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Molecular and Microbiological Characterization of Aerobic Vaginitis Among Women of Reproductive Age in Basrah, Iraq

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ABSTRACT

This study assessed the microbial and molecular aspects of aerobic vaginitis (AV) in women of reproductive age who visited Obstetrics and Gynecology Clinics in Basrah, Iraq, in 2024. Symptomatic women contributed 100 vaginal swab samples, in addition to 50 control samples. For bacterial characterization, traditional culture and PCR assays for the 16S rRNA gene were applied. Among patients, 93% had positive bacterial infections, which included *E. faecalis* (36.9%), coagulase-negative staphylococci (19.8%), *E. coli* (8.5%), and K. *pneumoniae* (3.4%). Furthermore, the bacterial profile indicated the presence of aerobic vaginitis, which suggested the presence of classic bacterial vaginosis, since it was devoid of anaerobic BV-associated organisms. *E. faecalis* and coagulase-negative staphylococci were the most predominant bacterial isolates in culture. Most at-risk socio-demographic groups were 16-35 years of age. The results indicate that frequent vaginal douching, past infections, and wrong use of antibiotics are some of the main causes. More educated people seem to be less likely to get sick. The findings recommend a focus on public health education to deter detrimental vaginal hygiene practices and encourage appropriate reproductive care, consequently diminishing the prevalence of vaginal dysbiosis and its related reproductive complications.

1. Introduction

Bacterial vaginosis (BV) is a complex medical condition that occurs when the normal vaginal lactobacilli are altered. The vaginal ecology of healthy women is typically balanced, as Lactobacillus species are the most prevalent type of bacteria [1,2]. By producing organic acids and other antibacterial substances, these bacteria inhibit the growth and spread of different germs [3,4,5]. When there are fewer Lactobacillus species in the vagina, anaerobic bacteria can proliferate [6]. Additionally, anaerobic bacteria, such as *Gardnerella vaginalis*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Mobiluncus species*, and *Prevotella species*, proliferate excessively [7, 8, 9].

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Microbes such as *Candida albicans, E. coli, Staphylococcus epidermidis, Pseudomonas aeruginosa, Lactobacillus spp., Klebsiella pneumoniae, Enterococcus faecalis, and Streptococcus spp. are often associated with vaginitis [10, 11, 12*].

BV is the most prevalent vaginal illness that affects women of childbearing age [9]. Some of the clinical signs include thin, uniform vaginal discharge; a foul odor, a fishy smell; the presence of clue cells, lactobacilli, and polymorphonuclear leukocytes [13,14]. BV has been linked to adverse outcomes during pregnancy, such as miscarriage, membranes breaking too soon, preterm birth, and low birth weight [15]. The huge development of vaginal anaerobes is linked to the increased synthesis of proteolytic carboxylase enzymes. These enzymes break down vaginal peptides into amines, which become volatile and smell noxious at high pH, notably trimethylamine. Bacteria that cause BV first stick to the vaginal epithelium, then multiply, and finally form a thick biofilm on the cells that make up the vaginal epithelium [16]. This biofilm makes the infection difficult to treat because it creates a favorable home for anaerobic bacteria. and preterm birth (PTB). The advancement of culture-independent molecular methodologies, including broad-range 16S ribosomal DNA (rDNA) PCR, quantitative real-time PCR (qPCR) assays, denaturing gradient gel electrophoresis (DGGE), and 454 pyrosequencing, has enhanced the identification of uncultivable anaerobes, such as BVAB1, BVAB2, BVAB3, *Eggerthella* spp., *Megasphaera* type 1, *Leptotrichia* spp., and *Sneathia* spp., in BV-positive women [4]. Analysis of vaginal microbiota using culture-based approaches has frequently failed to account for several anaerobic species. Analyses using the 16S rRNA gene sequence have found more than one anaerobic species [17]. Any woman can experience the swelling, itching, burning, and pain that accompany a vaginal infection. women may develop an infection due to the weakening of the body's natural defenses, triggered by stress, anxiety, sleeplessness, a poor diet, or sexual intercourse with an infected partner [2]. If such microorganisms are strictly anaerobic and are non-recoverable or poorly recovered by conventional culture methods, they remain unidentifiable during culture analysis [11]. Other studies have employed broad-range *16S rRNA* gene PCR, a cultivation-independent method, to characterize the vaginal bacterial community. This molecular approach has discovered a large number of fastidious uncultivated bacterial species [9].

The present study aimed to determine the causative pathogens of bacterial vaginosis and their prevalence among women attending Basrah Women's and Children's Hospital for Obstetrics and Gynecology and outpatient clinics in Basrah City, and to detect the presence of some BV-related bacteria by molecular and bacteriological methods.

2. Materials and Methods

2.1. Data collection

This research was carried out from January 2024 to December 2024 in the Biology Department at Basrah University. The research advances following the clearance of the Institutional Ethics Committee at Basrah Women's and Children's Hospital for Obstetrics and Gynecology, as well as outpatient clinics in Basrah City. Sociodemographic and clinical data were collected from patients using a validated questionnaire, as illustrated in Appendix 1. The questionnaire consisted of multiple enquiries regarding various parameters, including residence, age, education, occupation, year of marriage, chronic illnesses, history of bacterial vaginosis, vaginal hygiene practices, and abortion, among others. The questionnaire also encompasses other clinical symptoms, including abnormal vaginal discharge, lower back discomfort, genital burning or itching, genital ulcers, dyspareunia, and dysuria according to [18].

Sample Collection and Processing

A total of 150 samples were collected, 100 samples from women diagnosed with vaginosis and 50 samples from healthy women patients as a control. All patients were between 18 and 50 years of age. The sample included high vaginal swabs from married females. A specialized gynecologist utilized Amies transport medium to acquire samples from the lateral posterior vaginal fornix experiencing moderate to severe vaginitis. Swabs are put into the upper vagina and twisted to collect exudate from both the upper and lower vaginal walls; an endocervical swab must also be obtained. A

vaginal speculum is required to obtain a clear view of the cervix, and the swab has to be inserted and rotated around the cervix's introitus before being withdrawn without contamination from the vaginal wall [19].

The samples were thereafter delivered immediately to the microbiology laboratory at Basrah University in accordance with established laboratory protocols. For the isolation and identification of bacteria, freshly prepared media (nutrient agar, MacConkey Agar, blood agar, chocolate agar, and mannitol salt agar) were used. The medium was inoculated with a swab and incubated aerobically for 24 hours at 37°C. Plates with no growth were re-incubated for 24 hours and were only classified as negative after that. All isolates were purified through sub-culturing, and Gram staining was done for the differentiation of isolated bacteria, which was later examined under 100X oil immersion. The isolated and purified bacteria were identified through their morphological and colonial characteristics. The remaining isolated bacteria were further identified using the VITEK-2 Compact system from bioMérieux. VITEK-2 cards were inoculated with less than 18 hours of freshly cultured bacteria, following the instructions provided by the manufacturer. Additionally, the swabs were frozen at -20°C for later use in molecular diagnosis [20,21].

2.2. Molecular diagnosis

High vaginal swabs underwent DNA extraction by following the guidelines set by the Wizard® Genomic DNA Purification Kit (Promega, USA). A Nanodrop spectrophotometer was then used to evaluate the concentration and purity of the DNA samples before proceeding to PCR. The DNA samples are stored at -20°C until the PCR assay. A PCR assay was then conducted to detect the bacteria associated with bacterial vaginosis using 16S rRNA-specific primers as outlined in Table 2. The PCR mixture volume for 16S rRNA amplification was 25 µL, comprising 13 µL of premaster mix (Bioneer, Korea), 1 µL of each primer (10 pmol), 2 µL of genomic DNA (5-50 ng/mL), and the remainder filled with nuclease-free water. The PCR thermocycler conditions were conducted as in the reference, as shown in Table 1 [21]. The conventional PCR thermocycler system was similar for each gene utilized for the detection of other bacterial species, as delineated in Figure 1. The PCR products were analyzed via electrophoresis on a 1.5% agarose gel for 45 minutes, followed by ethidium bromide staining and visualization under a UV transilluminator [22], as shown in Figure 2.

Table 1. Amplification conditions of 16sRNA genes

Steps	Temperature	Time	No. of cycles
Initial Denaturation	95C	5 min	1
Denaturation	95C	30sec	
Annealing	55C	30sec	35 cycles
Extension	72C	1min	
Final extension	72C	5min	1
Hold	4C	Forever	-

Table 2. Universal 16S rRNA primers.

Primers	Sequence of Primers	Length(bp)
27 F	5-AGAGTTTGATCCTGGCTCAG-3	20
1492 R	5-GGTTACCTTGTTACGACTT-3	19

2.3. Ethical approval

The ethical statement received approval from the ethics committee of the College of Science, University of Basrah. Moreover, implicit assent from each participant was obtained. Information obtained from the patient will be maintained with absolute confidentiality, encompassing all enquiries related to socio-demographic, reproductive, and sexual histories, behavioral characteristics, and clinical features.

3. Results and Discussion

Among 100 vaginal swabs collected from women patients attending Obstetrics and Gynecological governmental hospitals and outpatient clinics, 93 (93%) of them showed positive bacterial vaginosis according to microbiological analysis, as presented in Figure 1. At the same time, the rest 7 (7%) of the samples were represented as negative. The identified bacteria (*E. faecalis, E. coli*, and *Klebsiella*) can be described as aerobic and facultatively anaerobic and do not include the primary BV pathogens. The data gathered in this study and the comparisons made with various publications lead to the conclusion that the occurrence of bacterial vaginosis in this site confirms its classification as a BV endemic area. Our data lacks the hallmark anaerobic bacteria that define classic BV, as all of them are absent. Aerobic vaginitis is a clinically separate entity. It has an inflammation that is a reaction to the excess of aerobic bacteria, usually of intestinal origin. The results we obtained fit well with the microbiological profile of AV.

The microbiological identification results comprised the isolation of Gram-positive and Gram-negative bacteria. *Enterococcus faecalis* was the main causative agent, with 36.9% of the cases of bacterial vaginosis (BV) 65, followed by Coagulase-negative staphylococci (CoNS) with 19.8% (35) of the cases, and *E. coli* (15, 8.5%), *Klebsiella pneumoniae* (6, 8.96%), *Serratia marcescens* (2, 1.1%), and *Morganella morganii* (2, 1.1%). Some rare bacteria had the lowest infection rate of 1 (0.6%) each. Our study demonstrates a particular bacterial profile that is considerably different from the typical Bacterial Vaginosis (BV) profile.

The predominance of *Enterococcus faecalis* (36.9%) and Coagulase-negative staphylococci (19.8%) strongly indicates Aerobic Vaginitis (AV) rather than traditional BV. According to a 2022 study by Jahic *et al.*, published in Mater Sociomed, the causative agents of AV are primarily *Enterococcus faecalis, Escherichia coli*, Group B Streptococcus, and *Staphylococcus aureus*, with *E. faecalis* being the most frequently isolated pathogen, at approximately 31% [23].

The current study agrees with the principle of prevalence and in a higher prevalence rate than that obtained in Nepal by Ranjit *et al.*, with values of (24.4%, 24% and 33% respectively). The variation in the obtained results among published articles is considered a normal event, which may be due to several factors, such as geographic distribution, population size, data analysis, behavioral differences, and socioeconomic status as described by Ranjit *et.al.* (2018) [24]. Reasons for the High Prevalence of *E. faecalis* include its intrinsic resistance to multiple antibiotics, including cephalosporins and clindamycin. This resistance enables it to survive and proliferate when other bacteria are eliminated by antibiotic therapy; additionally, it can form a biofilm. Recent studies demonstrate that *E. faecalis* has exceptional biofilm-forming capabilities. A 2024 global meta-analysis found that biofilm-producing *E. faecalis* strains are increasingly prevalent in healthcare settings, with biofilms protecting them from antimicrobials and host immune responses [25].

Gut-Vaginal Translocation is the other reason; the gastrointestinal tract serves as a reservoir for *E. faecalis*. Poor hygiene practices, sexual activity, and anatomical proximity facilitate translocation from the intestinal tract to the vaginal environment. Vaginal Dysbiosis, the reduction or absence of protective Lactobacilli, creates an ecological niche that *E. faecalis* readily occupies. Furthermore, this bacterium thrives in the altered pH environment (>5.0) [26].

Coagulase-negative Staphylococci (CoNS), particularly *Staphylococcus epidermidis*, which constitutes 19.8% of isolates in the current study, this finding is consistent with the AV literature, that S. *epidermidis* accounts for 10-20% of the AV isolates. These bacteria are normally skin flora, but turn into pathogenic bacteria under specific circumstances, such as poor hygiene, tight clothing, and other factors that can encourage their pathogenicity. These opportunistic pathogens are primarily activated when normal vaginal flora is disturbed. They produce biofilms, which are pivotal in their persistence against treatment, and contribute to resistance to treatment [27].

The gram-negative bacteria most frequently isolated are *E. coli*, followed by *Klebsiella pneumonia*, which are 8.5% and 3.4% respectively. *E. coli* is the most common gram-negative pathogen in aerobic vaginitis; it can cross the intestines and vaginal border in cases of disrupted flora. Its pathogenic mechanisms involve gut-vaginal translocation via poor hygiene, opportunistic pathogenesis through disrupted lactobacilli flora, biofilm formation to ensure persistence, and the presence of virulence factors such as adhesins and toxins [28]. *Klebsiella pneumoniae* comprises 3.4% of cases and is a significant but somewhat less common opportunistic pathogen. This prevalence is due to the severe

dysbiosis and the antibiotic resistance linked to the opportunistic pathogenicity of *Klebsiella pneumoniae*. These factors are polysaccharide capsule protection and ESBL production, and biofilm formation. This condition leads to considerable risks of nosocomial infections [29]. Several enteric bacteria were found during our research, comprising *Klebsiella pneumoniae* (3.4%), *Morganella morganii* (1.1%), *Serratia marcescens* (1.1%), *Shigella* spp. (1.1%), *Citrobacter freundii* (1.1%), and *Salmonella enterica* (0.6%). The abnormal varietal composition of vaginal flora AV, as indicated here, and bacterial vaginosis BV differ in the bacterial profiles. AV flora also suggests a disruption of the vaginal barrier and disruption of the immune defenses, likely as a result of exposure in the healthcare setting, such as past antibiotic exposure, which can select for resistant bacteria [30].

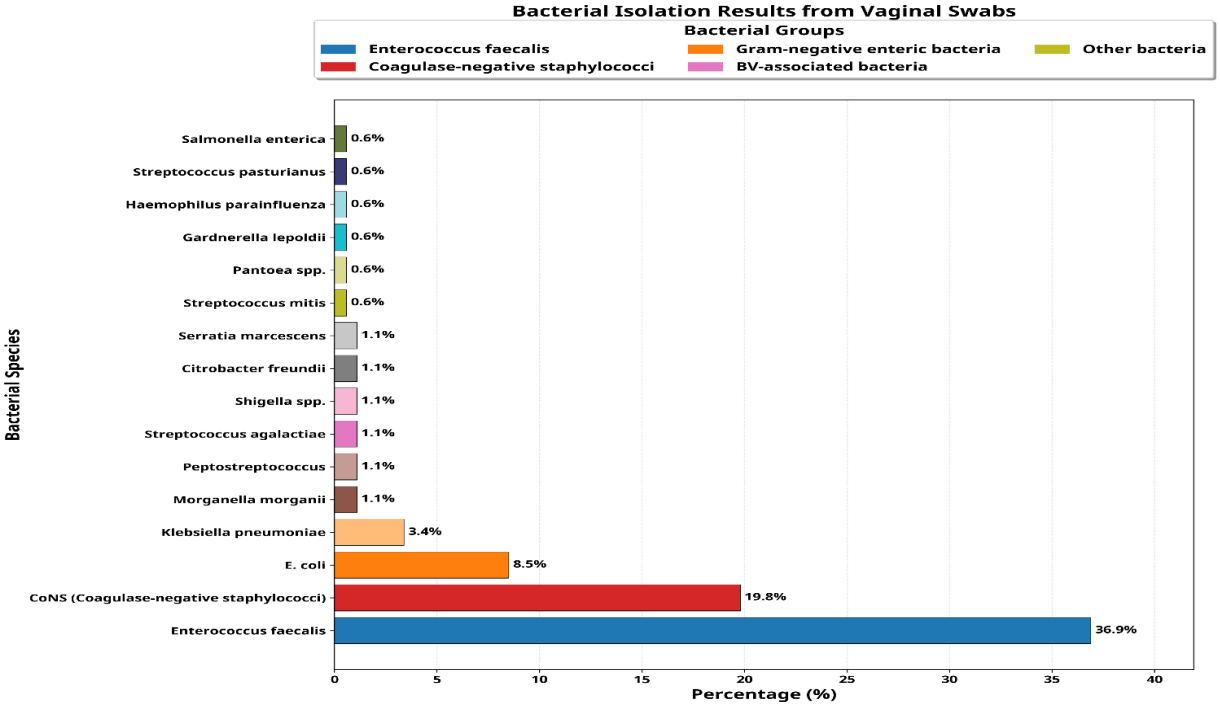

Fig. 1. Prevalence of bacterial vaginosis from all positive samples in the study

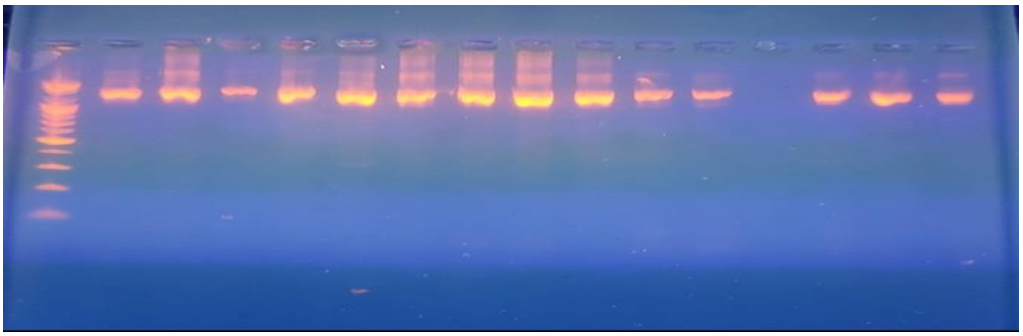

Fig. 2. T 2: Agarose gel electrophoresis image that showed PCR product analysis for the 16S rRNA gene (Marker ladder 2000-100bp)

The demographic and clinical profiles of our findings share notable similarities and discrepancies with the existing literature on the risk factors for bacterial vaginosis. Our cohort comprises mostly women in their reproductive years; 67% of our study population is between 16 and 35 years. This aligns closely with findings from Zahra *et al.* (2025) [31]. whom also identified women aged 25-30 as having the highest prevalence of BV in the sample population, setting it at 53%. The age distribution reflected in our research is consistent with this demographic since this appears to be the most predominant age range linked to the prevalence of bacterial vaginosis. This alignment is also consistent with other contemporary studies, which indicate women in the reproductive age

demographic are at the highest risk of acquiring bacterial vaginosis along with other sexually transmissible infections [32]. Our participants' educational background, with 83% literacy, is higher than in the more recent studies, which pointed to low education as a significant risk factor for BV. For instance, Kan *et al.* (2025) [33] noted that lower education or income was associated with higher Nugent-BV scores during pregnancy, while Chukwu *et al.* (2025) [34] demonstrated a considerable socioeconomic gradient in BV prevalence (OR = 3.10). Such results might account for the moderate BV prevalence observed during our research compared to findings where lower education and socioeconomic conditions were present. This indicates that some health literacy may explain the decreased prevalence of BV in our population.

The study population exhibited a high prevalence of behavioral risk factors. High-risk factors from more recent literature are a cause for concern. The 68% reported history of prior infections in our cohort also accounts for the association of recurrent vaginal infections and susceptibility to BV. Most concerning is the 62% prevalence of vaginal douching, which is higher than that reported in recent literature and is in direct alignment with the statement from WHO (2024) that "vaginal cleansing and douching can increase the risk of developing BV [35].

Wireko et al. (2024) found that 41% of cases link vaginal douching to the development of bacterial vaginosis (BV). CDC guidelines (2021) support this, suggesting that vaginal douching may increase the risk of BV recurrence. It is concerning that a large number of people in our study (31%) had recently taken antibiotics, which could be a cause of dysbiotic bacterial vaginosis [36].

Considering that 75% report abnormal vaginal discharge and 69% report symptoms such as odor, burning, pain, and itching, this population is mostly symptomatic and, therefore, seeking care, which explains the high identification levels for vaginal bacterial infections. This type of clinical presentation is also comparable to the findings of Gilbert *et al.* (2025) [37] concerning the impact of nutritional and immune factors and symptoms of BV. The 15% pregnancy rate in this population aligns with recent findings, including Sethi *et al.* (2025) [38], who indicate that the risk of developing BV during pregnancy was highest among women and takes into account the hormonal changes associated with pregnancy that affect the vaginal microbiome.

The 20% rate of previous miscarriages in this population may explain the increasing body of literature linking bacterial vaginosis (BV) to adverse pregnancy outcomes, particularly the recent findings associating BV with preterm birth and other pregnancy complications. Recent global literature demonstrates the multiple high-risk characteristics present in our study population, which explain the rising prevalence of BV [39]. The interplay of high rates of douching, antibiotics, and prior infections synergistically promotes vaginal dysbiosis and the subsequent onset of BV. The value of public health education campaigns emphasizing vaginal cleanliness is becoming increasingly evident. Douching is a significant modifiable risk factor for bacterial vaginosis. While public education on douching should be designed as risk communication, the gaps in risk factor education on douching, antibiotics, and integrated reproductive health care should be identified in targeted interventions, as Zhang *et al.* (2025) [40] and others have pointed out. Prioritizing engagement with the public and healthcare providers on these issues is crucial.

4. Conclusion

The study reveals that vaginal infections diagnosed as bacterial vaginosis in the examined population were consistent with aerobic vaginitis (AV), characterized by the predominance of Enterococcus faecalis and other aerobic intestinal bacteria. This differentiation is clinically significant as AV is resistant to metronidazole, the first line of treatment for BV, and requires a different treatment regimen. The high prevalence of AV among women in Basrah may be linked to behavioral factors such as vaginal douching, antibiotic misuse, and previous infections. Effective management of AV requires accurate diagnosis, targeted antibiotic therapy, and improved public awareness about maintaining vaginal health and avoiding harmful hygiene practices.

Appendix A: Socio-demographic parameters associated with the prevalence of bacterial vaginosis

characters	Frequency	characters	Frequency
Age		Chronic Diseases	
16-20	30 (30%)	Yes	18 (18%)
26-35	37 (37%)	No	82 (82%)
36-45	23 (23%)	Previous Infections	
>46	10 (10%)	Yes	68 (68%)
Place of Residence		No	32 (32%)
Urban	84 (84%)	Use of Douches, Vaginal, or Baths	62 (62%)
Rural	16 (16%)	Use of Certain Antibiotics or Medications	31 (31%)
Educational Attainment		Use of Contraceptives	32 (32%)
Literate	83 (83%)	Abnormal Vignale Discharge	75 (75%)
Illiterate	17 (17%)	Odor, Burning, Pain, Itching	69 (69%)
Marriage Age		Pregnancy	15 (15%)
1 to 5	30 (30%)	Previous miscarriage	20 (20%)
6 to 10	15 (15%)		
11 to 15	19 (19%)		
>15	36 (36%)		

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الخصائص الجزيئية والميكروبيولوجية لالتهاب المهبل الهوائي لدى النساء في سن الإنجاب في البصرة، العراق

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معلومات البحث

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الكلمات المفتاحية

التهاب المهبل الهوائي، *Enterococcus faecalis*، التهاب المهبل البكتيري، 16 S rRNA، النساء في سن الإنجاب، التشخيص الجزيئي.

الملخص

قيمت هذه الدراسة الجوانب الميكروبية والجزيئية لالتهاب المهبل الهوائي (AV) لدى النساء في سن الإنجاب اللواتي زرن عيادات التوليد وأمراض النساء في البصرة، العراق، في عام 2024. ساهمت النساء اللاتي يعانين من الأعراض بـ 100 عينة مسحة مهبلية، بالإضافة إلى 50 عينة تحكم. لتوصيف البكتيريا، تم استخدام طرق الزرع التقليدية واختبارات PCR لجين S rRNA16. بين المرضى، كان 93% منهم مصابين بعدوى بكتيرية إيجابية، والتي شملت *E. faecalis* (36.9%)، المكورات العنقودية السلبية التخثر (19.8%)، E. coli (8.5%)، و K. *pneumoniae* (3.4%). علاوة على ذلك، أشار الملف البكتيري إلى وجود التهاب المهبل الهوائي، مما يوحي بوجود التهاب المهبل البكتيري الكلاسيكي، حيث كان خاليًا من الكائنات الحية المرتبطة بالتهاب المهبل البكتيري اللاهوائي. كانت بكتيريا *E. faecalis* والعنقوديات السالبة للتخثر هي الأكثر انتشارًا في الثقافة. كانت الفئات الاجتماعية-الديموغرافية الأكثر عرضة للخطر تتراوح أعمارها بين 16-35 عامًا. تشير النتائج إلى أن الغسل المهبلي المتكرر، والعدوى السابقة، والاستخدام الخاطئ للمضادات الحيوية هي بعض الأسباب الرئيسية. يبدو أن الأشخاص الأكثر تعليماً أقل عرضة للإصابة بالمرض. توصي النتائج بالتركيز على التثقيف الصحي العام لردع الممارسات الضارة لنظافة المهبل وتشجيع الرعاية التناسلية المناسبة، مما يقلل بالتالي من انتشار خلل التوازن الميكروبي في المهبل والمضاعفات التناسلية المرتبطة به.

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