Prevalence of *Staphylococcus* spp. and *Candida* spp. in pregnant and non pregnant women in genital tract with detection of *nor*A and *sdr*M genes

A Thesis

Submitted to College of Science - University of Diyala in Partial Fulfillment of the Requirements for the Degree of Master in Biology.

By

Mustafa Salam Abdulkhaleq Noaman
B.Sc. Biology/Diyala university
(2017)

Supervised by

Assist. prof.Dr. Muthana A. Al-Mahdawi

2020 A.D   1442 A.H.

Chapter 2.   Literature Review

2.1 Vaginal infections

Worldwide, total disability-adjusted life years (DALYs) due to gynecologic diseases increased from 9292.8 thousands in 2005, to 10255.1 thousands in 2015 (kassebaum *et al.,* 2016). Vaginal infections with bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis are consider a global health problem for women (Go *et al.,* 2006). Vaginitis can be defined as an inflammation and infection of vagina commonly encountered in clinical medicine (Yenidunya *et al.,* 2012). Diverse spectrums of pathogenic microorganisms were observed in the vaginal microflora. Bacterial vaginosis, candidiasis and trichomoniasis are responsible for majority of vaginal infections in women during the reproductive age (Spinillo *et al.,* 1997; Yenidunya *et al.,* 2012).

Abnormal vaginal discharge, itching, burning sensation, irritation and felling discomfortable are frequent complaints among patients attending obstetrics and gynecology clinics. However, a number of vaginal infections present with few or no symptoms (Adeyeba *et al.,* 2003). Vulvovaginal candidiasis (VVC) consider one of the most frequent infections in women of reproductive age. Approximately 75 % of adult women have at least one episode of vaginitis by candida during their life time (Adeyeba *et al.,* 2003; Yenidunya *et al.,* 2012). Unfortunately, about 40 – 50 % of women who had a first episode is likely to present a recurrence and 5% may present a form of "recurring" characterized by at least three or more episodes of infection per year (Prospero, 2014).

Trichomonal vaginitis (TV) is the most common sexually transmitted disease (Prospero, 2014). It is caused by a parasitic protozoan *Trichomonas vaginalis* (Yenidunya *et al.,* 2012). Globally, TV affects approximately 57–

180 million people, with the majority living in the developing countries (Chalechale and Karimi, 2010). However, in the most cases TV is asymptomatic. In women, TV affects more frequently between 20 - 40 years old and is quite rare before puberty and postmenopausal age (Prospero, 2014). The symptoms of TV are mainly characterized by vaginal discharge with gray or greenish-yellow fluid rather frothy, foul-smelling, intense itching, edema cervix redness, the sensation of itching, dyspareunia and postcoital bleeding, pelvic pain and urinary symptoms (Yenidunya *et al.,* 2012 ; Prospero, 2014).

Bacterial vaginosis (BV) is the most common cause of abnormal vaginal discharge among women of reproductive age. The prevalence of BV is about 30 % in women of reproductive age (Yenidunya *et al.,* 2012; Prospero, 2014). BV is characterized by raised vaginal pH and milky discharge in which normal vaginal flora (Lactobacilli) is replaced by a mixed flora of aerobic, anaerobic and microaerophilic species. Anaerobic organisms like *Gardnerella vaginalis, Prevotella spp*., *Mycoplasma hominis, Mobiluncus spp.* colonize vagina predominantly in BV (Prospero, 2014; Lamichhane *et al.,* 2014).

Gonococcal infections are the second most common prevalent sexually transmitted bacterial infections causing substantial morbidity worldwide each year. Gonorrhea is a potent amplifier of the spread of sexually transmitted human immuno deficiency virus (HIV) (Hng *et al.,* 2009). Various studies across the world have shown that women with BV are more likely to be co-infected with, *T. vaginalis*, *N.gonorrhoeae* and HIV (Lamichhane *et al.,* 2014). Aerobic vaginitis has been identified for a smaller proportion of women whose microbiota (lactobacilli) is dominated by facultative anaerobic or aerobic bacteria especially *S.aureus*, group B streptococci, *E.coli* and *Klebsiella spp* (Lamichhane *et al.,* 2014; Prospero, 2014).

Vaginal infections are associated with a significant risk of morbidity in women. If untreated they can lead to pelvic inflammatory disease (PID), which can cause long-term sequelae, such as tubal infertility, ectopic pregnancy, reproductive dysfunction and adverse pregnancy outcomes (e.g., preterm labor and delivery and low birth weight). Cervical dysplasia, increased risk of postoperative infection, HIV and Herpes simplex virus (HSV) -1 acquisition and transmission are also resulted from vaginal infections(Castellano *et al.,* 2010 ; Yenidunya *et al.,* 2012 ; Eshete *et al.,* 2013). Moreover, BV propagates viral replication and vaginal shedding of the HIV and HSV-2 (Trabert and Mirse, 2007). Investigators have also reported epidemiologic associations between trichomonas infection and subsequent cervical neoplasia and carcinoma (Saleh *et al.,* 2014). Various etiologies of vaginal infection results in a number of gynecologic complications and amplify HIV and HSV-1 transmissions. (Trabert and Mirse, 2007)

2.2 Vaginal normal flora

The vaginal flora was first reported by Albert Döderlein, as early as (1892). Döderlein found that the microflora was homogenously colonized with Gram-positive rods, which were designated the name "Döderlein's bacilli". Later, these bacilli have been identified as *Lactobacillus spp.* Lactobacilli which consider the predominant micro-organisms of the vaginal microbiota and play a major role in the maintenance of a healthy urogenital tract by preventing the colonization of pathogenic microorganisms. In healthy women, the vaginal microflora is dominated by Lactobacillus species, at a level of 10^7 -10^8 CFU g^{-1} of fluid, which exert a significant influence on the microflora of the environment (Boris and Barbes, 2000).

Lactobacilli play an important role in maintaining the female genital tract by some mechanisms, such as producing lactic acid that decreases vaginal pH

and prevents the occurrence of pathogenic bacteria, hydrogen peroxide, bacteriocin and surface binding protein which in turn can reduce the risk of bacterial vaginosis. When lactobacilli decrease, there is a reduction of hydrogen peroxide and consequently, overgrowth of facultative anaerobes occurs and bacterial vaginosis happens as facultative anaerobes can displace lactobacilli then rise in vaginal PH (Stojanović *et al.,* 2012). Furthermore, changes in innate immunity are responsible for the conversion of normal vaginal flora such as lactobacilli to facultative anaerobes that cause bacterial vaginosis (Redelinghuys *et al.,* 2016).

In consequence, a depletion of vaginal lactobacilli has been directly associated with an increase in the incidence of the genital and urinary tract infections (Pavlova *et al.,* 2002). The Lactobacili have been shown to produce biosurfactants and collagen binding proteins that function in inhibiting the pathogens adhesion and displace the pathogensm (De Gregorio *et al.,* 2020).

2.3 Anatomy of vagina

The vagina is a fibromuscular virtual tube, designed as the female copulatory organ. It extends from the vestibule, between the labia minora, and the uterus, the anterior urethra and bladder, and the posterior anal canal and rectum (Larsson *et al.,* 1977). Depending on rectum and bladder content, the vagina describes a (90°) angle with respect to the uterus axis, ascending posterosuperiorly. Considered as a parallelepiped, the vagina is composed of a posterior wall, separated from the rectum by the rectouterine pouch and from the anal canal by the fibromuscular structure of the perineal body. Microscopic examination reveals that the vaginal structure comprises [1] the inner mucosal epithelial stratum, [2] a lamina propria containing thin-walled veins, [3] the intermediate muscularis stratum, and [4] the external adventitial layer, the mucosal epithelium is hormone-dependent (estrogen) and changes

during the menstrual cycle, having the potential for a basal, nonsexual, moisture, Glycogen is fermented by Döderlein's bacillus, lowering the vaginal pH. The lamina propria of the mucosa contains blood vessels contributing to the diffusion of the vaginal fluid across the epithelium, elastic fibers, lymphatic vessels, and nerves (Jannini *et al.,* 2006).

2.4 Amsel's clinical criteria

In clinical practice, the most commonly used criteria are the clinical criteria set by Amsel. The diagnosis is positive for BV if it meets at least three of the four criteria. These criteria are: 1) presence of a typical discharge; 2) pH > 4.5, 3) positive whiff test; and 4) presence of clue cells in the wet smear;(Amsel *et al.*, 1983)

These criteria are:

1. A typical discharge, with BV, is thin. A normal discharge is floccular.

2. The pH is measured using paper the pH indicator. The sensitivity of pH-measurement for BV diagnosis is high but it has low specificity.

The study by Hallén and Co-workers found a 98.8 % sensitivity and a 71 % specificity

(Hallen *et al.,* 1987). However, in the study by Amsel, only 81% of the women with BV had a pH >4.5(Amsel *et al.*, 1983). In a study by Eschenbach and Co-workers, 97% of the women with BV had an elevated pH(Eschenbach *et al.*, 1988).

3. An increase in pH quickly releases amines, such as trimetylamine, which dissolve as an acid when the pH is low in the discharge.

A trimetylamine sniff test / whiff test is performed to detect odor by adding a drop of 10-20 % potassium hydroxide (KOH) to the speculum discharge, or by placing a drop of discharge on a slide and microscope and adding a drop of 10-20 % potassium hydroxide mixture and then smelling,

TMA is responsible for the smell of spoiled fish and can be detected in women with BV in vaginal specimens. Smelling directly from the speculum is known as a whiff test, a method proposed by Gardner and Duke in 1955(Gardner and Dukes, 1955). Amsel *et al*. introduced the addition of potassium hydroxide to the sniff test in 1983 (Amsel *et al.,* 1983).

Reported sniff test studies indicate a very high sensitivity and specificity. Hallén and Co-workers study's showed a 95 percent sensitivity and a 100 percent specificity (Hallén *et al.,* 1987). In a study by Thomason and Co-workers, the sensitivity was 91.1% and the specificity was 61.2%(Thomason *et al.*, 1990).

4. The fourth criterion are the wet-mount clue cells. A small proportion of the vaginal discharge is mounted on a microscope slide and one drop of saline is applied. The specimen is then covered with a glass cover and examined under a microscope. When the patient has BV, a significant number of *Gardnerella* morphotype bacteria cover some of the epithelial cells. Clue cells are vagina epithelial cells whose boundaries are difficult to see because there are so many bacteria found on the cell surface. The clue cell was one of the clinical criteria Gardner and Duke introduced. These cells provided the clue to the diagnosis (Gardner and Dukes, 1955).

Amsel's clinical criteria are one of the gold standards for diagnosing BV (Eschenbach *et al.,* 1988). In treatment studies, it is common to say that the patient is "cured" if none of the four Amsel's clinical criteria are present, and "improved" if the patient has one or two of the Amsel's criteria (a typical discharge and a pH> 4.5). If the patient meets the third and the fourth criteria (a positive whiff test and the presence of clue cells), treatment is considered to have failed(Larsson and Forsum, 2005).

2.5 Bacterial vaginitis

Bacterial vaginosis is a syndrome that caused by a certain group of bacteria that change in the vaginal environment as a result of the replacement of the natural vaginal flora caused by the absence of lactobacilli producing hydrogen peroxide with the growth of mixed microorganisms such as aerobic, anaerobic, and vaginal permanent bacteria as anaerobic bacteria make up 1% of the vaginal flora in natural women (Bodean *et al.,* 2013). There is also an increase in the pH of the vaginal environment, as it reaches more than 4.5 and repeated colic occurs in the vagina due to repeated sexual intercourse and the use of vaginal washings and smoking, which causes a reduction in the normal flora of the vagina, It is replaced by an overgrowth of the aerobic and facultative anaerobic bacteria (Ronald *et al.,* 2011).

The most important symptoms of vaginitis in affected women is the feeling of discomfort, itching, irritation and redness, and intense vaginal discharge with an unpleasant odor that resembles fish smell and increases after sexual contact (Hantoushzadeh *et al.,* 2010). It is one of the most common problems that accompany women during the childbearing period (Allsworth and Peipert, 2011), the use of contraceptives and hormone therapy are factors that increase the severity of bacterial vaginosis (Curran, 2010), and that candidiasis, urinary tract infection, frequent sexual intercourse and multiple partners increase the chances of bacterial vaginosis (Verstraelen *et al.,* 2012), vaginal discharge is one of the most common symptoms in cases of vaginal infections (Sobel *et al.,* 2012). The secretions may be caused by physiological factors, as they are usually secreted from the bartholin gland, the endometrium, the cervix, or the fluid in the fallopian tubes, along with the cells of the vaginal walls, including those caused by inflammatory diseases or some inflammatory disease. These secretions are affected by hormonal

changes during the menstrual cycle of adult women (Van Schalkwyk and Yudin, 2015).

The change in the physical and chemical properties of these secretions leads to an impact on the types of organisms present in the vagina and their abundance. Vaginal secretions are anti-bacterial for many bacteria, depending on the components of these secretions (Hickey *et al.,* 2011)

2.5.1 Vaginitis caused by Gram positive bacteria

The quantitative and qualitative bacteriological studies are performed on the normal bacterial flora of the vaginal and cervical sites, lactobacilli, diphtheroids, staphylococci, streptococci, and occasionally members of enterobacteriaceae were the dominant aerobic group of bacteria. (Levison *et al.,* 1977).The dominant anaerobic groups are composed of Gram positive bacilli which include lactobacilli, peptostreptococci (which now incorporate peptococci), and Bacteroidaceae. Group B streptococci (GBS) constitute a potentially important subgroup within the streptococci (Ledger *et al.,* 1975).

2.5.1.1 *Staphylococcus spp.*

Alexander Ogston is considered the first person to call this bacterium staphylococcus in (1882) when he first saw it in the pus found in the abscesses, and then Rosenbach isolate it and grew it in pure culture in (1884), as described two types of strains belonging to this genus which are *Staphylococcus aureus* and *Staphylococcus albus* which currently known as *Staphylococcus epidermidis* (Liu *et al.,* 2011). The bacterial colony is (0.5–1.5) mm in diameter, occurring singly, in pairs, in tetrads, in short chains (3-4) cells, and characteristically dividing in more than one plane to form irregular grapelike clusters. Gram positive, nonmotile, nonflagellate, nonspore-forming cell wall contains peptidoglycan and teichoic acid. Usually uncapsulated or limited capsule formation. *Staphylococcacea* are aerobic or

Summary

A cross sectional study was conducted to assess the prevalence of Gram positive bacteria and fungi that cause infections among female genital tract. This study included 500 vaginal swabs that were collected from women in Al-Batoul Teaching Hospital in Diyala-Iraq for the period from October 2019 to February 2020. The samples included 333 pregnant and 167 non-pregnant women with age group ranging from 13 – 59 years old. The results of the routine diagnostic test, that was confirmed by using VITEK comparte 2 system showed that there was a diversity of bacterial and fungi species, where the *S. aureus* bacteria had the highest rate of isolation in 23.8%, followed by *E. coli*, *S. epidermidis*, *Klebsiella spp.*, *pseudomonas spp.*, and *S. saprophyticus* (16.3%, 12.6%, 9.3%, 2.8% and 1.8% respectively). *C. albicans* was the predominant fungi isolated which was (21.9%) followed by *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. krusei* (4.2%, 3.7%, 2.8% and 0.50% respectively). The investigation of virulence factors showed that *S. aureus* can produce haemolysin with percentage of 90%. The results showed that (62.7%) of *S. aureus* isolates have the ability to adhere on the surface of epithelial cells while *S. epidermidis* and *S. saprophyticus* can adhere by (77.7% and 75% respectively). In this study, the results showed a variation of resistance to the antibiotics, Trimethoprim was the most resisted antibiotic by *Staphylococci* (53.6%) followed by Erythromycin and Trimethoprim/sulfamethoxazole (48.7% and 46.8% respectively), while 97.5% of bacterial isolates were sensitive to Imipenem, followed by Vancomycin and Clindamycin (68.2% and 67% respectively). The results showed that Caspofungin was the most effective antifungal against *Candida spp.* (85.9%), while Fluconazole was the least effective antifungal against *Candida spp.* by (63.3%). The results of the molecular study showed that there is a wide distribution of *nor*A (94.54%) and *sdr*M (90.90%) genes

facultative anaerobes grows well in medium containing 10% NaCl, poorly in 15% NaCl (Duval *et al*., 2010). This bacteria is classified into two major groups which are coagulase positive staphylococci (COPS) represented by *Staphylococcus aureus* which is medically considered the most dangerous type , and coagulase negative staphylococci (CONS) represented by other types of staphylococci which *Staphylococcus epidermidis* medically represents the highest percentage among CONS , this is due to its possession of many virulence factors and its ability to form a biofilm on various surfaces (Namvar *et al.,* 2014).

Scientific classification of bacteria: (Holt *et al.,* 1994).

Domain:	Bacteria
Kingdom:	Eubacteria
Phylum:	Firmicutes
Class:	Bacilli
Order:	Bacillales
Family:	Staphylococcaceae
Genus:	*Staphylococcus*
Species:	*aureus* *epidermidis* *saprophyticus*