

**\*Vaginal Flora among Women with and without  
Bacterial Vaginosis**

Received :21/7/2014

Accepted :30/9/2014

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**Abstract**

This study has been conducted in Al-Diwaniya city through the period from December 2012 to December 2013, in order to investigate the prevalence of bacterial vaginosis, and to compare the composition of vaginal flora between women with bacterial vaginosis (BV) and those without the syndrome. Results showed that, according to Nugent's scores, 18 women had BV with a prevalence of 16.07%. Culturing out of vaginal swabs from women has been revealed that coagulase-negative staphylococci were the dominant microorganisms in women without BV followed by lactobacilli (frequency of 20.63 and 16.66% respectively), while in BV patients the dominant microflora were non-hemolytic streptococci and coagulase-negative staphylococci (frequency, 21.95 and 17.07% respectively). *Actinomyces israelii*, has been isolated from a BV patient with a cervical ulcer, this result can assumed as a new record in Iraq .

**Key words:** Vaginal flora, Bacterial vaginosis, Nugent's scores

**Microbiology Classification QR 75-99.5**

**\*The research is a part of PhD thesis in the case of the first researcher**

Vaginitis 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 (7). The most common vaginitis types worldwide is bacterial vaginosis (BV), which is characterized by alterations of the vaginal flora with acquisition of diverse communities of anaerobic and facultative bacteria and depletion of the usually dominant lactobacilli (8). The commonest presenting symptoms of women who have bacterial vaginosis is a malodorous vaginal discharge, which is not associated with itching or irritation (9).

Women with bacterial vaginosis have loss of many *Lactobacillus* species and acquisition of a variety of anaerobic and facultative bacteria (10), but it is not known whether the primary event initiating BV is the loss of key lactobacilli or acquisition of the complex bacterial communities found in this syndrome, these may be simultaneous processes (6). The exact etiological agents for bacterial vaginosis are unknown, however, it is thought that it is a polymicrobial syndrome (9).

This study was conducted in order to investigate the vaginal flora in both women with bacterial vaginosis and those without the syndrome.

### **Subjects and Methods**

A total of 112 women aged between 15-49 years, whom visiting the outpatient department in the Educational Hospital of

### **Introduction**

The microflora of the lower genital tract of healthy women is of interest because of its potential as a reservoir for infections both of the normally sterile upper genital tract and of the neonate during delivery (1). The increase in estrogen at the onset of puberty cause a thickening of the vaginal epithelium with a concomitant deposition of glycogen, lactobacilli are thought to metabolize glycogen and produce large amount of lactic acid (1,2). The resultant low pH would, therefore, select for acid tolerant microorganisms, predominately lactobacilli, and protect the vagina from colonization by pathogens (3).

Besides *Lactobacillus* spp., the bacterial flora in the genital tract is characterized by 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. (4,5).

Microbial communities in the human vagina undergo shifts in the representation, abundance, and virulence of key species over time that are influenced by factors which include age, hormonal fluctuations, underlying health conditions, use of medications, intravaginal washing practices and hygiene (6).

field where 0, no morphotypes; 1+, less than 1 morphotype; 2+, 1 to 4 morphotypes; 3+, 5 to 30 morphotypes; and 4+, more than 30 morphotypes.

The vaginal swabs in transport media were streaked out on a set of culture media, where each specimen was inoculated on blood agar plates and MRS agar plates, which incubated anaerobically using gas pack; chocolate agar plates, incubated under CO<sub>2</sub>; and MacConkey agar plates and blood agar, which were incubated under aerobic conditions. All plates were incubated at 37°C and for 24-48 hours, except MRS agar plates which were incubated for additional 24 hrs., i.e. for 24-72 hrs. After the incubation period, the identification of isolated bacteria was depended upon colonial morphology, cellular morphology and biochemical tests.

Differences in percentages of bacterial isolates between women with and without BV were calculated using Chi square at  $p < 0.05$ .

### **Results and Discussion**

According to Nugent's scoring system, the prevalence of bacterial vaginosis was 16.07% (18 out of 112 of the investigated women). Ninety four (83.92%) women were without BV according to Nugent's scores.

Culturing results have showed that in 94 women without BV according to Nugent's scores a total of 252 isolates belong to 15 microorganisms (species) have been

Maternity and Pediatrics, in addition to some private clinics in Al-Diwaniya city, were enrolled in this study. Informed consent was obtained from all subjects, women using intrauterine contraceptive devices and those who used antibiotics or vaginal creams (during the last two weeks) were excluded. By assistance of clinicians, a sterile unlubricated speculum was inserted into the vagina and specimens were collected from the lateral vaginal wall and posterior fornix using two sterile cotton tipped swabs.

Swabs were carefully removed to avoid contamination with microflora of the vulva and introitus. The first swab was used for the preparation of a smear for Gram's staining, and the second swab was placed into Amies transport medium for culture.

For scoring of vaginal flora, a vaginal swab was used to prepare a dry vaginal smear by rolling it along the middle of a glass slide. The smear was air-dried and fixed with methanol then Gram stained (11). The slides were examined under oil immersion objective 1000x magnification and evaluated for the following morphotypes: large Gram positive rods (*Lactobacillus* morphotypes), small Gram-variable rods (*G. vaginalis* morphotypes), small Gram negative rods (*Bacteroides* species morphotypes), small Gram-variable rods (*Mobiluncus* species morphotypes) and Gram positive cocci. Each morphotype was quantitated from 0 to 4+ with regard to the number of morphotypes per oil immersion

*Megasphaera*, and BVABs members of *Clostridiales*.

Secondly, although it was demonstrated that in women of reproductive age anaerobic bacteria outnumbered aerobic bacteria, the latter appeared to become more abundant with advancing age, onset of sexual activity, and parity (14). Thirdly, the investigated subjects were at different stages of their menstrual cycle, it was shown that strict anaerobes are more predominant in premenstrual period (15) i.e. the type of dominant microorganisms may vary through out the monthly cycle. Finally, previous 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 (14).

In women without BV, whom consisted a percentage of 83.03%, the most common isolated bacteria were coagulase-negative staphylococci (Table 1) where they were isolated from 55.91% of subjects in this group and at a frequency of 20.63% among other identified organisms (Figure 1).

identified, i.e. those women had a mean of 2.70 bacterial phenotypes per subject (ranged from 0, i.e. no growth, to 5). On the other hand, in patients with BV (18 women) the number of isolates were less than that in women without BV, where only 41 isolates represented 10 species were cultured out from subjects in this group, the mean of isolates per specimen was 2.27 (range, 0-3).

These results are unlike to what was concluded in most other studies that the diversity of bacterial population in women with BV is greater than that in healthy ones where the mean of isolated species per patient with BV was two times more using culture-dependant procedures (12), and about three times greater using molecular techniques (10,13).

This confliction in results with other reports can be explained through several points. Firstly, there was decreasing in numbers of isolated anaerobic bacteria which are dominant in bacterial vaginosis patients (9), most of these anaerobes are fastidious and require selective enriched media, and sometimes they are not easy to identify using traditional biochemical tests. In addition, a number of organisms that have been shown to be associated with BV are uncultivable e.g. species of *Eggerthella*,

**Table (1) Frequency of isolated microflora and percentage of positive women**

Microorganisms	No. of isolates	<u>Percentage of women</u>	
		With BV	Without BV
<i>Actinomyces israelii</i>	1	5.55 (ND)	-
<i>Candida sp.</i>	41	22.22 *	39.78
Coagulase-negative staphylococci	59	38.88 **	55.91
Diphtheroids	4	-	4.30 (ND)
<i>Enterobacter sp.</i>	3	-	3.22 (ND)
<i>Enterococcus sp.</i>	4	-	4.30 (ND)
<i>Escherichia coli</i>	33	33.33 *	29.03
<i>Klebsiella pneumoniae</i>	22	16.66 *	20.43
<i>Lactobacillus spp.</i>	47	27.77 **	45.16
<i>Micrococcus sp.</i>	5	5.55 *	4.30
<i>Proteus mirabilis</i>	10	-	10.75(ND)
<i>Pseudomonas aeruginosa</i>	9	-	9.67 (ND)
<i>Staphylococcus aureus</i>	7	-	7.52 (ND)
$\alpha$ -hemolytic streptococci	9	5.55 *	8.60
$\beta$ -hemolytic streptococci	9	22.22 **	5.37
Non-hemolytic streptococci	30	50.00 **	22.58

\* No significance difference.

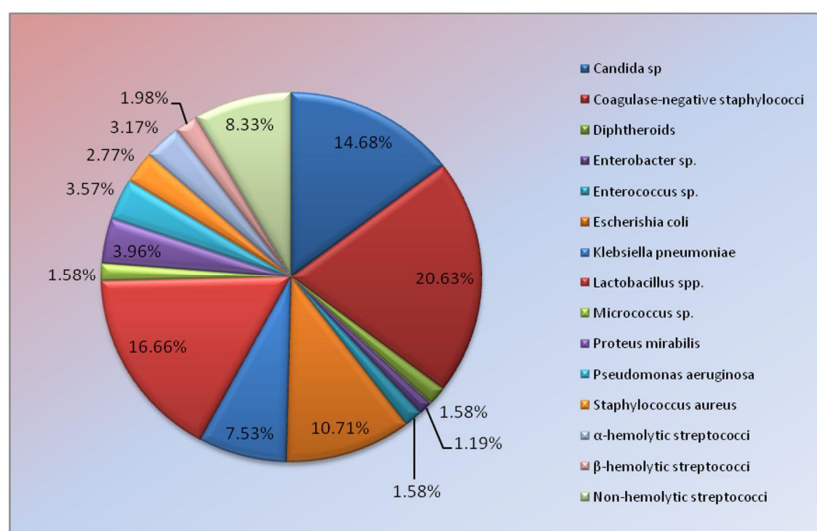
\*\* Significant difference.

(ND) Not determined.

(-) Not isolated.

reproductive age (1,2). These microorganisms are mainly consist of *Staphylococcus epidermidis* with a prevalence up to 95% in vaginal flora (14).

Coagulase-negative staphylococci, in addition to streptococci, are the most prevalent facultative anaerobic bacteria that found in the vagina of women of



**Figure (1) Frequency of microorganisms in women without bacterial vaginosis**

present but in small quantities. Previous investigations showed that *Lactobacillus* spp. were isolated at high percentage, reached to 89.00% of women with BV using culture-dependant and independent methods (12,19).

A number of *Enterobacteriaceae* members has been isolated during this study, of these *E. coli* was the prevalent one in women without BV (29.03%). This finding supports the previous studies showing that this species is the dominant among other enteric bacteria in the healthy vagina of reproductive-age women (14,20).

In addition to *E. coli*, other isolated species of *Enterobacteriaceae* family members were, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter* spp. with a prevalence of 20.43, 10.75, and 3.22% respectively. Colonization of the introitus with *Enterobacteriaceae* species is a predisposing factor for urinary tract

The genus *Lactobacillus* came secondly after coagulase-negative staphylococci in BV-negative women with a prevalence of 45.16%, i.e. it has not been isolated from more than half (54.83%) of women in this group. It is possible that women lacking lactobacilli were colonized with species that could not be detected on Rogosa agar like *L. iners*, which has been described as one of the most common *Lactobacillus* species in the vagina (16), this species cannot be cultured on common lactobacilli agar (17). Recent studies have revealed that a significant proportion of apparently healthy women lack appreciable numbers of lactobacilli (18,5).

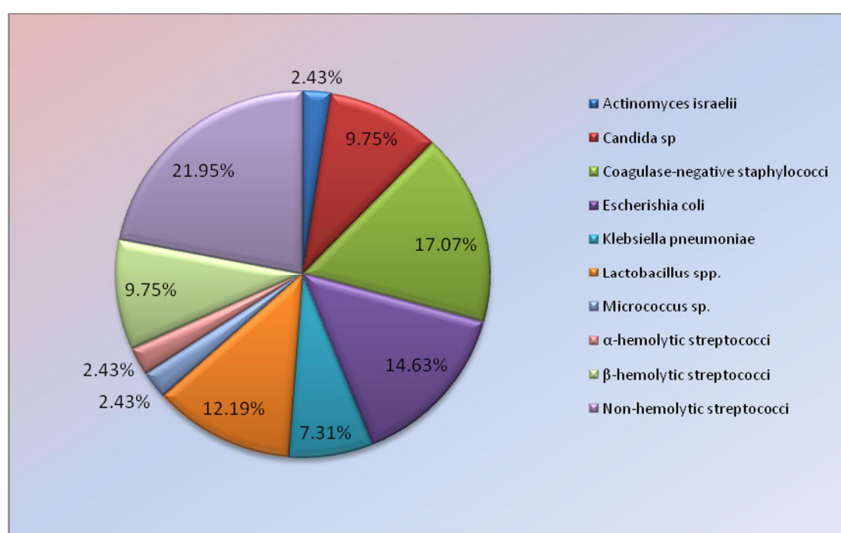
Lactobacilli have also been isolated from patients with BV with a prevalence of 27.77% as shown in table (2). This is acceptable as in bacterial vaginosis there is a depletion of the usually dominant *Lactobacillus* flora, i.e. lactobacilli can be

from samples with no lactobacilli have been detected. *E. coli* was isolated more likely from women lacking *Lactobacillus* species than those colonized with these organisms (23). This can be due to the potential role of some substances produced by lactobacilli such as hydrogen peroxide, where it was found that the absence of H<sub>2</sub>O<sub>2</sub>-positive lactobacilli may be important in the pathogenesis of recurrent UTI by facilitating *E. coli* introital colonization (24).

infection in women (14), where species like *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, and *Enterobacter* spp. have been implicated in such a condition (21,22).

In women with BV the only isolated members of the family *Enterobacteriaceae* were *E. coli* and *K. pneumoniae*, and again with the dominance of *E. coli* (33.33 vs. 16.66%) as indicated in Table (1).

*E. coli* was more frequent in subjects with BV than those without (Figure 2), in addition in the latter group of women, most *E. coli* isolates (70.37%) were recovered



**Figure (2) Frequency of microorganisms in bacterial vaginosis patients**

although they are sometimes within the recorded range, from other reports in which the prevalence of α-hemolytic streptococci in vaginal flora was higher (8 - 38%) than that of non-hemolytic ones, range between 0 and 32%, (1,14).

Beta-hemolytic streptococci have been isolated at a frequency of 9.75 and 1.98%

Streptococci have been isolated from both women with and without BV with the dominance of non-hemolytic (γ-hemolytic) ones in both groups, where the percentage of isolation was 8.33% in the first subjects group vice versa 21.95% in patients with BV. Alpha- hemolytic streptococci followed non-hemolytic types in samples from women without BV at a frequency of 3.17%. These results are slightly differ,



frequency of 3.57%. It was found that *Pseudomonas* is a common microbe in the healthy human premenopausal vaginal tract (4), yet with low prevalence (0-3%) in vaginal flora (14).

Based on the available data, a newly recorded species in Iraq, *Actinomyces israelii*, has been isolated from a patient with BV, who suffering from abnormal yellow discharge with a pH of 5.5 and she had a cervical ulcer on clinician examination. *A. israelii* is the most common species in humans and is even considered as a common occasional commensals of the oropharynx, the gastrointestinal tract, and the vagina (28), and this species is responsible for 90% of actinomycetes infections (29).

Intrauterine contraceptive devices (IUD) users may have a high prevalence of genital *A. israelii* and other *Actinomyces* types, which can be pathogenic or commensals under different circumstances, *A. israelii* may infect 1.6-11.6% of IUD users worldwide (30). IUDs induce mild inflammatory changes in the endometrium with necrosis that creates an anaerobic environment that favors growth of *A. israelii* and other anaerobes (28).

*Candida sp.* has been recovered from 39.78% of women without BV. Although yeasts may be transiently recovered from female genital tract, they are not considered normal flora (2). The typical rate of yeast carriage varies among populations and increases both after puberty and during

respectively from patients with BV and those without.

Of  $\beta$ -hemolytic streptococci, group B (*S. agalactiae*) has been found most frequently where it present in the vagina of one in three women although at low density as compared with other microbes (25).

*S. aureus* has been isolated only from women without BV whom constituted a percentage of 7.52% with isolation frequency of 2.77%. This species has been found in less than 5% of healthy women (1), with a prevalence ranging from 0 to 25% in vaginal flora (14).

Menstrual toxic shock syndrome (TSS) is a serious illness that afflicts women of premenopausal age worldwide and it arises from vaginal infection by *S. aureus* and concurrent production of toxic shock syndrome toxin-1 (TSST-1). This syndrome is a systemic illness characterized by extensive T-cell proliferation throughout the body and this lead to systemic inflammation and concurrent health problems, including rash formation, multiple organ failure, and potentially death (26).

It was suggested that women colonized by *S. aureus* and with aerobic vaginitis, but not BV, may be more susceptible to menstrual TSS, where *in vitro* studies demonstrated that *S. agalactiae* and *Enterococcus* spp. significantly induce TSST-1 production (27).

*Pseudomonas aeruginosa* has been isolated only from subjects without BV at a



The remaining isolated microorganisms include diphtheroids and *Micrococcus spp.*, both of them have been isolated from BV-negative women at a frequency of 1.58%, and only the latter has been cultured from one subject with BV. Diphtheroids are non-hemolytic, catalase positive, pleomorphic Gram-positive bacilli, they are part of the normal commensals flora of skin and mucous membranes but occasionally cause infections (29).

*Micrococcus spp.* are normal flora in skin, mucosa, and oropharynx (35).

Cultivation studies provide critical insights about the phenotypic characteristics of microbes and allow for the experimental manipulation of these organisms and the testing hypotheses about pathogenesis and virulence factors (6). Another advantage of these approaches is that some bacteria are more likely to be detected by cultivation when present in low concentrations. For example, Verhelst *et al.* have reported that of the 38 bacterial species identified from subjects with and without BV, using cultivation and cultivation-independent methods, 5 were detected by cultivation alone (36).

pregnancy, which suggests an important role for host physiology in cases of vaginal candidiasis (14). It was found that *Lactobacillus* colonization is associated with a nearly 4-fold increase in the likelihood of symptomatic vulvovaginal candidiasis, VVC (31).

Yeast infection can develop if the natural balance of microorganisms in vagina becomes upset to that the yeast proliferates (32). About 75 percent of women have VVC at some time in life (7). Risk of this infection is increased in women who use oral contraceptive pills, a diaphragm and spermicide, or an IUD. Other risk factors include young age at first intercourse, intercourse more than four times per month and receptive oral sex. The risk of VVC is also increased in some women who have diabetes, are pregnant or are taking antibiotics (7,32).

*Candida sp.* was also isolated from four patients with BV, which gave a prevalence of 22.22%. Yeast vaginitis and BV may occur as a mixed infection with symptoms and signs of both clinical entities being present simultaneously (33), alternatively, BV may develop after vaginal candidiasis episodes (34).

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\* الفلورا المهبليّة بين النساء المصابات بالتهاب المهبل البكتيري والنساء غير المصابات

تاريخ القبول 2014/9/30

تاريخ الاستلام 2014/7/21

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الخلاصة

اجريت الدراسة الحالية في مدينة الديوانية للفترة من شهر كانون الاول 2012 ولغاية شهر كانون الاول 2013 من اجل دراسة انتشار التهاب المهبل البكتيري، بالإضافة الى مقارنة تركيب الفلورا المهبليّة في النساء المصابات بالتهاب المهبل البكتيري و النساء غير المصابات بالمرض. بينت النتائج بانه وفقا لنظام نوكنت للعد وجد ان 18 امرأة كانت مصابة بالتهاب المهبل بنسبة انتشار 16.07%. اظهرت نتائج زرع المسحات المهبليّة ان المكورات العنقودية السالبة لاختبار التخثر كانت اكثر الاحياء المجهرية سيادة في النساء غير المصابات تلتها عصيات الحليب بنسب تردد 20.63% و 16.66% على التوالي. اما في النساء المصابات بالالتهاب فكانت الفلورا السائدة هي المسبقيات غير الحالة للدم و المكورات العنقودية السالبة لانزيم التخثر و بنسب تردد 21.95% و 17.07% على التوالي. تم خلال الدراسة عزل النوع *Actinomyces israelii*، من امرأة مصابة بالتهاب المهبل البكتيري و تعاني من قرحة في عنق الرحم، هذه النتيجة يفترض انها تسجيل لأول مرة لهذا النوع في العراق

الكلمات المفتاحية: الفلورا المهبليّة، التهاب المهبل البكتيري، نظام العد لنوجنت

Microbiology Classification QR 75-99.5

\* البحث مستل من اطروحة دكتوراه للباحث الاول