

**Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Thi-Qar
College of Science
Department of Biology**



**External genital tract infections among women
association with some hormones in Thi-Qar
provice-Iraq**

A Thesis

**Submitted to the College of Science, University of Thi-Qar
in a Partial Fulfillment of the Requirements for the Degree of
Master in Biology/ Microbiology**

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B. Sc. (2014 -2015)

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1439 A.H

2017 A.D

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا ۚ إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ))

صَدَقَ اللَّهُ الْعَلِيِّ الْعَظِيمِ

سورة البقرة

(الآية ٣٢)

Dedication

To which their ardor crowded before their souls

To which their carnage relegated sacrificing to their
country

To the souls of our heroes martyrs

And to my father and all people which support me , I
gift the product of this labor.

Safa

Acknowledgments

I want first to thank Allah for helping me and giving me strength, willingness and patience to finish what I have started.

I would like to express my gratitude, faithful appreciation and deep respect to my Supervisors Dr. Bassim Abdul Hussein Jarullah and Dr. Muhannad Abdul Razzaq Gadei for their suggestion of the project, supervision, help, continuous advice and efforts for helping me in my work.

I'd also like to thank Prof. Dr. Mohammed A. Aouda the dean of Science College, for his help and support all the time.

I am grateful to Asst. prof Dr Haider Radhi the head of Biology Department, College of Science, Thi-Qar University, for his encouragement and care.

I would like to express my special thanks and respects to Prof. Dr. Khalid Al-Fartosi for his help and support.

I express my special thanks to the all members and staff in the clinic microbiology in Bint AL-huda teaching hospital, for their help in the collection of specimens and work on them ., I also have the honor to thank Dr. Zeinab Ali Kazem for providing me with continuous assistance ., I wish her a health and success always ., I cannot find any word to express my sincere appreciation and gratitude to my family for their help and support.

Abstract

The current study was conducted in Thi-Qar,Iraq to determine the main types of microorganisms that cause female genital tract infection and the extent of the effect of the level of sex hormones (prolactin, progesterone and estrogen) on the presence of infection , also study of some factors that may have a relationship to the presence of infection such as age, diabetes,take contraception and abortion cases .

About 245 samples were collected for both vaginal swabs and blood (where take a vaginal swab and blood from every woman) , where 150 women were pregnant and 95 women were non-pregnant women , aged 16-45 year,the samples of our study were collected from women revisions to the consultancy clinic in Bint Al Huda teaching hospital for child birth and children and the consultancy clinic in Al- Hapupy teaching hospital in Al- Nasiriyah city for the period from the beginning of october 2016 until the end of february 2017 and prepared questionnaire form for each woman to obtain their information .

The study showed asignificant differences ($p<0.05$) in prevalence of infection among pregnant and non-pregnant women ,where was the prevalence of vaginitis is higher in pregnant women than in non-pregnant women where 150 cases were positive for culture in pregnant women compared to 95 cases in non-pregnant women . The isolates were subjected to biochemical and morphological tests where it was found that *Candida albicans* isolated by (30.6%) followed by *Streptococcus agalactiae* (14.3%), *Staphylococcus aureus* (11%) , *Escherichia coli* (10.2%) , *Staphylococcus epidermidis* (8.6%) , *Gardnerella vaginalis* (7.8%) ,*Klebsiella pneumoniae* (6.1%) , *Proteus mirabilis* (4.5%), *pseudomonas aeruginosa* (3.7%) , *Trichomonas vaginalis* (2.4%), *Enterobacter cloacae* (0.8%) .

Among non-pregnant women, most were women who had a reproductive tract infection within the leuteal phases , where our study included 58 women at leuteal phase , 20 women were in follicular phase , 17 women within ovulation phase of the menstrual cycle .The study showed that *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* , *Staphylococcus*

Abstract

epidermids , *Enterobacter cloaceae* were isolated from women in follicular phase , the diversity of infections was lower in women in the ovulation stage, where they were isolated only *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* ,and from women with leuteal phase was obtained *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* , *Staphylococcus epidermids* , *Proteus mirabilis*, *Pseudomonas aeruginosa* , the precence of all type of pathogens in our study was associated with elevated estrogen and progesterone levels except the growth of *Staphylococcus epidermids* , *Proteus mirabilis*, *Pseudomonas aeruginosa* , , *Enterobacter cloaceae* was associated with normal range of each hormones ,as for the prolactin hormone did not have a clear effect as the levels ranged between natural and high for all women in the three phases. Pregnancy was accompanied by high levels of estrogen, progesterone and prolactin hormones, this is accompanied with existence *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* , *Streptococcus agalactiae* , *Trichomonas vaginalis*.

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List of Abbreviations

A\A	Acid\Acid
AIDS	Aquired immunodeficiency syndrome
ALK\A	Alkaline \Acid
BV	Bacterial vaginosis
CD₄	Clusters of different
DNA	Deoxyribonudeic acid
ESBLs	Extended-spectrum beta-lactamases
FRT	Female reproductive tract
HPV	Human papillomavirus
HSV	Herpes simplex virus
Ig	Imunoglobulins
IL-2	Interlukine
IL-2R	Interlukine receptors
LPS	Lipopolysacharide
MR-VP	Methyl red voges
NK	Natural killer
PMNS	Polymorponuclear leukocytes
RBCs	Red blood cells
RTI	Reproductive tract infection
STI	Sexually transmitted infection
Th1-Th2	T helper 1-T helper 2
TSI	Triple Sugar Iron

VEC	Vaginal epithelial cells
VVC	Vulvovaginal Candidaiasis
WBCs	White blood cells

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Questionnaire

Name:

Age:.....

Pregnant or non pregnant :.....

Stage of pregnancy :.....

Type of nutrition :.....

Other diseases:.....

Associated symptoms:.....

Take contraception:.....

Abortion cases:

Chapter One

Introduction

1-1 Introduction :

Genital tract infection is one of the most common diseases in women during pregnancy and childbirth (Virginia *et al* .,2000) .

The genital tracts of women consist of specific type of bacteria which is called *Lactobacillus* (Babay and Babay,1999), these bacteria live peacefully , it does not cause damage , but in fact microflora protects the vagina from infection with harmful microbe (Larsen,1993), where it produce a disinfectant substance called “lactic acid” by its fermentation of the glycogen in the cell, contributes to the maintenance of the acidity of the vagina, as well as its production of hydrogen peroxide H_2O_2 and which help to prevent pathogenic bacteria from the settlement of the vagina and the incidence of inflammation in it (Brooks *et al* .,1998).

The environment of the vagina often prevents infection by maintaining a balance between the microflora and those that cause infection , genital tract infection can produce from defect the vaginal environment because excessive intake of antibiotic (Robert,1990) , pregnancy , diabetes , Acquired immunodeficiency syndrome (AIDs) , anatomical changes , vaginal doching , hormonal imbalance (Ness *et al* .,2002; Ramzan *et al* ., 2004; Pitt *et al* .,2005; Lan *et al* .,2008) . an inaccurate diagnosis of the condition of the woman with vaginitis and rushing to take treatment without a sensitivity test (Weber *et al*.,2002).

The excessive use of antibiotics is a cause" of producing resistant bacterial strains, and resistance is usually spread (Keren and Chan,2002)increase infection rates not only because of its multiple resistance to antibiotics , but they have many risk factors that increase their severity ,and their ability to attach red blood cells to humans and adherent epithelial cells(Jasmina *et al*.,2001). there are many causes of vaginitis involving bacteria that cause bacterial vaginitis, which reflect the imbalance and interference between the types of anaerobic bacteria that cause inflammation ,compared to the shortage in *Lactobacillus* which are considered normal flora presence in vagina(Romanik and Martirosian,2004) Yeast and fungus are one of causes , while parasites as well as viruses are considered another causative (William,

1997).Female genital tract (uterus, cervix, and vagina) mucosal tissues are regulated by several factors commonly produced in the mucosa, as well as by estrogen and progesterone(Witkin *et al.*, 2007). Innate and adaptive mucosal immunity in the FGT are regulated by the female sex hormones estrogen and Progesterone (Bouman *et al* ,2005; Butts and Sternberg ,2008) hormones regulate the transport of Igs, the levels of cytokines, the expression of TLR genes(Aflatoonian *et al.*,2007; Aflatoonian and Fazeli ,2008) and the distribution of immune cells and antigen presentation in the genital tissues during the reproductive cycle(Wira *et al* .,2005) ,as the innate and adaptive immune systems are under hormonal control, and immune protection in the FRT varies with the phases of the menstrual cycle(Wira and Kaushic ,1996). As the Immune protection is dampened during the secretory phase of the cycle to optimize conditions for fertilization and pregnancy, which creates a ‘window of vulnerability’ during which potential pathogens can enter and infect the FRT (Begum and Ashwini, 2012)

The hormonal changes play a role in change the natural flora in the vagina Leading to inflammation , where the normal level of estrogen necessary to maintain vaginal balance , on the basis that this hormone stimulates and activates the growth and integration of the vaginal epithelial membrane (Reid *et al.*,2004) , in normal women,estrogen supports vaginal epithelium in glycogen accumulation which in turn helps in maintenance of vaginal pH(Lehman,2009).As that the reproduction of lactobacilli depends on sex hormones oestrogen levels (Galask,1988;Miller *et al.*,2000) the numbers of these bacteria may change during the menstrual cycle depending on change of estrogen level . As acidification by vaginal secretion stimulates the growth of lactobacilli. If the level of this hormone is changed, the number of lactobacilli will be reduced and vaginal environment changes to become an appropriate place for pathogens .

1-2 The aims of study :

According to the importance of genital tract infections and their prevalence in our community, the present study was designed to investigate the following

1-Identification of major microorganisms that cause inflammation in the female reproductive system .

2- The relationship between sex hormones levels and the type of infection in the reproductive system.

3- Study the factors that may have an impact on infection such as age, diabetes, take contraception , abortion cases.

4- Determination of resistance to antibiotics for isolated organisms in the study .

Chapter Two

Literature

Review

2-1 History of Causes of Genital Tract Infection :

The main causes of female genital tract infections are fungi (especially *Candida albicans*) , bacterial vaginosis , *Trichomonas vaginalis*, virus (Biswas,1993). First recognized to *Candida* vaginitis at least since 1792 , when Frank showed it developing contemporary with aphtha of the mouth (Frank,1792;Haussman,1875) .

In 1875 Haussman observed that fungus spores occurred in the mouths of the babies whose mothers had vaginal thrush and he was proved to be the same fungus (Haussman.,1875). In 1973 Carroll et al and hisc showed that the isolates of *Candida albicans* concurrent with vaginitis in 840% of cases (as confirmed by direct examination of the vagina) and that in 14% it linked with other signs or symptoms of morbidity of the genital tract , also in 1973 Hurllely was said that *Candida albicans* , is not part of the normal flora of vagina and its presence indicates morbidity , as said that *Candida albicans* is often part of the intestinal flora , it may occasionally be isolated from the vagina , particularly if the clinical specimen is badly taken (Hurley *et al* .,1973) .

The composition of the normal vaginal flora first described by Doderlien in 1892 (Doderlein , 1894) , Bacterial vaginosis or non specific vaginitis was first reported in 1955 by Gardner and Dukes , who described the unique clinical signs , symptomus and distinctive nature of the vaginal discharge associated with it , they also described a new causative organisms, which have variously been named *Haemophilus vaginalis* (Gardner and Dukes,1955) , and subsequently rename and reclassified as the *Corynebacterium vaginalis*(Zinnemann and Turner,1963), only member of a new genus *Gardnerella vaginalis* vaginitis(Greenwood and Picket.,1980) , later on it was found that this microorganism is present in the vaginas of 40-50 % of patients without BV. As well as in those cured of bacterial vaginosis (Spiegel *et al* .,1980 ; Totten *et al*.,1982). Furthermore , bacteria other than *Gardnerella vaginalis* (Blackwell *et al* .,1983) . in 1982 vaginitis named anaerobic vaginosis (Black well and Barlow ,1982) then the name of bacterial vaginosis which was given in 1984(Mardh and Taylor,1984) . Donne was first identified

Trichomonas vaginalis, who noted there is motile microorganisms in the purulent, frothy leukorrhea of women, presenting with vaginal discharge and genital irritation. However, research on this organism did not begin until the 20th century (Donne, 1836).

In 1830s, recurrent genital herpes was described and 60 years later was identified as a "vocal disease" – a sexually transmitted infection (STI). The virus itself was not identified until the 1950s. In 1971, it was proposed that two different types of HSV caused infection. HSV-1 commonly causes labial or pharyngeal infection and transmission is primarily by nongenital contact. HSV-2 typically affects the genital area and is transmitted by intimate sexual contact. However, both viruses are capable of causing either genital or oral pharyngeal infections that appear identical on examination. In the United States, HSV infection is one of the most common STIs and is the leading cause of genital ulcers (Roizman and Whitley, 2001). In 1974, Zur Hausen published his first report attempting to find HPV DNA in cervical cancer and genital wart (Zur Hausen and Meinhof, 1974). Harald Zur Hausen's theory of HPV aetiology for cervical cancer was supported by later work by Meisels and Fortin (Meisels and Fortin, 1976), who had described koilocytotic atypical cells as the maintenance of papilloma virus-induced cytopathic change in cervical dysplasia.

2-2 Anatomy of the female genital tract :

The female genital system composed of a variety of organs and epithelial surfaces. The external genital organs include vulva (the mons pubis, labia majora, labia minora, Bartholin's glands, and clitoris). The internal genital organs form a pathway (the genital tract). This pathway consists of the vagina, the uterus, the fallopian tubes (oviducts) and the ovaries (Hall, 1990; Cole, 2006).

The vagina is a narrow, muscular but elastic organ about 10 cm long in an adult woman. It is the terminal portion of the female genital tract and its cavity is continuous proximally with that of the uterus at the external os of the cervix. It connects the external genital organs to the uterus (Stevens and Lowe, 2005). The uterus is a thick-walled,

muscular organ having a narrow cavity which is continuous with the peritoneal cavity through the uterine tubes and with the perineum. through the vagina . it is about the size and shape of a pear , with the narrow portion directed inferiorly . The cervix is the lower part of the uterus , which protrudes in to the upper end of the vagina . the channel through the cervix is lined with glands that secrete mucus (Llewellyn *et al.*,2004).

The 2 fallopian tubes or oviducts , which are about 5 to 7 cm long , extend from the upper edges of the uterus toward the ovaries . The tubes do not directly connect with the ovaries the end of each tube flares into a funnel shaped infundibulum with fingerlike extensions . called fimbriae . They are lined with tiny hair projections (cilia). The cilia and the muscles in the tubes wall propel an oocyte downward through the tube to the uterus(Llewellyn *et al.*,2004; Cole,2006).

The ovaries are usually pearl coloured , oblong . and measure 3 cm x 1.5 cm x 1.5 cm . the ovaries aren't attached to the fallopian tubes but to the outer layer of the uterus via the ovarian ligaments . the ovaries are uncovered in the peritoneal cavity. thus , the ovary is the only organ in the human body which is totally invaginated in to the peritoneum , making it the only inter peritoneal organ(Paavonen,1983;Kierszenbaum ,2002) .

2-3 Female Genital tract infections :

The female reproductive system is a pathway to enter many microorganism , especially vagina , where it is a warehouse of the various microorganism that cause the epidemic or may be sexually transmitted such as syphilis , AIDs , gonorrhea and viral hepatitis (Harithi et al ., 1999). the genital tracts of women consist of specific type of bacteria which is called lactobacillus(Babay and Babay,1999) , make up 70% of the natural flora of the vagina , these bacteria live peacefully , it does not cause damage , but in fact microflora protects against infection with harmful microbe(Larsen.,1993).

Where it produce a disinfectant substance called “lactic acid” By its fermentation of the glycogen in the cell, contributes to the maintenance of the acidity of the vagina, as well as its production of hydrogen peroxide H_2O_2 and which help to prevent pathogenic bacteria from the settlement of the vagina and the incidence of inflammation in it (Brooks et al .,1998).The environment of the vagina often prevents infection by maintaining a balance between the microflora and those that cause infection . There are several types of female genital tract infection ,Cervicitis especially caused by *Chlamydia trachomatis* , *Neisseria gonorrhoeae* (Vandepitte et al ., 1991) , and vaginitis which is caused by *Trichomonas vaginalis* which constitutes 1/3 of all causes .

Vaginitis which occurs by *Candida albicans* which are frequent in pregnant women, diabetes, and contraceptive users that increase susceptibility (Sobel et al ., 1998) another cause is viral infections such as the Human papilloma virus (HPV) and Herpes simplex (Vazques and Sobel.,1995;Dennerstein .,2001; Metchell.,2004), bacterial vaginosis or may be called nonspecific vaginitis where they carry large numbers of diverse bacteria instead of *lactobacilli* such as *Gardnerella vaginalis* , *Escherichia coli* , *Streptococci* and *Staphylococci* (Edwards ., 2004)*Klebsiella* , *Proteus* , *Acinetobacter* and *Pseudomonas* spp (Syed andBraverman .,2004),Common symptoms seen are vaginal discharge, itching, irritation, unpleasant odor and discomfort.(Egan and Lipsky ., 2000) .

2-4 The major causes of genital tract infection :

2-4-1 Bacterial causes:

The vagina is a complex system which contain a mixture of microorganisms (Larsen and Monif ., 2001) This distinctive environment undergoes major changes in all stages of life, from birth to the age of puberty and menopause (Romero ., 2014).

Females are more susceptible to urinary tract and vaginal infections due to short urethra and anatomical and physiological proximity to the anal canal (Puri and Malhotra, 2009). Naturally vaginal flora contains a wide range of microorganisms. The vagina of a healthy premenopausal woman is occupied by *Lactobacillus* spp (Islam *et al* ., 2009) *Lactobacilli* are Gram positive rods that produce secretions in vitro having antimicrobial properties, with acidolin, lactacidin, lactacin B and hydrogen peroxide. *Lactobacillus* strains producing H_2O_2 play a vital role in maintaining the microenvironment of the vagina and in the inhibition of overgrowth of organisms that are potentially pathogenic (Reid *et al.*, 1990; Klebanoff *et al* ., 1991).

The pathogenesis of BV condition is unclear. It is known to involve the substitution of the predominant beneficial microorganism (*Lactobacillus* spp) with other anaerobic microorganism (*G. Vaginalis*) and a corresponding increase in vaginal pH (>4.5) (Johnson *et al.*, 1984) . Worldwide frequency of BV is 3.6-40% reported in different population. Prevalence of BV varied based on the surveyed population, ranging from 4% in developed countries to 61% in the third world countries, with 14% is the mean prevalence in developed and developing region.

Today bacterial vaginosis is the most common vaginal disease in the United States. In USA BV is 26-37% prevalent whereas 4-37% in general population of European countries (Vanita *et al.*, 2012) . . Bacterial vaginosis is the most prevalent cause of vaginal discharge or malodor , bacterial vaginosis tends to produce a white , gray , yellowish turbid discharge with a foul or fishy odor that becomes stronger when the

discharge becomes alkaline (eg., after coitus or washing with soap). Vulvar pruritus or irritation may be present , but redness or edema is not usually marked(Amsel *et al* .,1983) .

2-4-2 Fungal causes :

Fungi are considered to be eukaryotic organisms with approximately 50,000 species, of fungi live in nature, including 80 species of molds and yeast,many diseasesare caused to both human and animal (Jawetz *et al.*, 2004).

The genus of *Candida* is part of the natural plant of the body, is found naturally in many areas of the body, including the oral cavity and the respiratory tract of healthy people as well as the presence in the vagina (Lewis, 2000). But the transformation of yeast from a co-living organism into a pathogen as opportunistic as a result of what the *Candida* possesses from harmful factors such as adhesion to the surface of the epithelial cells and the production of enzymes digesting fats and proteins and the formation of the germination tube(Panagod *et al* ., 2001).

Candida albicans take a chances of weak the body 's immunity to become pathogens as appportunistic . where they are more virulent in people who suffer from immune weakness as acquired immunodeficiency syndrome (AIDs) , malignam tumor , diabetes , pregnancy , excessive intake of antibiotic medications or weak the immunity such as cortisone (Robert , 1990) .The reason for this is thought to be due to increased oestrogen levels, glycogen and other substrates in the vagina (Sobel, 2007). Increased level of *Candida* spp may cause an imbalance in normal flora of the vaginal, thereby decreasing lactobacillus dominance(Jefferson, 2012; Cassone, 2015). Several studies around the world have shown VVC infection range between 10.0 to 55.0% (Mohanty *et al.*, 2007;Ahmad & Khan, 2009;Okonkwo & Umeanaeto, 2010; Olowe *et al.*, 2014). Vulvovaginal candidiasis (candidosis) is the most common cause of vaginitis (Vazques and Sobel ,1995;Dennerstein ,2001).

Most cases of acute vulvovaginal candidiasis are caused by *C. albicans* (89%) and *C. glabrata* (5%). Other species of the *Candida* genus, such as *C. tropicalis*, *C. parapsilosis*, and *C. krusei*, are estimated to constitute less than 1% of each, and *Saccharomyces cerevisiae* 1-2% [Ferrer.,2000;Edwards.,2004; ACOG.,2006; Petersen .,2006).*Candida* may be isolated from vaginal secretion in approximately one-fourth of asymptomatic women(Ferrer , 2000) .but fungal vaginitis is often accompanied with thick , white ,cottage cheese –like vaginal discharge that tends to cling to the vaginal wall(Geiger *et al* .,1995) . vulvar pruritus with or without burning , dyspareunia , redness ,edema and possibly excoriation .

2-4-3 Parasitic causes :

Trichomonas Vaginalis is a parasitic protozoan , motile, about 9 by 7 μ m in size, ovoid, flagellated . The organism has a cytoplasm, large nucleus and a highly developed golgi complex (Hashimoto *et al.*, 1964). The organism has five flagella, four in the anterior area and the fifth forms part of the undulating membrane (Ovcinnikov *et al.*, 1975). The quivering motility characteristic of the parasite is due to flagella and the undulating membrane (Honigberg & King, 1964).

T.vaginalis is composed of tubulins and actin fibers which is the cytoskeleton. Its nucleus is at the anterior portion and surrounded by a porous nuclear envelope (Hashimoto *et al.*, 1964). The axostyle is a slender hyaline, rod-like structure which starts at the nucleus and bisects the organism longitudinally. It protrudes through the end of the protozoan, terminating in a sharp point (Honigberg & King, 1964). It uses the axostyle to anchor the whole body to the vaginal walls. Under light microscopy, granules can be seen in the *T.vaginalis* .

There are two sets of these granules: paracostal and paraxostylar. Glycogen granules are also present in *T. vaginalis* and can be observed by transmission electron microscopy. It contains lysosome-like structures such as phagosomes and demonstrates hydrolase activity (Ovcinnikov *et al.*, 1975). *Trichomonas vaginalis* is the cause of trichomoniasis ,

a sexually transmitted disease (STD) of worldwide importance (Hohne , 1916). *Trichomonas vaginalis* is an obligate parasite in that it lacks the ability to synthesize many macromolecules denovo , particularly purine , pyrimidines , and many lipids . these nutrient are acquired from the vaginal secretion or through phagocytosis of host and bacterial cell (Heine and McGregor,1993) . Data on the incidence and prevalence of trichomoniasis is limited worldwide (WHO, 2004).

Few studies on the prevalence of trichomoniasis shows varying findings depending on the geographical locations, methods used in sample analysis and the selected group of people used for the studies. In the United States, a large population based survey spanning a period of 5 years recorded a prevalence of 3.1% in women aged 14-49 (Sutton *et al.*, 2007). Another recent study published in 2012 using a very accurate tool Aptima Combo 2 assay (Gen-Probe), found trichomoniasis prevalence rate of 8.7% in women aged 18 – 89 (Ginocchio *et al.*, 2012). An Asian country study (Iran) on trichomoniasis prevalence using culture and wet mount as their diagnostic tools found a lower prevalence of 2% in both male and females aged 16 – 60 (Arbabi *et al.*, 2014). Studies in Africa on trichomoniasis prevalence amongst women shows rate from 9.9% to 41.4% (Crucitti *et al.*, 2011; Francis *et al.*, 2014) using various diagnostic methods. In Ghana, cross sectional surveys amongst female sex workers in Ghana's two biggest cities Accra and Kumasi found very high prevalence of 31.4% (Deceuninck *et al.*, 2000). A similar survey recently conducted in a STI clinic in Kumasi had a lower rate of 5% using wet mount (Agyarko-Poku, 2011).

In pregnant women attending ANC in Kumasi, the prevalence of *T.vaginalis* was 5.4% using latex agglutination method with wet mount and culture as the gold standard (Adu-Sarkodie *et al.*, 2004). Signs of infection in symptomatic women include vaginal discharge 42%, odor 50% ,edema or erythema 22-37%, and colpitis macularis , strawberry cervix . other complaints may include dysuria and lower abdominal pain . nearly half of all women with trichomonas vaginalis are asymptomatic (Schwebke and Burgess .,2004). The classic symptoms associated with infection by trichomonas

vaginalis include a yellowish –green frothy discharge ,pruritis , dysuria , dyspareunia(Levett ,1980). According to studies prevalence of Trichomoniasis is between 2 to 6% (Adu-Sarkodie *et al.*, 2004; Apea-Kubi *et al.*, 2006; Agyarko-Poku, 2011).

2-4-4 Viral causes :

2-4-4-1 Human papillomavirus (HPV) :

It is a variety of viruses that a family of deoxyribonucleic acid (DNA) viruses, which infect the skin and mucous membranes of humans and a group of animals. There are more than 100 types of HPV. Some of these species cause benign epidermal embolism, or papillae. Papilloma viruses associated with the appearance of common boils are transmitted by normal skin contact. Another group of some 30 species is transgenically transmitted.

Genital HPV is common, with estimates that about 75% of women (in the West) are infected with one or more types of HPV during their lifetime (Baseman and Koutsky, 2005). condylomata acuminata or venereal warts Is the simplest evidence of an infection HPV, Although a large number of virus types may cause genital warts However, types 6 and 11 cause about 90% of cases (Greer ,1995 ; Gearheart *et al*, 2004) . HPV infection is very common. At least half of people who are sexually active will get HIV at some point in their lives. But many do not know it and do not have any symptoms. Some types of the virus are associated with cervical cancers and others, some of which occur in the folds or papillae of the sexual organs. These include the name of the virus. Some types of HPV also cause the common papillae that you can find in other areas of the body such as the hands or feet (Georheart *et al* ., 2004) .

HPV lives in the mucous membranes, such as those in the genital area, or on the skin. If genital papillae appear, they are an indicator of HPV infection. Genital papillae take many different forms so they can be shaped like cauliflower. It can be small or large. It can be on the anus, cervix . Genital papillae can appear weeks or even months after

sexual intercourse with someone infected with the HPV virus. This person may not know that he or she is infected and responsible for the transmission of HPV. Some types of HPV are associated with cancer, including cervical, vulvar, anal. They can cause cancerous changes in tissue cells without causing any symptoms(Greer *et al* .,1995).

2-4-4-2 Herpes simplex virus (HSV) :

Causing Genital herpes disease , it is a very contagious disease, transmitted by sex..Herpes simplex virus They are two types of viruses Herpesviridae Which affect humans .Herpes simplex virus type 1 (HSV-1) and herpes simplex virus (HSV-2). The majority of cases of oral herpes are caused by HSV-1 and most cases of genital herpes are caused by HSV-2; however, Type-1 or Type-2 can cause infection in any area, either genital or oral (Rayan and Ray,2004).Herpes simplex virus enter the body through small cracks located in the skin or in the tissues that secrete the mucus. Sex is the most common way to transmit nninfection. Genital herpes, which affects the genitals, is a very common disease that can affect both men and women(Guptaetal , 2007).Symptoms of genital herpes include Small red warts, ulcers in areas close to the genitals and anus , itching , pain , rash , buttocks and femur.

The first sign of genital herpes is, mostly, pain or itching, which appears after a few weeks of sex with an infected partner. A few days later, small red warts or blisters may appear. These warts and bursts then explode into open wounds (ulcers) from which liquid may ooze or blood may bleed. In the end, these wounds heal and heal(Geoch *et al* .,2006). These lesions may appear in women in the vaginal area, in the external area of thegenitals, buttocks, anus or cervix. Peeing may be accompanied by aches during the period in which these wounds appear, and aches and excess sensitivity may appear in the genital area until the infection disappears and the disease disappears. Herpes is different from one person to another. Signs and symptoms can appear and then reappear, bitter and repetitive, over a few years. There are people who have recurrent bouts every year.

However, the frequency of seizures decreases over time in many people with the disease(Herpes simplex , 2006) .

2-5 Sexual hormones and their role in maintaining the health of the vagina :

Sex steroid hormones are produced in the gonads (Labrie *et al* ., 2005) , estrogen is a female hormone that secrete from the ovary as the primary sex hormone. Its secretion stops when the female reaches menopause,there are 3 type of estrogen (estradiol , estraiol , estrone) ,estrogen controls the regularity of the ovulation process and regulated menstrual cycle and thus the ability to reproduce (Rayan,1982) .

Progesterone is a female hormone secreted from the corpus luteum in the ovaries in the last two weeks of the menstrual cycle of the female after ovulation ,also excessive secretion in the placenta during pregnancy , it works by fertilizing the uterus to the fertilized egg (Doderlein and Albert,1892) Prolactin is a peptide hormone secreted from the frontal lobe of the pituitary gland and is linked to the process of breastfeeding as it acts as a catalyst to generate milk from the breast gland (Larsen *et al* ., 2007) .Sex hormones especially estrogen are important for maintaining a healthy vaginal mucosa (Kingsberg *et al* ., 2013) . The vagina extend from the vulva to the uterine cervix in the female genital tract . the vaginal wall is composed of vaginal mucosa , lamina propria also called stroma , a smooth muscle layer , and an adventitia layer, all controlled by estrogen hormone (Junqueira *et al* ., 2005) , steroid hormone receptors are expressed in the vaginal wall (Hodgins *et al* ., 1998) .

Estrogen levels influence steroid hormone receptor expression in vaginal tissue (Gebhart *et al* ., 2001 ; Berman *et al* ., 2003) . The vaginal epithelium prevents colonization by microbes and their entrance into deeper tissue by secretion of vagina , this secretion transudate through the vaginal epithelium and from the cervical mucus with additional fluids from the endometrium, uterine tubes, and vestibular glands (Paavonen ,1983). The vaginal secretion is a mixture of several components including

ions Na^+ , Ca^{2+} , Cl^- , proteins, peptides, glycoproteins, lactic acid, acetic acid, glycerol, urea, and glycogen, exfoliated cells, the vaginal secretion also contains antimicrobial components of the immune system and leukocytes (Paavonen, 1983; Cole, 2006). Some of vaginal component use to protect the vagina from colonization by microbes, which all of vaginal component depending on the absolute levels of oestrogens and progesterone (Huggins and Preti, 1981; Kierszenbaum, 2002; Junqueira, 2005). As the Cervical mucus which has a pH of approximately 8.0, is the main contributor to the vaginal secretion and physically prevents microbes from attaching to the mucosal surface (Moghissi, 1976). Mucus consists of water (92-98%), glycoproteins (mucins), ions, antimicrobial proteins and polypeptides such as lactoferrin, lysozyme, immunoglobulins, and defensins (Cohen *et al.*, 1984; Cole, 2006).

The secreted mucus in the FRT is under hormonal control, and changes in viscosity can be seen during the menstruation cycle (Gipson *et al.*, 1999; Holmes, 1999; Wiggins *et al.*, 2001). The capacity of microbes to degrade mucin molecules by using microbial enzymes, (mucinases, sialidases) is often a fundamental step in disruption of the defensive mucosal barrier, as the constitute direct interfaces between internal and external environments (Holmes, 1999; Wiggins *et al.*, 2001). At fertile age the normal pH in the vagina ranges from 3.5 to 4.5, with a typical value of 4.2 (Owen and Katz, 1999), the vaginal epithelium release glycogen, the role of glycogen in maintaining a balanced vaginal ecosystem was first documented as early as the 1890's (Menge and Kronig, 1897) and recognized since the 1930's (Cruickshank *et al.*, 1934).

Glycogen is a natural energy source that is found in many places in the body – in particular it is well known that it supports positive vaginal health through the anaerobic metabolism mechanism, the vagina is an environment that is nutrient-rich and full with microbiota (Doderlien and Albert, 1892; Antoonio *et al.*, 1999; Borges *et al.*, 2014). This dynamic ecosystem continuously influences vaginal health during a women's life and natural stages (menstrual, pregnancy and menopausal) (Cailloutte *et al.*, 1997; Rampersaud *et al.*, 2012). When healthy, this ecosystem consists of microorganisms that

colonize surfaces with positive bacteria, the most common and “friendly” of which are *Lactobacilli* (Boskey *et al.*, 2001; Mironsef *et al.*.,2014). In reproductive-aged women this natural process is stimulated by the female hormone estrogen, which initiates the release of glycogen from the walls of the vagina, which is then metabolized by *Lactobacilli* to produce lactic acid . This balances the pH between 3.5 and 4.5 (Haya *et al.* ., 2014), providing natural resistance to potential health risks such as bacterial vaginosis, vaginitis and common anaerobic pathogens (Boris *et al.* ., 2000; Witkin *et al.* ., 2007; Srinivasan *et al.* 2015). In addition, this acidic environment promotes the growth of more *Lactobacillus* strains allowing the cycle that creates this protective environment to continue (Boskey *et al.* .,1999 ; Spear *et al.* ., 2015 ; Mironsef *et al.* ., 2016). During the course of natural life cycles, the levels of estrogen change and the shedding of the vaginal epithelium reduces the readily available glycogen.

2-6 Factors contributing to increased susceptibility of the FRT to infection :

There are many factors that have a role in the reproductive tract infections , Pregnancy is one of the most important factors that increase susceptibility of reproductive tract to infection where pregnancy is accompanied with high sexual hormones, especially estrogen , This will cause a malfunction in the conversion glycogen to the lactic acid, which in turn change the acidic environment of the vagina, in addition to a change in the production of hydrogen peroxide which has a role in preventing the colonization of the pathogens and thus will allow the infection (Galask , 1998 ; Brooks *et al.* .,1998) .

The genital tract infection of non-pregnant women may be attributed to a number of factors, including that take of contraceptive , the females that using various contraceptive methods had approximately 1–7 fold increase in risk of acquiring RTIs compared to women not take contraceptive users . Contraceptive methods with the use of hormone have been reported make immunity was low. It is possible to reduce immunity and give

rise to RTIs during the early weeks of contraceptive usage (Enomoto et al ., 2007) . The use of contraceptives also precipitates the glycogen in the epithelial cells of vagina, which results in change pH of the vagina and makes it an environment suitable for the growth of microbiology, especially yeast (Khider .,1985;Spinillo *et al.*, 1993). Excessive intake of antibiotics contributes to the killing of normal flora in the body, which encourages colonization of pathogens in the vagina (Willott .,1975;De-leon *et al.*, 2002). Many researchers agreed that Change of sexual partner, a recent pregnancy, use of an intrauterine contraceptive device and antibiotic treatment have been identified as plausible causes of BV (Hay, 2004; Smart, 2004). BV has been associated with, premature birth (Hay *et al.*, 1994) and with abortion (Donders *et al.*, 2009; Rocchetti *et al.*, 2011; Tavo,2013) .

Some studies have suggested that women who have been exposed to abortions often suffer from genital tract infections where germs can enter the uterus from the vagina and the organs used, or it is possible to enter the bacteria as a result of miscarriage or examination a sample of the endometrium (Gilchrist *et al* .,1995) , Post-abortion infection is not well defined as one particular type of infection in studies of medical abortion(Smart ,2004) . The most common type associated with abortion is endometritis, which is an infection of the lining of the, uterus, and genital tract. It may involve infection of urinary system and reproductive organs , germs may enter the uterus from vagina or the instruments used, causing pelvic inflammatory infection. If it grows complicated, it may lead to pain for the rest of the life. In serious cases, it may lead to sterility (Rocchetti *et al* ,2011) .

Other studies have reported that some bacterial infections cause serious health complications on the mother and fetus, of which the infections are Streptococcal bacterial vaginosis where the pregnant woman is at risk of being born and the fetus is exposed to a serious infection on the brain, lungs and bones (Sharma *et al* ., 2014). Other studies have indicated that 31.6% of pregnant women with vaginitis are at risk of miscarriage. The researchers believe that vaginitis caused by the growth of microbes in

the vagina may cause endometriosis, which causes a narrowing of the blood vessels that supply the fetus with oxygen and food thus aborting the fetus (Fregusson *et al* ., 2006).researchers report that although inflammation does not affect women's fertility and their ability to pregnant , it can prevent continued pregnancy (Munk *et al* ., 2011). the old is one of responsible factors of heterogeneity of the natural flora of the vagina causes periodic appearance of certain pathogenic organisms(Acikgoz *et al* .,2002), the incidence of vaginal infections increases in women with early age (16-30), This is due to the fact that these ages represent the early years of marriage in which sexual activity increases, adding to the arrival of reproductive hormones to their highest levels , in terms of advanced age, sexual activity decreases and the pH of the vagina returns to a low acidity that lead to decrease influmation (Al-Any , 2005) .

Diabetes is also one of the factors that promote the risk of infection with pathogens due to increase the proportion of sugar in the vagina, making it suitable environment for the growth of pathogens (Jeanette *et al* ., 2005) . where diabetes is one of most diseases that weaken the immunity and makes them more susceptible to germs, especially urinary tract (Zamanzad & Moezzi,2006) . Most with diabetics also suffer from Neurogenic bladder which lead to a complete emptying of the urine due to the presence of glucose in the urinemaking it a suitable medium for growth and reproduction of microbes. because of the anatomical nature of the vagina where the slot of the urinary tract close to the slot of the vagina, which increases the rate of transmission of these bacteria from its original location to the vagina as women with diabetes also have sugars in epithelial cells of their vagina that promotes the growth and reproduction of these microbes(Jeanette *et al* .,2005).

The vagina has special health standards characterized by natural acidity As well asA large group of healthy bacteria that help to prevent infection and maintain the function of the vagina (Russel,1999) .Dweik says there are no scientific studies available to confirm this information but the scientific experiment proved that some type of food have a

negative effect on vaginal odor such as garlic, warm spices, red meat because they reduce the degree of acidity of the vagina (Dweik,1990) .

Other studies have confirmed that fruits and vegetables support the health of the vagina as some vitamins and minerals found in fresh vegetables and fruits are particularly good, preventing dryness of the vagina. Citrus also increases the acidity of the vagina, in addition to containing vitamin C, which strengthens the immunity of vagina . Yogurt is rich in good and active bacteria which have a good effect on the vaginal environment which maintains a healthy and balanced pH ., also, vaginal membranes require a lot of water to function properly ,they need it to remain moist (Russel,1999) . Sugars are considered as nutrients for bacteria and yeast , because these organisms .simply love the environment sugary it is recommended to follow a low-carbohydrate diet to reduce its growth(Dweik ,1990; Russel ,1999 Zamanzad & Moezzi,2006).

2-7 Immunity of female genital tract :

Immune defense against female reproductive tract infection depends on vaginal flora , In addition to local component of innate immunity and acquired immunity. The innate immune system not only provides the early defence against infections but also instructs the adaptive immune system to respond to different microbes (Zinnemann and Turner ,1963). Where The innate immune system is capable of rapid elimination of microbes by locally secreted antimicrobial factors, phagocytosis and by activation of inflammation via enhanced phagocytosis by local macrophages and recruitment of phagocytes (neutrophils, monocytes)(Seifer *et al* .,2001 and Janeway , 2005).

This innate immunity also includes cells, such as dendritic cells and natural killer (NK) cells. Bacterial cells or accurate amounts of bacterial cell wall products at the invasion site , induce secretion of cytokines and chemokines from macrophages. Macrophages are the most efficient phagocytes, while neutrophils are the most abundant ones, appearing early at sites ofinfection. NK cells are specialised in attacking virus-infected host cells. Dendritic cells are phagocytising bacteria or virus particles and then carrying degraded

pathogen antigens to peripheral lymphoid organs, where they activate the adaptive immune system. The activation of the acquired immune system, which is the activation of lymphocytes T and B, the T cells are involved in cell-mediated immune responses, and B cells in humoral responses (secretion of antibodies). The T cells are either cytotoxic (CD8⁺ T cells), killing virus-infected cells, or they activate (CD4⁺ T cells) other cells (macrophages, B-cells). The cervico-vaginal epithelium, together with tissue-associated phagocytes (macrophages and neutrophils), represent the first line of the cellular host defence against microbes. The epithelial cells not only provide an essential physical and chemical barrier against infections, they can also actively participate in the defence against infection by their ability to secrete cytokines and chemokines (Basso *et al.*, 2005 and Wira *et al.*, 2005). Macrophages and neutrophils represent the first line of the cellular host defence against microbes.

The epithelial cells not only provide an essential physical and chemical barrier against infections, they can also actively participate in the defence against infection by their ability to secrete cytokines and chemokines (Basso *et al.*, 2005 and Wira *et al.*, 2005). Contains the highest numbers of macrophages and T cells (CD4⁺ and CD8⁺ lymphocytes). Numerous neutrophils are also present in this region. Few helper CD4⁺ T lymphocytes, NK cells, macrophages, or dendritic cells are found within the vaginal epithelium. Most of the CD4⁺ T cells and macrophages are located in the vaginal submucosa, the distribution of immune cells differs somewhat between the vagina and ectocervix in that more CD4⁺ T cells, NK cells, and dendritic cells are present in the epithelium of the ectocervix. Few CD4⁺ and CD8⁺ T cells are found in the epithelium and submucosa of endocervix. Macrophages are found in the epithelium, as well as the submucosa of endocervix, while dendritic cells are absent.

Neutrophils are "more or less" present in the endocervical epithelium and can be found in high numbers. Antibodies (IgM, IgG, IgA), which are produced by local B lymphocytes are secreted into the tissue and partly transferred to the mucosal fluid. Antibodies help block attachment of microbes to the epithelium (secretory IgA) and

neutralise and eliminate microorganism and microbial toxins (IgG and IgM) in the tissue or lumen of mucosal organs. In human cervical mucus, there are higher levels of IgG than of IgA, which determines the specific humoral defence of the mucosal surface (Mestecky and Fultz,1999 and Mestecky and Russell,2000). Immunohistochemical examination of tissue sections has indicated that the endocervix contains higher numbers of Ig-secreting cells than the ectocervix, fallopian tubes, and vagina (Mestecky and Fultz.,1999).

2-8 The relationship between sex hormones and immunity of female genital tract:

Sex hormones are steroid hormones. Due to their lipophilic nature, steroid hormones can diffuse across the cell membrane; classical steroid hormone receptors can thus be found intracellularly and have direct genomic effects (Beato and Sanchez,1996). More recently, also non-genomic effects of steroid hormones have been described, which are most likely regulated via more recently described membrane receptors for steroid hormones(.Marino *et al* ., 2006; Dosiou *et al* .,2008). An alternative explanation is that by their lipophilic nature, sex steroids can alter membrane properties by integrating into the membrane, thereby changing the function of the immune cells(.Lamche *et al* ., 1990).

Classical intracellular estrogen receptors are present in human monocytes (.Suenaga *et al* .,1998 and Phiel *et al* .,2005) human neutrophils(Molero *et al* .,2005) human dendritic cells(Komi and Lassila,2000) murine peripheral NK cells (Curran *et al* .,2001) human Tc lymphocytes and B lymphocytes(Cohen *et al* .,1983 and Stimson,1988) .There is no evidence that classical intracellular progesterone receptors are expressed on resting lymphocytes(Mansour *et al* ., 1994 and Szekeres.,2002), monocytes(Schust *et al* ., 1996),neutrophils(Aerts *et al* ., 2002) NK cells, B lymphocytes, Treg cells and dendritic cells. Various studies, however, demonstrated that activated lymphocytes, for instance lymphocytes during pregnancy, can upregulate progesterone receptors(Szekeres,1995;;Barakonyi *et al* .,1999;Polgar *et al* .,1999; Szekeres ,2002) .

More recently membrane-bound progesterone receptors have been shown to be present on resting lymphocytes (Szekeres *et al.*, 1990; Dosiou *et al.*, 2008). The most effects of sex hormones on the immune response lie in the effects of these hormones on the numbers of circulating immune cells. In the peripheral blood, about 65% of the leukocytes are granulocytes (90% neutrophils), 5-10% is monocytes, and 30% are lymphocytes (85-90% T lymphocytes and 10-15% B lymphocytes).

Sex hormones have been shown to affect these cell numbers by affecting proliferation or apoptosis of the cells (England, 1975). Although total white blood cell counts did not differ between males and females, an increase in white blood cells counts was observed in the luteal phase (and during pregnancy) as compared with the follicular phase of the ovarian cycle (Bain and England, 1975 and Elenkov *et al.*, 2001). This may be largely due to an increase in granulocyte numbers in these reproductive conditions (Apseloff *et al.*, 2000; Bouman *et al.*, 2001; Veenstra *et al.*, 2002). This suggests a role for progesterone and/or estrogen in increasing the numbers of granulocytes. In addition, various studies point to the monocyte numbers decrease in the presence of estrogen, and increased numbers of monocytes in menopausal women as compared with women in the follicular phase of the ovarian cycle (Bouman *et al.*, 2004).

2-9 Treatments

2-9-1 Treatment of Bacterial vaginosis:

Metronidazole and Clindamycin have been the conventional drug for treating BV (Livengood *et al.*, 1994; Sobel *et al.*, 2001). It has been the recommended drug for BV treatment by the Centre for disease Control and Prevention (CDC). These drugs are only effective in about 60% to 80% in BV cases (Livengood *et al.*, 1994; Paavonen *et al.*, 2000; Eriksson *et al.*, 2005). A study by Paavonen *et al.*, in 2000 had metronidazole cure rate of 80% and that of clindamycin to be 68.1% in BV infected women (Paavonen *et al.*, 2000). The use of probiotics as a means to improve the vaginal flora and prevent BV has shown mixed results. While some studies have shown beneficial effect (Anukam *et al.*,

2006), some other did not (Eriksson *et al.*, 2005). A lot of treatments are used to eliminate bacterial vaginitis such as aztreonam, cefotaxime, doxycycline, imipenem, ceftriaxone, cefoxitin, penicillin, ampicillin, amikacin, chloramphenicol, gentamicin, and other (Shamim *et al.*, 2008; MONIKA *et al.*, 2014; Okiki *et al.*, 2015). The most effective chemotherapeutic agents against bacterial vaginosis were imipenem 96.0%-100% (Mumtaz *et al.*, 2002).

2-9-2 Treatment of candidiasis

Vulvovaginal candidiasis is grouped into complicated and uncomplicated candidiasis (Sobel, 2007). Short oral or topical therapy such as azole, itraconazole, clotrimazole, butoconazole, nystatin, miconazole, terconazole, ketoconazole, boric acid, tioconazole and single dose fluconazole are used for the treatment of uncomplicated candidiasis (Pappas *et al.*, 2004). For complicated candidiasis, treatment needs seven days or more antimycotic therapy either topical therapy or as two 150mg doses of fluconazole. Non-*albicans* species are best treated with topical boric acid or flucytosin (Sobel, 2007). A systematic review publication recommended imidazole for treatment in symptomatic pregnant women suffering from candidiasis for a longer duration of about seven days (Young & Jewell, 2001).

2-9-3 Treatment of Trichomoniasis :

Metronidazole is the drug of choice for the effective treatment of Trichomoniasis. A single dose of 2g taken orally has a reported cure rate of 97% (Lossick, 1980). In pregnant women, there has been controversy regarding the adverse effect of metronidazole causing preterm delivery. However, CDC have not revised their recommended 2g single dose treatment regimen for pregnant women at any stage of the pregnancy (CDC, 2010). Metronidazole may display some level of resistance to treatment in about 2.5 to 5% in all cases of Trichomoniasis (Schmid *et al.*, 2001). Higher doses of the drug can overcome this resistance (Lossick *et al.*, 1986). Resistant strains of TV can be solved with

a 2g oral dose of tinidazole. The drug has been shown to have equal efficacy to metronidazole 90 to 100%(Sawyer *et al* ., 1976;Manorama & Shenoy, 1978) .

Chapter three

Materials and

Methods

3-1 Materials

3-1-1 :Equipment and apparatus:

The equipments and instruments that were used in the present study with their remarks are listed in table (3-1).

Table(3-1) : General Laboratory Equipments

No.	Equipment	Company	Country
1	Autoclave	Gemmy	Taiwan
2	Compound light microscope	Olympus	Japan
3	Conical flasks	BBL	USA
4	Disposable petri dishes	Al-Hani	USA
5	Disposable Syringes	Superestar	India
6	Electric Oven	Sartorius	Germany
7	Ependorff tubes	Sigma	England
8	Ichroma device	Boditech Med Inc	Korea
9	Incubator	Memert	Germany
10	MicroCentrifuge	Hettich	Germany
11	Refrigerator	Concord	Lebanon
12	Sensitive electronic Balance	Ohaus	U.S.A
13	Slides and cover slides	Superestar	India
14	Sterilized cotton swabs	SterellinLtd	England
15	Standard wire loop (1 μ)	Himedia	India
16	Test tubes	Superestar	India
17	Water distillatory	GFL	German

18	water bath	Memert	Germany
19	pH strip (range 0-6)	Sigma	USA.
20	Ichroma device	Best LAB	Korea

3-1-2 Chemical materials:

The Chemical materials that were used in the present study are listed in table (3-2)

Table (3-2) chemical material

No .	Chemical materials	Company	Country
1.	Acetone alcohole	BDH	(USA)
2.	Crystal violet	BDH	(USA)
3.	Iodine	Mastdiagnostic	(USA)
4.	Safranine	Sigma	(England)
5.	Hydrogen peroxid (70%)	SDI	(Iraq)
6.	Methyl red (C₁₅H₁₅N₃O₂)	BDH	(USA)
7.	giemsa stain	Hi Media	(India)
8.	Potassium hydroxide(KOH)	BDH	(USA)

3-1-3 Kits:

The kits with their remarks that were used in the present study are listed in table (3-3) .

Table(3-3) Kits

No.	Kit	Company	Country
1	Chroma Progesterone test kit	Boditech Med Inc	Korea
2	Chroma prolactin test kit	Boditech Me Inc	Korea
3	Chroma Estrogen test kit	Boditech Me Inc	Korea
4	API <i>Candida</i>	BioMerieux	France
5	API <i>Staphylococcus</i>	BioMerieux	France
6	API 20 <i>Streptococcus</i>	BioMerieux	France
7	API 20 <i>Enterobacteriaceae</i>	BioMerieux	France

3-1-4 culture media:

The culture media with their remarks that were used in the present study are listed in table(3-4).

Table (3-4): Culture media

No.	Culture media	Company	Country
1	Blood Agar	Accumix	(India)
2	Brain –Heart Infusion Agar	Mast diagnostic	(USA)
3	Chocolate Agar	Prepared in Lab.	
4	kligler Iron Agar	Himedia	(India)
5	MaCconkey Agar	Himedia	(India)
6	Manitol Salt Agar	Himedia	(India)

7	Malt Extract Agar	Himedia	(India)
8	Methyl red –Voges Proskauer Broth	Oxoid	(UK)
9	Muller Hinton Agar	Himedia	India
10	Nutrient agar	Himedia	(India)
11	Peptone Water	Himedia	India
12	Sabouraud Dextrose Agar	Oxoid	England
13	Sabouraud Dextrose broth	Oxoid	England
14	Simmon 's Citrate Agar	Himedia	(India)
15	Urea agar	Himedia	(India)

3-1-5Antibiotics and Antifungals:

The antibiotics and antifungals that were used in the present study with their remarks are listed in table(3-5).

Table(3-5): Antibiotics and Antifungals

No.	Antibiotic	Concentration	company	country
1	Amoxicillin(AML)	30 µg	Mast	UK
2	Aztreonam(ATM)	30 µg	Mast	UK
3	Cefotaxime(CTX)	30 µg	Mast	UK
4	Doxycycline(DO)	30 µg	Mast	UK
5	Imipenem(IPM)	10 µg	Mast	UK
6	Piperacillin/ (TPZ) tazobactam	10 µg	Mast	UK
No.	Antifungals	Concentration	Company	contry
1	Fluconazole(Fu)	25 µg	Himedia	(India)
2	Ketonazole(Kt)	15 µg	Himedia	(India)
3	Nystatin(Ns)	50 µg	Himedia	(India)

3-2 Methods

3-2-1 Preparation of Culture media

All used media mention in 3-1 except Blood agar , chocolate agar and urea agar were prepared according to the directions of the manufacturing companies, and were dissolved in D.W, then sterilized by autoclave at 121°C for 15 min. under 15 psi.

3-2-2 Preparation of Reagents and Solutions :

3-2-2-1 Preparation of reagents

- **Oxidase test reagent:**

Prepare 1 g of Tetra methyl-P-phenylene diamine hydrohydrate chloride in 90 ml of distilled distilled water and then complete to 100 ml. It was used to detect the susceptibility of bacteria to the production of oxidase (MacFaddin, 1985) .

- **Catalase test reagent**

Attended a 3% concentration of hydrogen peroxide H_2O_2 and was used to detect the ability of bacterial isolates to produce catalase (MacFaddin ,1985) .

- **Kovac's reagent:**

Dissolve 5 g of para-dimethyl aminobenzaldehyde in 75 ml isopropyl alcohol using a water bath and then complete to 100 ml with Hcl acid. The color of the detector is pale yellow, then stored in a dark bottle in the refrigerator.(MacFaddin ,1985).

- **Methyl red reagent:**

Prepare by dissolve 0.1 g of the methyl red in 300 ml of methyl alcohol at 95% concentration and then complete the volume to 500 ml by adding 200 mL distilled water(MacFaddin , 1985) .

- **Vogas- Proskaur reagent:**

This reagent consists of:

1) Alpha nepthol 5%

Prepare by dissolve 5 g of alpha nepthol in 100 ml ethyl alcohol at 95% concentration.

2)Potassium hydroxide KOH 40%

Prepare to dissolve 40 g of potassium hydroxide in 100 ml of distilled water(MacFaddin , 2000)

3-2-2-2 Preparation of Solutions:

- **Normal Saline Solution**

Prepare the solution by dissolving 0.85 g of sodium chloride in 90 ml distilled water, complete in size to 100 mL, and sterilized with a autoclave(MacFaddin, 2000) .

3-2-3 Design of study :

Samples of vaginal swabs and blood collected from women revisions to the consultancy clinic in Bint al huda teaching hospital for child birth and children and the consultancyclinic in al habouby teaching hospital in al nasiriyah city for the period from the beginning of october 2016 until the end of february 2017, the suspect they are suffering from a vaginal infections based on the presence of clinical sign and symptoms that diagnosed by a professional doctor. the number of samples are 245 appointed for each of the vaginal swabs and blood (where taken vaginal swab and blood of every woman). The women s' age ranging from 16 to 45 year divided into three age groups (16-29),(30-39),(40-45) year and collected the required information from them .

3-2-4Collection of specimens

The vaginal swab samples are taking by using a special machine to open the vaginal called "sim 's speculum" then insert sterile cotton swab to (2cm) in distance

inside the vagina and spin gently toward the wall of the vaginal before pulling swab and then placed in the carrier media (stuart transport media) until arrival to the lab and work on them (Holt *et al* .,1994) . Blood samples collected by the conventional way of taking blood from the vein.

3-2-5 Direct examination

The vaginal swabs are directly examined to check the presence of each of the clue cell , yeast cell , WBCs , *Trichomonase vaginalis* and epithelial cell by working stuck to the sample by using an aqueous salt solution (normal saline) where the drop of stuck is placed on glass slide and covered by the cover slide and examined under the large lens of the microscope(Atlas ,1995).

3-2-6 Specimens Culture:

The vaginal swabs are cultivation on blood agar , macconky agar , chocolate agar and sabouraud dextrose agar to diagnose all kinds of microbes could exist within it , then incubated at 37 ° C for 24-18 hours, and incubated dishes that did not appear to grow within 24 hours for another 24 hours before counting negative result.

3-2-7 Identification of Microbes :

Developing colonies were identified as dependent on

3-2-7-1 Phenotypic characteristics

The morphological characteristics of the developing colonies were observed in their forms, color and surface of the colonies, the presence of distinctive odors, their strength and translucency, the pattern of blood hemolysis on the blood agar medium, and the lactose fermentation of the macconky agar medium(Winn *et al* ., 2006).

3-2-7-2 Microscopic diagnosis

The colonies of microbes that growth on media were examined microscopically by taking the colonies by loop and fixing them to observe the forms of the microbial cells (Atlas,1995) .

- **Gram's Stain**

The slide was placed on a rack of stain and the specimen covered with crystal violet and Left to stand for 1 minute then it was washed briefly in tap water shaking off the excess. The specimen was covered with iodine solution and let stand for 1 minuteIt was washed with water and shaking off the excess. The slide was tilted at 45° angle and decolorized with the acetone-alcohol solution until the purple colour stopped running then washed immediately with water shaking off excess. The specimen was covered with neutral red and let to stand for 30 seconds to 1 minute. It was washed with water, shaking off excess, and gently blotted dry. The smear was ready to be read by using oil immersion lens (Christian *et al* ., 1996).

- **Giemsa stain**

The prepared smear was fixed by immersion in methanol for 1 minute and allowed to dry. Then It was stained with giemsa stain (Hi Media Laboratories, India), diluted 1 part to 19 parts of 1/15M phosphate buffer, pH 7.2 for 10 min and scanned for *Trichomonas vaginalis* at 100X magnification (Mason *et al* .,1976) .

3-2-7-3 Biochemical tests

A series of biochemical tests were performed to diagnose the microbial isolates under study, as follows :

1) Catalase test

Transfer part of a 24-hour-old colony by means of sterile wooden sticks to a clean glass slide and then add a drop of H₂O₂ solution at 3% concentration. The result is positive with bubbles of oxygen gas(MacFaddin ,1985) .

2) Oxidase test

Transferring part of a 24-hour-old colony by means of a sterile wooden stick to a filter paper saturated with oxidase, and the violet color within 10 seconds is evidence of the test positive (MacFaddin, 1985).

3) Urease test

In this test, the sterile Urea agar was used to irrigate a fraction of the pure bacterial colony to be tested and then incubated at 37 ° C for 24-48 hours. The positive result is indicated by changing the color of the medium from yellow to pink (MacFaddin, 2000).

4) Sugar fermentation & gas production test:

The tubes containing the Kliger's Iron Agar (KIA) were pelleted and plotted on the slanted surface of the bacterial plant and incubated at 37 ° C for 24 hours. Changing the color of the medium from red to yellow is evidence of the bacteria's ability to ferment glucose and lactose, while the production of hydrogen sulfide gas is in the form of a black deposit below the solid medium. (MacFaddin, 1985).

5) Coagulase test :

It is used to differentiate between *Staphylococcus aureus* and other types of *Staphylococcus* bacteria. The test uses a rabbit plasma that has been vaccinated with *staphylococcus*. The tube is incubated at 37 ° C for an hour and a half. If the result is negative, the test is passed to 18 hours. If the result is positive, the plasma will clot and appear as a lumped piece. If negative, plasma is not changed (MacFaddin, 2000).

6) Germ tube formation was done according to Kurtzman and Fell ., 1998).

1) 0.5 ml of egg albumin was mixed with a part of *Candida* colony and incubated at 37°C for 3 hr.

2) Drop of the albumin was placed on a slide and covered by slip.

3) The drop was examined under microscope (40X) to observe the presence of germ tube originating from the blastoconidium without any constriction at the point of origin and without obvious swelling along the length of the filaments which indicates a germ tube positive (*Candida albicans*).

7) IMViC group of tests include :

• Indol production test :

In this test, Peptone water was used which inoculated part of the pure bacterial colony to be tested and incubated the tubes at 37 ° C for 18-24 hours. After that, several drops of the Kovac reagent were added to each tube with good shaking. The appearance of a red ring showed evidence of positive examination and the ability of the bacteria to analyze the amino acid tryptophan and tryndophan production (MacFaddin ., 2000) .

• Methyl red test

This test was performed by injecting the tubes containing the MR.VP. Medium with a fraction of the pure bacterial colony to be tested and incubated at 37 ° C for 24-48 hours. After the end of the incubation period, 5 drops of red reagent were added. The positive result of the appearance of red indicates the production of acid, while the survival of the yellow color represents the negative result (MacFaddin ,1985; Collee *et al* ., 1996).

• Voges – Proskauer test:

The tubes containing the MR.VP. Medium with a fraction of the pure bacterial colony to be tested and then incubated at 37 ° C for 24-48 hours. After that, add 1 mL

of reagent to each tube with the silent stirring and then leave for 10-15 minutes ,The positive result was indicated by the appearance of red(MacFaddin ,1985 and Baron *et al .*, 1994) .

- **Citrate utilization test :**

In this experiment, the median of Simon Street was tilted with a fraction of the pure bacterial colony to be tested and incubated at 37 ° C for 24-48 hours. The positive effect of the test was indicated by a change in the color of the medium from green to blue, indicating that the bacteria consumed the jackets as a single source of carbon (MacFaddin ,2000 ; Winn *et al .*, 2006) .

3-2-7-4 Motility test:

Taken by sterile loop one diameter of distilled water , it is placed on the glass slide,the loop is then sterilized by a benzene lamp and then cool down,very few bacterial colonies are taken to be tested ,mixed with distilled water ,it is carefully placed on the sterile slide that special to motility test, the check is done gradually from little power to great power if movement is observed, the test is positive,if you do not notice movement, the test is negative(MacFaddin., 2000).

3-2-7-5 Hemolysis:

inoculate blood agar with pure bacterial culture and incubated in 37 C to24 hourse , The emergence of transparent regions around bacterial colonies developing indicates the susceptibility of bacteria to secretion hemolysin (Cowan,1985; Zunino *et al .*,1999;Dulczak and Kirk , 2005) .

3-2-7-6 CAMP factor :

Down the center of a blood agar plate , make a single straight line streak of beta-hemolysin producing *Staphylococcus aureus*,inoculate a streak of the beta-hemolytic streptococcus to be identified perpendicular to the staphylococcal streak.after

incubation, the growth of the two organisms will not be touching. The streptococcal streak should be 3 to 4 cm long. Incubate the plate at 35°C in ambient air for 18-24 hours (Wilkinson., 1977).

3-2-7-7 pH measurement :

The pH was measured by Place a paper of pH directly and contact with Sterilized vaginal swab loaded with discharge (Gaillouette et al., 1997).

3-2-7-8 Whiff test for amines :

A swab of the vaginal fluid was dipped in 1 ml of 10% KOH and mixed for 3–5 minutes. The test was interpreted as being positive, i.e. suggestive of *G. vaginalis*, if a fishy odour was noted (Gutman et al., 2005).

3-2-7-9 Api 20 E diagnosis system

The 20 Eapi strips were used to diagnose Enterobacteriaceae family in final form. This tape contains 20 tubes for specific biochemical tests. The work method includes the following steps:

A)Preparation of bacterial suspension:

A 5 mL saline solution was sprayed with 1-4 colonies of bacteria to be diagnosed by swab with continuous blending to obtain a concentration of 1.5×10^8 cell / ml compared to the standard (0.5) standard turbidity solution.

B)Api Inoculation of the api strip

After preparation airborne bacterial and using a clean and dry pipette the amount of 0.12 mL of bacteria stuck each test tubes and Javhqah in the amount of vaccine bacterial reached 0.28 mL pipeline tests CIT, VP, GEL, and add mineral oil sterilized (Sterile mineral oil) to the tests underlined that included URE, ADH, LDC, ODC, H₂S.

C)ApI Incubating the strip in its chamber

Place the api tape inside a special case or cover in the kit after placing a few drops of water in the hole of the cover , and then incubate the api tape at 37 ° C for 24-48 hours.

D)Addition of reagents

A drop of the TDA reagent to the test of the removal of the amino acid group of tryptophan (tryptophan deaminase), turning the color brown indicates the positive result .A drop of the JAMES detector was added to the indole production test.

The appearance of a red ring indicating the positive result.add a drop of each VP1 + VP2 detector, respectively, to the voges - Proskauer test, turning the color to pink indicates the positive result.A drop of the NIT1 detector and a drop from the NIT2 detector were added to the GLU test, turning the color to blue indicates the positive result .

E)Reading of Result

The bacteria were identified by the code number, which is formed after marking (+) or (-) for each of the biochemical tests in the api tape. Each bacteria has its own numeric code, and the numbers were compared to tape papers

3-2-7-10 API 20 *Streptococcus* :

The 20 strep strips were used to diagnose most of streptococcaceae family in final form. This tape contains 20 tubes for specific biochemical tests. The work method includes the following steps:

a)Preparation of the inoculum:

Open an ampule of API Suspension medium (2 ml) as indicated in the paragraph “warnings and precautions” or use any tube containing 2 ml of distilled

water without additives . using a swab , harvest all the culture from the previously prepared subculture plate make a dense suspension with a turbidity greater than 4 McFarland. This suspension must be used immediately after preparation .

b)Preparation of the strip

Prepare an incubation box (tray and lid) and distribute about 5 ml of distilled water or demineralized water (or any water without additives or chemicals) into the honey combed wells of the tray to create a humid atmosphere ,remove the strip from its individual packaging . place the strip in the incubation box.

c)Inoculation of the strip

In the first half of the strip (tests VP to ADH), distribute this suspension , avoiding the formation of bubbles .For the tests VP to LAP : distribute approximately 100ML into each cupule .

For the ADH test :fill the tube only. In the second half of the strip (tests RIB to GLYG):Open an ampule of API GP medium as indicated in the paragraph ”warnings and precautions” and transfer the rest of the suspension into it (appr.0.5 ml) . mix well.

Distribute this new suspension into the tube only.Fill the cupule of the underlined tests (ADH to GLYG) with mineral oil to form a convex meniscus.Place the lid on the tray .Incubate at 36 C +- 2 C in aerobic conditions for 4-4 1\2 hours to obtain a second reading if required .

d)Reading the strip :

After the 4.30 hours of incubation :Read the reactions by referring to the reading table in this package insert and record them as +or – on the result sheet . Each type of *Streptococcus* has its own numeric code, and the numbers were compared to tape papers.

3-2-7-11 API *Staphylococcus*

API Staph is a standardized system for the identification of the genera *Staphylococcus*, the API staph strip consists of 20 microtubes containing dehydrated substrates.

A) Preparation of the inoculum :

Open an ampule of API staph medium as indicated in the paragraph “ warnings and precautions” Prepare a homogeneous bacterial suspension with a turbidity equivalent to 0.5 McFarland. It is recommended to use young culture (18-24 hours old). This suspension must be used immediately after preparation.

B) Preparation of the strip

Prepare an incubation box (tray and lid) and distribute about 5 ml of distilled water or demineralized water (or any water without additives or chemicals) into the honey combed wells of the tray to create a humid atmosphere.

C) Inoculation of the strip

Using a pipette to fill the microtubes with the inoculated API Staph medium. Only fill the tube portion of the microtubes, not the cupules, to avoid the formation of bubbles at the base of the tubes.

Tilt the strip slightly forward and place the tip of the pipette against the side of the cupule. Ensure an aerobiosis in the ADH and URE tests by filling the cupules with mineral oil to form a convex meniscus, close the incubation box, incubate at 36°C ± 2°C for 18-24 hours.

D) Reading the strip

After the incubation period, develop the reactions by adding 1 drop of each of the following reagents and table: VP test: VP1 and VP2 reagents. Wait 10 minutes. A

violet –pink color indicates a positive reaction . a pale pink or light pink color obtained after 10 minutes should be considered negative .

NIT test :NIT 1 and NIT 2 reagents , wait 10 minutes . a red color indicates a positive reaction . PAL test :ZYM A and ZYM B reagents. Wait 10 minutes . A violet color indicates a positive reaction.

3-2-7-12 API candida

Api candida is a standardized system for the identification in 18-24 hours of yeasts . the Api candida strip consists of 10 tubes containing dehydrated substrates , which enable the performance of 12 identification tests (sugar acidification or enzymatic reactions) .. The work method includes the following steps:

a)preparation of the inoculum :

Open an ampule of API NaCl 0.85% medium (2ml) using a pipette or swab , take one or several well isolated , identical colonies and prepare a suspension with a turbidity equivalent to 3 McFarland compare with a turbidity control or measure using a densitometer . It is recommended to use young cultures (18-24 hours old). Homogenize the yeast suspension . this suspension must be used immediately after preparation .

b)preparation of the strip :

prepare the incubation box (tray and lid) and distribute about 5 ml of water without additives or chemicals added in to the honey combed wells of the tray to create a humid atmosphere . , remove the strip from its individual packaging . place the strip in the incubation box.

c)Inoculation of the strip

Distribute the prepared yeast suspension in to the tubes only , avoiding the formation of bubbles .Cover the first 5 tests (GLU to RAF) and the last test (URE) with mineral oil (underlined tests) immediately after inoculating the strip.

d)Reading the strip

After the 18-24 hours of incubation :Read the reactions by referring to the reading table in this package insert and record them as +or – on the result sheet . Each type of candida has its own numeric code, and the numbers were compared to tape papers .

3-2-8 Antibiotic Susceptibility Testing :**3-2-8-1 Antibiotic Susceptibility Testing for bacteria :**

The pharmacological sensitivity of bacterial isolates was examined in a CD-based method, based on bauer and his group (1966) and CLSI (2012) transfer 2-4 colonies of bacteria to a test tube containing 5 ml of salt solution to reduce growth,comparison of growth in the tube with the standard McFarland tube (0.5) .

The cotton swab was submerged in salt solution , the excess was removed by pressing the internal aspects of the tube , the bacteria were deployed on the center of muller-hinton agar in a planning manner for more than once,and in different directions for the purpose of ensuring the dissemination of bacteria to be tested sensitivity equally, the dishes were left for 15 minutes at room temperature to ensure moisture absorption .

The tablets of antibiotics mentioned in paragraph (3-5) were developed by nine tablets in dish measuring 100 millimeters .and 12 tablets in a 150-millimeter dish ,the distance between each disk is 20 millimeters from the center of the first disk to the center of the other disc .the dishes were incubated at 37 m for 18 hours for all types of antibiotics,the diameters were measured and compared with the standard values listed in CLSI (2012) .

3-2-8-2 Antibiotic Susceptibility Testing for *Candida albicans*

Antifungal susceptibility testing- antifungal susceptibility testing of the isolates was performed by three antifungal drugs disk nystatin(50Mg),fluconazole(25Mg), ketoconazole (15Mg).

Antifungal susceptibility testing was performed by disk diffusion method using mueller-hinton agar + 2% glucose and 0.5 µg/ml methylene blue dye (GMB) medium as per CLSI guidelines (C.L.S.I. document M44-A2,2009.). 0.5 McFarland standard was used to standardize the inoculum density.

C.albicans was used as quality control. all the culture media, antifungal disk, and control strains were obtained from media laboratories, india.the antifungal susceptibility of the *Candida albicans* isolates was interpreted as sensitive (S), dose dependent-susceptible (DDS) and resistant (R). The results were interpreted as per the clinical and laboratory standards Institute (CLSI) guidelines.

3-2-9 Blood Sample Procedure :

3-2-9-1 Measurement of hormones:

The concentration of progesterone,estrogen and prolactin in the blood serum have been measured by using the “ ichroma” device with kit ready-analysis of these hormone by following the instructions supplied with the kit for hormone progesteron .

3-3 Statistical Analysis :

The statistical analysis proceeded in groups of study, descriptive statistics analyzed by using one-way analysis of variance (ANOVA) were performed using means and standard deviations (SDs) with LSD test for continuous variables ($p < 0.05$) was considered to be significant, and X^2 distribution (P-value 0.05) was considered to be significant. All analyses were performed with the statistical

package for the social sciences SPSS for windows (version 17.0, SPSS Inc, Chicago, III).

Chapter four

Results

4-1 Type and number of isolates taken during the study:

A total of 245 vaginal swabs were collected from 150 pregnant women and 95 non-pregnant women in age average 16 - 45 years . the pathogens isolated in this study are shown in table (4-1) .

They included 75 isolate of *Candida albicans* which were predominant in pregnant women 55(36.7%) than non pregnant women 20(21.1%) , as obtained 35 isolate of *Streptococcus agalactiae* were isolated from pregnant women only ,other are 27 isolate of *Staphylococcus aureus* and 25 isolate of *Escherichia coli* which were isolated from 20 (13.3%) and 15(10.0%) pregnant women and 7(7.4%) , 10(10.5%)from non pregnant women respectively.

As isolated 21 *Staphylococcus epidermidis* only from non pregnant women , *Gardnerella vaginalis* which also obtained in a higher rate of isolates from pregnant women 12(8.0%) than non pregnant women 7(7.4%) while *Klebsiella pneumoniae* was isolated in nearly equal proportions from the pregnant and non pregnant women with 7(4.7%) , 8(8.4%) respectively , *Proteus mirabilis* and *Pseudomonas aeruginosa* were isolate only from non pregnant women 11(11.6%) and 9(9.5%).finally ,*Trichomonas vaginalis* , *Enterobacter cloacae* was isolate from pregnant women 6(4.0%) , (0%) and (0%) , 2(2.1%) from non pregnant women respectively,for women infected with *C.albicans*,*S.agalactiae*,*S.epidermidis*,*P.mirabilis*, *P.aeruginosa*,*T.vaginalis* the study recorded there was a significant difference at level ($P<0.05$) between the rate of infection in pregnant women and non pregnant women while for women infected with *S.aureus*, *E.coli*, *G.vaginalis* , *K.pneumonia*, *E.cloacae* there were no significant difference at level ($P<0.05$) between the rate of infection in pregnant women and non pregnant women .

Table (4-1) organisms isolated from the vaginal swabs samples

Pathogens	case						X ²
	Pregnant %		Non pregnant %		Total %		
	+ve	-ve	+ve	-ve	+ve	-ve	
<i>C.albicans</i>	55(36.7)	95(63.3)	20(21.7)	75(78.9)	75(30.6)	170(69.4)	6.67
<i>S.agalactia</i>	35(23.3)	115(76.79)	0(0.0)	95(100)	35 (14.3)	210(85.7)	25.86
<i>S. aureus</i>	20(13.3)	130(86.79)	7(7.4)	88(92.6)	27(11.0)	218(89.0)	2.11
<i>E.coli</i>	15(10.0)	135(90.0)	10(10.5)	85(89.5)	25(10.2)	220(89.8)	0.01
<i>S.epidermids</i>	0(0.0)	150(100)	21(22.1)	74(77.9)	21(8.6)	224(91.4)	36.26
<i>G.vaginalis</i>	12(8.0)	138(92.0)	7(7.4)	88(92.69)	19(7.8)	226(92.2)	0.032
<i>K. pneumonia</i>	7(4.7)	143(95.3)	8(8.4)	87(91.6)	15(6.1)	230(93.9)	1.42
<i>P. mirabilis</i>	0(0.0)	150(100)	11(11.6)	84(88.4)	11(4.5)	234(95.5)	18.18
<i>P. aurgenosa</i>	0(0.0)	150(100)	9(9.5)	86(90.5)	9(3.7)	236(96.3)	14.75
<i>T.vaginalis</i>	6(4.0)	144(96.0)	0(0.0)	95(100)	6(2.4)	239(97.6)	3.89
<i>E. cloacae</i>	0(0.0)	150(100)	2(2.1)	93(97.9)	2(0.8)	243(99.2)	3.18
Total	150(61.2)		95(38.8)		245(100.0)		

Tab. X²: (df:1 ; P_value: 0.05) = 3.841

4-2 Identified of microbes that isolated in this study :

Many of biochemical and morphological tests performed to identify the microbes isolated during our study . the characteristic of *Candida albicans* colonies on blood agar was convex , creamy white , dull surface while it was white to cream soft colonies on sabouraud dextrose agar and creamy colonies on malt extract agar (figure 4-2). under microscope *Candida albicans* was oval to spherical shape (figure4-3) , its gave positive result in gram stain , germ tube formation (figure4-4) , API Candida(7512). *Streptococcus agalactiae* showed pin point colonies with B-hemolysis on blood agar (figure 4-5) , on nutrient agar had shown small circular colonies and it was in chain (pairs, small cocci) , not motile under microscope , it's positive to gram stain , CAMP factor , API 20 strep(3462415) and negative to coagulase , catalase . *Staphylococcus aureus* distinguished in golden yellowish colonies on manitol salt agar (figure 4-5), it was white golden yellow colonies , B-hemolysis on blood agar and it was gray white or yellowish colonies on nutrient agar . under microscope it was in clusters shape (cocci), not motile , positive to gram stain , catalase , urease ,coagulase , API staph(6336151). While the colonies of *staphylococcus epidermidis* was pink on mannitol salt agar (figure4-5) , on blood agar it was alpha hemolysis and on nutrient agar it was white colonies , under microscope it was pairs in shape , not motile , it was positive to gram stain , catalase ,API Staph (2706013) and it was negative to coagulase . *Gardnerella vaginalis* was growth on chocolate agar by use candle jar. it's colonies was small , circular , convex , gray . under microscope it was bacilli and gave negative result to catalase , coagulase , oxidase , motility . The characteristic of *Escherichia coli* colonies on blood agar was white to brown colonies and pink colonies on macConky agar (figure 4-6)while it was circular , with a convex, elevation and a smooth surface on nutrient agar . when do microscopic examination *Escherishia coli* , had shown as a gram negative bacilli shape , motile , positive to catalase, indol, methyl red , API 20 E (3144533) and negative to oxidase , urease, citrate, voges proscaure while the result of TSI test was Alk\A with gas . The colonies of *Klebsiella pneumonia* was mucoid on

blood agar , on macConky agar it was pink mucoid colonies (figure 4-7) . under microscope it was negative gram bacilli ,not motile , negative to oxidase, indol , methyl red and positive to catalase , urease, voges proscaure, citrate , ,API 20 E (5014733) , the result of TSI test was A\A\gas .

Enerobacter cloaceae appeared in pink colonies on macConky agar , under microscope it was gram negative bacilli , motile , it was positive to catalase, citrate , voges proscaure , API 20 E (3115573) , negative to urease , indol , methyl red and gave A\A\gas in TSI test.

Pseudomonas aeruginosa had shown as a grayish colonies ,B-hemolysis on blood agar , on nutrient agar *Pseudomonas aeruginosa* appeared in smooth large , greenish blue colonies while on macConky agar it gave pale colonies with brown melanin pigment , it was gram negative bacilli , motile under microscope ,it was positive to oxidase , catalase, citrate , API 20E (1314475) and negative to urease, indo, voges proscaure methyl red , and when use TSI test gave A\A, no gas .

The colonies of *proteus mirabilis* appeared pale on macConky agar and white colonies with a smell like fish and swarming phenomenon(figure 4-8). While it was small colonies on nutrient agar . in microscopic examination *Proteus mirabilis* appeared gram negative rod , motile , it was positive to urease , catalase, indol , citrate , methyl red , API 20 E(0132000) and negative to oxidase, voges proscaure .the result of TSI test was A\A\H₂S\no gas.

By using giemsa stain *Trichomonas vaginalis* was seen and diagnosed,both the internal and external structures of the organism were clearly visualized. The former stained dark blue with a red nucleus and the latter was sharply outlined ,showing clearly the flagella and the undulating membrane (figure 4-9).

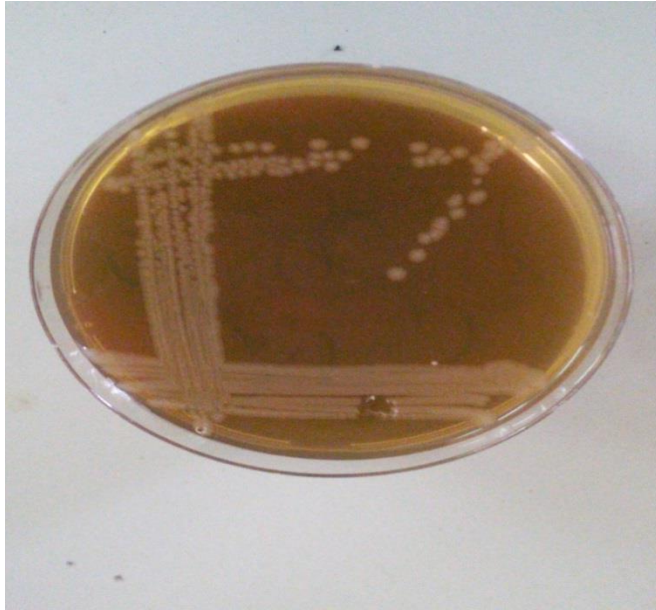


Figure (4-2) : *Candida albicans* on malt extract agar .



Figure (4-3): oval shape of *Candida albicans* under microscope .

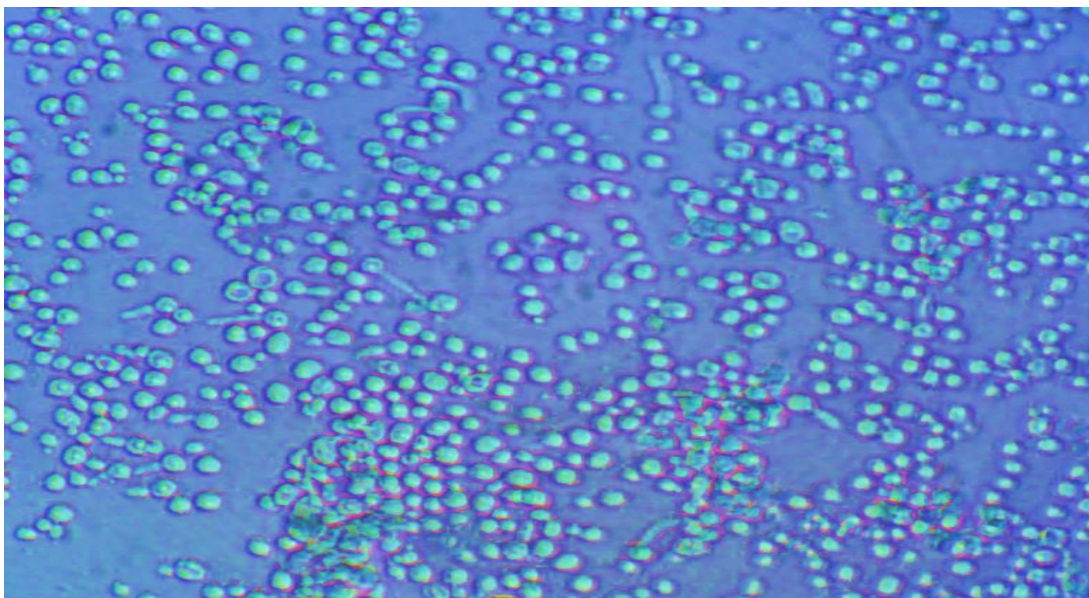


Figure (4-4): Germ tube formation by *Candida albicans* .

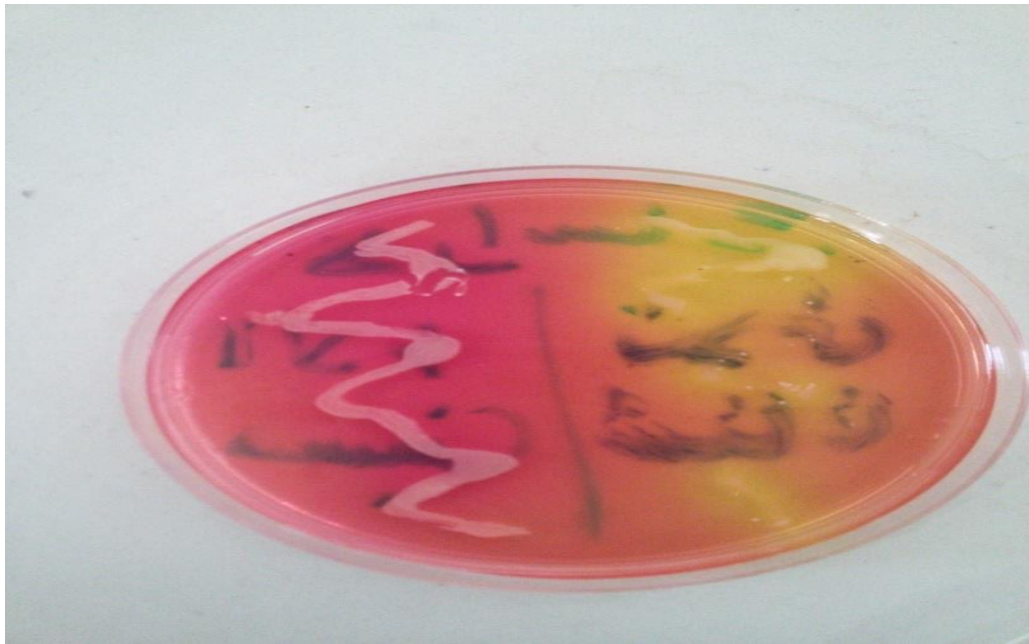


Figure (4-5):*Staphylococcus aureus* and *Staphylococcus epidermidis* on manitole salt agar

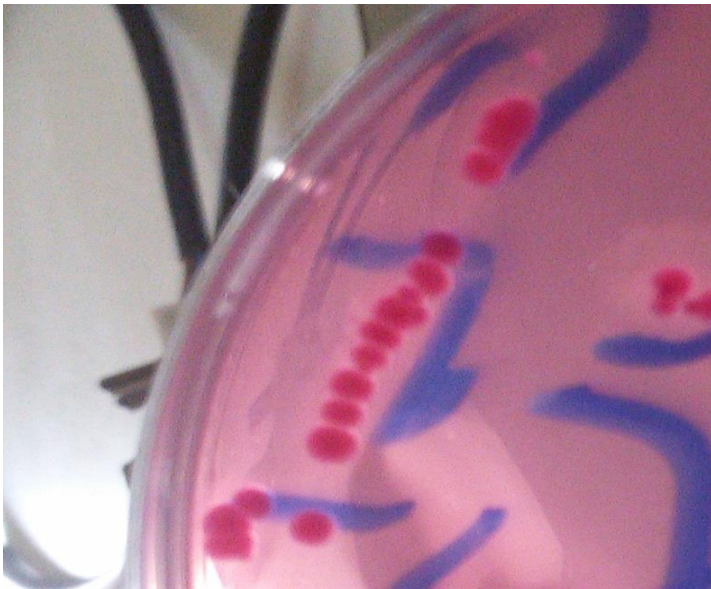


Figure (4-6): colonies of *Escherichia coli* on MacConky agar .



Figure (4-7): *Klebsiella pneumoniae* on MacConky agar.



Figure (4-8): swarming phenomenon of *Proteus mirabilis* on blood agar .

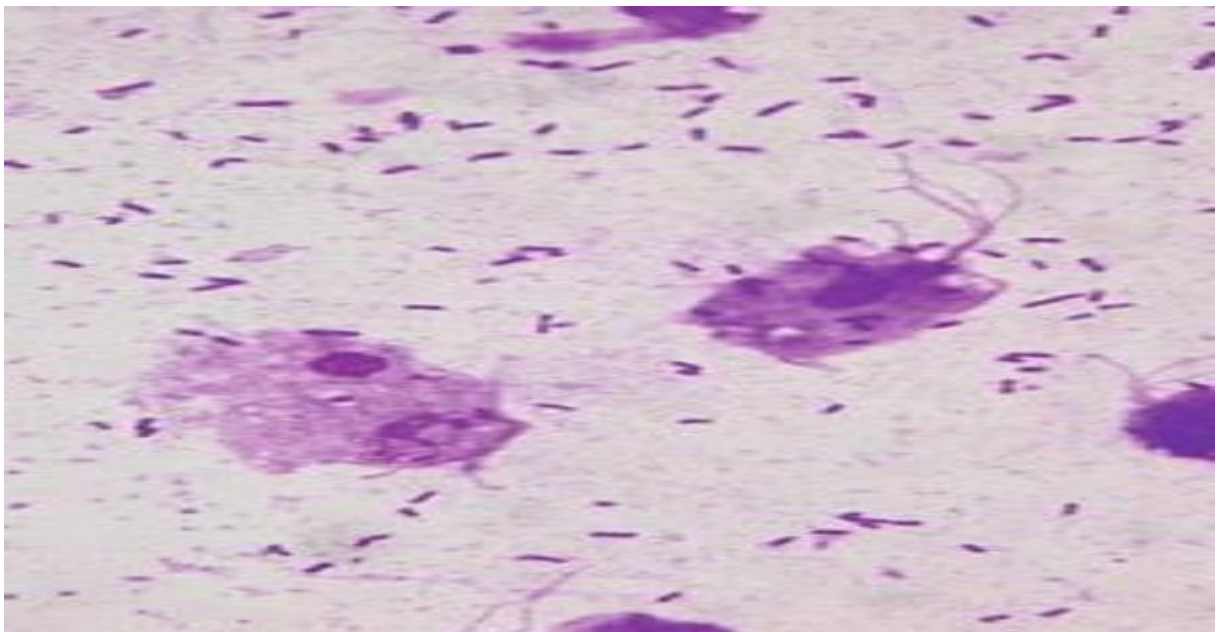


Figure (4-9) : *Trichomonas vaginalis* staining with giemsa stain.

4-3 Microscopic examination of vaginal swab samples:

The results of this study were based on the presence of pus cells , red blood cells (RBCs) , epithelial cells , clue cells , which their presence indicate to infection with bacteria, fungi , *Trichomonas vaginalis* which all appeared in wet smears as shown in table (4-2) . Where the pus cells were found at a rate of (6-10) cells in 94 sample and in rate (1-5) cells in 81 sample , while the number of samples declined that possessed the large numbers, where it was in 35 sample at rate (11-20)cell , as well as it was in rate greater than 21 cells in 35 samples . The epithelial cells were present at the rate of (1-5) cells in 97 sample while they formed more than 21 cells in 45 sample only . As well as the presence of RBCs in rate (1-5) cell in 147 sample while it was more than 21 cells in only three samples . clue cells were present only in 19 sample and at a rate of (1-5) cell and their presence was associated mainly with presence of *Gardnerella vaginalis* . *Trichomonas vaginalis* has been found in rate (1-5) cell in 6 samples only after dye with giemsa stain .

Table (4-2) Microscopic examination of vaginal swab samples

Type of cell and microbes	Rate of cells							
	1-5		6-10		11-20		More than 21	
	No.	%	No.	%	No.	%	No.	%
Pus Cells	81	33	94	38.4	35	14.3	35	14.3
Epithelial cells	97	39.6	50	20.4	53	21.6	45	18.4
RBCs	147	60	—	—	5	2	3	1.2
Clue cells	19	7.8	—	—	—	—	—	—
<i>T. vaginalis</i>	6	2.4	—	—	—	—	—	—

4-4 Indications for vaginitis :

Among 245 women there were 205 women has symptomatic lower genital tract infection and 40(16.3%) women has asymptomatic lower genital tract infection , where white thick discharge and itching accompanied with candidiasis infection .White to gray discharge(thin), itching and odour accompanied with Bacterial Vaginosis while Trichomoniasis associated with yellowish to green discharge and itching . The pH values associated with the bacterial infection were >4.5 , positive to whiff test , in wet smear was there few WBC and clue cell . while candidiasis infections associated with $\text{PH} \leq 4.5$,negative to whiff test , WBC ranged from few to many .trichomoniasis associated with $\text{PH} > 4.5$, positive to whiff test , many WBC table(4-3) .

Table (4-3) Indications for Vaginitis

Indicator	Result	Type of infection
White thick discharge	69 (28.2%)	Candidiasis
White to gray discharge,thin	50 (20.4%)	Bacterial Vaginosis
Yellowish to Green discharge	6(2.4%)	Trichomoniasis
Odour	45(18.4%)	Bacterial Vaginosis
Itching	35(14.3%)	Candidiasis
		Bacterial Vaginosis
		Trichomoniasis
No symptoms	40(16.3%)	Bacterial vaginosis
Vaginal pH	> 4.5	Bacterial Vaginosis
	≤ 4.5	Candidiasis
	> 4.5	Trichomoniasis
KOH test	Positive	Bacterial Vaginosis
	Negative	Candidiasis
	Positive	Trichomoniasis
NaCl wet mount	Clue cell/few WBCs	Bacterial Vaginosis
	Few to many WBCs	Candidiasis
	many WBCs	Trichomoniasis

4-5 Relationship between nutrition and type of infections :

The information obtained from women shows that carbohydrate intake a lot found a common factor among women infected with *Candida albicans* and women infected with *Klebsiella pneumonia* as well as women infected with *E-coli* while red meat intake a lot was also a common factor among women with *S. aureus* and women with *S.agalactiae* , as for women that infected with other microbes eat food almost evenly . table (4-4)

Table (4-4) Nutrition and type of infection

Pathogen	Type of nutrition
<i>Candida albicans</i>	Carbohydrate
<i>E-coli</i>	
<i>Klebsiella Pneumoniae</i>	
<i>Streptococcus agalactiae</i>	Red meat
<i>Staphylococcus aureas</i>	
Other pathogen	Normal nutrition

4-6 Diabeties and type of infection :

Samples were take from 15 women with diabetes during the study , Among them 9 (60%) women infected with *Candida albicans* which recorded the highest incidence followed by *Escherichia coli* 5(33.3%). the lowest incidence was for *Klebsiella pneumonia* 1(6.7%) , the results showed no significant difference at level ($P>0.05$) between the infected and non infected women with diabeties as shown in table(4-5).

Table (4-5) Diabetes and type of infection

Pathogen	case						X ²
	Diabeties		non diabeties		Total		
	%		%		%		
	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	
<i>C. albicans</i>	9 (60)	6 (40)	66 (66)	34 (34)	75 (65.2)	40 (34.8)	0.207
<i>E.coli</i>	5 (33.3)	10 (66.7)	20 (20)	80 (80)	25 (21.7)	90 (78.3)	1.36
<i>K. Pneumonia</i>	1 (6.7)	14 (93.3)	14 (14)	86 (86)	15 (13)	100 (87)	0.618
Total	15(13.0)		100(100)		115(100)		

Tab. X²: (df:1 ; P_value: 0.05) = 3.841

4-7 Relationship between female reproductive tract infections and oral contraceptive users :

The use of oral contraceptives is one of the most important reasons for vaginitis. This is evident in the results of this study, where 58 women with vaginitis who were taking oral contraceptives were among 95 women , the highest incidence of infection was associated with the use of oral contraceptives was *Candida albicans* 18(31.0%) , the lowest incidence of infection was *E.cloaca* which included only 2(3.4%) women taking oral contraceptives , for women infected with *C.albicans*, *E.coli*, *G.vaginalis* ,*S.epidermidis* the study recorded there was a significant difference at level (P<0.05) between the rate of infection in women taking oral contraceptives and those that not take oral contraceptive while for women infected with *S.aureus*, *K.pneumonia*, *P.aurgenosa* ,*p.mirabilis*, *E.cloacae* there were no significant difference at level

($P < 0.05$) between the rate of infection in women taking oral contraceptives and those that not take oral contraceptive. table (4-6).

Table (4-6) Relationship between FRT infection and contraceptive users

Pathogen	case						X ²
	take contraception %		not take contraception %		Total %		
	+ve	-ve	+ve	-ve	+ve	-ve	
<i>C. albicans</i>	18 (31.0)	40 (69.0)	2 (5.4)	35 (94.6)	20 (21.1)	75 (78.9)	8.92
<i>E.coli</i>	10 (17.2)	48 (82.8)	0 (0.0)	37 (100)	10 (10.5)	85 (89.5)	7.13
<i>G.vaginalis</i>	7 (12.1)	51 (87.9)	0 (0.0)	37 (100)	7 (7.4)	88 (92.6)	4.81
<i>S. aureus</i>	5 (8.6)	53 (91.4)	2 (5.4)	32 (86.5)	7 (10.5)	85 (88.5)	0.57
<i>K. pneumoniae</i>	3 (5.2)	55 (94.8)	5 (13.5)	32 (86.5)	8 (8.4)	87 (91.6)	2.03
<i>S. epidermidis</i>	5 (8.6)	53 (91.4)	16 (43.2)	21 (56.8)	21 (22.1)	74 (77.9)	15.72
<i>P.aerugenosa</i>	3 (5.2)	55 (94.8)	6 (16.2)	31 (83.8)	9 (9.5)	86 (90.5)	3.21
<i>P. mirabilis</i>	5 (8.6)	53 (91.4)	6 (16.2)	31 (83.8)	11 (11.6)	84 (88.4)	1.27
<i>E. cloaca</i>	2 (3.4)	56 (96.6)	0 (0.0)	37 (100)	2 (2.1)	93 (97.9)	1.30
Total	58(61.1%)		37(38.9%)		95(100%)		

Tab. X^2 : (df:1 ; P_value: 0.05) = 3.841

4-8 Abortion and genital tract infection :

The study showed that there were 42 women who had previous abortions suffering from vaginitis from 245 women ,of these women were 12(27.3%) infect with *Streptococcus agalactiae* they represent the highest proportion , and *Klebsiella*

pneumonia represent lower proportion which were 1(2.3%), while *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter cloacae* were infected women who not had previous abortions. For women infected with *C.albicans*, *E.coli*, *S.aureus*, *K.pneumoniae*, *T.vaginalis* the study recored no significant difference at level ($P>0.05$) between women subjected to previous abortion and women not subjected to a previous abortion while in women infected with *S.agalactiae*, *G.vaginalis* and other bacteria where there was a significant difference at level ($P<0.05$) between women subjected to previous abortion and women not subjected to a previous abortion table(4-7).

Table (4-7) Abortion and genital tract infection

Pathogen	case						X ²
	With abortion No. (%)		Without abortion %		Total %		
	+ve	-ve	+ve	-ve	+ve	-ve	
<i>S.agalactiae</i>	12 (27.3)	32 (72.7)	23 (11.4)	178 (88.6)	35 (14.3)	210 (85.7)	7.38
<i>C.albicans</i>	10 (22.7)	34 (77.3)	65 (32.3)	136 (67.7)	75 (30.6)	170 (69.4)	1.57
<i>E.coli</i>	8 (18.2)	36 (81.8)	17 (8.5)	184 (91.5)	25 (10.2)	220 (89.8)	3.72
<i>G.vaginalis</i>	7 (15.9)	37 (84.1)	12 (6.0)	189 (94.0)	19 (7.8)	226 (92.2)	4.98
<i>S. aureus</i>	4 (9.1)	40 (90.9)	23 (11.4)	178 (88.6)	27 (11.0)	218 (89.0)	0.204
<i>K. pneumoniae</i>	1 (2.3)	43 (97.7)	14 (7.0)	187 (93.0)	15 (6.1)	230 (93.9)	1.383
<i>Other bacteria</i>	0 (0)	44 (100)	23 (12.7)	158 (87.3)	43 (10.2)	202 (89.8)	6.22
<i>T. vaginalis</i>	2 (4.5)	42 (95.5)	4 (2)	197 (98)	6 (2.4)	239 (97.6)	0.98
Total	44(18.0)		201(82.0%)		245(100%)		

Tab. X²: (df:1 ; P_value: 0.05) = 3.841

4-9 Microbial infection according to age groups:

Women's ages in this study ranged from (16-45) year, divided into three age groups (16-29) , (30-39) ,(40-45) ,the study recorded significant differences at level ($P < 0.05$) between the three ages, the results of our study showed that women in the age group (16-29) year were more susceptible to infections of the external reproductive system where 131 of women were suffered from genital tract infection included in this age group, while women in the age group (40-45) year less prone to infection where was only 34 of women suffered from genital tract infection. *Candida albicans* was predominant in the three age groups with highest rate among other isolates table (4-8).

Table (4-8) Percentage of infections according to age groups:

Pathogen	Age groups							
	16-29 %		30-39 %		40-45 %		Total %	
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
<i>C. albicans</i>	39 (29.8)	92 (70.2)	25 (31.3)	55 (68.8)	11 (32.4)	23 (67.6)	75 (30.6)	170 (69.4)
<i>S.agalactiae</i>	21 (16.0)	110 (84)	14 (17.5)	66 (82.5)	0 (0)	34 (100)	35 (14.3)	210 (85.7)
<i>S. aureus</i>	14 (10.7)	117 (89.3)	9 (11.3)	71 (88.8)	4 (11.8)	30 (88.2)	27 (11.0)	218 (89.0)
<i>E.coli</i>	15 (11.5)	116 (88.5)	7 (8.8)	73 (91.2)	3 (8.8)	31 (91.2)	25 (10.2)	220 (89.8)
<i>G.vaginalis</i>	10 (7.6)	121 (92.4)	8 (10)	72 (90)	1 (2.9)	33 (97.1)	19 (7.8)	226 (92.2)
<i>K. pneumonia</i>	10 (7.6)	121 (92.4)	5 (6.2)	75 (93.8)	0 (0)	34 (100)	15 (6.1)	230 (93.9)
<i>T. vaginalis</i>	6 (4.6)	125 (95.4)	0 (0)	80 (100)	0 (0)	34 (100)	6 (2.4)	239 (97.6)
Other bacteriae	16 (12.2)	115 (87.8)	12 (15)	68 (85)	15 (44.1)	19 (55.9)	43 (17.6)	202 (82.4)
Total	131(49.6%)		80(30.3%)		34(20.1%)		245(100%)	
X ² (Cal)	72.029							

Tab. X^2 : (df:14 ; P_value: 0.05) = 23.685

4-10 The relationship between hormones and infections:

In our study 245 blood samples were collected from women, 150 women were pregnant, 58 women at leuteal phase , 20 women were in follicular phase , 17 women within ovulation phase of the menstrual cycle .*Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* , *Staphylococcus epidermids* , *Enterobacter cloaceae* were isolated from women in follicular phase as shows in table (4-9) .

the study recorded increase in the level of progesterone and estrogen hormones for *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* , there were also significant differences ($P < 0.05$) between the level of progesterone hormone in infected women with *Candida albicans* and women infected with *Escherichia coli* , also there was a significant differences ($P < 0.05$) between the level of progesterone hormone in infected women with *Candida albicans* and women infected with *Gardnerella vaginalis* ,as recorded Significant differences ($P < 0.05$) between the level of progesterone hormone in women infected with *Candida albicans* , *Staphylococcus aureus* and each of *Staphylococcus epidermids* , *Enterobacter cloaceae* .

For estrogen hormones there were significant differences ($P < 0.05$) between the level of this hormone for each of women infected with *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* and each of *Staphylococcus epidermids* , *Enterobacter cloaceae* as existence significant differences ($P < 0.05$) between the level of estrogen hormone for women infect with *Staphylococcus aureus* and women infect with *Escherichia coli*. while the level of prolactin hormone ranged from normal to high with all infection except *Staphylococcus epidermids* , *Enterobacter cloaceae* , its presence was accompanied with normal levels of prolactin hormone.Observed precence of significant differences

($P < 0.05$) between the level of this hormone for women infect with *Staphylococcus aureus* and each of *Gardnerella vaginalis* , *Klebsiella pneumonia*, as precence significant differences ($P < 0.05$) between the level of this hormone for women infect with *Gardnerella vaginalis* , *Klebsiella pneumonia* and each of *Staphylococcus epidermids* , *Enterobacter cloaceae* .Table (4-10) shows that the diversity of infections was lower in women in the ovulation stage, where they were isolated only *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , the results also showed a rise in both estrogen and progesterone hormones , the progesterone elevation was slight while the rise in estrogen was apparent , where as prolactin had no apparent effect,all the infections were accompanied by normal levels of this hormone . the study recorded simple significant differences ($p < 0.05$) between the level of estrogen and progesterone hormones for women infected with *Staphylococcus aureus* and the level of this hormones for women infect with *Escherichia coli*, while there were no significant differences between other infections .

Candida albicans , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* , *Staphylococcus epidermids* , *Proteus mirabilis*, *Pseudomonas aeruginosa* isolated from women with leuteal phase .Table (4-11) shows that the precence of infection with *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* was associated with elevated estrogen and progesterone levels while prolactin was within normal limits and the growth of *Staphylococcus epidermids* , *Proteus mirabilis*, *Pseudomonas aeruginosa* was associated with normal range of each hormones , Significant differences ($p < 0.05$) were observed between the level of both estrogen and progesterone hormones for each of women infect with *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* and women infect with *Staphylococcus epidermids* , *Proteus mirabilis*, *Pseudomonas aeruginosa* , there were no significant differences ($P > 0.05$) between the level of prolactin hormone for women infect with the types of infection.

Pregnancy was accompanied by high levels of estrogen, progesterone and prolactin hormones, this is accompanied with existence *Candida albicans* , *Staphylococcus aureus* , *Escherichia coli* , *Gardnerella vaginalis* , *Klebsiella pneumonia* , *Streptococcus agalactiae* , *Trichomonas vaginalis* ,the study recorded no significant differences between all isolates relative to progesterone , as for hormone prolactin, there were significant differences ($P < 0.05$) between the level of this hormone for women infect with *Candida albicans* and each of women infect with *Escherichia coli* , *Trichomonas vaginalis* , and the absence of significant differences $P > 0.05$ between the level of prolactin hormone for women infect with other infections . as for estrogen the results showed significant differences($P < 0.05$) between the level of this hormone in women infect with *Escherichia coli* and women infect with each of *Candida albicans* , *Klebsiella pneumonia* , *Gardnerella vaginalis* . There was also a significant difference ($P < 0.05$) between the level of estrogen hormone in women infect with *Streptococcus agalactiae* and women infect with *Klebsiella pneumonia* for estrogen hormone as shows in table (4-12) .

Table (4-9)The relationship between hormones and infections in follicular phase of menstrual cycle.

Hormons Pathogens	Progsteron mean±SD	Prolactin mean±SD	Estrogen mean±SD
<i>Candida albicans</i>	2.46 ± 0.15 ^a	17.60 ± 3.12 ^{bcd}	186.03 ± 12.30 ^{ab}
<i>Staphylococcus aureus</i>	1.86 ± 0.21 ^{ab}	13.06 ± 2.65 ^{cd}	174.10 ± 6.90 ^b
<i>Escherichia coli</i>	1.26 ± 0.35 ^{bc}	21.56 ± 3.35 ^{abc}	196.96 ± 5.15 ^a
<i>Gardnerella vaginalis</i>	0.76 ± 0.15 ^{bc}	26.36 ± 2.05 ^a	191.40 ± 2.20 ^{ab}
<i>Klebsiella pneumonia</i>	0.70 ± 0.10 ^c	25.10 ± 1.70 ^{ab}	179.66 ± 11.75 ^{ab}
<i>Staphylococcus epidermids</i>	0.30 ± 0.08 ^c	15.56 ± 1.75 ^{cd}	71.10 ± 25.40 ^c
<i>Enterobacter cloaceae</i>	0.40 ± 0.10 ^c	12.50 ± 7.20 ^d	43.26 ± 11.65 ^d
L.S.D.	1.15	8.66	18.42

* ANOVA : Analysis of variance *Values are mean ± (SD) * Difference letter in superscript represent a significant difference ($p \leq 0.05$) between the level of hormones and the type of microorganism that causes the infections . *L.S.D.:Least significant difference . *The same letters refer to non significant difference . *The different letters refer to significant difference .

Table (4-10)The relationship between hormones and infections in mid (Ovulationphase) of menstrual cycle .

Hormones Pathogen	Progsteron mean±SD	Prolactin mean±SD	Estrogen mean±SD
<i>Candida albicans</i>	9.96 ± 0.95 ^{ab}	19.0±3.80 ^{ab}	544.50±40.90 ^{ab}
<i>Staphylococcus aureus</i>	10.46 ± 1.05 ^a	11.76±1.95 ^b	594.10±72.90 ^a
<i>Escherichia coli</i>	7.16±0.65 ^b	21.20±2.66 ^a	517.0±6.0 ^b
<i>Gardnerella vaginalis</i>	9.0±0.30 ^{ab}	14.16±1.35 ^{ab}	552.50±54.50 ^{ab}
L.S.D	3.20	7.35	75.89

* ANOVA : Analysis of variance *Values are mean ± (SD) * Difference letter in superscript represent a significant difference ($p \leq 0.05$) between the level of hormones and the type of microorganism that causes the infections . *L.S.D.:Least significant difference . *The same letters refer to non significant difference . *The different letters refer to significant difference .

Table(4-11)The relationship between hormones and infections in leuteal phase of menstrual cycle .

Hormones Pathogens	Progesterone mean±SD	Prolactin mean±SD	Estrogen mean±SD
<i>Candida albicans</i>	25.50 ± 2.10 ^b	18.99 ± 1.35 ^{ab}	264.50 ± 44.50 ^a
<i>Staph. aureus</i>	30.30 ± 1.80 ^a	11.70 ± 1.90 ^{ab}	287.0 ± 11.0 ^a
<i>E.coli</i>	26.06 ± 4.45 ^b	20.16 ± 5.35 ^a	254.50 ± 20.50 ^{ab}
<i>Gardnerella vaginalis</i>	23.56 ± 1.45 ^b	12.36 ± 3.05 ^{ab}	268.50 ± 43.50 ^a
<i>Klebsiella pneumonia</i>	26.20 ± 2.30 ^{ab}	13.56 ± 3.61 ^{ab}	221.50 ± 4.50 ^b
<i>Staphylococcus epidermids</i>	17.20 ± 1.40 ^c	9.66 ± 2.85 ^b	115.16 ± 35.85 ^c
<i>Proteus mirabilis</i>	16.40 ± 3.30 ^{cd}	16.96 ± 3.05 ^{ab}	57.70 ± 9.80 ^d
<i>Pseudomonas.aeruginosa</i>	12.30 ± 2.90 ^d	19.30 ± 3.88 ^{ab}	105.36 ± 18.45 ^c
L.S.D.	4.15	9.88	39.59

* ANOVA : Analysis of variance *Values are mean ± (SD) * Difference letter in superscript represent a significant difference ($p \leq 0.05$) between the level of hormones and the type of microorganism that causes the infections . *L.S.D.:Least significant difference . *The same letters refer to non significant difference . *The different letters refer to significant difference .

Table (4-12)The relationship between hormones and infections in pregnant women:

Hormones Pathogens	Progesterone mean±SD	Prolactin mean±SD	Estrogen mean±SD
<i>Escherichia coli</i>	43.46±5.05 ^a	70.26±17.75 ^a	2872.66±78.50 ^a
<i>Staph. aureus</i>	34.26±5.75 ^a	66.86±16.15 ^{ab}	2517.66±265.50 ^{ab}
<i>Streptococcus agalactiae</i>	85.96±18.05 ^a	59.80±15.20 ^{ab}	2215.0±385.0 ^{ab}
<i>Trichomonas. Vaginalis</i>	88.86±20.15 ^a	70.36±14.65 ^a	1822.0±277.0 ^{abc}
<i>Gardnerella vaginalis</i>	83.06±16.95 ^a	65.36±17.65 ^{ab}	1610.0±139 ^{bc}
<i>Candida albicans</i>	34.50±10.50 ^a	26.36±2.05 ^b	1555.66±220.0 ^{bc}
<i>Klebsiella pneumonia</i>	87.27±19.70 ^a	33.06±1.35 ^{ab}	1079.0± 89.0 ^c
L.S.D.	65.48	40.97	1201.05

* ANOVA : Analysis of variance *Values are mean ± (SD) * Difference letter in superscript represent a significant difference ($p \leq 0.05$) between the level of hormones and the type of microorganism that causes the infections . *L.S.D.:Least significant difference . *The same letters refer to non significant difference . *The different letters refer to significant difference .

4-11 Antibiotic susceptibility of bacterial isolates:

The sensitivity of the isolates that cause vaginitis was tested to 6 antibiotic as shown in the table(4-13) where the results of the study showed that bacteriae *Streptococcus agalactiae* was found high sensitivity to wards piperacillin/tazobactam 35(100%) and imipenem 35(100%), while it was high resistant to aztreonam. Regarding to *Staphylococcus aureus* were found high sensitivity to wards imipenem 26(96.3%) and piperacillin/tazobactam 24(88.9%), while showed high resistance to amoxicillin 10(37%) , aztreonam 5(18.5%) .

As for the isolates of *Escherichia coli* has shown the high sensitivity towards imipenem 24(96%) , piperacillin/tazobactam 23(92%) while the high resistance was to amoxicillin 7(28%) and doxycycline 7(28%) .either bacteria *Gardnerella vaginalis* has shown the high sensitivity to imipenem and amoxicillin 18(94.7%),15(78.9%) Respectively .While the high resistance to doxycycline 9(47.5%), aztreonam 5(26.3%) . *Klebsiella pneumoniae* showed high sensitivity to imipenem 15(100%) , piperacillin/tazobactam 14(93.3%) . and high resistance to amoxicillin 2(13.3). on other hand *Proteus mirabilis* Was the high sensitivity to imipenem 11(100%), piperacillin/tazobactam 9(81.8%) , and the high resistance to cefotaxime 3(27.3%) , aztreonam 2(18.1%) .*Pseudomonas aeruginosa* shown the high sensitivity to imipenem 8(88.9%) , piperacillin/tazobactam 7(77.8%) . and high resistance to amoxicillin, doxycycline .The two isolates of *Enterobacter cloacae* showed high sensitivity to Imipenem 2(100%) , and high resistance to amoxicillin where the percentage of resistance 0%.

Table (4-13)Percentage of Sensitivity of Aerobic Vaginal Isolates to various Antibiotics.

Antibiotics	<i>E-coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Strep-agalactiae</i>
Penicillin group:					
Piperacillin/tazobactam	23(92%)	14(93.3%)	7(77.8%)	24(88.9%)	35(100%)
Amoxicillin	7(28%)	2(13.3)		10(37%)	29(82.9%)
Cephalosporins:					
Cefotaxime	19(76%)	11(73.3%)	4(44.4%)	20(74.1%)	32(91.4%)
Monobactams:					
Aztreonam	13(52%)	10(66.7%)	5(55.6%)	5(18.5%)	
Imipenem	24(96%)	15(100%)	8(88.9%)	26(96.3%)	35(100%)
Miscellaneous:					
Doxycycline	7(28%)	8(53.3%)		13(48.1%)	21(60%)

Antibiotics	<i>Proteus mirabilis</i>	<i>Gardnerella vaginalis</i>	<i>Enterobacter cloacae</i>
Penicillin group:			
Piperacillin/tazobactam	9(81.8%)	11(57.9%)	1(50%)
Amoxicillin	5(45.4%)	15(78.9%)	0(0%)
Cephalosporins:			
Cefotaxime	3(27.3%)	13(68.4%)	1(50%)
Monobactams:			
Aztreonam	2(18.1%)	5(26.3%)	1(50%)
Imipenem	11(100%)	18(94.7%)	2(100%)
Miscellaneous:			
Doxycycline	4(36.4%)	9(47.5%)	1(50%)

4-12 Antifungal susceptibility test for *Candida albicans* :

Sensitivity was tested for all isolates of by using antifungal agents nystatin , ketonazole, fluconazole , the results recorded significant differences at level ($P < 0.05$) between this antibiotic where the results showed nystatin one of the best therapeutic options as the ratio of resistance (0%) ,while the resistance ratio of ketonazole were (34.2%) , and fluconazole showed highest resistance were (65.8%) . as for the diameters of zone's of inhibition the nystatin recorded highest inhibition diameters which were (20-30) , while fluconazol recorded lowest rate of diameters of inhibition zone which were (7-14) .

Table (4-15) rate of diameters of inhibition zones to *Candida albicans* resulting from use of antifungal agents

Pathogen	diameters of inhibition zones (Mm)	Sensitivity					
		Resistance %		Sensitivity %		Total %	
		R	Non	S	Non		
Nystatin	20-30	0 (0.0)	79 (100)	75 (51.4)	71 (48.6)	75 (33.3)	150 (66.7)
Ketonazol	16-29	27 (34.2)	52 (65.8)	48 (32.9)	98 (67.1)	75 (33.3)	150 (66.7)
Fluconazol	7-14	52 (65.8)	27 (34.2)	23 (15.8)	123 (84.2)	75 (33.3)	150 (66.7)
Total		79 (35.1%)		146 (64.9%)		225 (100%)	

Tab. X^2 : (df:1 ; P_value: 0.05) = 3.841

Chapter Five

Discussion

5-1 Female reproductive tract infections :

In our study, the results showed that the highest number of women had genital tract infection with symptoms, while a few women had genital tract infection without symptoms, where 205(83.67%) of the 245 women had symptomatic lower genital tract infection , and 40 (16.3%) women had asymptomatic lower genital tract infection. this result agreed with earlier study by Olartan (2006) in Nigerian university that reported that a small number of women had genital tract infection without symptoms , where the results of his study showed that a prevalence of asymptomatic genital tract infection in only 10 % of women ,and agreed with study of Anhe *et al .*, (1996) that reported a prevalence of 20.12 % asymptomatic vaginitis among women in Honnoi , Vietnam .

Fleury (1981) reported that BV is the leading cause of vaginal symptoms such as vaginal discharge and pruritus in developed communities, the most prevalent symptoms in our study were vaginal discharge (51%) , vaginal odour (18.4%), genital itching (14.3%) , this result agreed with finding of Geisler *et al.*, (2004) that (46%) of the 296 women with genital infection had vaginal discharge , (28%) of the women suffered from vaginal odour , and 15% of the women suffered from itching .

The results of our study showed that there precence significant differences ($P<0.05$) between the rate of infection in pregnant women and non- pregnant women, where 150 isolates were obtained from pregnant women and 95 isolated from non-pregnant women ,this agreed with (AL-Zuhairi ,2001; Dybas *et al .*, 2005) which found that the rate of infection in pregnant women is higher than the rate of infection in non-pregnant womem , Dybas *et al .*, 2005 found that the percentage of bacterial infections was (37.30%) for pregnant women and (20%) among non--pregnant women , and the percentage of *Candida albicans* infection was (28.57%) (6.66%) pregnant women and non- pregnant women respectively and also *Trichomonas vaginalis* infected (5.55%) pregnant women (3.33%) non- pregnant women , this is due to in pregnant

women hormones imbalance especially estrogen, this will lead to a change in the acidity of the vagina , thus allowing infect with microbes(Lehman.,2009).

5-2 Microbial isolated from the vaginal swabs during study:

Candida albicans were the most prevalent among organisms isolated in our study 75(30.6%) and among bacterial isolates the highest ratios that isolated during our study It was *Streptococcus agalactiae* ,*Staphylococcus aureus* , *Escherichia coli* 35(14.3%), 27(11%), 25(10.2%) respectively , this result is in line with reports from other studies (Geiger *et al* .,1995; Joishy *et al* ., 2005).

The appearance of a germ *Streptococcus agalactiae* at high rates may be due to the fact that it forms part of the normal flora of the vagina ,it is a common cohabitation in the lower genital tract for women without any clinical symptoms(Huet *et al* .,1993) but its rate increases during pregnancy because of its opportunistic nature as it causes diseases to certain people, including pregnant women, which confirms nature opportunistic(Lerner *et al* .,1977). The results also agreed with the work of Gilbert *et al.*, (2002) were found the microorganisms associated with vaginal infection and hence BV were found to be mainly group BStreptococci, *S. aureus* , *E. coli* and this similar to the findings of (Onile ,1980) which in its study *Streptococcus agalactiae* constituted the highest percentage(19.3%) of bacterial isolates of a total 150 vaginal swabs , this result not agreed with some study that showed *staphylococcus aureus* as the predominate species (Mumtaz *et al* .,2008; Fan *et al* ., 2013)and also not agreed with (Verner *et al* ., 2016) which found *Escherichia coli* was the predominant . Mahdi and Maysoon (2001) reported the isolation of *S. aureus* (10%) and *E. coli* (8%) as part of vaginal microorganisms associated with BV this is close to the rates that found in our study (11.1%) *Staphylococcus aureus* , (10.2%) *Escherichia coli*.in our study found that (8.6%) of *Staphylococcus epidermidis* isolated from this area , due to contact the swab with the skin while taking the sample. The ratio of *Gardnerella vaginalis* in this study were 19 (8.6%) . As for the intestinal bacteria that cause

vaginitis such as *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Enterobacter cloacae* were 25 (10.2%), 15 (6.1%), 11 (4.5%), 9 (3.7%), 2 (0.8%) respectively, they form part of the normal flora of the gastrointestinal tract and because of the anatomical nature of the vagina, the slot of rectum is close to the slot of the vagina which increases the rate of transmission of these bacteria from its original location to the vagina. The 6 (2.4%) Trichomoniasis reported in the present study was much lower than 5.4% reported by Adu sakordie et al and 10.6% as stated in Balaka et al. the report of Wyker and Gillenwater, 1995 explained the presence of *Trichomonas vaginalis* in small percentage in vaginal secretions, but it is considered an indicator of the infection although without symptoms because it is a sexually transmitted disease, other studies conducted in the United States of America (USA) found the rates of prevalence trichomoniasis to be between 3.1% and 8.7% (Sutton et al., 2007; Ginocchio et al., 2012) this is close to results in our study.

5-3 Diagnostic Tests:

According to the approved diagnostic systems (Koneman et al., 1997) depending on the biochemical and morphological test the isolates were diagnosed in our study. Wet smear were performed for vaginal secretions to determine the type of infection, where the number of pus cells, clue cells, epithelial cell, RBC cells was calculated as shown in table (4-4) according to (Geisler et al., 2004) which he showed estimation of the numbers of pus cells is necessary to assess the condition of disease whether bacterial or fungal vaginitis and even *Trichomonas vaginalis*. These researchers pointed out existence (0-1) pus cell evidence that the situation is normal, in existence (2-4) pus cells the case is medium, either in existence (7-8) pus cells indications presence of bacterial or fungal vaginosis. The result of our study showed that the WBCs found in small amount in samples taken from women with bacterial vaginitis, large amount in samples taken from women with *Trichomonas vaginalis* and WBCs ranged from few to many in samples were taken from women infected with candida

albicans , while clue cells it was found in samples taken only from women infected with *Gardnerella vaginalis* .

Vaginal secretions considered as evidence of the abnormal state of the ecosystem of the vagina, in our study white thick discharge accompanied with presence of *Candida albicans* , white to gray discharge (thin) accompanied with presence of bacteria , yellowish to green discharge accompanied with presence of *Trichomonas vaginalis* , this is in addition to the unacceptable smell which are given by these secretions after adding KOH where gave the smell of fish pathogens are present in large numbers in the vagina of women with vaginitis (Amsel *et al.*, 1983) , our study showed that both bacterial infections and *Trichomonas vaginalis* gave positive result to whiff test while fungal infection gave negative result , this agrees with (Schmidt and Hausen , 2000).

The *Trichomonas vaginalis* and types of bacteria can elaborate amines, including triethylamine, putrescine, and cadaverine while *Candida albicans* can't elaborate this compounds , this compounds responsible for the odor noticed by affected patients , when vagina become alkaline , these compounds become more volatile and are easily smelled (Larsen, 1993) vaginal environment (pH) determined with a suitable indicator paper by placing secretions on it , the result of the examination was the pH elevated in women with bacterial vaginosis and in women with trichomoniasis , while pH decrease in women infected with candidiasis table (4-5) , in the healthy adult female, each mL of vaginal fluid contains more than 10⁵ lactobacilli, principally *Lactobacillus crispatus* and *Lactobacillus jensenii* (Giorgli *et al.* , 1987), Normal lactobacillary flora is considered an important host defense. these organisms elaborate hydrogen peroxide, which limits the growth of anaerobic bacteria and other pathogens (Hawes *et al.* , 1996). Such lactobacilli also maintain the normal acidity of the vagina at a pH of around 4 to 4.5 .

5- 4 Distribution Microbial infection according to age groups :

The results are shown in the table(4-10) there were significant differences ($P<0.05$) between the three age groups , where the results showed the rate of female genital tract infection was declined with advance age , as the result recorded increase in the rate of infection among women in age group (16-29) and decreases in women with age group (40-45). the increase in the rate of infection in the age group (16-29) years may be attributed to the fact that most women in this age group are pregnant , this result was agreed with results of many researchers (Gentry and Price,1985;Little *et al.*, 2000) , and also agrees with (Stamm *et al.* , 1989) were showed young women are more prone to infections than any other age this is due to hormonal changes in early age this is in addition to the fact that vaginal infections occur more often in the fertility age this is confirmed by our study.

Some researchers have previously reported that bacterial vaginosis is more common among younger women (Bukusi *et al.*, 2006) while others like Morris *et al.* , (2001) and Jones *et al.* , (2007) found that risk of vaginitis increases with age , (Acikgoz *et al.* ,2002) said that the old is one of responsible factors of heterogeneity of the natural flora of the vagina causes periodic appearance of certain pathogenic organisms, the reason for the high incidence of vaginal infections in the age group (16-29) year because these ages represent the early years of marriage where sexual activity increases, adding to the arrival of reproductive hormones to their highest levels due to change in PH of vagina to be suitable for the growth of microbes , as for age group (40-45) year and above where sexual activity decreases , the pH of vagina is back to low acidity (Alany,2005) .

5-5 Nutrition and genital tract infection :

Table (4-4) show that the infection with *C.albicans* , *K.pneumoniae* , *E.coli* linked with women who eat a lot of carbohydrates , this result was agreed with Russell, who stated that eating a lot of carbohydrates often leads to increase sugar in

blood and thus imbalance in the vaginal environment that makes women more receptive to infection with inflammation and irritable yeast infection (Russell, 1999). The results also showed that women who ate a lot of red meats were infected with *S.aureus*, *S.agalactiae*. The isolation of these types of bacteria from women who eat a lot of red meat has no clear explanation, but previous studies have reported that red meats are one of the types of food which reduce the acidity of the vagina (Dweik, 1990; Russell, 1999). The results also showed that women who ate food in a balanced manner were infected with the rest of the bacterial species, where the food factor did not play a role in causing infection.

5-6 Diabetes and genital tract infection:

As for the relationship between diabetes and infection, our study showed no significant differences ($P > 0.05$) between the infected and non-infected with diabetes, where only 15 women with diabetes suffer from genital infection, as the results of the study showed that there are three pathogens associated with the female reproductive system for women with diabetes, these pathogens were *Candida albicans*, *Escherichia coli* and *Klebsiella pneumoniae*. Diabetes is also considered an encouraging factor to be infected with *Candida albicans* due to the increase in the amount of sugar in the vagina for those infected with this disease, making it an environment suitable for the growth of yeast (De-leon *et al.*, 2002) this is consistent with the results of our study. and also *Escherichia coli* and *Klebsiella pneumoniae* were isolated from women with diabetes 5 (20%), 1 (6.7%) respectively table (4-7). The appearance of the infection with *Escherichia coli* and *Klebsiella pneumoniae* in diabetic women due to most people with diabetes suffer from urinary tract infection with *Escherichia coli*, *Klebsiella* (Hansen *et al.*, 1998; Geerlings *et al.*, 2000) where diabetes is one of the most diseases that weaken the immunity and makes them more susceptible to germs, especially urinary tract (Zamanzad & Moezzi, 2006). Most with diabetics also suffer from neurogenic bladder which leads to a complete emptying of the urine due to the presence of glucose in the urine making it a suitable

medium for growth and reproduction of microbes (Jeanette *et al* .,2005). because of the anatomical nature of the vagina where the slot of the urinary tract close to the slot of the vagina, which increases the rate of transmission of these bacteria from its original location to the vagina as women with diabetes also have sugars in epithelial cells of their vagina that promotes the growth and reproduction of these microbes.

5-7 Relationship between FGT infections and contraceptive users :

The study showed that there were significant differences ($P<0.05$) between women taking oral contraceptives and women who did not take oral contraceptives for women infected with *C.albicans*, *E.coli*,*G.vaginalis*,*S.epidermids* , the females that using various contraceptive methods had approximately 1–7 fold increase in risk of acquiring RTIs compared to women not take contraceptive users (Sharief , 1998) , the result of our study agreed with other previous reports(Sharief ., 1998; Ghazal *et al* ., 2004) . contraceptive methods with the use of hormone have been reported make immunity was low. it is possible to reduce immunity and give rise to RTIs during the early weeks of contraceptive usage (Enomoto *et al* ., 2007) . the use of contraceptives also precipitates the glycogen in the epithelial cells of vagina, which results in change pH of the vagina and makes it an environment suitable for the growth of microbiology, especially yeast (Khider ,1985;Spinillo *et al.*, 1993). As that the high incidence of vaginitis can be attributed to the practice of vaginal douching , the use of antibiotics prior to collection of samples, delay in the cultivation of vaginal specimens (Ness *et al* ., 2002;Pitt *et al* .,2005; Lan *et al* ., 2008).

5- 8 Abortion and genital tract infection :

The present study documents that RTIs is common among women were subjected to previous abortions , where there was a significant difference at level ($P<0.05$) between women had previous abortion and women not subjected to a previous abortion for women infected with *S.agalactiae*,*G.vaginalis* ,our study found the highest percentage of infection associated with women who had previously

exposed to abortions was *Streptococcus agalactiae* 12(34.3%), the lowest proportion was for *Trichomonas vaginalis* 2(33.3%) and *Klebsiella pneumonia* 1(6.7%) while *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter cloacae* was not isolated from women who were exposed to previous abortions. Women who are exposed to abortions appear to be more likely to have RTIs than other women of childbearing age. Vietnamese studies have shown a high prevalence to RTIs (Phan *et al.*, 2002; Goto *et al.*, 2007; Lan *et al.*, 2008) of women requesting abortion, results are consistent with cohort study in terms of documentation that the presence of bacterial vaginitis is common in women who had an abortion (Hellberg *et al.*, 1999) and reported in a Swedish study where BV was found to be a risk factor for post-abortion upper genital-tract infection (Larsson *et al.*, 1989; Larsson *et al.*, 1992). The cause may be due mainly to the use of bacteria-contaminated devices when cleaning the uterus from the dead fetus.

5-9 The Relationship between the level of sexual hormones estrogen, progesterone, prolactin and infections:

There are a lot of studies on several aspects specific to infection with microorganism, but we did find a few studies on the relationship between the incidence of infection with microorganisms and influence of female sex hormones. Most studies have been carried out on laboratory animals. This study showed that among non pregnant women the proportion of women with vaginitis in the luteal phase of the menstrual cycle was more than ovulation phase and the follicle phase. This result agrees with (Kalo-Klein and Witkin, 1989), interpretation of this situation. It may be due to that the innate and adaptive immune systems are under hormonal control, and immune protection in the FRT varies with the phases of the menstrual cycle (Wira and Kaushic, 1996). As the immune protection is dampened during the secretory phase of the cycle to optimize conditions for fertilization and pregnancy, which creates a 'window of vulnerability' during

which potential pathogens can enter and infect the FRT (Begum and Ashwini., 2012) .

according to our study , the high estrogen level was associated with fungal vaginitis in each of the three stages of menstrual cycle in addition to pregnancy where pregnancy accompanies increase the level of estrogen , this accompanied by an increase in the glycogen content in the epithelial cells, which increases the activity of lactobacillus bacteria, which consumes glycogen resulting change in the pH of the vagina , this is one of the encouraging conditions for the colonization and spread of Candidiasis (Mahdi and Al-Hamdani.,1998).

Estrogen also plays a role in increasing the adhesion of *Candida albicans* to the vagina epithelial cells (VEC). thus encouraging the transformation from the yeast shape to the filamentous shape (King et al.,1980 ; Chaffin, and Stocco., 1983; Granger.,1992; Vazques and Sobel ., 2002) , as the epithelial cells represent a form of innate resistance against *C. albicans* at the vaginal mucosa. thus a reduced or absent epithelial cells mediated anti-Candida , in response to presence of estrogen may be an additional factor associated with estrogen , it is interesting to speculate that the resolution of infection in the absence of estrogen is due to epithelial cells mediated anti-Candida activity (sheet et al ., 1999) . the study also showed that the fungal infection was associated with high progesterone level , with respect to innate defenses , polymorphonuclear leukocytes (PMNs) were recently shown to have minimal effects on vaginal fungal burden in vivo (Black well et al., 1998; Fidel et al .,1999) despite their ability to kill *C. albicans* in vitro (. Odds.,1988) and their frequent presence in vaginal lavage fluid during infection. Interestingly, while PMN anti-Candida activity , in vitro showed that reduce in the presence of progesterone , but not estrogen (Nohmi et al.,1995) , the result of our study agrees with (Kalo-Klein and Witkin .,1989) which proved that the incidence of fungal infections was associated with elevated level of estrogen and progesterone and also the bacterial infection was companion with elevated estrogen and progesterone ,

this agrees with (Galask ., 1998) , the estrogen imbalance, especially in pregnant women, this will lead to a change in the acidity of the vagina, thus allowing infect with microbes . In normal women,estrogen supports vaginal epithelium in glycogen accumulation which in turn helps in maintenance of vaginal pH(Lehman .,2009). the female genital tract infection attributed to changes in the secretion levels of the steroid hormones estradiol and progesterone and the ability of some microbial species to use these hormones as growth factors. As that the Reproduction of lactobacilli depends on oestrogen levels (Galask., 1988;Miller et al .,2000) .

The numbers of these bacteria may change during the menstrual cycle depending on change of estrogen level "as acidification by vaginal secretion stimulates the growth of lactobacilli" , if the level of this hormone is changed, the number of lactobacilli will be reduced and vaginal environment changes to become an appropriate place for pathogens(Komman and Loesche,1982) . female genital tract (uterus, cervix, and vagina) mucosal tissues are regulated by several factors commonly produced in the mucosa, as well as by estrogen and progesterone(Witkin et al., 2007).

Innate and adaptive mucosal immunity in the FGT are regulated by the female sex hormones estrogen and Progesterone and have been reviewed in(Bouman et al .,2005; Butts and Sternberg.,2008) hormones regulate the transport of Igs, the levels of cytokines, the expression of TLR genes(Aflatoonian et al.,2007; Aflatoonian and Fazeli .,2008) and the distribution of immune cells and antigen presentation in the genital tissues during the reproductive cycle(Wira et al .,2005) . in some studies oestrogen has been showed to have a protective effect in autoimmunity, particularly in T cell mediated autoimmune conditions, through activation of ER- α signalling (Yang et al. 2010). high concentrations of oestrogen have been shown to have a dampening effect on immune responses as demonstrated in T cell , as oestrogen is able to decrease Th1 mediated responses

and increase Th2 immune responses by decreasing Th1 cytokines such as IFN- γ . as high concentrations of oestrogen have also been shown to decrease IL-2R expression and IL-2 production in activated T cells from healthy controls (McMurray et al. 2001), the hormone imbalance will affect the immunity of the reproductive system and allow the pathogens to grow and multiply there.

Proactin has no apparent effect on the presence of fungal and bacterial infection, as its level varies between normal and high. The study found that the parasite *Trichomonas vaginalis* was isolated only from pregnant women, where the parasite presence was accompanied by a rise in estrogen, progesterone and prolactin, the role of estrogen in influencing the parasite is unclear and controversial, according to an earlier study (Sugarman and Mummaw et al., 1988) the estrogen decrease the growth of parasite, and their attachment to mammalian cells in vitro, and acted as a chemo repellent, therefore this study didn't agree with the results of the current study. While the results of the current study agreed with the findings of the researcher (Inceboz et al., 2012) who has studied the relationship between the estrogen and *T. vaginalis* in vitro, which he found the high concentrations of estrogen promoted the reproduction of *T. vaginalis*, the researcher instructed the reason for this situation estrogen can change in pH and make it suitable for the growth of the parasite, increased parasite growth at the high concentration of this hormone is that it affects directly in its own hormone receptors on the surface of the parasite, which may play an important role in the growth and reproduction of the parasite, as previously proposed by (Ford et al., 1987).

The results also agreed with the findings of (Martinotti and Savoia, 1985) who found that the estrogen (17- α –Estradiol, 17- β -Estradiol), they were motivated for the growth of the parasite. As the results also agreed with (Markel and Voges, 2006) who suggested that the greater the concentration of progesterone inhibits *T. vaginalis* growth in vitro. (Baeten et al., 2001) found that women who use

contraception that enter progesterone in its composition reduce their incidence of *T. vaginalis*, and therefore did not agree with the results of the current study, the reason may be due to that not in all cases the concentration of hormones affect the incidence the parasite as can sometimes be the parasite is able to produce a change in the concentration of hormone of a host through a change to one of the immune response of the gland endocrine host paths after parasite infection (Klein, 2004). As well as for prolactin hormone, where the presence of the parasite was associated with elevated prolactin level. Pregnant Women mainly susceptible to infected with germs due to hormonal imbalances, anatomical changes (Ramzan *et al.*, 2004), the change in environment of vagina of pregnant women encourages the growth of germs, if the pregnant woman has diabetes, this will support the infection.

5-10 Sensitivity of bacterial isolates to antibiotics:

The current study showed that all the positive and negative gram bacteria were sensitive to imipenem, this result similar to report of (Shamim *et al.*, 2008), While it's different with (Cherpes *et al.*, 2005) where they found that their bacterial isolates was sensitive to imipenem in the rate (10-18%) only. As mentioned by Tariq *et al.*, 2006 that most of the *E. coli* and *Klebsiella pneumoniae* were sensitive to imipenem. As this result agreed with (Bradford, 2001; Paterson and Bonomo, 2005) were showed imipenem is effective against negative and positive bacteria, the effective of imipenem due to it is interferes with the ability of bacteria to build cell walls lead to breakdown and died bacterial cell and carbapenems (imipenem) are effective against ESBL producer strains (extended spectrum beta-lactamases (ESBLs) are enzymes produced by certain bacteria and effected against beta-lactam antibiotics, in the last decade there have also been numerous reports of production of extended-spectrum beta-lactamases (ESBLs) by *Proteus* spp and *Pseudomonas aeruginosa*, the carbapenems (imipenem) are generally not hydrolyzed by ESBLs (Philippon *et al.*, 1989). The gram positive isolates and *Gardnerella vaginalis* were resistance to aztreonam and gram negative isolate were sensitive to this antibiotic, except *proteus mirabilis* which

appeared resistance to aztreonam this result agrees with (Philippon *et al.* , 1989; Chanal *et al.*, 2000) which showed the ESBLs can confer resistance to the monobactam (aztreonam) .

The report of (Kobayashi *et al.* , 1992) also showed that the aztreonam effective against the negative gram bacteria , it has not useful activity against gram positive bacteria or anaerobes , this antibiotic act on prevent the formation of bacterial cell membranes of gram negative bacteria and it is an inhibitor of B-lactamase .As observed through the results of this study that all of the isolates were sensitive to piperacillin/tazobactam this result agrees with (Shamim *et al.* , 2008) , as agreed with Mumtaz *et al.*,2002 and Tariq *et al.*, 2006 reported that most of the gram negative rods were sensitive to piperacillin-tazobactam , the activity of piperacillin-tazobactam due to this antibiotic consist of two substance, piperacillin that work on inhibition of cell wall by bind with specific protein and therefore killing and preventing their reproduction , tazobactam that inhibition to B-lactamase (Eylt *et al.*,2012) , piperacillin/tazobactam has been successfully used to treat ESBL producing *P. mirabilis* infections in Italy (Luzzaro *et al.*, 2001).

Gardnerella vaginalis and *Streptococcus agalactiae* were sensitive to amoxicillin while all of gram negative bacteria were resistance to amoxicillin . For a long time, penicillin group have a main stay for the management of a variety of Staphylococcal infections, But the organism has gradually acquired resistance towards them evident from this study where only 37% of of the *Staphylococcus aureus* were sensitive towards amoxicillin in our study , and when penicillin combination with one of the β -lactamase inhibitors gives much better results (Akhtar *et al.* , 1997;Hafiz *et al* 2002) , clearly seen in this study (piperacillin-tazobactam 88.9%) ,this differed with Tariq *et al.*, 2006 that shown Each of *Staphylococcus* and *Streptococcus* were mostly sensitive to amoxicillin , this antibiotic killid bacteria by interfering in the process of building a bacterial cell wall , non active against bacteria that produce B-lactamase . The results also showed that gram positive

bacteria, *Gardnerella vaginalis* and gram negative bacteria except *Proteus mirabilis* and *Pseudomonas aeruginosa* have sensitive to cefotaxime, The ESBLs of *proteus mirabilis* and *Pseudomonas aeruginosa* can confer resistance to third generation cephalosporins such as cefotaxime, ceftriaxone and ceftazidime (Philippon *et al.*, 1989; Chanal *et al.*, 2000). This agrees with (Shamim *et al.*, 2008).

Cephalosporins are not recommended for the treatment of ESBL producing *P. mirabilis* isolates; failures have been observed (Luzzaro *et al.*, 2001) doxycycline shown different effect on bacteria where *streptococcus agalactiae*, *Klebsiella pneumoniae*, *Enterobacter cloacae* appeared sensitivity where doxycycline Inhibits protein synthesis in bacteria, without proteins the germs can't grow this stopped the spread of infection (WHO, 2007), while *proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Gardnerella vaginalis*, *staphylococcus aureus* appeared resistance to this antibiotic. the random use and unspecified for antibiotics will lead to the emergence of resistance cases as these antibiotics are prescribed to patients without conducting tests to investigate the pathogens and sensitivity test to these antibiotics as indicated by some statistics (Prescot *et al.*, 1990).

5-11 Sensitivity of *Candida albicans* isolates to antifungal :

Vulvovaginal candidiasis is one of the most common fungal infections among adult women during their lifetime (Parisa *et al.*, 2010 ;Ajitha and Maimoona, 2014). nystatin, fluconazole, ketoconazole are the antifungal drug that are widely used in the treatment of vulvovaginal candidiasis (Zahra *et al.*, 2012). The main agent of vulvovaginal candidiasis is *C. albicans*, the sensitivity patterns of *Candida albicans* isolates varies among studies in different countries (Kelen *et al.*, 2011). In our study all isolates of *Candida albicans* were offered for nystatin, fluconazole, ketoconazole, the results showed significant differences ($P < 0.05$) between these antibiotics, nystatin was the best antifungal, followed by ketoneazole, while fluconazole was less effect on *Candida albicans*, the appeared of nystatin in maximum sensitivity

(100%) to *C. albicans* quite agreed with (Emam *et al.*, 2012) that find same result, the nystatin gave higher diameters of the inhibition zone, was among (20-30) Mm this agreed with (Sheehan *et al.*, 1999; Al-Abeid *et al.*, 2004) that showed the nystatin is one of the most potent antifungal in the appearance of the highest areas of inhibition due to that nystatin act on inhibits of manufacturing of ergosterol which considered important in building the cell membrane of yeast, either inhibition zone of ketoconazole (16-29) Mm and the diameters of inhibition zone of fluconazole were (7-14) Mm.

Ketoconazole had clear effect where inhibited 64% of *C. albicans*, although some studies have reported that ketoconazole was of the most effective antifungal agents (Nawrat *et al.*, 2000; El-sayed and Hamouda, 2007; Dias *et al.*, 2011) however, since July 2013, the U.S. Food and Drug Administration (FDA) cleared that ketoconazole oral tablets can cause severe liver injuries, adrenal gland problems and harmful drug interactions with other medications. and should not be a first-line treatment for any fungal infection. However, the topical formulations of the drug have not been associated with such side effects (FAD Drug safety communication, 2013).

While fluconazole had shown (30.6%) sensitivity against *C. albicans*, this result not agrees with (Babin *et al.*, 2013) (76%), and the study done by (Ajitha *et al.*, 2010; Emam *et al.*, 2012) that showed higher sensitivity rate of 93.3% and 96.7% respectively, also (Al-Abeid *et al.*, 2004) found only 6% of *C. albicans* isolates were resistant to fluconazole, our study agreed with a study conducted in Brazil reported a resistance to fluconazole was (32%) (Dota *et al.*, 2011), the resistance rose to 16% in India reported (Babin *et al.*, 2013), while not found fluconazole resistance or a very low level of resistance (0.6%) among vaginal *C. albicans* isolates was reported in other studies from Australia and Kuwait, respectively (Ellis, 2011; Alfouzan *et al.*, 2015).

Conclusion and Recommendation

Conclusion

1-The current study shows that the rate of female genital tract infection in pregnant women was higher than non-pregnant women .

2-The study records a high rate of female genital tract infection among women in the age group (16-25) while the rate of infection is lower in women at the age group (40-45).

3- Existence of a relationship between infection and taking oral contraception , previous abortion.

4- The study shows that there is a relationship between infection and the level of estrogen and progesterone, where the infection is accompanied by the rise of these hormones , while there is no relationship between infection and the level of hormone prolactin .

5- The study finds that among the antibiotics that used in the study against bacterial isolates is imipenem , where it was effective against all the positive and negative gram bacteria . Nystatin is also the best antifungal agent, while fluconazole is the least effective .

Recommendation

Through the findings , this study recommends the following :

1-The need to pay attention to the necessary tests when the emergence of symptoms of genital tract infection female, especially culture and non-description of treatments for patients without a sensitivity test in order not to allow the emergence of strains of resistance between microorganisms causing the infection of the reproductive system .

2-Spread health awareness among women and urge attention to personal hygiene to reduce the spread of infection of the female reproductive system .

3-Due to the prevalence of female genital tract infection among women, we propose do extensive studies on the female genital tract infection and the factors that can help it to occur .

References

References

- Acikgoz, Z.C.; Ozturk, T.N.; Gamberzada, S.; Ark, E. & Gocer, S. (2002).** Retrospecti
vemicrobiologic evaluation of vaginal cultures. *Mikrobiol. Bul.*, 26(1):23-9.
- ACOG Practice Bulletin. (2006).** Clinical management guidelines for
obstetrician-gynecologists, Number 72, May 2006: Vaginitis. *Obstet Gynecol*,
107(5):1195-1206.
- Adu-Sarkodie, Y., Opoku, B. K., Danso, K. A., Weiss, H. A., & Mabey, D. (2004).**
Comparison of latex agglutination, wet preparation, and culture for the detection of
Trichomonas vaginalis. *Sexually transmitted infections*, 80(3), 201-203.
- Aerts JL, Christiaens MR, Vandekerckhove P. (2002).** Evaluation of progesterone
receptor expression in eosinophils using real-time quantitative PCR. *Biochim
Biophys Acta*; 1571(3): 167-72.
- Aflatoonian, R. Tuckerman E, Elliott SL, Bruce C, Aflatoonian A, Li TC, Fazeli A.
(2007).** Menstrual cycle-dependent changes of Toll-like receptors in
endometrium. *Hum. Reprod.* 22, 586–593 .
- Aflatoonian, R. and Fazeli, A. (2008).** Toll-like receptors in female reproductive tract
and their menstrual cycle dependent expression. *J. Reprod. Immunol.* 77, 7–13 .
- Ahmad, A., & Khan, A. U. (2009).** Prevalence of *Candida* species and potential risk
factors for vulvovaginal candidiasis in Aligarh, India. *European journal of
obstetrics, gynecology, and reproductive biology*, 144(1), 68-71.
- Ajitha Reddy, Maimoona Mustafa. (2014).** Phenotypic identification of *Candida* species
and their susceptibility profile in patients with genitourinary candidiasis
International Journal of Advanced Research Volume 2, Issue 12, 76-84.

References

- W. Alfouzan, R. Dhar, H. Ashkanani, M. Gupta, C. Rachel, Z.U. (2015).** KhanSpecies spectrum and antifungal susceptibility profile of vaginal isolates of *Candida* in KuwaitJ Mycol Med, 25 (1) , pp. 23-28.
- Al-Abeid HM, Abu-Elteen KH, Elkarmi AZ, Hamad MA. (2004).** Isolation and characterization of *Candida* spp. in Jordanian cancer patients: prevalence, pathogenic determinants, and antifungal sensitivity. *Jpn J Infect Dis.*;57 (6):279-84.
- Al-Ani , Zina Hashim Shihab . (2005) .** epidemiologic study to vaginal infection in Baghdad thesis of master ,college of Education , University of Baghdad .
- Amsel, R., Totten, P.A., Spiegel, C.A., Chen, K.C.S., Eschenbach, D. and Holmes, K.K.,(1983).** Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. *Am. J. Med.*, Vol.74, pp.14-22.
- Andersch-Bjorkman Y, Thomsson KA, Holmen Larsson JM, Ekerhovd E, Hansson GC. (2007).** Large scale identification of proteins, mucins, and their O-glycosylation in the endocervical mucus during the menstrual cycle. *Mol Cell Proteomics*, 6(4):708- 716.
- Anh, P.T., Mai, T.P., and Phuong, H.T. (1996).** Prevalence of lower genital tract infections among Vietnamese women attending a maternal and child Health Centre in Hanoi, Vietnam. *Southeast Asian J. Trop. Med. Public Health*, 27:193 – 195.
- Antonio, May A. D. and Hawes, Stephen E. (1999).**the Identification of Vaginal *Lactobacillus* Species and the Demographic and Microbiologic Characteristics of Women Colonized by These Species.The Journal of Infectious Diseases, Vol. 180, pp. 1950-1956.

References

- Anukam, K., Osazuwa, E., Ahonkhai, I., Ngwu, M., Osemene, G., Bruce, A. W., & Reid, G. (2006).** Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14: randomized, double-blind, placebo controlled trial. *Microbes and infection / Institut Pasteur*, 8(6), 1450-1454.
- Apea-Kubi, K. A., Sakyi, B., Yamaguchi, S., & Ofori-Adjei, D. (2006).** Bacterial vaginosis, *Candida albicans* and *Trichomonas vaginalis* infection in antenatal and gynaecological patients in Ghana. *Tropical Journal of Obstetrics and Gynaecology*, 22(2), 108-112.
- Apseloff G, Bao X, LaBoy-Goral L, Friedman H, Shah A. (2000).** Practical considerations regarding the influence of the menstrual cycle on leukocyte parameters in clinical trials. *Am J Ther*; 7(5): 297-302.
- Arbabi, M., Fakhrieh, Z., Delavari, M., & Abdoli, A. (2014).** Prevalence of *Trichomonas vaginalis* infection in Kashan city, Iran (2012-2013). *Iranian journal of reproductive medicine*, 12(7), 507-512.
- Atlas ,R.M. (1995).** Principles of microbiology . Mosby , Missouri 590 pp.
- Babay, H.A. and Babay, F.A., (1999).** Vaginal microflora and its association with obstetric and gynecological disorders and other illnesses. *Saudi Medical Journal*, Vol.20(8),pp.621-625.
- D. Babin, S. Kotigadde, P. Rao, T.V. (2013).** RaoClinico-mycological profile of vaginal candidiasis in a tertiary care hospital in Kerala *Int J Res Biol Sci*, 3 (1) , pp. 55-59.

References

- Baeten, J.M., Nyange, P.M. Richardson, B.A. Lavreys L, Chohan B, Martin HL Jr, Mandaliya K, Ndinya-Achola JO, Bwayo JJ, Kreiss JK. (2001).** Hormonal contraception and risk of sexually transmitted disease acquisition: results from a prospective study. *Am J. Obstet Gynecol.*, 185: 380–5 .
- Bain BJ, England JM. (1975).** Variations in leucocyte count during menstrual cycle. *Br Med J*; 2(5969): 473-5.
- Balaka B, Agbere A, Kpemissi E, Baeta S, Kessie K, Assimadi K. (1998).** Evolution de la mortalité neonatale précoce en dix ans (1981-82 & 1991-92) au CHU de lome : quelle politique de santé néonatale pour demain? *Méd Afr Noire.* 45:430–434. (Fre).
- Bartlett, J. G., and B. F. Polk. (1984).** Bacterial flora of the vagina: quantitative study. *Rev. Infect. Dis.* 6(Suppl. 1): S67-S72.
- Bartlett, J. G., A. B. (1977).** Onderdonk, E. Drude, C. Goldstein, M. Anderka, S. Alpert, and W. M. McCormack.. Quantitative bacteriology of the vaginal flora. *J. Infect. Dis.* 136:271-277.
- Baseman J.G. and Koutsky L.A. (2005).** The epidemiology of human Papilloma virus infections . *Journal of clinical virology* , 32(1): 516-24
- Basso B, Gimenez F, Lopez C (2005).** IL-1beta, IL-6 and IL-8 levels in gynecologic infections. *Infect Dis Obstet Gynecol*, 13(4):207-211.
- Beato M, Sanchez-Pacheco A. (1996).** Interaction of steroid hormone receptors with the transcription initiation complex. *Endocr Rev*; 17(6): 587-609.

References

- S. Begum and S. Ashwini, (2012).** “Study of immune profile during different phases of menstrual cycle,” International Journal of Biology and Medicinal Research, vol. 3, pp. 1407–1409.
- Berkow R . (1992).**The Merck Manual of Diagnosis and Therapy. Sixteenth Edition :Merck Research laboratories.
- Berman JR, Almeida FG, Jolin J, Raz S, Chaudhuri G, Gonzalez-Cadavid NF. (2003).** Correlation of androgen receptors, aromatase, and 5-alpha reductase in the human vagina with menopausal status. Fertil Steril. Apr;79(4):925-31.
- Black C A, Eysers F M, Russell A, Dunkley M L, Clancy R L, Beagley K W. (1998).**Acute neutropenia decreases inflammation associated with murine vaginal candidiasis but has no effect on the course of infection. Infect Immun. ;66:1273–1275.
- Blackwell A, Barlow D (1982)** Clinical diagnosis of anaerobic vaginosis: a practical guide. Br J Vener Dis 58:387–393.
- Blackwell , A.L, Fox ,A.R., Phillips , I & Barlow , D. (1983).** Anaerobic vaginosis (non specific vaginitis): Clinical ,microbiological , and therapeutic findings . Lancet 2, 1379-1382.
- Biswas MK (1993)** Bacterial Vaginosis. Clin Obstet Gynecol 36:166–176.
- Boris, Soledad and Barbes, Covadonga (2000).** Role Played by *Lactobacilli* in Controlling Population of Vaginal Pathogens., Microbes and Infection, Vol. 2, pp. 543-546.

References

- Boskey, E R, Cone RA, Whaley KJ, Moench TR. 9, (2001)** .origins of Vaginal Acidity: High D/L Lactate Ratio is Consistent with Bacteria Being the Primary Source., Human Reproduction, Vol. 16, pp. 1809-1813.
- Boskey, E R, Telsch KM, Whaley KJ, Moench TR, Cone RA. 10, (1999).** acid Production by Vaginal Flora In Vitro is Consistent with the Rate and Extent of Vaginal Acidification., Infection and Immunity, Vol. 67, pp. 5170-5175
- A . Bouman M.J. Heineman , and M. M .Faas .(2005).**”Sex hormones and the immune Sponse in humans , “Human Reproduction Update , Vol. 11 no .4 ,PP. 411-423,.
- Bump, R. C., F. P. Zuspan, W. J. Buesching, L. W. Ayers, and T. J. Stephens. (1984).** The prevalence, six month persistence, and predictive values of laboratory indicators of bacterial vaginosis (nonspecific vaginitis) in asymptomatic women. Am. J.Obstet. Gynecol. 150:917-924.
- Butts, C.L. & Sternberg, E.M. (2008).** Neuroendocrine factors alter host defense by modulating immune function. Cell. Immunol. 252, 7–15 .
- Bonventre , C.R. Buncher , and A . Beiting . (1982).** The epidemiology of genital colonization with *Staphylococcus aureus* .Ann . Intern. Med . 96:940 - 944.
- Borges, Sandra, Silva, Joana and Teixeira, Paula.(2014).** The Role od Lactobacilli and Probiotics in Maintiaing Vaginal Health., Archives Gynecology Obstetrics, Vol. 289, pp. 479-489.
- Bouman A, Schipper M, Heineman MJ, Faas MM. (2004).** Gender difference in the non-specific and specific immune response in humans. Am J Reprod Immunol; 52(1): 19-2.

References

- Bouman A, Moes H, Heineman MJ, de Leij LF, Faas MM. (2001).** The immune response during the luteal phase of the ovarian cycle: increasing sensitivity of human monocytes to endotoxin. *Fertil Steril*; 76(3): 555-9.
- Boskey ER, Cone RA, Whaley KJ, Moench TR. (2001).**Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. *Hum Reprod*, 16(9):1809-1813.
- Brooks, A.F., Butel, A.S. and Morse, T.A., (1998).** Medical Microbiology. 21st ed.Appelton and Lange.
- Cassone, A. (2015).** Vulvovaginal *Candida albicans* infections: pathogenesis, immunity and vaccine prospects. *BJOG* : an international journal of obstetrics and gynaecology, 122(6), 785-794. doi: 10.1111/1471-0528.12994.
- Cailloutte,James,CCharlesF.SharpJr.MD^aGrenith J.ZimmermanPhD^bSubirRoyMD^c**
6. (1997).Vaginal pH as a Marker for Bacterial Pathogens and Menopausal Status. American Journal of Obstetrical and Gynecology, Vol. 176, pp. 1270-1277.
- CDC. (2010).**FDA licensure of quadrivalent human papillomavirus vaccine (HPV4, Gardasil) for use in males and guidance from the Advisory Committee on Immunization Practices (ACIP). *MMWR*;59:630–2.
- Chaffin,W.L.and D.M.Stocco. (1983).**Cell Eall protein of *Candida albicans*.*Can.J.Microbiol.*29:1439-1444.
- Chanal C, Bonnet R, De Champs C, Sirot D, Labia R, Sirot J. (2000).** Prevalence of β -lactamases among 1,072 clinical strains of *Proteus mirabilis*: a 2-year survey in a French hospital. *Antimicrob Agents Chemother.* 2000;44:1930–1935. doi: 10.1128/AAC.44.7.1930-1935..

References

- Cherpes, T.L.; Melan, M.; Kant, J.A.; Cosention, L.A.; Meyn, L.A; Hillier, S.L. (2005)**
.Genital tract shedding of herpes simplex type 2 in woman ;effect of hormonal contraception, bacterial vaginosis & vaginal group B *Streptococcus* colonization. Clin. Infect. Dis. 40(10); 142-8.
- Chen YH, Chen YS, Liu JW, Lu MC, Lam C, Liu CY, Hsueh PR. (2007).** Rates of susceptibility of carbapenems, ceftobiprole, and colistin against clinically important bacteria collected from intensive care units in: Results from the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART). Microbiol Immunol Infect.. 2015 Jan 10 Epub ahead of publication.
- Chen K, Amsel R, Eschenbach D A, Holmes K K. (1982).** Biochemical diagnosis of vaginitis: Determination of diamines in vaginal fluid. J Infect Dis 145:337-345.
- Christian L Rampellos, Morris c, Ralote v. (1996).** Bacterial Vaginosis and prevalence in Italian population of asymptomatic women and diagnostic aspects. Euro J. Epidemiology 383-390.
- Chow, A.W., k.H. Bartlett, R. Percival –Smith , and B .J .Morrison . (1984).** Vaginal colonization with *Staphylococcus aureus* , positive for toxic –shock marker protein , and *Escherichia coli* in healthy women . J . infect. Dis .150:80-84.
- Chow, A. W., Percival-Smith, R., Bartlett, K. H., Goldring, A. M. & Morrison, B. J. (1986).** Vaginal colonization with *Escherichia coli* in healthy women. Determination of relative risks by quantitative culture and multivariate statistical analysis. *Am J Obstet Gynecol* 154, 120±126.
- Clinical and Laboratory Standards Institute. (2012).** M02-A11. Performance standards for antimicrobial disk susceptibility tests; approved standard: eleventh edition. Clinical and Laboratory Standards Institute, Wayne, PA.

References

- Clinical and Laboratory Standards Institute. (2009).** Performance standards for antimicrobial susceptibility testing; 19th informational supplement. CLSI document M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA.
- Cohen MS, Black JR, Proctor RA, Sparling PF. (1984).** Host defences and the vaginal mucosa. A re-evaluation. *Scand J Urol Nephrol Suppl*, 86:13-22.
- Cohen JH, Danel L, Cordier G, Saez S, Revillard JP. (1983).** Sex steroid receptors in peripheral T cells: absence of androgen receptors and restriction of estrogen receptors to OKT8-positive cells. *J Immunol*; 131(6): 2767-71.
- Cole AM. (2006).** Innate host defense of human vaginal and cervical mucosae. *Curr Top Microbiol Immunol*, 306:199-230
- Collee, J. G. ;Fraser, A. G. ;Marmino, B. P. and Simons, A. (1996).** Mackin and McCartney Practical Medical Microbiology.14th ed., The Churchill Livingstone, Inc. U. S. A.
- Cowan,A. S. T.(1985).** Cowan and steel's manual for identification medical bacteria .2nd ed. London , Cambridge University Press ,UK.
- Crucitti, T., Jespers, V., Mulenga, C., Khondowe, S., Vandepitte, J., & Buve, A. (2011).** Non-sexual transmission of *Trichomonas vaginalis* in adolescent girls attending school in Ndola, Zambia. *PloS one*, 6(1), e16310. doi: 10.1371/journal.pone.0016310 .
- The Biology of the Vagina in the Human Subject. Cruickshank, Robert and Sharman, Albert. 2.(1934).** *Journal of Obstetrics and Gynecology*, Vol. 41, pp. 190-207.

References

- Curran EM, Berghaus LJ, Verneti NJ, Saporita AJ, Lubahn DB, Estes DM. (2001).** Natural killer cells express estrogen receptor-alpha and estrogen receptor-beta and can respond to estrogen via a non-estrogen receptor-alpha-mediated pathway. *Cell Immunol*; 214(1): 12-20.
- Deceuninck, G., Asamoah-Adu, C., Khonde, N., Pépin, J., Frost, E. H., Deslandes, S., Asamoah-Adu, A., Bekoe, V., & Alary, M. (2000).** Improvement of Clinical Algorithms for the Diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* by the Use of Gram-Stained Smears Among Female Sex Workers in Accra, Ghana. *Sexually transmitted diseases*, 27(7), 401-410.
- De- leon , E.M.; Jacober , S.J.; Sobel , J.D. and Fox man , B.(2002).** Prevalence and risk factors for vaginal *Candida* colonization in women with type land diabetes *Infect .Dis , 2: 1-11 infect .immune. 68(7): 3840-3847.*
- Dennerstein , G.(2001).** The treatment of *Candida* vaginitis and vulvitis .*Aust. preser .;24:62-64.*
- L.B. Dias, M.C. Melhem, M.W. (2011).** SzeszsVulvovaginal candidiasis in Mato Grosso, Brazil: pregnancy status, causative species and drugs tests *Braz J Microbiol*, 42 pp. 1300-1307.
- Doderlein , Albert . (1892).** Das scheidensekret and seine bedeutung fur das puerperalfieber leipzigis . n,.
- Doderlein A . (1892) .** Das Scheidensekret und seine Bedeututung Fur das Puerperalfieber . Leipzig , Germany . : verlag von Eduard Besold .

References

- Donders GG, Van Calsteren K, Bellen G, Reybrouck R, Van Den Bosch T, Riphagen I, Van Lierde S. (2009).** Predictive value for preterm birth of abnormal vaginal flora, bacterial vaginosis and aerobic vaginitis during the first trimester of pregnancy. *BJOG*;116:1315–1324 .
- Donne A. (1836).** Animalcules observes dans Les matieres purulentes et al produit des Secretions des organes genitaux de l' homme et de la femme . CR Behd . séances Acad Sci ; 3:385-6
- Dosiou C, Hamilton AE, Pang Y, Overgaard MT, Tulac S, Dong J , Thomas P, Giudice LC. (2008).** Expression of membrane progesterone receptors on human T lymphocytes and Jurkat cells and activation of G-proteins by progesterone. *J Endocrinol*; 196(1): 67-77.
- K. Dota, A. Freitas, M. Consolaro, T.I.A.(2011).** Svidzinski Challenge for clinical laboratories: detection of antifungal resistance in *Candida* species causing vulvovaginal candidiasis *Science*, 42 (2) , pp. 87-93.
- Dulczak, S. and Kirk, G. (2005).** Overview of evaluation, diagnosis, and management of urinary tract infections. *Urol Nurs*. 25(3):185-91
- Dybas,I.;Sidor-Wojtowicz,A.and Montewka,K.M.(2005)** .Bacterial flora and Mycosis of the vaginal in women .*J.Med . Microbiol*.76(5):385-90 .
- Edwards L.(2004).** The diagnosis and treatment of infectious vaginitis .*Dermatol Ther*. .17:102-10.
- Egan ME , Lipsky MS. (2000).** Diagnosis of vaginitis. *Sep* 1;62(5):1095-104.

References

- Elenkov IJ, Wilder RL, Bakalov VK, Link AA, Dimitrov MA, Fisher S et al. (2001).** IL-12, TNF-alpha, and hormonal changes during late pregnancy and early postpartum: implications for autoimmune disease activity during these times. *J Clin Endocrinol Metab*; 86(10): 4933-8.
- Ellis D. (2011).** Antifungal susceptibility profile (Australian antifungal susceptibility data for *Candida* isolates from recurrent vulvovaginal candidiasis (2007–2009) using the CLSI M44-A2 disc susceptibility standard for yeasts). *Mycology online*. The University of Adelaide. CRICO Provider N. 00123M.
- H. El-sayed, A.(2007).** Hamouda *Candida albicans* causing vulvovaginitis and their clinical response to antifungal therapy *Egypt J Med Microbiol*, 16 (1) , pp. 53-62.
- Emam, H, Farrell, P and Abdelaal, M (2012)** Causes of Delay in GCC Construction Projects:A Critical Review. In: Okeil, A (Ed.) “The 1st International Conference of CIB Middle East and North Africa Conference”, 14-16 December 2014, Abu Dhabi, UAE.CIB Middle East and North Africa Research Network, 607-621.
- Enomoto LM, Klobardanz KJ, Mark DG, Elizabeth D, Weinberg A. (2007).**Ex vivo effect of estrogen and progesterone compared with dexamethasone on cell-mediated immunity of HIV-infected and uninfected subjects. *J Acquir Immun Defic Syndr* ;45:137–45.
- Eriksson, K., Carlsson, B., Forsum, U., & Larsson, P. (2005).** A double-blind treatment study of bacterial vaginosis with normal vaginal lactobacilli after an open treatment with vaginal clindamycin ovules. *Acta dermato-venereologica*, 85(1), 42-46.

References

- Eschenbach D A, Hillier S, Critchlow C, Stevens C, DeRouen T, Holmes K K. (1988).** Diagnosis and clinical manifestations of bacterial vaginosis. *Am J Obstet Gynecol* 158:819-828.
- Eschenbach D A, Bekassy S, Blackwell A, Ekgren J, Hallén A, Wathne B. (1985).** The diagnosis of bacterial vaginosis (Working group recommendations). *Scand J Urol Nephrol*:260-261.
- Eylt , Yeung , Gore J G. (2012).** Aretrospective analysis of the incidence of clostridium Difficile associated Diarrhea with Meropenem and piperacillin tazopactum , Auersperg EV. *International Journal of collaborative research on internal medicine and public health* . 4(8) :1567-1576.
- Fan W., Chen J., Wang X., Cai R., Tan Q., Chen Y., Qingsong Yang,Shanming Zhang,Yun Wu,Zilu Yang,Xi-Ai Wang,Yiping Zhong(2013).** Electrophysiological correlation of the degree of self-reference effect. *PLoS ONE* 8:e80289 10.1371/journal.pone.0080289.
- FDA Drug Safety Communication.(2013).** FDA limits usage of Nizoral (ketoconazole) oral tablets due to potentially fatal liver injury and risk of drug interactions and adrenal gland problems.
- Ferrer J: Vaginal candidosis (2000).** epidemiological and etiological factors. *Int J Gynaecol Obstet*, 71 Suppl 1:S21-27
- Fidel P L, Jr, Luo W, Steele C, Chabain J, Baker M, Wormley F L.(1999).** Analysis of vaginal cell populations during experimental vaginal candidiasis. *Infect Immun.* ;67:3135–3140.
- Fleury, F.J. (1981).** Adult vaginitis. *Clin Obstet Gynaecol.* 24(2): 407-38

References

- Ford, L.C.H.A., Hammill, R.J., Delange, D.A., Bruchner, F., Suzuki-Charez, H.L., Mickus, and Lebberz, T.B., (1987).** Determination of estrogen and androgen receptors in *Trichomonas vaginalis* and the effects of anti-hormones. Am. J. Obstet. Gynecol., 156: 1119-1121 .
- Francis, S. C., Ao, T. T., Vanobberghen, F. M., Chilongani, J., Hashim, R., Andreasen, A., Watson-Jones, D., Chagalucha, J., Kapiga, S., & Hayes, R. J. (2014).** Epidemiology of Curable Sexually Transmitted Infections among Women at Increased Risk for HIV in Northwestern Tanzania: Inadequacy of Syndromic Management. PloS one, 9(7), e101221.
- Gaillouette, J.C.; C.F. Sharp; G.J. Zimmerman and S. Roys. (1997).** Vaginal PH as a marker for bacterial pathogens and menopause. Am. J. Stet. Gynecol. 176:1270-1277.
- Galask RP. (1988).** Vaginal colonization by bacteria and yeast. Am J Obstet Gynecol, 158(4):993-995.
- Gardner HL, Dukes CD. (1955).** Haemophilus vaginalis vaginitis : a newly defined specific infection previously classified non-specific vaginitis . AMJ obstet Gynecol ; 69:962-976.
- Gebhart JB, Rickard DJ, Barrett TJ, Lesnick TG, Webb MJ, Podratz KC, Spelsberg TC. (2001).** Expression of estrogen receptor isoforms alpha and beta messenger RNA in vaginal tissue of premenopausal and postmenopausal women. Am J Obstet Gynecol. Dec;185(6):1325-30; discussion 30-1.

References

- Geerling , S.E.; Brouwer, E.C.; Van-Kessel, K.C.; Gastra, W.; Stolk, R.P. and Hoepelman, A.I.(2000)** Cytokine secretion is impaired in women with diabetes mellitus. *Eur.J.Clin. Invest.*, 30 : 955-1001 (Medline).
- Geiger AM, Foxman B, Sobel JD. (1995).** Chronic vulvovaginal candidiasis: Characteristics of women with *Candida albicans*, *C.glabrata* and no candida. *Genitourin Med* 75:304-7.
- Geisler, W.M.; Yu S.; Venglarik M. and Schwebke S.R. (2004).**Vaginal leukocyte counts in women with bacterial vaginosis relation to vaginal and cervical infections, *Sex Transm. Infect.*, 80 (5): pp.401-405.
- Gentry , L .o. and price , M.F. (1985) .** Urinary and genital Candida infections . In candidiasis infections . In candidiasis , Bodey G.,P.and Fainstein V. (eds.) Raven press new York : 169-179.
- MC Geoch DJ, Rixon FJ, Davison AJ(2006) .**” Topics in herpes virus genomic and evolution “ virus Res.117(1): 90-104 .
- Georheart P.A. ,Randall T.C. Buckley R.M.J R (2004).** Human Papillomavirus . *emedicine* , Decembe.2004.
- Ghazal S, Musmar M, AL-Tell MAI. (2004).** Effect of IUD (intrauterine device) on reproductive tract infection (RTI) in the Northern West Bank. *Middle East J Fam Med*;2:1–12.
- Gilbert, D. T., Driver-Linn, E., & Wilson, T. D. (2002).** The trouble with Vronsky: Impact bias in the forecasting of future affective states. In L. Feldman-Barrett & P.

References

Salovey (Eds.), *The wisdom of feeling* (pp. 114-143). New York: Guilford.

Ginocchio, C. C., Chapin, K., Smith, J. S., Aslanzadeh, J., Snook, J., Hill, C. S., & Gaydos, C. A. (2012). Prevalence of *Trichomonas vaginalis* and coinfection with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in the United States as determined by the Aptima *Trichomonas vaginalis* nucleic acid amplification assay. *Journal of clinical microbiology*, 50(8), 2601-2608. doi: 10.1128/JCM.00748-12.

Giorgi A, Torriani S, Dellaglio F, Bo G, Stola E, Bernuzzi L. (1987) .Identification of vaginal *lactobacilli* from asymptomatic women. *Microbiologica*;10:377–384.

Gipson IK, Spurr-Michaud S, Moccia R, Zhan Q, Toribara N, Ho SB, Gargiulo AR, Hill JA, 3rd. (1999). MUC4 and MUC5B transcripts are the prevalent mucin messengerribonucleic acids of the human endocervix. *Biol Reprod*, 60(1):58-64.

Goto A, Nguyen QV, Pham NM, Kato K, Cao TP, Le TH, Hoang QK, Le TQ, Nguyen BT, Katsube M, Ishii S, Yasumura S. (2005). Prevalence of and factors associated with reproductive tract infections among pregnant women in ten communes in Nghe An Province, Vietnam. *J Epidemiol.* ;15:163–72. doi: 10.2188/jea.15.163.

Granger,S.E.(1992).The aetiology and Patholog of Vaginal Candidiasis.*Brit.J.Clin.Pract.*46.

Greenwood JR, pickett MJ. (1980). Transfer of *Haemophilus vaginalis* Gardner and Dukes to a new Genus , *Gardnerella* :*G. vaginalis* (Gardner and Dukes) comb . nov . *Int J syst Bacteriol* ; 30 : 170-8 .

Greer CE, Wheeler CM , Ladner MB, Beutner K, Coyne MY ,Liang H , Langenberg A, Yen TS , Ralston R. (1995) . Human papillomavirus (HPV) type

References

distributed and Serological response to HPV6- virus- like Particle in Patients with genital Warts .Jurnal of Clinical Microbiology 33(8):2058-2063.

Grossman, J. H., III, and R. L. Adams. (1979). Vaginal flora in women undergoing hysterectomy with antibiotic prophylaxis. Obstet. Gynecol. 53:23-26.

Gupta R , Warren T, Wald A(2007). “ Genital herpes “.Lancet . 370(9605): 2127 -37.

Gutman RE, Peipert JF, Weitzen S, Blume J (2005) Evaluation of clinical methods for diagnosing bacterial vaginosis. Obstet Gynecol 105:551–556.

Haffar, A. A. M., P. A Fuselier, and C. J. Baker. (1983). Species distribution of non-group D alpha-hemolytic *streptococci* in maternal genital and neonatal blood cultures. J. Clin. Microbiol. 18:101-103

Hallén A, Pålson C, Forsum U. (1987). Bacterial vaginosis in women attending STD clinic: diagnostic criteria and prevalence of Mobiluncus spp. Genitourin Med 63:386-389.

Hall –Craggs ECB. (1990). Anatomy as a Basis for Clinical Medicine .Second Edition Urban & Schwarzenberg .

Harithi ., L., Roebuck , K.A ., Olinger , G.G ; Landay , A ; Sha, B.E ; Hashemi F.B. and Spear, G.T (1999).Bacterial vaginosis associated microflora isolated from the female genital actirates HIV. I expression , J. Acquird immune . Defic.

Hashimoto, M., Komori, A., Kuramasu, T., Yokoyama, Y., Kawase, N., & Nakamura, T. (1964). Electron Microscopic Studies on the Fine Structure of

References

Trichomonas Vaginalis. Journal of the Japanese Obstetrical & Gynecological Society, 11, 162-166.

Haussman D (1875) Parasites des organes sexuels femelles de l'homme et de quelques candida animaux , avec une notice sur developpement de l'Oidium albicans (Robin).

Hawes SE, Hillier SL, Benedetti J, Claire E. Stevens, Laura A. Koutsky, Pal Welncr-Hanssen, and King K. Holmes (1996). Hydrogenperoxide-producing lactobacilli and acquisition of vaginal infections. J Infect Dis; 174:1058–1063.

Hay P. (2004) .Bacterial vaginosis and miscarriage. Curr Opin Infect Dis; 17:41–44.

P.E. Hay, R.F. Lamont, D. Taylor-Robinson, D.J. Morgan, C. Ison and J. Pearson, (1994). Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage, Br Med J 308, p. 295.

Haya, Javier, Africa G, Carlos L, Maher B, Lara H. (2014) .Importance of Lactic Acid in Maintaining Vaginal Health: A Review of Vaginitis and Vaginosis Etiopathogenic Bases and a Proposal for a New Treatment., Open Journal of Obstetrics and Gynecology, Vol. 4, pp. 787-799.

Heine P, McGregor JA. (1993). *Trichomonas vaginalis* :Are emerging pathogen . clin Obstet Gynecol; 36:137-44.

Herpes simplex “.(2006). Derm Net NZ – New Zealand Dermatological Society “.

References

- Hillier SL., (1993).**Diagnostic microbiology of bacterial vaginosis. Am J Obstet Gynecol,. 169: p. 455-459.
- Hillier, S.L., Kroh, M.A., Rabe, L.K., Klebanoff, S.J. and Eschenbach, D.A., (1993).** TheNormal vaginal Flora, H₂O₂-Producing *Lactobacillis*, and Bacterial Vaginosis . Clinical Infectious Diseases, Vol.16 (Suppl 4), S273-81.
- Hodgins MB, Spike RC, Mackie RM, MacLean AB. (1998).** An immunohistochemical study of androgen, oestrogen and progesterone receptors in the vulva and vagina. Br J Obstet Gynaecol. Feb;105(2):216-22.
- Hohne O . (1916).**Trichomonas vaginalis also haufiger erreger ciner typischen colpitis purulenta . Centralblatt gynaekol; 40:4-15.
- Holmes KK. (1999).**Sexually transmitted diseases. 3rd edition. New York: McGraw-Hill, Health Professions Division; .
- Holt, J. G.; Krieg, N. R.; Sneath, P. H. A.; Staley, J. T. and Williams, S. T. (1994).** Bergey's manual of determinative bacteriology. 9th ed. Williams & Wilkins: Baltimore, Maryland; pp. 20 & 527-558.
- Holst E, Wathne B, Hovelius B ,Mardh PA . (1987).** Bacterial vaginosis :Microbiological and clinical findings . Eur J clin Microbiol;6:536-41 .
- Honigberg, B. M., & King, V. M. (1964).** Structure of Trichomonas Vaginalis Donn'e. The Journal of parasitology, 50, 345-364.
- Huggins GR, Preti G. (1981).** Vaginal odors and secretions. Clin Obstet Gynecol,24(2):355-377.

References

- Hurley R, Leask G S , Faktor J A and de Fonseka CI (1973).** Journal of obstetrics and Gynaecology of the British commonwealth 80 , 252.
- Inceboz, T., Yalcin, G., Bayrakal, V., Inceboz, U. and Derici, Y., (2012).** Investigation of in vitro effects of estrogens and selective estrogen receptor modulators (serms) on *Trichomonas vaginalis*. AfricanJournal of Microbiology Research. ISSN 1996-0808. 6(43): 7078-7083 .
- Islam A, Safdar A, Malik A. (2009).**Bacterial vaginosis. J Pak Med Assoc.; 59(9):601-42.
- Janeway C. (2005).**Immunobiology : the immune system in health and disease. 6th edition. NewYork: Garland Science.
- Jawetz, E.; Melnick, J.L. and Adelberg, E.A.(2004).** Medical Microobiology, 23th ed. Appelton and Lang. U.S.A.
- Jean SS, Lee WS, Yu KW, Liao CH, Hsu CW, Chang FY, Ko WC, Chen RJ, Wu JJ, Tariq N, Jaffery T, Ayub R, Alam AY, Javid MH, Shafique S. (2006).**Frequency and antimicrobial susceptibility of aerobic vaginal isolates. J Coll Physicians Surg Pak;16(3):196–99.
- Jefferson, K. K. (2012).** The bacterial etiology of preterm birth. Advances in applied microbiology, 80, 1-22. doi: 10.1016/B978-0-12-394381-1.00001-5 .
- Johnson, A., Ison, C., Hetherington, C., Osborn, M., Southerton, G., London, W., Easmon, C., & Taylor-Robinson, D. (1984).** A study of the susceptibility of three

References

species of primate to vaginal colonization with *Gardnerella vaginalis*. British journal of experimental pathology, 65(3), 389.

Joishy B., Ordonez-Ercan D., Dasgupta P., Chellappan S. (2005). Induction of human metallothionein 1G promoter by VEGF and heavy metals: differential involvement of E2F and metal transcription factors. *Oncogene*. 24:2204–2217
10.1038/sj.onc.1208206.

Jones, F.R., Miller, G., Gadea, N. Meza, R., and Leon, S. (2007). Prevalence of bacterial vaginosis among young women in low income populations of coastal Peru. *Int J STD AIDS*, 18:188-92.

Junqueira LC, Carneiro J, editors. (2005). Basic Histology: text and atlas. 11th ed:McGraw-Hill Medical Publishing Division.

Kalo-Klein A, Witkin S S. (1989). *Candida albicans*: cellular immune system interactions during different stages of the menstrual cycle. *Am J Obstet Gynecol.* ;161:1132–1136.

Kelen F. D. Dota, Alessandra R. Freitas, Marcia E. L. Consolaro, Terezinha I. E. Svidzinski. (2011). A Challenge for Clinical Laboratories: Detection of Antifungal Resistance in *Candida* Species Causing Vulvovaginal Candidiasis. *Journal of Labmedicine* February Volume 42 Number 2.

Kierszenbaum AL. (2002). Histology and cell biology : an introduction to pathology. St. Louis, Mo.: Mosby.

King, K.D.; J.C. Lee and A.L. Moriris. (1980). Adherence of *Candida albicans* and other *Candida* species to Mucosal epithelial Cells. *Infect, Immune*. 27:667-674

References

- Kingsberg SA, Wysocki S, Magnus L, Krychman ML. (2013).** Vulvar and vaginal atrophy in postmenopausal women: findings from the REVIVE (Real Women's Views of Treatment Options for Menopausal Vaginal ChangEs) survey. *J Sex Med.* Jul;10(7):1790-9.
- Klebanoff SJ, Hillier SL, Eschenbach DA, Waltersdorff AM. (1991).**Control of the microbial flora of the vagina by H₂O₂ generating lactobacilli. *J Infect Dis*; 164:94–100.
- Klein, S.L., (2004).** Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunology.*, 26: 247–264. Volume 26, Number 6/7.
- Kobayashi , Y., Uchida , H.; Kawakami Y. (1992).** “synergy with aztreonam and arbicasin or to bramycin . against pseudomonas aeruginosa isolated from blood “ *J Antimicrob chemother* ; 30(6) : 871 -872.
- Koch A L. (1994).** Growth measurement. In: Gerhardt P, Murray R G E, Wood W A, and Krieg N R editors. *Methods for general and molecular bacteriology*. Washington DC: American Society for Microbiology.
- Komi J, Lassila O. (2000).** Nonsteroidal anti-estrogens inhibit the functional differentiation of human monocyte-derived dendritic cells. *Blood*; 95(9): 2875-82.
- Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenberger, P.C. and Winn, W.C.W.,(1997).** “Color Atlas and Textbook of Diagnostic Microbiology”. 5th. ed. J. B.Lippincott-Raben Publ., Philadelphia.
- Kornman, K. S. and Loesche, W. J. (1982)** Effects of estradiol and progesterone on *Bacteroides melaninogenicus* and *Bacteroides gingivalis*. *Infect Immun*, 35, 256-263.

References

- Kurki T Ssivonens A, Beukemen Or, Savia E, ylinkorkala O, (1992).**Bacterial vaginosis in early pregnancy and pregnancy outcomes.Obst Gynecol res.173-177.
- Kurtzman,C.P and Fell,J.W.(1998).**The Yeasts, A Taxonomic Study.4th .ed.Elsevier,USA.P:454.
- Labrie F et al . (2005) .** is denydroepiandrosterone a hormone J. Endocrinol . 187 , 169 - 196.
- Lamche HR, Silberstein PT, Knabe AC, Thomas DD, Jacob HS, Hammerschmidt DE. (1990).** Steroids decrease granulocyte membrane fluidity, while phorbol ester increases membrane fluidity. Studies using electron paramagnetic resonance. Inflammation; 14(1): 61-70.
- Lan PT, Lundborg CS, Phuc HD, Sihavong A, Unemo M, Chuc NT, Khang TH, Mogren I. (2008).** Reproductive tract infections including sexually transmitted infections: a population-based study of women of reproductive age in a rural district of Vietnam. Sex Transm Infect.84:126–32. doi: 10.1136/sti.2007.027821.
- Larsen P, Shikar V, Gregg C, Mak G, Chojnacki A, Yong VW, Weiss S. (2007).** " White Matter Plasticity and enhanced reyelination in the maternal CNS " . J neurosci , 27(8) : 18: 12-23.
- Larsen B.(1993).** Vaginal flora in health and disease. Clin ObstetGyneco.;36:107–121.
- Larsen , B., and R .p . Galask .(1982).** vaginal microbial flora : composition and influences of host physiology . ANN . Intern . Med .96:926-930.
- Larsen B, Monif GR. (2001).**Understanding the bacterial flora of the female genital tract. Clin Infect Dis. Feb 15; 32(4):e69-77.

References

- Larsson P G, Forsum U. (2005)** . Bacterial vaginosis--a disturbed bacterial flora and treatment enigma. *Apmis* 113:305-316
- Larsson PG, Bergman B, Forsum U, Platz-Christensen JJ, Pahlson C. (1989).** Mobiluncus and clue cells as predictors of PID after first-trimester abortion. *Acta Obstet Gynecol Scand.*68: 217-20.
- Larsson PG, Platz-Christensen JJ, Thejls H, Forsum U, Pahlson C. (1992).** Incidence of pelvic inflammatory disease after first-trimester legal abortion in women with bacterial vaginosis after treatment with metronidazole: a double-blind, randomized study. *Am J Obstet Gynecol.* 166 (1 Pt 1): 100-3.
- Lehman R. (2009).**Clinical approach to recognizing and managing a patient with vaginal atrophy: a guide for physician assistants. *Internet Journal of AcademicPhysician Assistants.*; 8(1).
- Levett PN . (1980).** A comparison of five methods for the detection of *Trichomonas vaginalis* in clinical specimens . *Med lab Sci* ;37: 85-8.
- Levison, M. E., L. C. Corman, E. R. Carrington, and D. Kaye. (1977).** Quantitative microflora of the vagina. *Am. J. Obstet. Gynecol.* 127:80-85.
- Lewis, R. E.; Klepser, M. E. & Pfuller, M. A.,(2000).** Invitro pharmacodynamiccharacteristics of flucytosine determined by time-kill methods.*Diagn. Microbiol. Infect. Dis.* 36:101-105.
- Lindner, J. G. E. M., F. H. F. Plantema, and J. A. A. Hoogkamp- Korstanje. (1978).** Quantitative studies of the vaginal flora of healthy women and of obstetric and gynaecological patients. *J. Med. Microbiol.* 11:233-241.

References

- Linnemann , C.C., Jr., J.L. Staneck ,S.Hornstein ,T. p. Barden , J. L. Rauh , P .F . Schlievert , P. M ., M.T .Osterholm , J.A. Kelly , and R.D. Nishimura. (1982).** Toxin and enzyme characterization of *Staphylococcus aureus* isolates from patients
- Little C.H., Georgious , G.M ; Marceglia , A; Dgedgebe , H. Cone , R.E. and Mazze , D (2000) .**Measurement of T cell derived dantigen binding molecules and immunoglobulin G specific to *Candida albicans* Mannan is sera of patient with recurrent vulvovaginal candidiasis.
- Llewellyn-Jones D, Oats J, Abraham S. (2004).**Llewellyn-Jones fundamentals of obstetrics and gynaecology. 8th edition. Edinburgh ; New York: Mosby.
- Livengood, C. H., 3rd, McGregor, J. A., Soper, D. E., Newton, E., & Thomason, J. L. (1994).** Bacterial vaginosis: efficacy and safety of intravaginal metronidazole treatment. American journal of obstetrics and gynecology, 170(3), 759-764.
- Lossick, J. G. (1980).** Single-dose metronidazole treatment for vaginal trichomoniasis. Obstetrics and gynecology, 56(4), 508-510.
- Lossick, J. G., Muller, M., & Gorrell, T. E. (1986).** In vitro drug susceptibility and doses of metronidazole required for cure in cases of refractory vaginal trichomoniasis. The Journal of infectious diseases, 153(5), 948-955
- Luzzaro F, Perilli M, Amicosante G, Lombardi G, Belloni R, Zollo A, Bianchi C, Toniolo A. (2001).** Properties of multidrug-resistant, ESBL-producing *Proteus mirabilis* isolates and possible role of beta-lactam/beta-lactamase inhibitor combinations. Int J Antimicrob Agents.17:131-5.

References

- MacFaddin, JF. (2000).** Biochemical Tests for the Identification of Medical Bacteria, 3rd ed. Philadelphia: Lippincott Williams & Wilkins.
- MacFaddin, J.F., (1985).** Biochemical tests for Identification of medical bacteria. Williams and Wilkins, Baltimore, USA. 285p.
- Mahdi , N. k. and AL-Hamdani, M.M.(1998).** sexually transmitted diseases among women with habitual abortion. East . Med .H.J.;4; 343-349 .
- Mansour I, Reznikoff-Etievant MF, Netter A. (1994).** No evidence for the expression of the progesterone receptor on peripheral blood lymphocytes during pregnancy. Hum Reprod. 9(8): 1546-9.
- Manorama, H. T., & Shenoy, D. R. (1978).** Single-dose oral treatment of vaginal trichomoniasis with tinidazole and metronidazole. The Journal of international medical research, 6(1), 46-49.
- Mardh P-A, Taylor –Robinson D. (1984).** Bacterial vaginosis . Scand J Urol Nephrol suppl . 86.
- Markel, E.K. and Voges, M. (2006).** Medical Parasitology. Ninth Edition. Pp 57-58.
- Marino M, Galluzzo P, Ascenzi P. (2006).** Estrogen signaling multiple pathways to impact gene transcription. Curr Genomics.7(8): 497-508
- Martinotti, M.G., and Savoia, D.(1985).**Influenza di alcuntarononisteroideisullacresscita du *Trichomonas vaginalis*. G. Batteriol. Virol. Immunol. Microbiol.,78: 52-59 .
- .Mason PR, Super H, Fripp PJ. (1976).** Comparison of four techniques for the routine diagnosis of *Trichomonas vaginalis* infection. J Clin Path .29:154–57.

References

- McMurray, RW, Ndebele, K, Hardy, KJ and Jenkins, JK (2001).** "17-beta-estradiol suppresses IL-2 and IL-2 receptor." *Cytokine* 14(6): 324-333.
- Meisels A, Fortin R. (1976).** Condylomatous lesions of the cervix and vagina .I. Cytologic patterns . *Acta Cytol* .20(6):505-9.
- Menge, C and Kronig, B. (1897).** Bakteriologie des weiblichen Genitale Kanals. Leipzig : S.n.,.
- Mestecky J, Russell MW. (2000).** Induction of mucosal immune responses in the human genital tract. *FEMS Immunol Med Microbiol*.27(4):351-355.
- Miller L, Patton DL, Meier A, Thwin SS, Hooton TM, Eschenbach DA. (2000).** Depomedroxyprogesterone-induced hypoestrogenism and changes in vaginal flora and epithelium. *Obstet Gynecol*, 96(3):431-439.
- Mirmonsef, Paria, Anna L. Hooton, Douglas Gilbert, Casey J. Gioia, Danijela Maric, Thomas J. Hope, Alan L. Landay, Gregory T. Spear 4. (2016) .** Glycoge Levels in Undiluted Genital Fluid and Their Relationship to Vaginal pH. Estrogen and Progesterone., *PLoS ONE*, Vol. 11, p. e0153553.
- Mironsef, Paria, Anna L. Hooton, Douglas Gilbert, Derick Burgad, Alan Landay, Kathleen M. Weber, Mardge Cohen, Jacques Ravel, Gregory T Spear , (2014).** Free Glycogen in Vaginal Fluids is Associated with Lactobacillus Colonization and Low Vaginal pH., *PLoS ONE*, Vol. 9, p. e102467.
- Moghissi KS. (1976).** Postcoital test: physiologic basis, technique, and interpretation. *Fertil Steril*, 27(2):117-129.

References

- Mohanty, S., Xess, I., Hasan, F., Kapil, A., Mittal, S., & Tolosa, J. E. (2007).** Prevalence & susceptibility to fluconazole of *Candida* species causing vulvovaginitis. *The Indian journal of medical research*, 126(3), 216-219.
- Molero L, Garcia-Duran M, Diaz-Recasens J, Rico L, Casado S, Lopez-Farre A. (2002).** Expression of estrogen receptor subtypes and neuronal nitric oxide synthase in neutrophils from women and men: regulation by estrogen. *Cardiovasc Res*; 56(1): 43-51.
- Monika Bigos, Maria Prosniewicz, Agnieszka Seraficka, Malgorzata Wasiela , (2014).** Vagina is not a significant reservoir of methicillin-resistant *S. aureus* in pregnancy, *Archives of Perinatal Medicine* 20(2), 108-112.
- Morris, M. Nicoll, A., Simms, I., and Wilson, J. (2001).** Bacterial vaginosis: A public health review. *BJOG*, 108:439-50 .
- Mumtaz S, Akhtar N, Hayat A. (2002).** Antibigram of aerobic pyogenic isolates from wounds and abscesses of patients at Rawalpindi. *Pakistan J Med Res*.41(1):16–18.
- U. Nawrat, K. Grzybek-Hryncewicz, A.(2000).** KarpiewskaSusceptibility of *Candida* species to antimycotics determined by microdilution method *Mikol Lek*, 7. pp. 19-26
- Ness RB, Hillier SL, Richter HE, Soper DE, Stamm C, McGregor J, et al . (2002).** Douching in relation to bacterial vaginosis, *lactobacilli*, and facultative bacteria in the vagina. *Obstet Gynecol.*, 100: 765-10.
- Nohmi T, Abe S, Dobashi K, Tansho S, Yamaguchi H. (1995).** Suppression of anti-*Candida* activity of murine neutrophils by progesterone in vitro: a possible

References

mechanism in pregnant women's vulnerability to vaginal candidiasis. *Microbiol Immunol*.39:405–409.

Odds F C. (1988). *Candida and candidosis*. Baltimore, Md: University Park Press. Chronic mucocutaneous candidosis; pp. 104–110.

Okiki Pius A., Amiegheme Nosazena E. and Oyinloye Josiah M. A.(2015). Evaluation of microorganisms associated with vaginal infections, Nigeria, 6(9): 79-83.

Ovcinnikov, N. M., Delektorskij, V. V., Turanova, E. N., & Yashkova, G. N. (1975). Further studies of *Trichomonas Vaginalis* with transmission and scanning electron microscopy. *The British journal of venereal diseases*, 51(6), 357-375.

Osborne, N. G., R. C. Wright, and L. Grubin. (1979). Genital bacteriology: a comparative study of premenopausal women with postmenopausal women. *Am. J. Obstet. Gynecol.* 135: 195-198.

Owen DH, Katz DF. (1999). A vaginal fluid simulant. *Contraception*, 59(2):91-95.

Paavonen, J., Mangioni, C., Martin, M. A., & Wajszczuk, C. P. (2000). Vaginal clindamycin and oral metronidazole for bacterial vaginosis: a randomized trial. *Obstetrics and gynecology*, 96(2), 256-260.

Paavonen J. (1983). Physiology and ecology of the vagina. *Scand J Infect Dis Suppl*, 40:31-35.

References

- Panagoda, G.J.; Ellepola, A.V. & Samaronyake, L.P.(2001).** Adhesion of *Candida* parapsilosis to Epithelial & a crylic surface correlates with surface hydrophobicity-Mycoses. 44(1-2):29-35.
- Pappas, P. G., Rex, J. H., Sobel, J. D., Filler, S. G., Dismukes, W. E., Walsh, T. J., and Edwards, J. E. (2004).** Guidelines for treatment of candidiasis. Clinical Infectious Diseases, 38(2), 161-189.
- Parisa B , Abdolvahab A, Mohammad A , Shakiba E. (2010).** Distributions and Antifungal Susceptibility of *Candida* Species from Mucosal.Sites in HIV Positive Patients Archives of Iranian Medicine, Volume 13, Number4, July.
- Petersen EE.(2006).** Infections in obstetrics and gynecology : textbook and atlas. Stuttgart ; New York: Thieme.
- Phan TL, Elias C, Nguyen TL, Bui TC, Nguyen HP, Gardner M. (2002).** The prevalence of reproductive tract infections in Hue, Vietnam. Stud Fam Plann. 2002;33:217–26. doi: 10.1111/j.1728-4465.00217.x.
- Phiel KL, Henderson RA, Adelman SJ, Elloso MM. (2005).** Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations. Immunol Lett; 97(1): 1
- Philippon A, Labia R, Jacoby G. (1989).** Extended-spectrum beta-lactamases. Antimicrob Agents Chemother,33:1131-1136.
- Plitt SS, Garfein RS, Gaydos CA, Strathdee SA, Sherman SG, Taha TE. (2005).** Prevalence and correlates of *chlamydia trachomatis*, *neisseria gonorrhoeae*,

References

- trichomonas vaginalis* infections, and bacterial vaginosis among a cohort of young injection drug users in Baltimore, Maryland. Sex Transm Dis.32: 446-53. 21291.59.
- Polgar B, Barakonyi A, Xynos I, Szekeres-Bartho J. (1999).** The role of gamma/delta T cell receptor positive cells in pregnancy. Am J Reprod Immunol41(4): 239-44.
- Prescott, L.M., Harley, J.P. and Klein, D.A.(1990).** “Microbiology”. 3rd. ed. McGraw–Hill Comp. Inc., London .
- Pudney J, Quayle AJ, Anderson DJ. (2005).**Immunological microenvironments in the human vagina and cervix: mediators of cellular immunity are concentrated in the cervical transformation zone. *Biol Reprod*,73(6):1253-1263
- Puri R, Malhotra J. (2009).**Recurrent urinary tract infection (UTI) in women. South Asian Federation of Obstetrics and Gynecology. Jan-Apr; 1(1):1.
- Rampersaud, Ryan, Randis, Tara M and Ratner, Adam J. (2012).**Microbiota of the Upper and Lower Genital Tract., Seminars in Fetal and Neonatal Medicine, Vol. 17, pp. 51-57.
- Ramzan, M.; Bakhsh, S.; Salam,A.; Khan, G.M. and Mustafa, G.(2004)** Risk factors in urinary tract Infection, Department of pathology women Medical collage, Abbottabad .
- Redondo-Lopez V, Cook RL, Sobel JD.**Emerging role of *lactobacilli* in the control and maintenance of the vaginal bacterial microflora. Rev Infect Dis 1990;12:856–72.
- Reid G, Bruce AW, McGroarty JA, Cheng KJ, Costerton JW.**Is there a role for lactobacilli in prevention of urogenital and intestinal infections? Clin Microbiol Rev 1990; 3:335–44.

References

- Robert, G.D. (1990).** Laboratory method for basic Mycology .In Baily & Scotts: Diagnostic Microbiology (Ed. E.J.Barron & S.M.Finegold).The C.V.Mosby Co.
- Rocchetti TT, Marconi C, Rall VLM, Borges VTM, Corrente JE, Da Silva MG. (2011).** Group B streptococci colonization in pregnant women: risk factors and evaluation of the vaginal flora. Arch Gynecol Obstet.283:717–721.
- Roizman B, Whitley RJ . (2001).** The nine ages of herpes simplex virus . Herpes .8:23-7.
- Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Nikita L, Galuppi M, Lamont RF, Chaemsaitong P, Miranda J, Chaiworapongsa T, Ravel J (2014).** The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. Microbiome. Feb 3; 2(1):4.
- Ryan KJ, Ray CG (editors) (2004).** Sherris Medical Microbiology .4th;555-62 .MCGraw Hills.
- Ryan, R. M. (1982).** Control and information in the intrapersonal sphere: An extension of cognitive evaluation theory. Journal of Personality and Social Psychology, 43, 450–461.
- Sautter, R. L., and W. J. Brown. (1980).** Sequential vaginal cultures from normal young women. J. Clin. Microbiol. 11: 479-484
- Sawyer, P. R., Brogden, R. N., Pinder, R. M., Speight, T. M., & Avery, G. S. (1976).** Tinidazole: a review of its antiprotozoal activity and therapeutic efficacy. *Drugs*, 11(6), 423-440.

References

- Schmid, G., Narcisi, E., Mosure, D., Secor, W. E., Higgins, J., & Moreno, H. (2001).** Prevalence of metronidazole-resistant *Trichomonas vaginalis* in a gynecology clinic. *The Journal of reproductive medicine*, 46(6), 545-549.
- Schmidt, H. and Hansen, J.G. (2000).**Diagnosis of bacterial vaginosis by wet mount identification of bacterial morphotypes in vaginal fluid. *Int. J. STD. AIDS.*, 11(3): pp.150-155.
- Schust DJ, Anderson DJ, Hill JA. (1996).** Progesterone-induced immunosuppression is not mediated through the progesterone receptor. *Hum Reprod*, 11(5): 980-5.
- Schwebke JR ,Burgess D. (2004).** Trichomoniasis .*clin Microbiol Rev.* 17(4):794-803.
- Seifer DB, Samuels P, Kniss DA. (2001).**The physiologic basis of gynecology and obstetrics.Philadelphia: Lippincott Williams & Wilkins.
- Sharief M. (1998).** Genital infections among women using various contraceptive methods in Basra, Iraq. *Eastern MediterrHealth*,4:487–92.
- Shamim Mumtaz , Mumtaz Ahmad ,Irum Aftab , Naeem Akhtar , Masood ul Hassan (2008).**AEROBIC VAGINAL PATHOGENS AND THEIR SENSITIVITY PATTERN *J Ayub Med Coll Abbottabad*.20(1).
- Sheehan, D. J. ; Hitchcock, C. A. & Sibely, C. M.(1999).** Current and emerging azole antifungal agent. *Clin. Microbiol.* 12 : 40 – 79
- Sobel JD, Chaim W. (1996).**Vaginal microbiology of women with acute recurrent vulvovaginal candidiasis. *J Clin Microbiol*, 34(10):2497-2499.

References

- Sobel, J., Peipert, J. F., McGregor, J. A., Livengood, C., Martin, M., Robbins, J., & Wajszczuk, C. P. (2001).** Efficacy of clindamycin vaginal ovule (3-day treatment) vs. clindamycin vaginal cream (7-day treatment) in bacterial vaginosis. *Infectious diseases in obstetrics and gynecology*, 9(1), 9-15.
- Sobel, J. D. (2007).** Vulvovaginal candidosis. *Lancet*, 369(9577), 1961-1971. doi: 10.1016/S0140-6736(07)60917-9.
- Sobel JD , Faro S, Force RW, Foxman B, Ledger WJ, Nyirjesy PR, Reed BD, Summers PR. (1998).** Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. *Am J Obstet Gynecol*.Feb;178(2):203-11.
- Srinivasan, Sujatha, Martin T. Morgan, Tina L. Fiedler, Danijel Djukovic, Noah G. Hoffman, Daniel Raftery, Jeanne M. Marrazzo, David N. Fredricks. 2, (2015).** Metabolic Signature of Bacterial Vaginosis., *mBio*, Vol. 6, pp. 204-215.
- Smart S. (2004).** Social and sexual risk factors for bacterial vaginosis. *Sex Transm Infect*;80:58–62.
- Spear, Greg T, Mary McKenna, Alan L. Landay, Hadijat Makinde, Bruce Hamaker, Audrey L. French .(2015).** Effect of pH on Cleavage of Glycogen by Vaginal Enzymes. 7, *PLoS ONE*, Vol. 10, p. e0132646.
- Spiegel, C. A., R. Amsel, D. E. Eschenbach, F. Schoenknecht, and K. K. Holmes. (1980).** Anaerobic bacteria in nonspecific vaginitis. *N. Engl. J. Med.* 303:601-607.
- Spinillo , A; Pizzol , G.; Colonna , L.; Nicola , S.; De-seta , F and Guaschino , S . (1993).** Epidemiologic characteristic of women with idiopathic recurrent vulvovaginal candidiasis *obstes Gynecol.*,81 (5);721-727.

References

- Stamm, W.E., Martin, S.M. and Bennett, J.V.(1989)** . Epidemiology of noscomial infection due to gram-negative bacilli : aspects relevant to development and use of vaccines. J. of Infect. Dis., 136: pp.151-159
- Stevens A, Lowe JS.(2005).** Human histology. 3rd edition. Philadelphia ;Elsevier/Mosby .
- Stimson WH. (1988)** .Oestrogen and human T lymphocytes: presence of specific receptors in the T-suppressor/cytotoxic subset. Scand J Immunol. 28(3): 345-50.
- Sugarman, B. and Mummaw, N. (1988).**The effect of hormones on *Trichomonas vaginalis*. J Gen Microbiol.,134: 1623–8 .
- Sutton, M., Sternberg, M., Koumans, E. H., McQuillan, G., Berman, S., & Markowitz, L. (2007).** The prevalence of *Trichomonas vaginalis* infection among reproductive-age women in the United States, 2001–2004. Clinical Infectious Diseases, 45(10), 1319-1326.
- Suenaga R, Evans MJ, Mitamura K, Rider V, Abdou NI. (1998).**Peripheral blood T cells and monocytes and B cell lines derived from patients with lupus express estrogen receptor transcripts similar to those of normal cells. J Rheumatol.25(7): 1305-12.
- Syed TS, Braverman PK. (2004).** Vaginitis in adolescents. Adolesc Med Clin.;15(2):235–51.
- Szekeres-Bartho J, Philibert D, Chaouat G. (1990).** Progesterone suppression of pregnancy lymphocytes is not mediated by glucocorticoid effect. Am J Reprod Immunol. 23(2): 42-3.

References

- Szekeres-Bartho J. (1995).** Progesterone receptors on lymphocytes. Hum Reprod.10(3): 695-6.
- Szekeres-Bartho J. (2002).** Immunological relationship between the mother and the fetus. Int Rev Immunol.21(6): 471-95.
- Tashjian, J. H., C. B. Coulam, and J. A. Washington II. (1976).** Vaginal flora in asymptomatic women. Mayo Clin. Proc. 51: 557-561.
- Tavo V. (2013).** Prevalence of Mycoplasma hominis and Ureaplasma urealyticum among women of reproductive age in Albania. Med Arch.67:25.
- Thomason J L, Gelbart S M, Anderson R J, Walt A K, Osypowski P J, Broekhuizen F F. (1990).** Statistical evaluation of diagnostic criteria for bacterial vaginosis. Am J Obstet Gynecol 162:155-160
- Thongngarm T, Jenkins JK, Ndebele K, McMurray RW. (2003).** Estrogen and progesterone modulate monocyte cell cycle progression and apoptosis. Am J Reprod Immunol; 49(3): 129-38
- Totten PA, Amsel R, Hale J, Piot P, Holmes KK. (1982).** Selective differential human blood bilayer media for isolation of *Gardnerella (Haemophilus) vaginalis* . J clin Microbiol ,15:141-147.
- Vanita DJ, Michal AE. (2012).** Preterm Labor. In: NMS Obstetrics and Gynecology. Samantha M, Joanna A, Dacarla MA, Lily A, Janice B, Thomas J et al. 7th edition,15, Lippincott Williams & Wilkins, Philadelphia,159167

References

- Vandepitte , J ; Engback , K ; Piot , Pand ,Heuch , C .C.(1991).** Basics laboratory procedure in clinical Bacteriology . world health organization , Geneva ., PP.37-42 .
- Vazques , J.A. and Sobel , J.D.(2002) .** Mucosal candidiasis . infect . Dis . Clin .N. Am ., 16:793-820.
- Vazques,J.A. and Sobel ,J.D.(1995).** Fungal Infection in diabetes. Infection disease clinics of North America., 9(1):97-116.
- .Veenstra van Nieuwenhoven AL, Bouman A, Moes H, Heineman MJ, de Leij LF, Santema J, Faas MM. (2002).** Cytokine production in natural killer cells and lymphocytes in pregnant women compared with women in the follicular phase of the ovarian cycle. Fertil Steril,77(5): 1032-7.
- Werner F, Kojonazarov B, Gaßner B, Abeßer M , Schuh K , Völker K , Baba HA , Dahal BK , Schermuly RT, Kuhn M (2016).** Endothelial actions of atrial natriuretic peptide prevent pulmonary hypertension in mice : Mar;111(2):22. doi: 10.1007/s00395-016-0541-x. Feb 24.
- Wiggins R, Hicks SJ, Soothill PW, Millar MR, Corfield AP.(2001).**Mucinases and sialidases:their role in the pathogenesis of sexually transmitted infections in the female genital tract. *Sex Transm Infect*, 77(6):402-408.
- Willmott , F, E . (1975) .** Genital yeasts in female patients attending a venereal disease clinic , Br . J. ven .Dis ., 51(119) : 119 -122.
- Wilkinson HW (1977).** "CAMP-disk test for presumptive identification of group B *Streptococci*". J. Clin. Microbiol. **6** (1): 42–5.

References

- Winn, C.W., W.E. Koneman, S.D. Allen, W.M. Janda, G.W. Procop, P.C. Schreckenberger and G.A. Woods, (2006).** Mycoplasmas and Urea plasmas. *Konemans color Atlas and Textbook of diagnostic microbiology 6th Edn.*, pp: 1023-1054.
- Wira CR, Grant-Tschudy KS, Crane-Godreau MA. (2005).** Epithelial cells in the female reproductive tract: a central role as sentinels of immune protection. *Am J Reprod Immunol*, 53(2):65-76
- Wira C R, Kaushic C. (1996).** Mucosal immunity in the female reproductive tract: effect of sex hormones on immune recognition and responses. In: Kiyono H, Ogra P L, McGhee J R, editors. *Mucosal vaccines: new trends in immunization*. New York, N.Y: Academic Press. pp. 375–386.
- Witkin, S.S., Linhares, I.M. & Giraldo, P. (2007).** Bacterial flora of the female genital tract: function and immune regulation. *Best. Pract. Res. Clin. Obstet. Gynaecol.* 21, 347–354 .
- . Witkin, Steven S, Linhares, Lara Moreno and Giraldo, Paulo. 3, (2007).** Bacterial Flora of the Femal Genital Tract: Function and Immune Regulation, *Best Practice & Research Clinical Obstetrics Gynaecology*, Vol. 21, pp. 347-354.
- World Health Organization (WHO), (1994).** Global program on AIDS management of sexually transmitted disease. Geneva: world health organization .
- World health organization WHO.(2007) .** Doxycycline hyclate : final text to replace published monograph in the international pharma copeia.
- WHO, W. H. O. (2005).** Sexually transmitted and other reproductive tract infections: a guide to essential practice

References

- Wyker, A. and Gillenwater, J. (1995).** Method of Urology. 1st ed., The Williams and Wilkins company, Baltimore., pp.199-201.
- Yang, YH, Ngo, D, Jones, M, Simpson, E, Fritzemeier, KH and Morand, EF (2010).** "Endogenous estrogen regulation of inflammatory arthritis and cytokine expression in male mice, predominantly via estrogen receptor alpha." *Arthritis Rheum* 62(4): 1017-1025.
- Young, G., & Jewell, D. (2001).** Topical treatment for vaginal candidiasis (thrush) in pregnancy. *Cochrane Database Systematic Review*, 4.
- Zahra Salehei , Zahra Seifi, Ali Zarei , Mahmoudabadi A. (2012).** Sensitivity of Vaginal Isolates of Candida to Eight Antifungal Drugs Isolated From Ahvaz, Iran. *Jundishapur J Microbiology*.5(4):574-577.
- Zamanzad,B. & Moezzi, M.(2006)** prevalence of Asymptomatic Bacteruria and Associated Host Factors in women with Diabetes type 2 shahre-Kord University of Medical Science, Iran. *J Res Health Sci*, Vol 6, No 1 pp. 14- 20.
- Zinnemann K, Turner GC. (1963).** The taxonomic position of “ *Haemophilus vaginalis*” (*Corynebacterium vaginal*) . *J pathol Bacteriol*. 85 :213-9 .
- Zunino, P. ; Piccini, C. and Legnanifajard, C. (1999).** Growth, Cellular differentiation and virulence factor expression by *proteus mirabilis* in vitro and vivo. *J. Med .Microbiol*.48:34-527.
- Zur Hausen H, Meinhof W. (1974).** to detect virus –specific DNA in human tumors. I. Nucleic acid hybridizations with complementary RNA of human Wart virus . *Int J cancer*.13(5):650-6.

اجريت الدراسة الحاليه في محافظة ذي قار لتحديد الانواع الرئيسية للكائنات المجهرية التي تسبب اصابة الجهاز التناسلي للأناث ومدى تأثير مستوى الهرمونات الجنسية (البرولاكتين ، البروجسترون ، الاستروجين) على وجود عدوى ، كما دُرست بعض العوامل التي قد يكون لها علاقة بوجود عدوى مثل العمر،مرض السكري،تناول حبوب منع الحمل،وجود حالات اجهاض سابقاً.

تم جمع 245 عينة لكلا المسحات المهبلية والدم (حيث أخذت مسحه مهبلية وعينة دم من كل امرأة) وشملت العينات 150 امرأة حامل و95 امرأة غير حامل تتراوح اعمارهم بين 16-45 سنة، تم جمع مسحات المهبل وعينات الدم من النساء المراجعات للعيادة الاستشارية في مستشفى بنت الهدى التعليمي للولادة والاطفال والعيادة الاستشارية في مستشفى الحبوبى التعليمي لمدينة الناصرية للفترة من بداية اكتوبر 2016 وحتى نهاية فبراير 2017 وعدت استمارة استبيان للحصول على معلومات هؤلاء النساء .

ووجدت الدراسة فروق معنوية ($P < 0.05$) في انتشار العدوى بين النساء الحوامل وغير الحوامل حيث كان انتشار التهاب الجهاز التناسلي الخارجي أعلى لدى النساء الحوامل عنه في النساء غير الحوامل ، كانت 150 حاله ايجابيه للزرع في النساء الحوامل مقارنة مع 95 حاله في النساء الغير حوامل، اخضعت العزلات للأختبارات البايوكيميائيه المورفولوجيه حيث وجد ان

[*Candida albicans*] كانت معزوله بنسبة (30.6%) تليها [*Streptococcus agalactiae*] كانت نسبتها [*Staphylococcus aureus*] (14.3%) ونسبتها (11%)، [*Escherichia coli*] وعزلت بنسبة (10.2%)، [*S.epidermidis*] (8.6%)، [*G. vaginalis*] وعزلت بنسبة كانت (7.8%)، [*Proteus mirabilis*] وهي بنسبة (4.5%)، [*Klebsiella pneumoniae*] (6.1%)، [*Pseudomonas aeruginosa*] وكانت بنسبة (3.7%)، واقل نسبة كانت ل [*Enterobacter cloacae* , *Trichomonas vaginalis*] (2.4%,0.8%)

تم جمع 245 عينة دم من النساء لقياس مستوى الهرمونات الجنسيه ،كانت من بين هؤلاء النساء 150 امرأة حامل و 58 امرأة في المرحلة اللوتيه و 20 امرأة في مرحلة الجرييه و 17 امرأة في مرحلة الإباضة من الدورة الشهرية. أظهرت الدراسة ان *Staphylococcus aureus* , *Klebsiella pneumonia* , *Staphylococcus epidermids* كانت *Enterobacter cloacae* , *Candida albicans* , *Gardnerella vaginalis* ، *Escherichia coli* عزلت من النساء في مرحلة الجرييات، . كان تنوع العدوى أقل في النساء في مرحلة الإباضة، حيث تم عزل *Candida albicans* ، *Gardnerella* ، *Staphylococcus aureus* *Escherichia coli* ، *vaginalis* فقط ضمن هذه المرحلة. *Klebsiella* , *Gardnerella vaginalis* , *Staphylococcus aureus* , *Staphylococcus epidermids* , *Proteus mirabilis* , *Pseudomonas aeruginosa pneumonia* ، *Escherichia coli* ، *Candida albicans* عزلت من النساء في المرحلة اللوتيه من دوره الشهرية. ورافقت جميع

الإصابات قيد الدراسة زيادة بمستوى هرمون البروجسترون و هرمون الاستروجين باستثناء بكتيريا *Enterobacter* ,
 Pseudomonas aeruginosa , *Proteus mirabilis* ، *Staphylococcus epidermids*، *cloaceae* التي
 صاحب وجودها مستويات طبيعيه لكلا الهرمونين ،بينما لم يكن لهرمون البرولاكتين تأثير واضح حيث تراوحت
 مستوياته بين الطبيعية والعالية لجميع النساء.وقد رافق الحمل مستويات عالية من هرمون الاستروجين والبروجسترون
 و البرولاكتين وتوافق هذا مع وجود اصابه, *Escherichia coli* , *Staphylococcus aureus* ،
 Gardnerella vaginalis, *Klebsiella pneumonia*، *Candida albicans*، *Trichomonas vaginalis*
 .*Streptococcus agalactiae*



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وزارة التعليم العالي والبحث العلمي

جامعة ذي قار

كلية العلوم

قسم علوم الحياة

عدوى المسالك التناسلية الخارجية بين النساء مرتبطة مع بعض الهرمونات في محافظة ذي قار-العراق

رسالة مقدمة الى

مجلس كلية العلوم في جامعة ذي قار

وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة/ الاحياء المجهرية

من قبل الطالبة

صفا عبد الوهاب وجيه اليعقوبي

بكالوريوس علوم \ علوم الحياة \ جامعة ذي قار

2015-2014

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