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# Microbial Profile Associated with Vaginosis

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**Abstract :** This project has been carried out through the period from October 2016 toApril2017 in attempt to investigate the vaginal flora in women with bacterialvaginosis. Vaginal samples were collected from 112 women attending the outpatient department in the Teaching Hospital of Maternity and Pediatrics in Al-Diwaniya city. Control group included 20 healthy women.
Vaginal swabs were collected carefully to evaluate the vaginal microbiota using two parameters represented by; Amsel's criteria represented by vaginal discharge, vaginal pH, clue cells, and amine odour and the culturing technique.
The results of culturing method revealed that a total of 292 different microbial isolates were obtained, among those 41isolates were *Candida* spp.which accounted for 14%. Among the bacterial isolates, coagulase-negative staphylococci represented the highest frequency, that accounted for 59 isolates (20.2%) followed by *Lactobacillus* spp.which recorded 47 isolates (16.1%), *Escherichia coli* 33 isolates (11.3), non-hemolytic streptococci 30 isolates (10.3%), *Klebsiella pnumoniae* 22 isolates (7.5%), *proteusmirabilis*10 isolates (3.4). *Pseudomonas aeruginosa*, alpha- and beta- hemolytic streptococci were also isolated in a frequencies of 9 isolates (3.1%) for each. *Staphylococcus aureus* represented 7 isolates (2.4%). *Diphtheroids* and *Enterococcus* spp. accounted for 4isolates (1.4%) for each. *Enterococcus* spp. accounted the lowest frequency 3 isolates (1.0%).
**Keywords** : APCVD ; $SnO_2$ thin film; optical properties; capillary tube.

## I. Introduction

The microflora of the lower genital tract of healthy women is of a great attention because of its potential as a reservoir for infections both of the normally sterile upper genital tract and of the neonates during delivery (Ison, 1990). The increase in oestrogen at the onset of puberty cause a thickening of the vaginal epithelium with a deposition of glycogen. Lactobacilli are thought to metabolize glycogen producing large amount of lactic acid (Forbes *et al*., 2007) resulting in low pH which therefore, selects the acid tolerant microorganisms, e.g. Lactobacilli, and then protect the vagina from colonization by pathogens (Boris and Barbés, 2000).

Besides *Lactobacillus spp*., the bacterial flora in women genital tract is a mixture of Gram-positive cocci and Gram-negative rods, such as *Streptococcus spp*.; *Staphylococcus spp*., and members of *Enterobacteriaceae*, mostly *Escherichia coli*; in addition to other anaerobic species e,g., *Bacteroides spp*., *Bifidobacterium spp*., *Fusobacterium spp*., *Peptococcus spp*., *Prevotella spp*. and *Veillonella spp*. (Hyman*et al*., 2005; Ravel*et al*., 2011).

Lactobacilli act as gatekeepers in maintaining the normal vaginal microflora by preventing overgrowth of pathogenic and opportunistic organisms (Witkin *et al*., 2007)by producing lactic acid, bacteriocins, and hydrogen peroxide (Reid and Bruce, 2001; Darouiche and Hull, 2012).

Microbial population in the human vagina undergo shifts in the representation, abundance, and virulence, since it is influenced by some factors such as age, hormonal fluctuations, underlying health conditions, use of medications, intravaginal washing practices and hygiene (Srinivasan and Fredricks, 2008).

vaginosis develops when the vaginal flora has been altered by introduction of a pathogen or by changes in the vaginal environment that allow pathogens to proliferate (Egan and Lipsky, 2000). The exact etiological agents for bacterial vaginosis(BV) is not well known yet, however, it is thought that it is a polymicrobial syndrome (Hay, 2002).

Vaginosis can clinically be diagnosed by Amsel's clinical criteria (Amsel, *et al*, 1983 and AL-Taweel, 2014) and confirmed by bacteriological culturing methods to identify the types and frequency of organisms involved in vaginosis.

## Materials and methods

**Materials**:

The common equipments, stains, chemicals and culture media were used through this project for isolation and identification of vaginal microbiota.

**Subjects:**

During a period of 7 months (from October 2016 to April 2017) a total of 112 women aged between 15 – 49 years who attendant the outpatients clinics in the Teaching Hospital of Maternity and Pediatrics in AL-Diwaniyacity were involved in this project. The cases of those women were clinically diagnosed as vaginosis by gynecologists using the Amsel's clinical criteria(Amsel, *et al*, 1983 and AL-Taweel, 2014). Twenty additional healthy women were also enrolled in this project as a control. Women using intrauterine contraceptive device or oral contraceptive pills and those women who used antibiotics or vaginal creams during the last 2 weeks were excluded.

**Sampling:**

By assistance of gynecologists, a sterile unlubricated spatula was inserted into the vagina and specimen were collected from the lateral vagina wall and posterior fornix using three sterile cotton swabs. Swabs were carefully removed to avoid contamination with microflora of vulvo and interoitus. One of three swabs was used for measuring the vaginal pH, the second was used for the preparation vaginal wet mount and the third swab was inserted in the Amies transport medium for culturing.

**Amsel's Criteria**

The clinical diagnosis of bacterial vaginosis was made when at least three out of four Amsel's criteria are present (Amsel *et al*., 1983). These criteria are as follow:

**1- Vaginal discharge:**

An evaluation of the nature of the vaginal discharge was made by the clinician during pelvic examination. Discharge was reported as positive for BV if it is thin, homogenous, and with milky colour (Easmon*et al*., 1992).

**2- Hydrogen ion potential (pH):**

The pH has been determined directly with the use of narrow range (3.5-6) pH strips (Himedia/India) which placed on the speculum after removing from vagina, or by touching the swab directly on to the pH strip (WHO, 2013). The colour change was matched with a colour coded guide provided by the manufacturer. A pH greater than 4.5 was considered as positive forBV.

**3- Wiff (sniff) test**

This test included a drop of 10% potassium hydroxide was placed on a glass slide and the swab with vaginal fluid was stirred in the KOH drop and immediately evaluated for the presence of a fishy odour which indicates a positive result for BV (Money, 2005).

**4- Clue cells**

The swab from vagina was extracted into 0.2 ml of physiological saline then a drop of this extract was placed on a clean glass slide and covered with a coverslip and examined at 400X magnification with a light microscope for the presence of clue cells ( > 20% of epithelial cells with indistinct borders due to adherent bacteria) (Money, 2005).

Clue cells were also detected during examination of Gram stained smears.

**Preparation of Culture Media**

Different types of culture media were used in this study. Those were routinely prepared according to the manufacturer instructions. The culturing results were recorded according to the referential references as follows:

**Blood Agar Medium**(MacFaddin, 2000).
**Brain-Heart Infusion Broth**(MacFaddin, 2000).
**Chocolate Agar Medium** (Forbes*et al.*, 2007).
**Columbia Agar Medium**(MacFaddin, 2000).
**De Man Rogosa Sharpe (MRS) Agar Medium**(MacFaddin, 2000).
**De Man Rogosa Sharpe (MRS) Broth Medium**(MacFaddin, 2000).
**Kligler′s Iron Agar**(MacFaddin, 2000).
**MacConkey Agar Medium**(Colleet *al*., 1996).
**Mannitol Salt Agar Medium**(MacFaddin, 2000).
**Nutrient Agar Medium**(MacFaddin, 2000).
**Nutrient Broth Medium**(MacFaddin, 2000).
**MR-VP Medium** (MacFaddin, 2000).
**Pepton Water Medium** (Forbes *et al*., 2007).
**Simmons Citrate Medium**(MacFaddin, 2000).
**Urea Agar Medium**(MacFaddin, 2000).

**Isolation of Microorganisms:**

High vaginal swabs were streaked out on a set of culture media, where each specimen was inoculated on blood agar plates and MRS agar plats, which incubated anaerobically using gas pack (Oxoid/England); chocolate agar plates, incubated under CO2; and MacConkey agar plates and blood agar, which were incubated under aerobic conditions. All plates were incubated at 37ºC and for 24-48 hours, except MRS agar plates which were incubated for additional 24 hrs.

**Identification of Isolated Microorganisms**

This procedure was depended upon colonial morphology, cellular morphology and biochemical tests, which were included the following tests:

**Catalase test as mentioned by** Forbes *et al*., (2007).
**Citrate Utilization Test**as recommended by Forbes*et al*., (2007).
**Coagulase Test:** This test was used to differentiate *Staphylococcus aureus* which is coagulase positive from coagulasenegative staphylococci (Forbes *et al*., 2007).
**Indol Test**: as indicated by Forbes *et al*.,( 2007).
**Kliger's Iron Test:** as indicated in Forbes *et al*.,( 2007).
**Methyl Red Test:** as mentioned byMac Faddin, ( 2000).
**Oxidase Test** as in Forbes *et al*.,( 2007).
**Urea Hydrolysis Test**(Forbes *et al*., 2007).

**Vogus-Proskauer Test**(MacFaddin, 2000).

## Results and discussion:

### Amsel's Clinical Criteria:

All the specimens investigated in this study were obeyed to the in addition to the clinician diagnosis. Accordingly, those specimens revealed positive testfor BV, since all of them resulted in positive results for at least 3 clinical criteria as indicated in table 1. Therefore, the specimens then were investigated by culturing on appropriate culture media for microbial analysis.

| Amsel's Clinical Criteria | Total subjects = 112 | |
|---|---|---|
| | No. positive ( % ) | No. negative ( % ) |
| Vaginal pH | 104 (92.9 ) | 8 ( 7.1) |
| Clue cells | 36 ( 32.1 ) | 76 ( 67.9) |
| Vaginal discharge | 74 (66.1 ) | 38 ( 33.9 ) |
| Wiff test | 62 ( 55.4) | 50 ( 44.6) |

The results shown in table 1 indicates that Wiff test and vaginal discharge are of remarkable indication on vaginosis since the clinically diagnosed patients women with vaginosis revealed positive testin high frequencies (100% and 92.9% respectively).

Although Amsel's Clinical Criteria are of great value in diagnosis BV easily and in short time, but they have some disadvantages, i.e., microbes implicated in BV are completely ignored. Moreover, there is a difficulty, somewhat to perform and judge the clue cells. However, Amsel's criteria are a combination of clinical and laboratorial observations, i.e,.discharge and pH are observed clinically while fishy odour and clue cells are tested in the laboratory, therefore, there is a need for both a clinician and a technician at the same time (Jakobsson, 2008).

### Vaginal pH

The results indicated that the most sensitive clinical criterion was vaginal pH, since all patients with BV had elevated pH between 4.8 and > 6.0. Munjoma (2004) obtained similar results since he reported that the positive test for vaginal pH was as high as 92 %. However, pH of vaginal discharge can be raised in response to several factors or during different situations, for example, Vaginal pH may elevate above 4.5 at the time of menstruation (Hay, 2002).

Other causes of increased vaginal pH may include infections such as trichomoniasis, atrophic vaginitis (Sobel, 1997), and desquamative inflammatory vaginitis (Forbes *et al.*, 2007). Also it was found that 25% of women with a pH above 4.7 had coccoid aerobic vaginitis (Donders *et al.*, 2011). pH may rise if the sample contains blood or if it was close to cervix, where cervical mucus has alkaline pH (Hay, 2002). In addition, douching also increases the vaginal pH (Brotman *et al.*, 2008).Amsel *et al*. (1983) in their original article defined a pH more than 4.5 as one of the four criteria for the diagnosis of BV.

### Clue Cells

The presence of clue cells in stained smears (figure-1)is a valuable indicative criterion for BV, although there is some difficulties in its detection, since they have not found in most patients clinically being diagnosed as BV patients. The microscopic analysis of clue cells is sometimes difficult, and this criterion was applied differently from mere existence to occurrence on 20% of the epithelial cells (Eschenbach *et al.*, 1988).

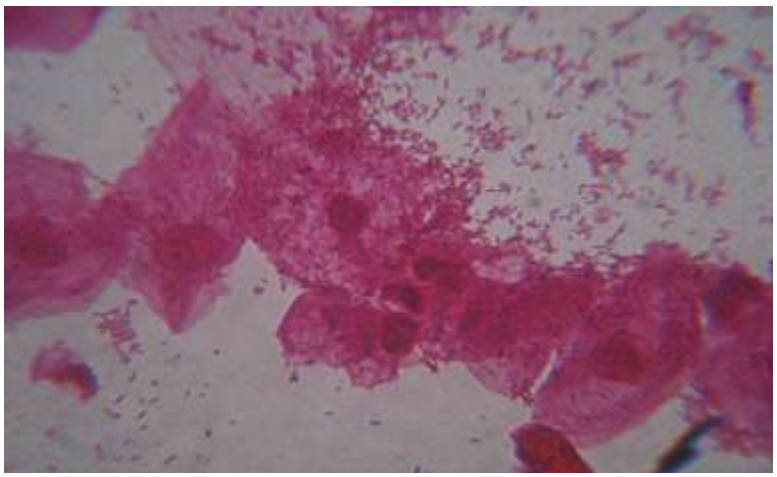

**Figure-1: Gram stained vaginal smear from a patient with bacterial vaginosis shows clue cells (1000X)**

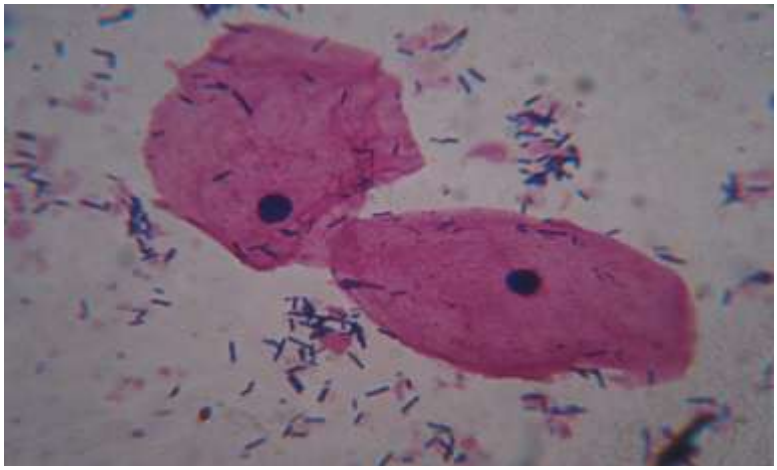

**Figure-2: Vaginal smear from a control woman with normal flora, dominance of lactobacilli and absence of other morphotypes (1000X)**

**Vaginal Discharge**

Regarding the vaginal discharge, it has reported that there is a variation in the decision of clinicians and technician to detect the discharge (Eschenbach *et al.*, 1988). Abnormal discharge is also associated with other infections rather than BV such as trichomoniasis and candidiasis (Adler *et al.*, 2004). On the other hand appearance of vaginal fluid may be altered by several factors including sexual intercourse and douching (Easmon *et al.*, 1992; WHO, 2013).

**Wiff Test**

This test, like other Amsel's criteria, is also depends on the investigator skill in detection the characteristic amine odour. Infection with *T. vaginalis* may give positive result for wiff test (Egan and Lipsky, 2000). Also false positive KOH tests can occur in women whose have had sexual intercourse recently (Spiegel, 1991). In addition, when wiff test gives a positive result, the sample become without amine odour due to volatility of amines quickly and completely (Hay, 2002).For these fluctuations in Amsel′s criteria, three of these criteria should be considered.

**Recovered Microorganisms Using Culture-Dependent Method**

Microorganisms detected in women with vaginosis indicated that there is a vast interaction between microorganisms colonize the vaginal environment. A total of 292 different isolates being identified in vaginal specimens as shown in table 2 below.

**Table -2: Types and frequencies of isolated organisms from women with vaginosis**

| **Microorganisms** | **No. Isolated microbe ( % )** |
|---|---|
| *Candida sp.* | 41 ( 14.0 ) |
| Coagulase-negative staphylococci | 59 ( 20.2 ) |
| Diphtheroids | 4 ( 1.4 ) |
| *Enterobacter sp.* | 3 ( 1.0) |
| *Enterococcus sp.* | 4 ( 1 .4 ) |
| *Escherichia coli* | 33 ( 11.3 ) |
| *Klebsiella pneumoniae* | 22 ( 7.5 ) |
| *Lactobacillus spp.* | 47 ( 16.1 ) |
| *Micrococcus sp.* | 5 ( 1.7 ) |
| *Proteus mirabilis* | 10 ( 3.4 ) |
| *Pseudomonas aeruginosa* | 9 ( 3.1 ) |
| *Staphylococcus aureus* | 7 ( 2.4 ) |
| α-hemolytic streptococci | 9 ( 3.1 ) |
| β-hemolytic streptococci | 9 ( 3.1 ) |
| Non-hemolytic streptococci | 30 ( 10.3 ) |
| Total | 292 (100) |

These isolates belong to 15 different groups. Coagulase-negative staphylococci was the dominant, since it accounted for 59 isolates (20.2%) followed by *Lactobacillus* spp.47 isolates (16.1). *Candida sp.* represented 41isolates (14.0) which indicates for the great diversity in microbial interaction between microorganisms in vaginal patients with vaginosis. Other different gram positive and gram negative were isolated and diagnosed as shown in table 2 above, among these, members of *Enterobacteriaceae*. Beside, healthy control women, revealed homogenous bacterial population, the most dominant bacteria was *Lctobacillus* spp. followed by coagulase-negative staphylococci (results are not shown). The diversity of bacterial population in women with BV is greater than that in healthy group where the mean of isolated species per patient with BV was two to three times more by using culture-dependant procedures (Puapermpoonsiri *et al.*, 1996, Fredricks*et al.*, 2007; Oakley *et al.*, 2008). However, there is some conflicting results being reported by others studies (Puapermpoonsiri *et al.*, 1996, Fredricks*et al.*, 2007; Oakley *et al.*, 2008) which may due to decreasing in the numbers of isolated anaerobic bacteria which are dominant in bacterial vaginosis patients (Hay, 2002), most of these anaerobes are fastidious and require selective enriched media, and sometimes they are not easy to identify using traditional biochemical tests. Moreover, a number of organisms associated with BV are uncultivable e.g. species of *Eggerthella*, *Megasphaera*, Clamembers of *Clostridiales*, *Mycoplasma*. Other reason can be attributed to the age of women, since in women of reproductive age anaerobic bacteria outnumbered aerobic bacteria, while the latter appeared to become more abundant with advancing age and onset of sexual activity(Larsen and Monif, 2001). Other possible reason for the fluctuation of microorganisms associated with vaginosis is the menstrual cycle, sine the subjects included in this study were at different stages of menstrual cycle. It has been shown that strict anaerobes are more predominant in premenstrual period (Domingue *et al.*, 1991) i.e. the type of dominant microorganisms may vary throughout the monthly cycle. However, some studies have suggested the concept that organisms of which there are a great number are readily found in cultures, whereas those species that are fewer in number may not be noticed during primary isolation (Larsen and Monif, 2001).

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