10% KOH test, and stained smears.

### *Trichomoniasis identification*

*T. vaginalis* infections were identified by jerky motility of the organisms in wet direct mount. Dried smears stained with Giemsa and Leishman stains were also prepared. All slides were examined under 40 X and 100 X of compound microscopy (Garcia and Ash, 1979; Yazar *et al.*, 2002).

### *Candidiasis identification*

VC was confirmed by direct wet preparation with 10% KOH which clarify the appearance of yeast cells or pseudohyphae and killed the majority of bacteria, dry smear for Gram staining, and culturing on Sabouraud's dextrose agar supplemented with chloramphenicol. The plates were incubated at 37C for 48hr. Then smears were prepared from each growth and stained with lactophenol cotton blue for confirming the characteristic of *Candida* as blue budding yeast cells (AL-Attraqhchi *et al.*, 2013).

Moreover, *Candida* isolates were identified by germ tube formation for distinguishing *C. albicans* from other non- *Candida albicans* species (Cheesbrough, 2006) and by culturing on Chrom agar as differential and selective medium for classifying *Candida* spp. which differentiate *Candida* species depending upon the specific color that produced by each one (Yadav and Prakash, 2016). As well as the antifungal activity of Nystatin was assessed using agar disc diffusion technique; three concentrations (16.6, 8.3, 4.15mg/ml) of the drug were tested against the clinical isolates of *Candida* spp. Diameters of the inhibition zone surrounding each disc was measured in comparison with zone Diameter Interpretive standards (Table. 2) (Maroszynska *et al.*, 2013).

#### Bacterial vaginosis identification

BV was assessed according to Nugent scoring system. Gram stained slides were prepared and examined under 100X; about 10-15 microscopic fields were checked for each samples, A Nugent score of 0-3 was considered as negative for BV and number of 4-6 account for intermediate, whereas a score of 7-10 was recorded as consistent with BV (Naravankhedkar *et al.*, 2015; Nugent *et al.*, 1991). In addition, the presence of Amsel *et al.* criteria for diagnosis of BV was observed in most of BV samples by presence of clue cells (the borders of epithelial cells intensively covered with bacilli after Gram staining), fishy amine odor or positive whiff test after adding 0%KOH solution, and presence of vaginal homogeneous white discharge (Amsel *et al.*, 1983).

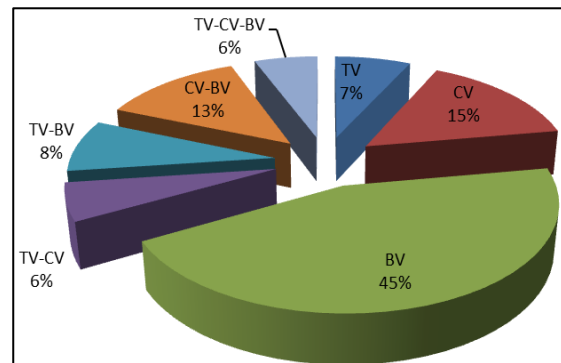
#### Statistical analysis

Chi-square test was used to analyze the data. If P value <0.05 so considered as statistically significant.

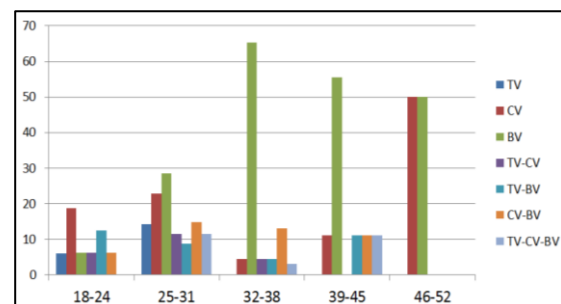
#### Results

The results of the current study revealed that amongst the 97 enrolled vulvovaginitis women, 85 (87.6%) cases were positive for etiologic agents and 12(12.3) with nonpathogenic agents. Among the positive patients, BV was the most common type of vulvovaginitis (45%), followed by VC (15%), *Trichomonas* vaginitis (TV) (7%), mixed infection of VC+BV (13%), TV+BV (8%) and (6%) for both of TV+VC and TV+VC +BV. The total mixed infections was about 33% Fig. 1.

Regarding the age groups, the highest percentage of infection with TV was in 25-31 years age interval which was 14.28%. Candidiasis also distributed remarkably in the age group 25-31years as 22.8% if we excluded the age group 46-52 years owing to few cases (just two). Moreover high percentage of BV was confirmed within 32-38 years age group 65.2%. As well as, maximum percentage of mixed infection with vaginitis pathogens was found in 25-31 age group. No significant differences was observed. Fig.2.



**Fig. 1.** Repartition of patients according to etiological agents Bacterial vaginosis (BV), Candidal vaginitis (CV), *Trichomonas* vaginitis (TV).

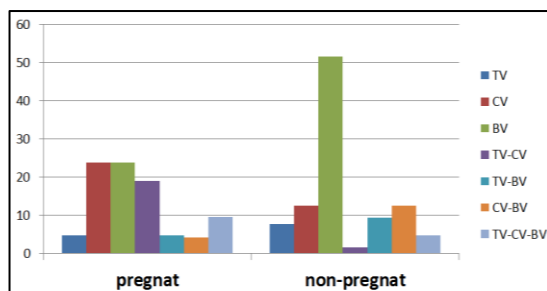


**Fig. 2.** Relationship between age groups and vulvovaginitis. Bacterial vaginosis (BV), Candidal vaginitis (CV), *Trichomonas* vaginitis (TV).

According to pregnancy out of 85 infected women included in this study, 21 showed to be pregnant and 64 non –pregnant women. The distribution of TV was higher in non-pregnant women 7.8% compared in pregnant women 4.7% as single infection, but when take in account the total infection with both single and mixed, the result showed that pregnant women had higher percentage 38.09% (8/21) than non-pregnant women 23.4% (15/64) with no significant differences.

VC was more prevalent in pregnant women 23.8% than in non-pregnant women 12.5%. Also the overall percentage of single and mixed infection of pregnant women with candidiasis was higher (14/21) 66.6% than that of non-pregnant women 31.2% (20/64).

Concerning bacteria, the present result showed that the non-pregnant women had high infection 51.5% in comparison with pregnant group 23.8%. Finally the total mixed infections were detected in pregnant women 47.6% more than that recorded in non-pregnant 28.1%. Fig. 3.



**Fig. 3.** Prevalence of pathological agents among pregnant and non-pregnant women. Bacterial vaginosis (BV), Candidal vaginitis (CV), *Trichomonas* vaginitis (TV).

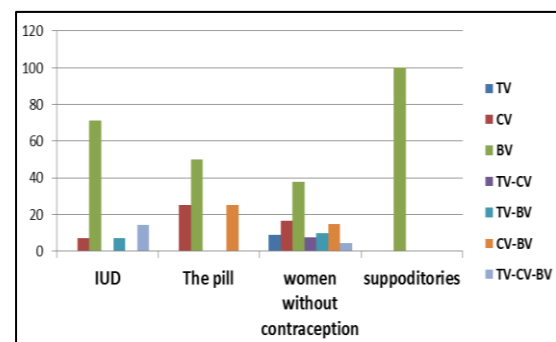
Moreover the current data showed just 19 women out of 85 women enrolled were used contraceptive methods. The most prevalent etiologic agent that detected in contraceptive user women was bacteria that reached 71.4% in Intra Uterine Device (IUD) user and 50% in women used oral pills. Beside one woman who used suppositories afflicted with BV.

No infection with trichomoniasis as single in contraceptives using women, but mixed infection with bacteria and Candida were detected in three cases. VC was recorded in low percentage as 7.1% in IUD user women for both of single and mixed infection of the three agents. Furthermore it was proved as a single infection in 1/4 (25%) and as mixed infection with bacteria 1/4 (25%) in pill user women Fig.4.

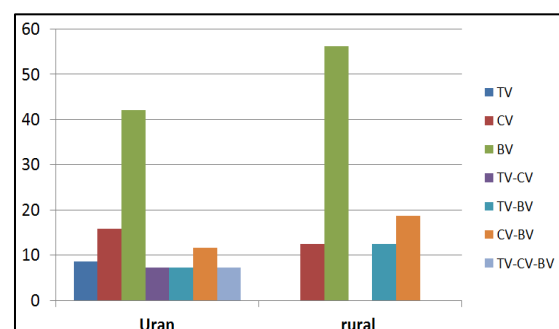
According to the residence of women, Within the 85 vulvovaginitis women, 69 and 16 of them lived in urban and rural area respectively. In women resident in urban area trichomoniasis was recorded in 8.6% as

single infection and 7.24% in mixed infection with both bacteria and candida. Whereas rural women showed 12.5% as mixed infection with bacteria. VC was distributed as 15.9% and 12.5% in urban and rural area respectively; also mixed infections with the other two pathogens were detected. Infection with the three pathological agents was identified in urban women only as 7.2% Fig. 5. Regarding BV, 56.2% in women of rural area more than that of women in urban location 42%.

In addition to, mixed infections were identified in 18 and 5 cases related to urban and rural area respectively. Statistically no significant differences were confirmed. Fig.5.



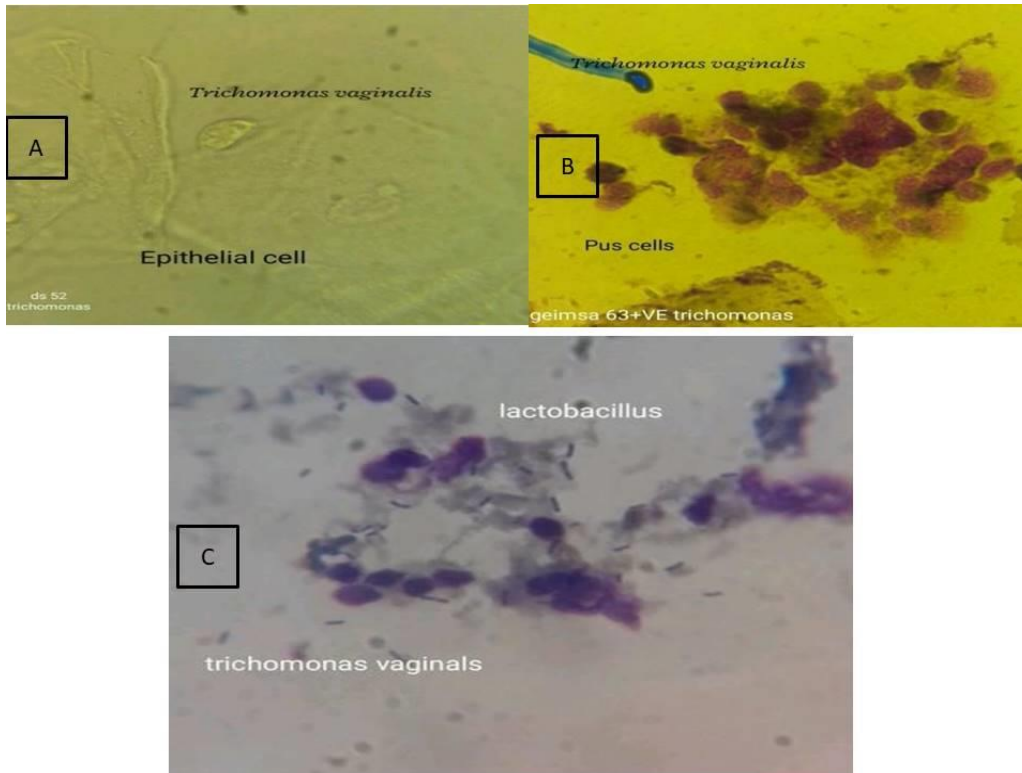
**Fig. 4.** Distribution of pathological agents according to contraceptive methods Bacterial vaginosis (BV), Candidal vaginitis (CV), *Trichomonas* vaginitis (TV)



**Fig. 5.** Distribution of pathogens according to residence of women Bacterial vaginosis (BV), Candidal vaginitis (CV), *Trichomonas* vaginitis (TV).

#### Identification of *Trichomonas vaginalis*

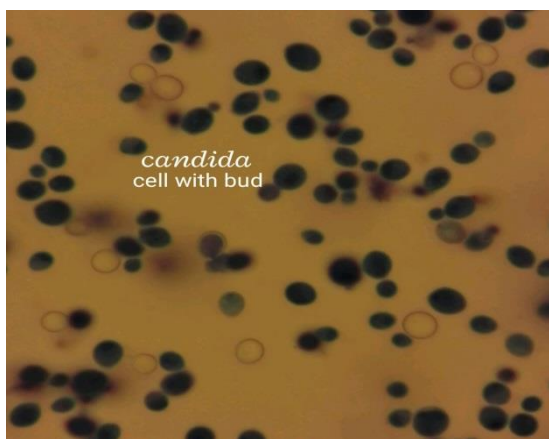
A total of 23 (27.05%) cases of trichomoniasis were recognized from 85 vulvovaginitis women by direct wet mounts (Fig.6.A) And dried smears stained with Giemsa and Leishman stains (Fig.6.B,C).



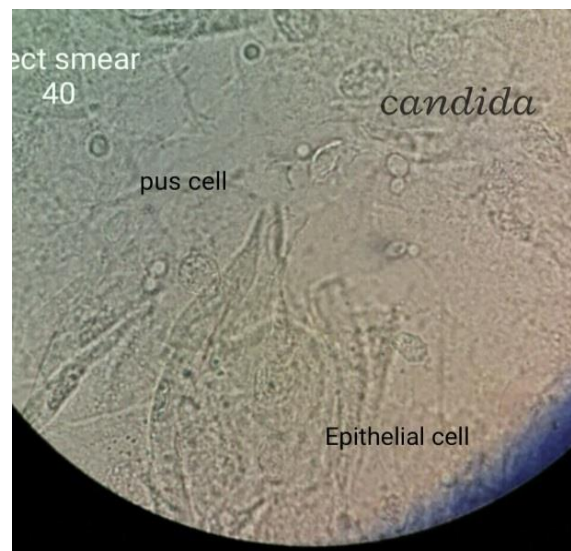
**Fig.6.** A. Lemon-shaped *T.vaginalis* in direct smear. B. stained with Giemsa stain C. Stained with Leishman stain.

*Identification of Candida spp*

*Candida spp* were identified microscopically which appeared as unicellular, spherical shaped with bud in, Gram and Lactophenol cotton blue stained preparations. Fig.7.A &B. Also multicellular pseudohyphae was detected in direct specimen examination Fig.8.A. Moreover *C.albicans* was confirmed by germ tube formation Fig.8.B. and differentiation among *Candida spp* was achieved by culturing on Chrom Agar medium Fig.9.



**Fig. 7.A** *Candida* cells with buds, A. Stained with Lactophenol cotton blue.



**Fig. 7.B.** *Candida* cells in direct smear of vaginal exudate.

The prevalence of *C. albicans* was recorded as 26 (76.47%) and the non albicans included, *C. galabrata* 5(14.7%), *C.Krusei* 2 (5.88%) and *C.dubliensis* 1(2.94%). Also the present results proved the sensitivity of *Candida spp* to all Nystatin concentrations compared to interpretive chart of diameter zone of growth inhibition for Nystatin. Table 2.

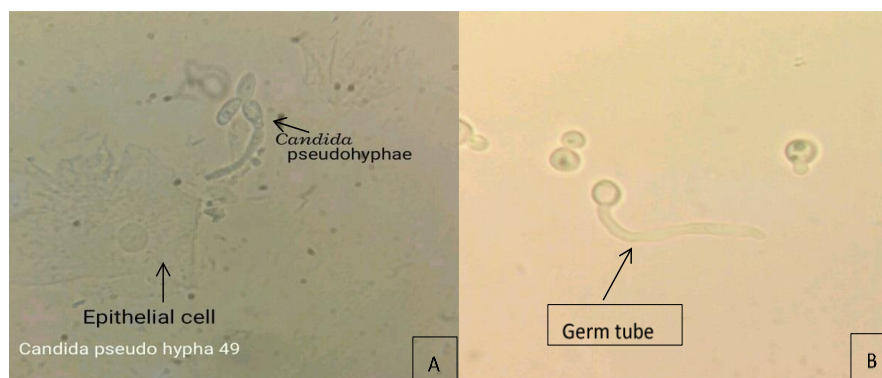


Fig. 8.A. *Candida pseudo hyphae*. B. Germ tube of *C.albicans*.



Fig. 9. *Candida spp* on Chrom Agar Medium, A-*C.albicans*, B-*C.dubliensis* C-*C.krusei*, D-*C.glabrata*.

Table 1. Characterization of *Candida spp* beside sensitivity to Nystatin.

Species	Number of isolate	%	Germ tube	Colony on Chrom agar	Sensitivity to Nystatin		
					Disc potency (µg/ml)	Diameter (mm)	Interpretive standard
<i>Candida albicans</i>	26	76.47%	Positive	green	16.6	21 mm	Sensitive (S)
					8.3	29 mm	S
					4.15	16 mm	S
<i>Candida glabrata</i>	5	14.70%	Negative	Dark pink or purple	16.6	20 mm	S
					8.3	23 mm	S
					4.15	17 mm	S
<i>Candida krusei</i>	2	5.88%	Negative	Pink center with white edge	16.6	24 mm	S
					8.3	20 mm	S
					4.15	16 mm	S
<i>Candida dubliensis</i>	1	2.94%	Positive	Pale color	16.6	23 mm	S
					8.3	20 mm	S
					4.15	17 mm	S

Table 2. Interpretive chart with diameter zone of growth inhibition for Nystatin.

Antifungal agent	Zone diameter interpretive standard (mm)		
	Resistant(R)	Intermediate(I)	Susceptible(S)
Nystatin	No zone	10-14	≥15

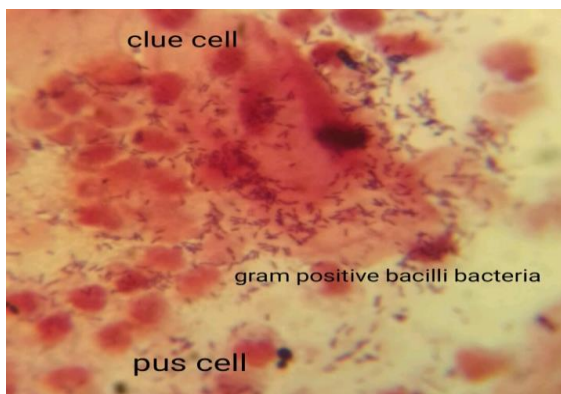
### Identification of Bacterial vaginosis

According to Nugent- scoring method, smear preparations of vaginal discharges were stained with Gram stain for detection and counting of bacterial cells, as well as clue cells were identified as epithelial cells that heavily covered with bacilli. Fig.10.A and B.

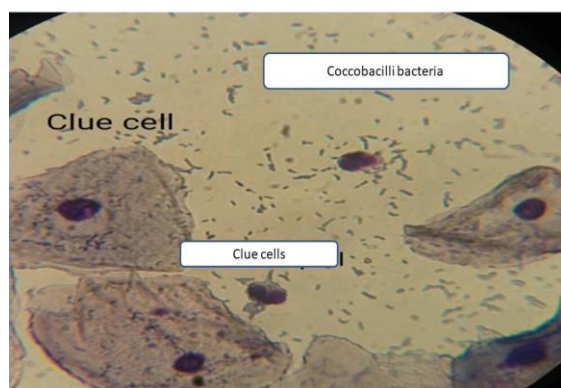
The current data showed that, out of 85 positive vulvovaginitis women 60 (70.5%) of them were infected with bacteria depending upon Nugent scoring with milky-white, homogenous discharges and most of them with amine odor after addition of 10% potassium hydroxide to it, 15(17.6%) considered as intermediate bacterial flora, and 10 (11.7) classified as normal bacterial flora. Table 3.

**Table 3.** Bacterial vaginosis by Nugent scoring.

Nugent scoring	Total +ve cases (n=85)	%
<4	10	11.76%
4-6	15	17.64%
7-10	60	70.58%



**Fig.10A.** Bacterial cells in smear preparation. Gram positive with clue cells and pus.



**Fig. 10B.** Clue cells and coccobacilli bacteria.

### Discussion

Laboratory diagnostic results of the vulvovaginitis women enrolled in the present study showed that high percentage (45%) of them had BV as a single infection. This prevalence was approximately similar to the result reported in Ethiopia (48.6%) (Bitew *et al.*, 2017). Whereas much higher than that documented in Canada (7.1%) (Pham *et al.*, 2012) and in India (17.3%) (Narayankhedkar *et al.*, 2015). Beside this, Olowe *et al.*, (2014) recorded it as (38%) in Nigeria. The present results suggested that large percentage of women of reproductive age is highly vulnerable to such infection. Lactobacilli are known to provide defence against infection by maintaining an acidic pH and presence of hydrogen peroxide in genital environment. In contrast, BV is a polymicrobial disease resulting in a decreased number of lactobacilli and an increase in pathogenic bacteria, mainly anaerobic or microaerophiles. These organisms include *Gardnerella vaginalis*, *Mobiluncus* spp., *Bacteroides*, *Prevotella* spp and *Mycoplasma* spp. (Hillier, 1993).

Percentage of trichomoniasis in the present study (7%) was lower than the other vaginal infections. However, this prevalence was seemed to be higher than that recorded by (Kadir & Fattah, 2010; Al-Saeed, 2011; Nouraddin and Alsakee, 2015) in Sulaimania, Dohok and Erbil respectively and consistent with (Khadir and Jerjis, 1999) in Kurkuk, whereas it was lower than that of (Al-Kaisi *et al.*, 2008) in Baghdad as 19.16%, (Khadir *et al.*, 1996) in Erbil as 10% and (Kharofa, 1999) in Mosul as 14%. As well as an overall high prevalence of 17.5% was documented by (Iwueze *et al.*, 2014) in Nigeria. These differences may be owing to variation in the sample size, diagnostic techniques, environmental hygiene, personal hygiene, socioeconomic level, study population and sexual activity (Wondemagegn *et al.*, 2015).

Regarding VC, the recent result showed 15% as single infection and 40% of both single and mixed infection which seemed to be higher than that recorded in Nigeria 14%; 20%; 36% (Emeribe *et al.*, 2015; Mbim *et al.*, 2017; Olowe *et al.*, 2014) respectively in Iran as 9.3% (Bonyadpour *et al.*, 2016) and in India as 30% (Narayankhedkar *et al.*, 2015). In contrast the present finding was lower than that in Baghdad which was 44.35% (Al-Attraqhchi *et al.*, 2013).



Progesterone and estrogens have direct effects on *Candida* cells, possibly contributing to VC. One direct effect of the hormones is the stimulation of estrogen and progesterone cytosolic receptors, which have been already identified in several *Candida* species. An estrogen-binding protein (EBP), that displays high affinity for estradiol and estrone was identified and characterized in *C. albicans* (Skowronski and Feldman, 1989; Wagner and Johnson, 2012) and an estrogen binding system was also detected in *C. glabrata* (Powell *et al.*, 1984). In addition, a corticosteroid-binding protein (CBP) that exhibits high affinity for corticosterone and progesterone, but low affinity for estrogens was identified in *C. albicans*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis* and *C. tropicalis* (Loose *et al.*, 1983; Skowronski and Feldman, 1989).

Concerning the age group, the most effected one with VC and trichomoniasis was 25- 30 year. These result agreed with previous studies whom concluded that this age group was the most susceptible to infectious vaginitis (Al-akeel *et al.*, 2013; Emeribe *et al.*, 2015; Kadir *et al.*, 2010 Nwadioha *et al.*, 2010; Yadav and Prakash, 2016;). Lowest infection with VC was reported in the age group (46-52) years and this is in accordance with the study of (Al-akeel *et al.*, 2013; Nwadioha *et al.*, 2010) in Saudi Arabia and Nigeria respectively. Several studies explained the factors that responsible for the low percentage of candidiasis infection in old women like the reduced effect of estrogen hormone, reaching menopause, less or no sexual activity, no contraceptive, and also have good vaginal immunity because they have low estrogen and corticoids (Nelson *et al.*, 2013). BV was higher in age group 32-38 years and this present result agree with the finding of (Nzomo *et al.*, 2013) followed by (25-31) years. It is worthwhile to mention these age groups represent the peak of child bearing ages and also may attributed to high sexual activity. Several researchers explained the high frequency of infection of these groups by using of these women drugs indiscriminately and contraceptive to prevent pregnancy (Nelson *et al.*, 2013). Moreover, Okungbowa *et al.* ( 2003) concluded that the women within this age group are vulnerable probably due to sexual promiscuity ,drug abuse and use of contraceptives.

Regarding pregnancy as a host related risk factor, the present result showed that trichomoniasis was most prevalent in pregnant women compared to non-pregnant. This finding agreed with the result of (Kadir *et al.*, 2010). In contrast, in Nigeria high percentage was detected in non-pregnant women compared to pregnant one but with non-significant differences (Iwueze *et al.*, 2014). The commensal dominant lactobacilli modulate the pathogenicity of this parasite (Phukan *et al.*, 2013). Recently some researchers proved that *T. vaginalis* is able to lyse T-cell and B-cells in vitro. Moreover they explained that this lysis of lymphocytes was mediated by contact – dependent and soluble factors (Mercer *et al.*, 2016).

Candidiasis in the current study was also documented in pregnant women (66.6%) more than that in non-pregnant group (31.2 %).This result was consistent with that of (Al-akeel *et al.*, 2013). Also it was recorded that up to 40%of pregnant women may have vaginal candidiasis globally (Alli *et al.*, 2011; Alo *et al.*, 2012).

The high percentage of vaginal candidiasis in pregnant women has been attributed to the high level of the sex hormones which lead to increase *Candida* attachment, hyphae formation and decrease vaginal responses (Goncalves *et al.*, 2016). In addition, Olowe *et al.*, (2014) concluded that the incidence of candidiasis is highly increased in recent time among pregnant women particularly due to the widespread use of antibiotics and immunosuppressive drugs. Moreover, Babic and Hukic, (2010) reasoned this by the increasing amount of glycogen in the vagina and raising level of estrogen hormones. This condition provides good sources of carbon, which provoke the growth and germination of *Candida* spp.

Identification of *Candida* species is essential due to the emergence of new pathogenic species and owing to the various antifungal susceptibility profiles. Regarding this aspect the present result showed that *C. albicans* represent 76.4% and the others as 23.5% of the vaginal isolates. This finding agree with the results of other researchers whom concluded that *C. albicans* is the most common cause (80%-90%), whereas other yeasts account for up to 20% of cases.

*C. glabrata* was also identified in the current study in 14.7% which is higher than that mentioned by (Geiger *et al.*, 1995; Ray *et al.*, 2007; Spinillo *et al.*, 1997;) which accounts as 10% of vaginal yeast isolates. Symptomatic vaginitis caused by this organism is associated with less intense itching and dyspareunia (Geiger *et al.*, 1995) than that caused by other *Candida* species, but the organism may be more difficult to eliminate with standard medications. (Ray *et al.*, 2007). In other study, Nyirjesy (2008) recorded that 70% of complicated cases of recurrent vulvovaginitis are caused by *C. albicans*. Whereas *C. glabrata* and *C. parapsilosis* represent 30%. (Nyirjesy *et al.*, 1995). Al-akeel *et al.*, (2013) found that *C. albicans* and *C. glabrata* constitute 80.3 and 12.7% respectively in vaginal specimens. Recently, Goncalves *et al.*, (2016) concluded that in spite of *C. albicans* is the main causative agent for VC, the identification of non-*Candida albicans* *Candida* species, particularly *C. glabrata*, as responsible for this disease, appears to be high.

In addition, the present results identified *C. krusei* and *C. dubliensis* in 5.8% and 2.9% respectively. Whereas other recorded *C. tropicalis* in 1% to 5% and concluded it may be associated with a higher rate of recurrence after standard treatments. (Horowitz *et al.*, 1985; Spinillo *et al.*, 1997).

According to the sensitivity of *Candida* spp to Nystatin, the present data showed that all species seemed to be sensitive for the three concentrations of Nystatin. These results agreed with the studies of (Nelson *et al.*, 2013; Toua *et al.*, 2013) in Kenya and Cameron respectively.

On the other hand, the present result of single BV showed that non- pregnant women were more infected than pregnant group. This result disagree with the result of (Afolabi *et al.*, 2016; Bahram *et al.*, 2009) in Nigeria and Iran respectively. The present findings may be related to the population size which included (21) pregnant women, while non-pregnant women were (64).

In general the recent total mixed infections were detected in pregnant women more than that recorded in non-pregnant ones with significant differences. This finding may be related to the abundant of reproductive hormones in pregnancy. Estrogen is responsible for increasing the vulnerability of pregnant women to vaginitis by reducing the ability of epithelial cells to inhibit the growth of *Candida* beside reduction of the immunoglobulins in the vaginal secretion (Anorlu *et al.*, 2004). Moreover, Progesterone has suppressive effects on the activity of neutrophils (Adad *et al.*, 2001). It is well known that the alteration of the vaginal environment PH toward alkalinity during pregnancy lead to overgrowth of the pathogens responsible for vaginitis due to reducing level of Doderlein bacilli which convert glycogen of the epithelial cells into lactic acid that keep the vagina acidic.

Bacteria were the most prevalent etiological agent that detected in contraceptive user women which reached 71.4% in intrauterine device users. Several researchers pointed out that IUD increase the likelihood of BV in women (Calzolari *et al.*, 2000; Madden *et al.*, 2012), whereas another in recent study found no correlation between the IUD contraception and the alteration of the vaginal microbiota composition. (Bassis *et al.*, 2017). Because of the few number of contraceptive users among the vulvovaginitis women included in the current study, no clear coloration was recognized between the disease and the type of contraceptive.

### Conclusions

Relatively high percentage of women whom enrolled in the current study was infected with the three etiological agents that responsible for vulvovaginitis particularly with bacteria. Routine culture of vaginal discharge must be performed for these patients; also drug susceptibility of bacterial isolates should be determined. Comprehensive healthcare education is needed to reduce this disease among women particularly those of child bearing age. Further studies with large number of infected women will be needed to highlight this important disease, which can be managed successfully.

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