Detection of the vaginal microflora in Pregnant and non-pregnant women using culture-independent method

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Abstract

Background: Bacterial Vaginosis (BV)is a disorder characterized by alterations of the vaginal flora with acquisition of diverse communities of anaerobic and facultative bacteria and depletion of the usually dominant lactobacilli.

Objective: To conduct and characterize the main bacterial players in BV affected pregnant and non- pregnant women.

Method: The BV work included collecting vaginal swabs from 158 women (18-45) years old.The samples were subjected in a laboratory to Amsel's clinical criteria (ACC) and Nugent score system (NSS). The clinical diagnosis of BV was comprehensive when at least three ACCs were present out of a total four criteria (pH of vaginal secretions(Vss),Vaginal discharge ,Clue cells and Wiff (sniff) test).

Results: The findings of the ACC and NSS revealed BV in 32 pregnant women(Pw) and 55 non pregnant women(Npw). In details, the ACC identified 39 (15 Pw and 24 Npw) positive samples. On the other hand, NSS unveiled that 48 (17 Pw and 31 Npw)with Vss were positive to BV. The bacterial isolates were distributed over the age categories (18-47 years old) of the Pw and Npw, in all age categories, (18-23, 24-29, 30-35, 36-41, and 42-47) year old, respectively.

Conclusion:The presented work, here, provides beneficial data obtained by using feasible techniques with high degrees of reliability to overcome setbacks generated from the use of cultivation techniques.

Keywords: vaginal microflora, Pregnant, Non-pregnant.

Iintroduction

Bacterial Vaginosis cause of foul vaginal discharge and is linked to the number of horrible health outcomes in reproductive age of femalessince has been associated with serious clinical obstetrical and gynecological sequalae, of these are infertility, endometritis, pelvic inflammatory disease (PID) , post-abortal sepsis, post-surgical abortion infections, post-hysterectomy infection, and increased risk of HIV and other STIs acquisition [1] .

BV is a clinically well-understood health condition manifested by the presence of a pH $\geq$ 4.5-vaginal discharge with properties of fishy-odor, gray to white, and homogenized adherent thin smear which is highly recognized after intercourse and menses [2] .

BV is the most prevalent vaginal disease that happens when useful *Lactobacillus* spp. decreases and different obligated or facultative anaerobic VBS elevated, which were usually available in very small amounts or absent in non-BV females [3.] This dysbiosis induces he BV pathological processes predominated by vaginal discharge which is an indicative sign for BV in 20 to 30% of females; however, risk can be higher as 50–60% in some active communities sexual behaviors [4,5].

Culture-dependent methods for BVidentification face potential obstacles represented by non-cultivability of a wide range of bacterial members which may affect the reality of the profile of BV causative agents and the functions of bacterial members [6].

Ecosystem of the vagina include several aerobic and anaerobic microorganisms coexist in a dynamic balance, this homeostasis results from complex interactions and synergies among the host and different microorganisms that colonize the vaginal mucosa [7].

Studies conducted in Iraq revealed that BV rates were between 28% and 37.5% among both pregnant and non-pregnant women [8].

The present BV-directed work was conducted aiming at understanding the real bacterial composition of the vaginal tract in pregnant and non-pregnant women (Pw and Npw, respectively) at reproductive age using primary scoring criteria non-cultivating.

Materials and Method

Subjects:

A total of 158 females, aged from (18-45) years old who visiting the outpatient clinics in the Maternity and Pediatrics Teaching Hospital during a period from November 2018 to April 2019 in Al-Diwaniya city, to evaluate of vaginal flora between women with and without bacterial vaginosis.Any participant having any of the following exclusion criteria was excluded from participation:-<18 years of age,Diabetes mellitus,Using of antimicrobial agents (orally or suppositories),Females during menses period and Females using intrauterine contraceptives.

Samples collection and preparation:-

By guidance of gynecologists, a sterile unlubricated speculum was inserted into the vagina and specimens were collected from the vaginal wall lateral areas and posterior fornix using sterile cotton tipped swabs and were carefully removed to avoid contamination with microflora of the vagina.The swabs were Amies-transport-media inserted and then transported to the designated facility, two swabs were taken from each women were used for gram stain preparation.

Amsel's Criteria

The clinical diagnosis of BV was comprehensive when at least three ACCs were present out of a total four criteria [9]. These criteria are as follows:

• **PH of vaginal secretions:** With a cotton swab the vaginal discharge were collected then transferred on a narrow range (3.5-6.0) PH strips for determined the PH directly. [10].

• **Vaginal discharge:** During pelvic examinationthe clinician will evaluate the nature of the vaginal discharge, in BV case the discharge have to be thin, homogenous, and with milky colour [11].

• **Clue cells:**A high 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 stained by Gram stain .The epithelial cells will be completely covered by the gram variable coccobacilli with indistinct borders due to adherent bacteria[12].

• **Wiff (sniff) test:** Adropletof 10% potassium hydroxide (KOH)was added to the vaginal discharge on a glass slideand immediately evaluated for the presence of a fishy smell which reveals a positive outcome [13].

Nugent's Criteria and Gram Staining

The stained slides were examined under oil immersion objective 1000x magnification and evaluated for the following morphotypes:-*Lactobacillus* morphotypes; longrods with Gram-+vecriteria,*G. vaginalis*morphotypes; small rods withGram-±ve,*Bacteroides*spp.morphotypes; small rods with Gram–veproperties, and *Mobiluncus*spp. morphotypes; small curved rods with Gram–velooking. Morphotypes were counted separately from 0 to 4+ in respect to thesingle-oil-immersion field morphotype numbersin which 0:nil morphotypes, 1+:<1 morphotype; 2+: 1-5 morphotypes, 3+: 5-30 morphotypes, and 4+:>30 morphotypes.These scores added up to yield a final score of (0-7) or more.The score (0-3) is considered as normal, (4-6) is considered intermediate, and the condition of BV is a score of (7-10) [14].

Results & Discussion

Although many BV-directed studies have been done, the picture of knowledge about the real vaginal bacterial composition in BV- or non-BV-affected women is not completely clear. Those studies either were focused on determining the major bacterial players in BV or characterizing the BV as a health condition with multiple bacterial members.

High vaginal swabs were collected from 158 women at different reproductive age groups, (18-45) years old ,a total of (53) pregnant women who had abnormal vaginal discharge during the study period

were recruited. Further than these women with atypical vaginal discharge, and (105)samples were taken from non-pregnant womencomplaints of abnormal vaginal discharge,odor , and itching or burning(Figure 1).
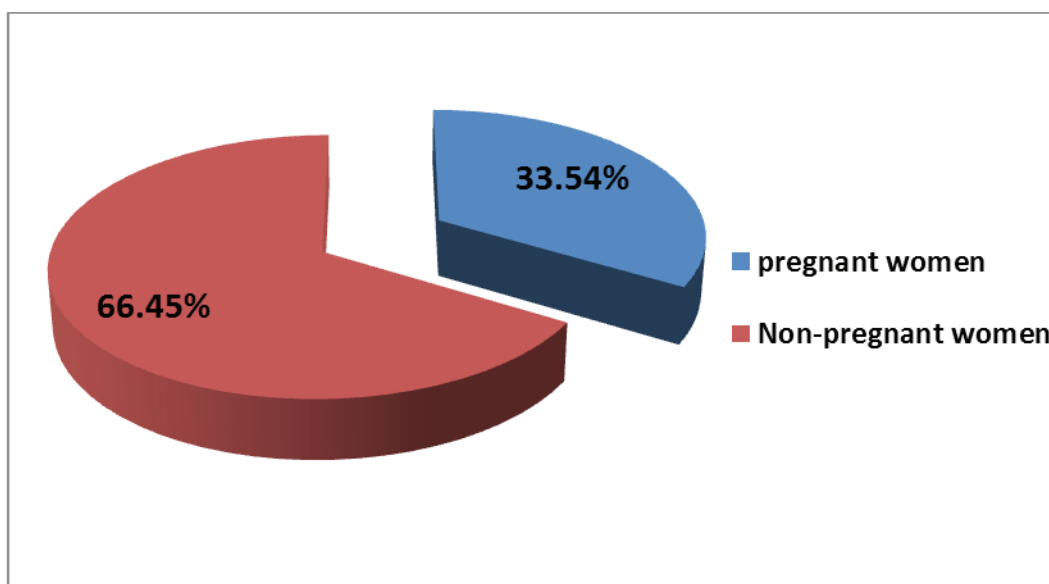

Figure (1): Distribution of bacterial vaginosis according to pregnant and non pregnant women.

Thirty two pregnant women had bacterial vaginosis giving an occurrence rate of (36.78%) these women were positive for bacterial vaginosis using both the clinical criteria and gram stain morphology, 15 (38.46%) were positive by using the Amsels clinical criteria alone while 17 (35.41%) pregnant women had bacterial vaginosis using the Nugent score system only as shown in Table (1), Table (2).

Table (1) the Nugent's score system

Score	Morphotype of Lactobacillus spp.	Morphotype of Gardnerella and Bacteroides spp.	Morphotype of curved bacteria
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

Table (2): Occurrence of bacterial vaginosis in pregnant and non pregnant women using culture-independent method.

Diagnostic tool	Total number	Positive number	Pregnant	Non-pregnant
Amsels clinical criteria	158	39(24.68)	15(38.46)	24(61.53)
Nugent score system		48(30.37)	17(35.41)	31(64.58)
Total	-----	87	32(36.78)	55(63.21)
X2		1.285(NS)	0.086(NS)	
P value		0.257	0.770	

NS : Non-significant difference at p≥0.05

The incidence rate of BV among pregnant women in this study (36.78%) falls within the range of previous studies (17.3 – 67.7%) [15] .but is higher than that of another studies which ranged from (3.54 – 25.1%) [16] ,considering local studies on the subject, the occurrence of (36.78%) is higher than the results reported by Al-Fadul [17] were (13.1%) in pregnant women only.

This differences could be related to the different populations studied and difference in the gestation period [18], the present study involved pregnant women who presented at reservation during the study period regardless of gestational age,or whether they were symptomatic or not at acceptance.

Nevertheless, bacterial vaginosis incidence during pregnancy has an importance since it was revealed to be related with numerous obstetric sequalae such as preterm labor, still births ,postpartum infections , premature rupture of membranes, abortion and low weight infants [19] .

Fifty five non-pregnant women had bacterial vaginosis giving an occurrence rate of (63.21%) these women were positive for bacterial vaginosis using both the gram stain morphology, and Amsels clinical criteria , 24 (61.53%) were positive by using the Amsels clinical criteria, whereas 31 (64.58%) non-pregnant women had bacterial vaginosis using the Nugent score system (Table 1).

Theincidence rate of (63.21%) among non-pregnant women is lower than the reported rate in a previous study were conducted in Iraq (86.9%) (Al-Fadul *et al*,.2007),but with higher rate reported in a foreign study in Egypt (33%) [20] ..Socio-demographic characteristics,sexual activity, reproductive health information, andbehavioral or genital hygiene have been identified as causesof variation in the prevalence rates of bacterial vaginosis among non-pregnant women [21]. Figure(2) showed no significant differences(p≥0.05) in the diagnostic tools that used in the diagnosis of BV among pregnant and non-pregnant women .
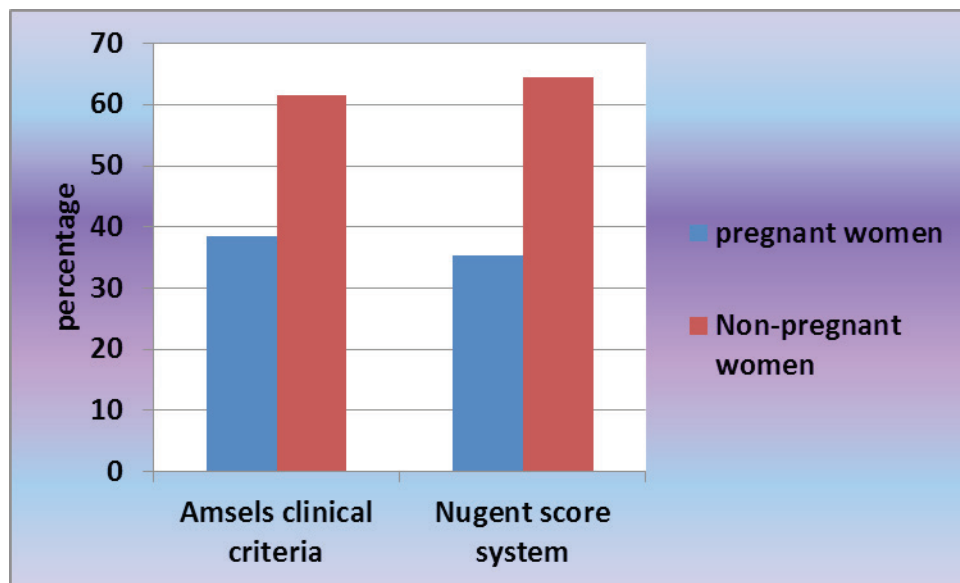

Figure (2):Diagnostic tools used in the diagnosis of BV among pregnant and non-pregnant women

In our study, The results of the Amsel's test shown that 39 (24.68%)subject of infected women gave positive results (15 were pregnant women and twenty four 24 were non- pregnant women) ,since they matched three of four Amsel′s clinical criteria.The most significant criteria for diagnosis of BV are as follows: presence of a thinhomogenous discharge, a pH of vaginal fluid greater than 4.5 and a positive KOH 10% test.(Wiff test). The attendance of clue cells in the wet smear upon

microscopic examination was reflected ,clue cells are critical component of Amsel's criteria for BV diagnosis [22].

The use of Amsels clinical criteria to diagnose BV has the advantage of rapid diagnosis at the point of care but requires assessment of vaginal pH and, more importantly, performance of microscopy of vaginal fluid by a sensibly skilled consultant or laboratory personnel.

The results of vaginal discharge inspection is homogenous , thin ,milky and adherent to the vaginal wall that exposed by clinicians or physician women help.

Definitely there is a diverse in the talent of clinicians to remark the vaginal discharge, abnormal discharge commonly associated with other vaginal infections rather than BV such as trichomoniasis and candidiasis,conversely presence of vaginal fluid may be enhanced by numerous factors as well as sexual intercourse, menstruation and douching [23].

In the present study vaginal pH was measured by using narrow range pH papers, by which the pH value is visually examined through the estimate of the test strip to a standard colour scale with fixed values, i.e. pH persistence rely on the examiner, Hence, there is no specific interpretation number for pH.

Wiff test were used in this study for detection of fishy odor associated with BV, and this testlike other Amsel's criteria, is also subjective and depends on the investigator variation in the ability to detect the characteristic amine odor,infection with *T. vaginalis* may give positive result for Wiff test,also false positive KOH test can occur in women whose have had sexual intercourse recently, finally, when Wiff test gives a positive result, the sample become without amine odor due to volatility of amines quickly and completely [27].

The presence of clue cells in stained smears was the most definite criterion among the other Amsels clinical criteria and they have not been detected in any subject without BV [14].

The overalloccurrence rate of bacterial vaginosis in thepresent study as determined by Gram-stain Nugent scoringcriteria was (30.37%),the reported rate of BV was well within thereported range, (8%–75%) [28] in some studies being conducted in Iraq, theoccurrence rate of bacterial vaginosis ranged from(37.5%) to (68.7%) by using Nugent′s scores as adiagnostic tool(8), but it's with higher rate reported by another study in India (24.3%) [29].

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicine, Iraq and all experiments were carried out in accordance with approved guidelines.

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