Studying the relationship between women hormonal activity and the distribution of bacterial vaginosis and bacteria's antibiotics susceptibility

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ABSTRACT

Sex hormones have a large influence on the structure and function of vaginal microbiota. A delicate balance between the vaginal microbiota and the host is one of the most important health reasons for women. The purpose of this study was to see if there were any links between female sex hormone levels and vaginal microbial diversity. A total of 100 vaginal swabs were obtained from female patients between the ages of 20 and 60 years who were suspected of having symptomatic and asymptomatic vaginal infections at the General Hospital and outpatient gynecological clinics, Samarra city, Iraq. The findings revealed that 70% and 25% of 100 high vaginal swabs with 95% positive cultures were from infected and healthy women, respectively. The most common organisms isolated belonged to Gram-negative bacteria and were *Escherichia coli* at 16 (16.84%) and in infected women were 9 (9.47%) and 7 (7.36%) in healthy women. The second most common isolated microorganisms from women with vaginitis were *Proteus vulgari*s at 8 (8.42%), *Acinetobacter* at 7 (7.36%), *Proteus mirabilis* at 7 (7.36%), and *Citrobacter* at 6 (6.31%). The highest percent of the isolates belonged to Gram-positive bacteria and were *Staphylococcus saprophyticus* at 5 (5.26%) and *Staphylococcus aureus* at 4 (4.21%) followed by *Enterococcus faecalis* at 4 (4.21%), *Bacillus stearothermophilus* at 3 (3.15%), *Bacillus megaterium* at 3 (3.15%), *Enterococcus faecium* at 1 (1.05%) and *Leuconostoc pseudomesenteroides* at 1 (1.05%), and *Lactobacillus* at 6 (6.31%). The greatest proportion of infection discovered in the age group 30 years was 36 (37.89%), while the least infected age group was 60 and above (2.1%). All of the bacteria that were isolated

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were susceptible to levofloxacin but resistant to metronidazole and amoxicillin-clavulanic acid. The relationship between isolated bacteria and sex hormone levels (estrogen and progesterone) revealed that sex hormone levels vary depending on the bacterial type. *Burkholderia cepacia* and *Lactobacillus* had the highest estrogen levels, while *Pseudomonas fluorescens* and *Aeromonas* had the highest progesterone levels.

INTRODUCTION

Vaginal inflammation, or vaginitis, is caused by a variety of infectious and noninfectious factors (Al-Marabeh *et al.*, 2017; Vieira-Baptista *et al.*, 2022). Bacteria, fungi, viruses, and parasites can all penetrate the vaginal area (Al-Marabeh *et al.*, 2017; Lin *et al.*, 2021). The three most common types of infectious vaginitis are bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomonal vaginitis (Abdul-Aziz *et al.*, 2019). Lactobacilli, which when present in sufficient numbers defend against pathogenic bacterial species, populate the healthy vaginal tracts of reproductive-aged women (Kumar *et al.*, 2011). As a result, lactobacilli deficiency disrupts the vaginal microbiota's homeostasis and increases the number of anaerobic organisms, which contributes to BV (Ventolini, 2016). Although the majority of cases of BV are asymptomatic, it is distinguished by the discharge of homogeneous grayish-white foul secretions, a fishy odor after intercourse or during menstruation, and an increase in vaginal pH to even more than 4.5 (Paladine and Desai, 2018). BV prevalence ranges from 8% to 51% depending on geographic region, socioeconomic class, and ethnicity (Kenyon *et al.*, 2013). Vaginal microbiota are essential for maintaining a healthy vaginal environment, and disruption of this system has been linked to poor vaginal health and other negative consequences (Greenbaum *et al.*, 2019; Kroon *et al.*, 2018). Vaginal microbiota are constantly changing and are influenced by hormonal changes, sexual activity, and hygiene (Huang *et al.*, 2014a, 2014b). Aside from that, hormone levels fluctuate at different times. As a result, the microbial population that lives in the human vaginal canal exhibits a wide range of homeostatic states (Ravel *et al.*, 2011). Although the vaginal microbiota's composition is constantly changing, it is heavily influenced by factors such as food, behavior, and the stages of a woman's reproductive cycle. The vaginal microbial ecology changes significantly during the menopausal stage (Vitali *et al.*, 2017). Several systemic and reproductive diseases have been linked to changes in female gonadal hormone levels (Zhang *et al.*, 2018). As a result of these challenges, novel treatment approaches have emerged (Al-Ghazzewi and Tester, 2016). Probiotics, which are living microorganisms made up primarily of *Lactobacillus* spp., are widely used to improve digestive health. They may, however, be useful in obstetrics or treating gynecological problems to build and maintain normal vaginal microbiota to treat conditions such as BV, VVC, and PTB (Akour, 2020). The primary goal of this study was to obtain a comprehensive picture of the organization and composition of the vaginal microbial population at various stages of gynecology. Other objectives included (a) determining whether there are any links between female sex hormone levels and vaginal microbial diversity and (b) comparing the pattern of community diversity in cases of BV (an abnormal disease characterized by microbial dysbiosis or imbalance followed by vaginal discharge) to that in a healthy state.

MATERIALS AND METHODS

Patients

Between November 2020 and March 2021, researchers conducted a cross-sectional study at the General Samarra Hospital in Samarra, Salah al-Din Governorate, Iraq (Fig. 1). During normal prenatal visits to the Gynecology and Obstetrics Clinic, nonpregnant women (*n* = 100) between the ages of 20 and 60 years were included with the suspicion of having symptomatic and asymptomatic vaginal infections. Patients' information, such as age, location, past abortion history, last menstrual cycle, and clinical signs and symptoms, was gathered through referral files and laboratory request forms. The Research Council Board of Samarra University's Faculty of Medical Laboratory Science gave its approval for the project.

Sample collection and transportation

A doctor collected three high vaginal swabs from each patient and examined them for sex hormones in Amie's transport medium. They were transported in under three hours to the Microbiology Laboratory Department of the University of Samarra's Faculty of Medical Laboratory Sciences.

Sample processing

Following submission to the Microbiology Laboratory, the samples were promptly analyzed for potential pathogenic microbe isolation and identification using conventional laboratory procedures (Abdul-Aziz *et al.*, 2019). One of the blood agar plates was filled with three high vaginal swabs from each patient. They were incubated aerobically for 48 hours at 37°C in a moist CO_2 environment. After that, each dish was placed in a sterile container containing 10 ml of freshly cooked beef medium (Al-Kafaween *et al.*, 2020 Al-Kafaween *et al.*, 2020a; Khleifat *et al.*, 2006; Khleifat *et al.*, 2019; Qaralleh *et al.*, 2019).

Diagnosis of BV

BV can be diagnosed clinically and/or microbiologically. The clinical diagnostic criteria published by Amsel *et al.* (1983) are still in use today. Three of the four criteria in Amsel's technique, a vaginal pH of more than 4.5, the presence of clue cells in the vaginal fluid, a milky, homogeneous vaginal discharge, and the emission of a fishy odor after adding 10% potassium hydroxide to the vaginal fluid, should all be documented (Al-kafaween *et al.*, 2021; Cheesbrough, 2000; Khleifat *et al.*, 2021).

Identification of genital microorganisms

To identify the bacteria, scientists employed the Gram stain, colony morphology, germ tube test, motility, and biochemical tests including catalase, coagulase, DNase, indole, lactose fermentation, citrate utilization, urease, Kligler Iron, oxidase, sugar fermentation, and an automated system (Al-Kafaween *et al.*, 2020b; Cheesbrough, 2000; Khleifat *et al.*, 2008).

Macroscopic and colonial morphology

The Nugent scoring method was used to diagnose BV using direct Gram stain smears (Brookheart *et al.*, 2019). The following morphotypes were found on the Gram smear: curved Gram-variable rods, small Gram-variable rods (*Gardnerella*
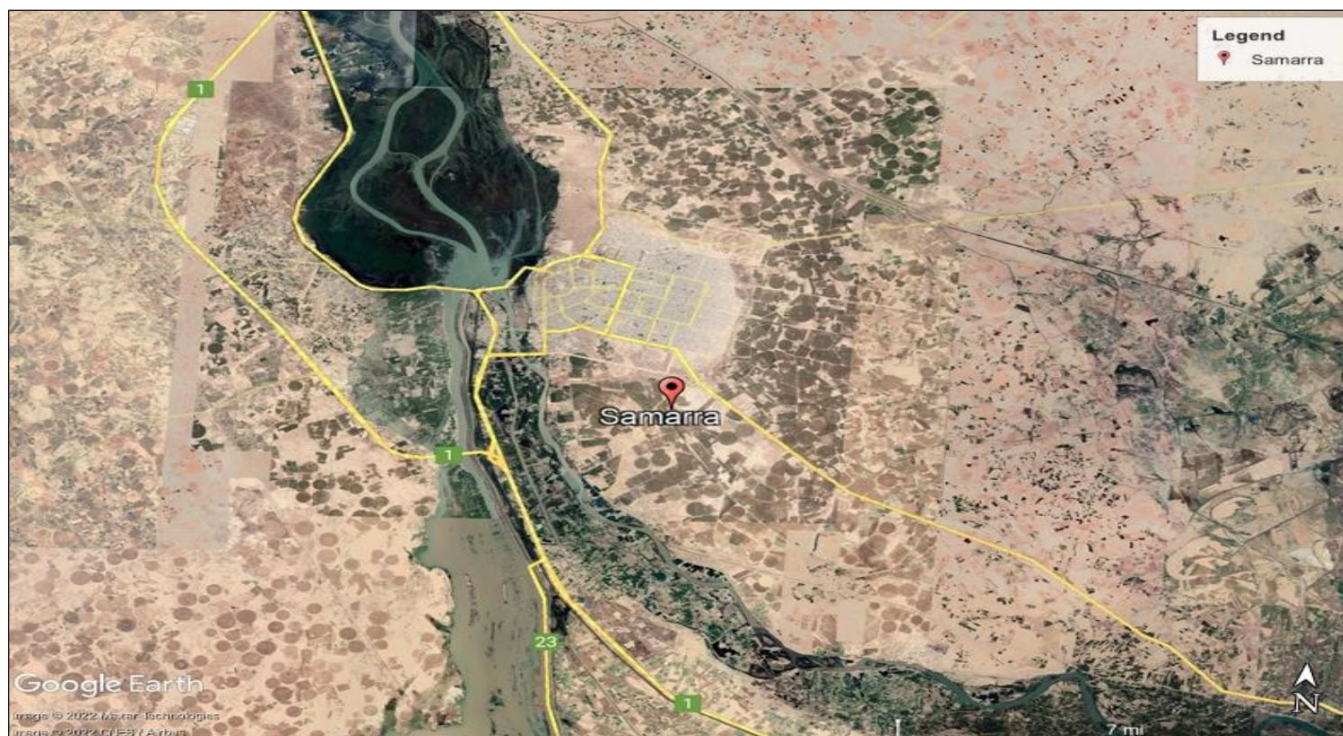
Figure 1. An image showing the areas of sample collection in the city of Samarra, Iraq.

vaginalis morphotypes), small Gram-negative rods (*Bacteroides* species morphotypes), large Gram-positive rods (*Lactobacillus* morphotypes), large Gram-negative rods (*Bacteroides* species morphotypes), and Gram-positive cocci (*Mobiluncus* species morphotypes). The pattern of typical vaginal flora does not include an increase in Gram-positive cocci. Normal was defined as a score of zero to three, while BV was defined as 4 to 10. Each initial positive culture yielded a single colony, which was identified using morphological characteristics (colony size, form, pigment hue, and nature). Bacterial smears stained with the Gram stain were used to analyze cellular morphological characteristics of bacterial cells, such as Gram response, form, organization, and spores. Biochemical tests were performed based on the Gram reaction (Khleifat and Abboud, 2003; Al-Kafaween *et al.*, 2020a; Al-kafaween *et al.*, 2021; Cappuccino and Welsh, 2018; Khleifat *et al.*, 2021; Al-kafaween *et al.*, 2021; Al-kafaween *et al.*, 2020b).

Biochemical tests

Indole test

This test was carried out by inoculating a peptone water medium with bacterial growth from the loop for 24–48 hours at 37°C. Drops of Kovac's reagent were added to the indole test (*p*-dimethylaminobenzaldehyde in amyl alcohol). A red color ring formed at the top of the soup, indicating a good response (Brown and Smith, 2014).

Methyl red test

Select bacterial colonies were injected into tubes of an MR-VP broth, which were then incubated at 37°C for 24–48 hours. After that, it was given five drops of methyl red reagent.

Good resultant full glucose hydrolysis is indicated by the presence and observation of a red color (Brown and Smith, 2014).

Voges–Proskauer test

Selected bacterial colonies were injected into the MR-VP broth tubes, which were then incubated at 37°C for 24–48 hours. After that, 0.6 ml alpha naphthol (reagent A) and 0.2 ml 40% KOH solution were added to the result (reagent B). The presence of acetoin or acetyl-methyl-carb after 15 minutes indicates a good result owing to partial hydrolysis of glucose, which produces acetoin or acetyl-methyl-carbinol (Brown and Smith, 2014).

Citrate utilization test

The bacterial colonies were inoculated and cultured for 24–48 hours at 37°C after the Simmons citrate slants were autoclave-sterilized. The organisms were able to use citrate as their only carbon source, as evidenced by the shift in medium color from green to blue (Abboud *et al.*, 2010; Cappuccino and Welsh, 2018; Tarawneh *et al.*, 2009).

Urease test

By inoculating the urea medium with bacterial growth, this test was done. At 37°C, the tubes were cultured for 24–48 hours. A good outcome was signified by the medium becoming pink (Al-Asoufi *et al.*, 2017; Cappuccino and Welsh, 2018; Khleifat *et al.*, 2021; Al-kafaween *et al.*, 2020a).

Motility test

This test was carried out by stabbing tested bacteria into a tube containing a semisolid medium and incubating it at 37°C for 24–48 hours. The spread of development beyond the stab line

was a sign of a successful outcome (Al-Kafaween *et al.*, 2020b; Tille, 2015).

Catalase test

Catalase is an enzyme that catalyzes the oxidation of hydrogen peroxide to produce oxygen. The chosen bacterial colonies were streaked on a nutritional agar medium and cultured at 37°C for 24 hours, after which the growth was transferred to a clean slide with a drop of 3% H_2O_2. A good outcome is indicated by the production of gas bubbles (Cappuccino and Welsh, 2018).

Oxidase test

This test requires the presence of a bacterial oxidase enzyme that catalyzes the transfer of electrons between electron donors in the bacteria and a redox dye (tetramethyl-*p*-phenylene-diamine dihydrochloride), which was reduced to a deep purple color. A strip of filter paper was soaked in a small amount of fresh-ly prepared reagent, and with a sterile wooden stick, the colony to be tested was picked up and spread on the filter paper. A bright deep purple color developed within 5–10 seconds, indicating a good outcome (Cappuccino and Welsh, 2018).

VITEK 2 compact system

This device was used to confirm isolates after diagnosing them in the usual ways. It is used to diagnose most types of bacteria through a diagnostic kit for the device. The kit contains 47 pits inoculated with a bacterial suspension of 24 hours and incubates for 24 hours, and the device records the color changes that occur as a result of the growth of bacteria.

Antibiotic susceptibility testing

All bacteria isolates were tested for susceptibility using the Clinical and Laboratory Standards Institute (CLSI) 2020 modified disc diffusion technique. Antibiotic resistance was evaluated using the CLSI 2020 standards and the six antibiotic discs AMC, AZM, DA, CN, LEV, and MET. The inoculums for this test were made by diluting the suspension with 0.5 McFarland standard tubes after adding the isolated colonies formed on a blood agar plate to 5 ml of a nutrient broth and incubating at 35°C for 18 hours (Tarawneh *et al.*, 2021; Tarawneh *et al.*, 2022). A swab of the growth was taken from a diluted solution and streaked across the surface of the medium multiple times on Muller-Hinton agar plates. Using a pair of sterile forceps, antibiotic discs were placed on each plate. The discs were positioned 15 mm from the plate's edge on a 15 cm plate. To ensure even contact with the medium, each disk was gently pushed into place. For 18–24 hours, the plates were kept at 37°C in an incubator. A ruler was used to measure the diameter of each zone (including the disc's diameter), and the results were recorded in mm. The results were then interpreted according to CLSI (Al-Essa *et al.*, 2021; Al-kafaween *et al.*, 2021; Al-Kafaween *et al.*, 2020a, 2020b; Clinical and Laboratory Standards Institute, 2017; Khleifat and Abboud, 2003; Mahmoud *et al.*, 2020; Tarawneh *et al.*, 2021, 2022).

Sex hormones assay

Estradiol and progesterone were measured using the Estradiol Test System and the Progesterone Test System (Monobind Inc., USA). These were performed in microplate wells, and the absorbance at 450 nm was recorded after 30 minutes of adding the stop solution according to the manufacturer's instructions.

Statistical analysis

The results were statistically analyzed using the SPSS statistical program by applying the analysis of variances test and chi χ^2 test, and the significant differences were compared using the Ducum test with multihull rouge probability levels of 0.05 and 0.01.

RESULTS AND DISCUSSION

Isolation and identification

In Samarra, Salah al-Din Governorate, Iraq, 100 high vaginal swabs were isolated from infected and healthy women and compared based on bacterial type (Fig. 1). The results showed that only 95 of the samples were positive cultures (95%), where 70 (70%) were infected and 25 (25%) were healthy, while 5 (5%) samples were negative cultures where 1 (1%) was infected and 4 (4%) were healthy. As shown in Figure 1, the statistical analysis revealed no significant differences in infection between infected and healthy people. There is only one reason for do not grow in negative samples because the patient may have taken antibiotics before they were given the sample, despite the fact that the verification of antibiotic doesn't quite absorbed before the sample is taken or may return the cause of another type of influential factors of infection, which is required to do some other techniques to identify such as virus, mycoplasma, chlamydia, and others. Our findings are consistent with previous studies in Iraq and Afghanistan (Razzak *et al.*, 2011; Wójkowska-Mach *et al.*, 2021), which revealed that 95% and 91.32%, respectively, of infected-healthy women had vaginitis and the results were bacterial culture positives and 5 samples gave culture negatives.

Other investigators, however, reported a lower percentage of infections from Erbil and Basrah (Iraq) reporting rates of 68.3% and 67.6%, respectively (Khalid and Yassin, 2017; Lhwak and Abbas, 2018). The high rate of positive BV cultures in our study is most likely due to the fact that we chose only women with complaints of abnormal vaginal discharge and vaginitis, and the differences between our results and others could be due to sample size, and our target populations were selected by physicians of only women patients with symptoms of vaginitis such as abnormal vaginal discharge, itching, burning, and lower abdominal pain.

The chi square (χ^2) test was used to find out statistical differences. *p* values less than 0.010 were considered statistically significant (*), whereas those more than > 0.010 were not. Statistical analysis showed that in Table 1 the incidence of microorganisms isolated showed culture positive 70 (70%), 12 (12.63%) were Gram-positive bacteria, while Gram-negative

Table 1. The incidence of Gram-positive and Gram-negative bacteria in vaginal infection in relation to infected and healthy women.

Group		No. of culture positives	Gram-positive bacteria	Gram-negative bacteria
Infected women	No.	70	12	58
	%	73.68*	12.63*	61.05*
Healthy women	No.	25	15	10
	%	26.31*	15.78*	10.52*
Total	No.	95	21	
	%	100*	22.105*	77.89*

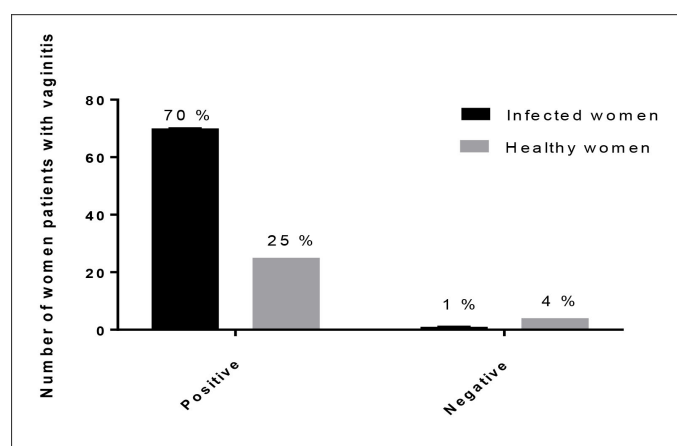*Pearson's χ^2 = 3.804, *p* value = 0.051.

Figure 2. Distribution of women patients with vaginitis in relation to infected and healthy women. Pearson's chi square = 6.649, *p* value = 0.010.

bacteria were 58 (61.05%) in infected women significantly higher compared to that from healthy women 15 (15.78%) were Gram-positive bacteria, while Gram-negative bacteria were 10 (10.52%) (*p* < 0.051). Our findings contradicted the findings of Essa and Hussein (2018) in Mosul (Iraq) and Erbil (Iraq), who found that the majority of vaginitis infections had Gram-positive bacterial infection.

The number and percentage of microorganisms isolated from 95 patients with vaginitis and the distribution of isolated microorganisms among infected and healthy women with vaginitis are shown in Table 2 and Figure 2. The most common organisms isolated belonged to Gram-negative bacteria and were *Escherichia coli* at 16 (16.84%): in infected women 9 (9.47%) and healthy women 7 (7.36%). Other results from Pakistan (13.7%) that were consistent with ours showed the same type of bacteria and the same percentage (Mumtaz *et al.*, 2008). The fact that this bacterium is part of the normal fecal flora and that different virulence factors contribute to their pathogenicity may explain the high prevalence of these bacteria in bacterial vaginitis, and the difference in results may be explained by the number of samples taken and the period (year) of the study. The second most common isolated

microorganisms from women with vaginitis were *Proteus vulgaris* at 8 (8.42%), *Acinetobacter* at 7 (7.36%), *Proteus mirabilis* at 7 (7.36%), *Lactobacillus* at 6 (6.31%), and *Citrobacter* at 6 (6.31%). The highest percentages of the isolates belonged to Gram-positive bacteria and were *Staphylococcus saprophyticus* at 5 (5.26%) and *Staphylococcus aureus* at 4 (4.21%) followed by *Enterococcus faecalis* at 4 (4.21%), *Bacillus stearothermophilus* at 3 (3.15%), *Bacillus megaterium* at 3 (3.15%), *Enterococcus faecium* at 1 (1.05%), and *Leuconostoc pseudomesenteroides* at 1 (1.05%). A study in Al-Diwaniya revealed high prevalence of isolated *S. aureus* (5.6%) and *S. saprophyticus* (4.8%) (Ranjit *et al.*, 2018). These findings contradicted a previous study conducted in Mosul, Iraq, which found that the prevalence of *S. saprophyticus* infection was 1.9 % (Khudhur Mohammed and Jawad, 2019). Most female vaginal infections are caused by bacteria that are endogenous to the female genital tract, as evidenced by the results presented above (Arianpour *et al.*, 2009).

The composition of the vaginal ecosystem is not static but changes over time and in response to endogenous and exogenous influences (Eschenbach *et al.*, 2001). The vaginal canal of women in labor has a vital role in the health of their newborns, including establishing their early gut flora (Gabriel *et al.*, 2018). Pathogens found in the blood of newborns with early-onset sepsis were found to be significantly linked to maternal vaginal infections with *E. coli* or *S. aureus* in a recent study (Kim *et al.*, 2017). The relation between the age of the patient and the highest percentage of infection was detected in the age group <30 years at 36 (37.89%) while the least infected age group was 60 and above at 2.1%. In Figure 3, statistical analysis showed that in a positive culture the incidence of infection in the age group < 30 years was significantly higher compared to other age groups at *p* < 0.008. Similar results (Dhabhai *et al.*, 2022) from India showed the highest prevalence of infection in ages > 30 years. The findings of this study agreed with those of Lamichhane *et al.* (2014) from Nepal, who found the highest prevalence of infection in the age group 20–29 years. Vaginal infections were found to be most common in young, sexually active females. The different patterns of infection observed in this study could be explained by current conditions in each country, such as health education, cleanliness, and medical coverage.

Table 2. Frequency of microorganisms from vaginal samples in infected and healthy women.

Stai	Bacteria isolated from HVS	Infected women (70)		Healthy women (25)		Total	
		No.	%	No.	%	No.	%
G⁺ᵛ	*Staphylococcus aureus*	3	3.15	1	1.05	4	4.21
	Staphylococcus saprophyticus	4	4.21	1	1.05	5	5.26
	Bacillus stearothermophilus	0	0	3	3.15	3	3.15
	Bacillus megaterium	0	0	3	3.15	3	3.15
	Enterococcus faecalis	3	3.15	1	1.05	4	4.21
	Enterococcus faecium	1	1.05	0	0	1	1.05
	Leuconostoc pseudomesenteroides	1	1.05	0	0	1	1.05
	Lactobacillus	0	0	6	6.31	6	6.31
	Escherichia coli	9	9.47	7	7.36	16	16.84
	Acinetobacter	7	7.36	0	0	7	7.36
	Proteus vulgaris	8	8.42	0	0	8	8.42
	Proteus mirabilis	5	5.26	2	2.10	7	7.36
	Aeromonas	2	2.10	1	1.05	3	3.15
	Pseudomonas fluorescens	3	%3.15	0	0	3	3.15
	Clostridium sporogenes	2	2.10	0	0	2	2.10
G⁻ᵛ	*Citrobacter*	6	6.31	0	0	6	6.31
	Klebsiella	5	5.26	0	0	5	5.26
	Serratia	5	5.26	0	0	5	5.26
	Methylobacterium	1	1.05	0	0	1	1.05
	Burkholderia cepacia	1	1.05	0	0	1	1.05
	Sphingomonas paucimobilis	3	3.15	0	0	3	3.15
	Rhizobium radiobacter	1	1.05	0	0	1	1.05
	Total	70	73.68**	25	26.31**	95	100**

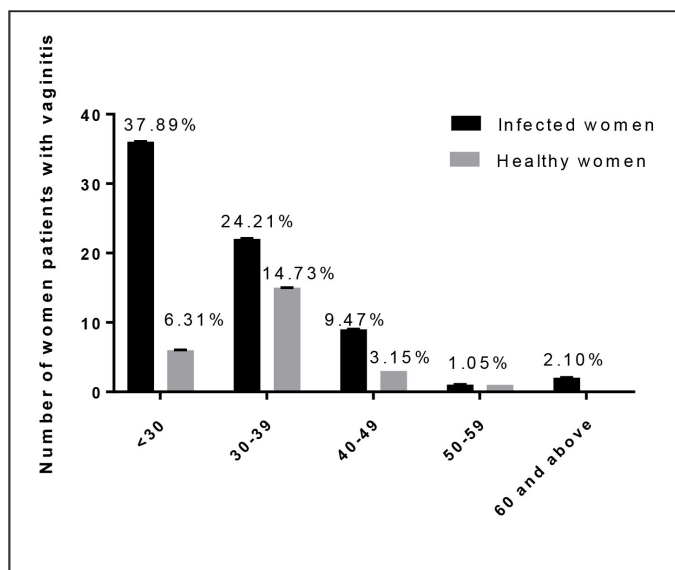** Pearson's $\chi^2 = 52.027$, *p* value = 0.0009.

Figure 3. Relation between age groups and positive culture of vaginal swabs in infected and healthy women. **Pearson's $\chi^2 = 8.299$, *p* value = 0.008.

Antibiotic sensitivity

Nineteen –five isolated thereafter were subjected to susceptibility testing according to the CLSI 2020 guidelines using different antibiotics, namely, levofloxacin, azithromycin, amoxicillin, clavulanic acid, clindamycin, metronidazole, and gentamicin, as shown in Table 3. Gram-positive bacteria like *S. aureus*, *S. saprophyticus*, and *B. stearothermophilus* were highly sensitive to levofloxacin, azithromycin, and gentamicin but highly resistant to amoxicillin-clavulanic acid, clindamycin, and metronidazole. The bacterium *E. faecalis* was found to be sensitive to levofloxacin, clindamycin, and gentamicin but not to amoxicillin-clavulanic acid, metronidazole, or azithromycin. *Enterococcus faecium* was found to be sensitive to amoxicillin-clavulanic acid, metronidazole, clindamycin, and gentamicin but sensitive to levofloxacin and azithromycin.

Gram-positive bacteria *S. aureus, S. saprophyticus*, and *B. stearothermophilus* showed high sensitivity to levofloxacin, azithromycin, and gentamicin and high resistance to amoxicillin-clavulanic acid, clindamycin, and metronidazole. *Enterococcus faecalis* showed high sensitivity to levofloxacin, clindamycin,

Table 3. Antibiotic susceptibility patterns of the bacteria isolated from vaginitis cases.

Bacteria isolated from HVS	AMC	AZM	DA	CN	LEV	MET
Escherichia coli	R	R	R	R	I	R
Acinetobacter	R	R	R	R	S	R
Bacillus stearothermophilus	R	S	R	S	S	R
Bacillus megaterium	R	R	R	R	S	R
Proteus vulgaris	R	R	R	R	S	R
Proteus mirabilis	R	R	R	S	S	R
Aeromonas	R	R	R	R	R	R
Staphylococcus aureus	R	S	R	S	S	R
Staphylococcus saprophyticus	R	S	R	S	S	R
Pseudomonas fluorescens	R	R	R	R	S	R
Clostridium sporogenes	R	S	R	S	S	R
Citrobacter	R	R	R	S	R	R
Klebsiella	R	S	R	R	I	R
Enterococcus faecalis	R	S	S	S	S	R
Enterococcus faecium	R	S	R	R	S	R
Serratia	R	R	S	R	R	R
Leuconostoc pseudomesenteroides	R	R	R	R	S	R
Methylobacterium	R	R	R	R	S	R
Burkholderia cepacia	R	R	S	S	R	R
Sphingomonas paucimobilis	R	R	R	R	S	R
Rhizobium radiobacter	R	S	S	S	S	R

I: intermediate; S: sensitive; R: resistant.

and gentamicin and high resistance to amoxicillin-clavulanic acid, metronidazole, and azithromycin. *Enterococcus faecium* showed high sensitivity to levofloxacin and azithromycin and high resistance to amoxicillin-clavulanic acid, metronidazole, clindamycin, and gentamicin. Gram-negative bacterial isolates: *E. coli* was intermediately resistant to levofloxacin and highly resistant to amoxicillin-clavulanic acid, metronidazole, azithromycin, clindamycin, and gentamicin. *Acinetobacter*, *P. fluorescens*, *Methylobacterium*, *P. vulgaris*, and *Sphingomonas paucimobilis* were found to be highly sensitive to levofloxacin and highly resistant to amoxicillin-clavulanic acid, metronidazole, azithromycin, clindamycin, and gentamicin. *Proteus mirabilis* was sensitive to levofloxacin and gentamicin but resistant to amoxicillin-clavulanic acid, metronidazole, clindamycin, and azithromycin.

Aeromonas showed high resistance to amoxicillin-clavulanic acid, metronidazole, levofloxacin, gentamicin, clindamycin, and azithromycin. *Clostridium sporogenes* showed high sensitivity to levofloxacin, gentamicin, and azithromycin and high resistance to amoxicillin-clavulanic acid, metronidazole, and clindamycin. *Citrobacter* was sensitive to gentamicin but resistant to amoxicillin-clavulanic acid, metronidazole, levofloxacin, clindamycin, and azithromycin. *Klebsiella* was highly sensitive to azithromycin, intermediate to levofloxacin, and extremely resistant

to amoxicillin-clavulanic acid, metronidazole, gentamicin, and clindamycin. *Serratia* was sensitive to clindamycin but resistant to amoxicillin-clavulanic acid, levofloxacin, metronidazole, gentamicin, and azithromycin. *Burkholderia cepacia* was sensitive to clindamycin and gentamicin but resistant to amoxicillin-clavulanic acid, levofloxacin, metronidazole, and azithromycin. *Rhizobium radiobacter* was extremely sensitive to clindamycin, levofloxacin, gentamicin, and azithromycin but extremely resistant to amoxicillin-clavulanic acid and metronidazole. All of the isolated bacteria were sensitive to levofloxacin but resistant to metronidazole and amoxicillin-clavulanic acid.

We believe that the varied susceptibility of bacteria to the investigated antibacterials is due to the random and widespread administration of these antibacterials, which leads to the development of bacterial resistance and virulence factors with a high ability to avoid antibacterial effects (Brocklehurst *et al.*, 2013; McDonald *et al.*, 2003). These findings suggest an increase in resistance to antibacterial drugs commonly used to treat vaginitis, as well as a public health concern about the rate at which bacteria develop resistance to antibacterial treatments. As a result, bacteriological culture and sensitivity testing must be used on a regular basis in the treatment of bacterial vaginitis.

Table 4. The relation between the isolated bacteria and sex hormone levels (estrogen and progesterone).

Bacterial isolation	Mean ± S.D	
	Estrogen	Progesterone
Escherichia coli	91.40 ± 8.5	5.85 ± 1.39
Acinetobacter	38.80 ± 4.4	0.04 ± 0.024
Bacillus stearothermophilus	71.60 ± 4.1	23.30 ± 3.6
Bacillus megaterium	28.50 ± 2.9	3.78 ± 0.45
Proteus vulgaris	63.10 ± 8.5	1.79 ± 0.526
Proteus mirabilis	29.2 ± 3.44	4.53 ± 1.60
Aeromonas	82.90 ± 7.1	39.30 ± 5.2
Staphylococcus aureus	398.00 ± 29.7	3.09 ± 1.20
Staphylococcus saprophyticus	106.50 ± 13.6	1.19 ± 0.886
Lactobacillus	477.00 ± 34.8	5.12 ± 1.82
Pseudomonas fluorescens	83.37 ± 3.50	79.30 ± 12.67
Clostridium sporogenes	15.96 ± 0.522	1.22 ± 0.0346
Citrobacter	70.20 ± 7.3	5.99 ± 1.74
Klebsiella	108.30 ± 24.6	0.88 ± 0.083
Enterococcus faecalis	101.60 ± 20.7	1.32 ± 0.470
Enterococcus faecium	6.11 ± 1.04	0.76 ± 1.74
Serratia	47.20 ± 8.8	5.33 ± 1.83
Leuconostoc pseudomesenteroides	52.86 ± 10.60	8.91 ± 1.60
Methylobacterium	79.62 ± 18.60	14.07 ± 1.72
Burkholderia cepacia	955.50 ± 25.60	3.74 ± 1.30
Sphingomonas paucimobilis	140.00 ± 9.2	6.66 ± 2.95
Rhizobium radiobacter	18.98 ± 3.60	0.37 ± 0.04
$p \leq$	0.023	0.041

Furthermore, appropriate healthcare measures that keep women's vaginas healthy and human public health are required.

The relation between age of patient and sex hormones levels (estrogen and progesterone)

The statistical analysis showed significant differences between the age groups of women and sex hormone levels (estrogen and progesterone). The high rate of estrogen was in the age group of 30–39 years, while the high rate of progesterone was in the age of 40–49 years; the estradiol level was negatively correlated with age, as shown in Figure 4. This result is in agreement with another result (Lei *et al.*, 2021), where it was found that the estradiol level was negatively correlated with age. It is important to mention that sex hormone levels are affected by many factors, including age and health status, among which age is considered the main factor (Del Río *et al.*, 2018). According to our research's epidemiological data, approximately 20% of girls would therefore experience severe depression at some point in their lives. Premenstrual, perinatal, and premenopausal phases, for example, can all lead to

or worsen depression in some women (Bromberger and Epperson, 2018). According to new research, sex hormones play a role in the physiological and pathological development of depression in women (Lei *et al.*, 2021; Slavich and Sacher, 2019). Nonetheless, more research into the relationship between depression and female sex hormones is required.

The relation between isolated bacteria and sex hormone levels (estrogen and progesterone)

As shown in Table 4, statistical analysis revealed significant differences in the type of bacteria isolated and sex hormone levels (estrogen and progesterone), indicating that sex hormone levels vary depending on the type of bacteria. *Burkholderia cepacia* and *Lactobacillus* had the highest estrogen rates, while *P. fluorescens* and *Aeromonas* had the highest progesterone rates. This study's findings are similar to previous studies in that they tend to establish a link between hormone levels and vaginal bacteria diversity (Kaur *et al.*, 2020). Hormonal contraception has been linked to a lower risk of presenting BV
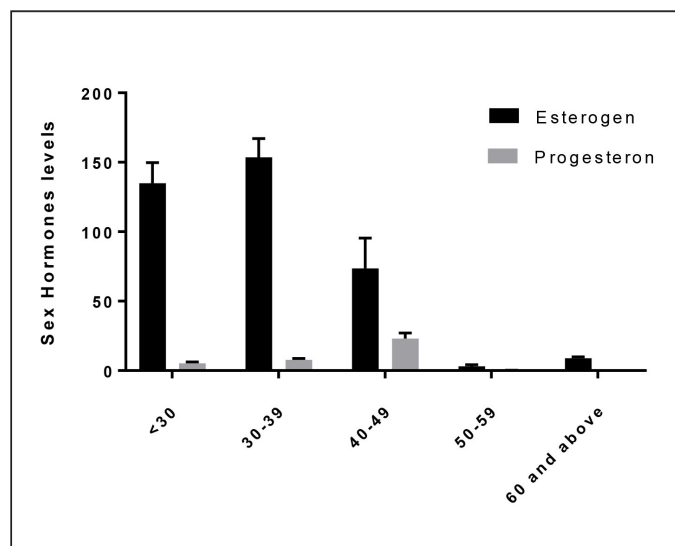
Figure 4. The relation between the age of women and sex hormone levels (estrogen and progesterone). $p \leq 0.050$ estrogen, $p \leq 0.037$.

in the same age group of women because it increases estrogenic stability (Romero *et al.*, 2014). In postmenopausal women, a decrease in estrogen causes a rise in pH, allowing gut bacteria to proliferate (Neggers *et al.*, 2007). Although most studies on the link between contraception and BV have been cross-sectional, a recent longitudinal study of 3,077 reproductive-age women examined whether hormonal contraception use was associated with BV diagnosis over the course of a year (Riggs *et al.*, 2007). Injectable hormonal contraceptives (Depo-Provera) may aid in preventing pH imbalances (Brabin *et al.*, 2005). Vaginal pH is known to have an impact on vaginal flora. Estradiol and progesterone also regulate the innate and adaptive immunity of the female reproductive tract (Beagley and Gockel, 2003). Researchers examined estrogen and progesterone levels in women at various stages of their reproductive and menopausal lives to see if there was a relationship between circulating gonadal hormones and vaginal microbial diversity. The main objective of this data collection was to generate a preliminary framework and hypothesis to explain observed differences in microbial diversity across a variety of reproductive and menopausal (sub)phases. This study's findings appear to suggest a link between hormone levels and microbial diversity in the vaginal canal. For example, gonadal maturation causes a rise in gonadal hormone levels during the start of puberty (Tanner stage II) (Auriemma *et al.*, 2021). In this period, vaginal bacterial diversity is also increased, as previously mentioned. Although gonadal hormone levels grow during puberty (Tanner stages II–V), bacterial diversity decreases. The surge in hormone levels that occurs at the start of puberty is likely to result

in increased glycogen accumulation in the vaginal walls. As a result, vaginal bacteria will have an ample supply of resources to grow and multiply. As gonadal maturation continues, hormone-stimulated glycogen synthesizes acids in the development of the *Lactobacillus* community in the vaginal canal. Lactic acid lowers the pH of the vaginal environment, preventing the growth of other anaerobic bacteria. As a result, despite increased progesterone/estrogen levels, the latter stages of puberty are characterized by limited bacterial diversity.

This hormone surge causes an overabundance of glycogen to be deposited in the vaginal epithelial walls, resulting in a rise in microbial diversity (Auriemma *et al.*, 2021; Mirmonsef *et al.*, 2014). The hostile reaction triggered by *Lactobacillus* growth is likely to contribute to a vaginal environment that is less varied and stable. Interestingly, prior research has found that the variety of vaginal bacteria is reduced during the first trimester. Trimester of preterm deliveries, despite the increased bacterial diversity in terms of results (Haque *et al.*, 2017). Given the link between hormone levels and vaginal microbial variety, the absence of vaginal microbial diversity in preterm pregnancies is most likely due to a severe hormonal imbalance and/or an impaired mother immunological response. Furthermore, the vaginal microbiome of pre- and perimenopausal women is less varied, with *Lactobacillus* dominating. However, the postmenopausal group has a higher level of variety. Menopause is marked by a drop in the levels of circulating gonadal hormones (Yun *et al.*, 2022). As a result, reduced hormone levels in the postmenopausal stage are likely to lead to a drop in *Lactobacillus* colonization and, as a result, an increase in anaerobic microbial communities in the vaginal canal. Surprisingly, hormone surge periods are linked with greater vaginal microbial diversity. During these times, the vaginal microbiota's composition shifts from anaerobic bacteria to a *Lactobacillus*-dominated community (puberty, pregnancy, and menopause) according to previous experimental findings. Estrogen hormone replacement treatment has been shown to enhance *Lactobacillus* colonization in the vaginal environment in postmenopausal women (Vitali *et al.*, 2017).

CONCLUSION

The study showed that microbiome diversity in the vagina VMB depends on the level of hormones in women in the Iraq region. The high rate of estrogen was in the age group 30–39 years, while the high rate of progesterone was in the age group 40–49 years; the estradiol level was negatively correlated with age. The statistical analysis showed significant differences between the age groups of women and sex hormone levels (estrogen and progesterone). As it is known, the formation of vaginal microbiota is completely continuous, but it may be affected by several factors such as diet, behavior, psychological state, and other requirements of life.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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AUTHORS' CONTRIBUTION

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

ETHICAL APPROVAL

The ethical statement for this study was approved by the Ethical Committee of Samarra Hospital [Reference No. 2020/20] in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All experiments were performed in accordance with the approved guidelines.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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