

ORIGINAL ARTICLE

Evaluation of the Causative Microorganisms and Antibiogram Results in Women with Vaginal Swab Cultures Through Prediagnosis of Vaginitis

Vajinit Ön Tanısı ile Vajinal Sürüntü Kültürü Alınan Kadınlarda Etken Mikroorganizmaların ve Antibiyogram Sonuçlarının Değerlendirilmesi

¹Samet KIRAT , ²Nurullah ÇİFTÇİ 

¹Kafkas University School of Medicine, Department of Obstetrics and Gynaecology, Kars, Türkiye
²Kafkas University, School of Medicine, Department of Medical Microbiology, Kars, Türkiye

Correspondence

Samet KIRAT
Kafkas University School of Medicine, Department of Obstetrics and Gynaecology, Kars, Türkiye

E-Mail: sametkirat1989@hotmail.com

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ABSTRACT

Objective: Bacterial vaginosis is a common cause of vaginitis worldwide and is associated with serious diseases such as increased risk of preterm birth, sexually transmitted infections, and pelvic inflammatory disease. In this study, we aimed to evaluate the demographic data, growth of pathogenic microorganisms, and antibiotic susceptibility of women who underwent vaginal swab cultures with a preliminary diagnosis of vaginitis.

Methods: Vaginal swab samples from 314 women admitted to the Department of Obstetrics and Gynecology of Kafkas University were included in the study. Women with immunosuppressive diseases, gynecologic malignancies, and the use of intrauterine devices (IUD) were excluded from the study. Vaginal swab samples were sent to the Medical Microbiology laboratory immediately after collection with a transport swab. When the specimens arrived at the laboratory, they were Gram stained without waiting, inoculated on 5% sheep blood, eosin methylene blue, Sabouraud dextrose agar, and Chukulatamsi media, and incubated at 37 °C for 24-48 hours. Microorganisms were identified using conventional and biochemical methods, and antibiotic susceptibility tests were performed using the Kirby-Bauer disk diffusion method, according to the EUCAST guidelines.

Results: The median age of 314 women included in this study was 39 years (20-69). In total, 73.6% (n=231) were premenopausal, 24.5% (n=77) were postmenopausal and 1.9% (n=6) were pregnant. The most common complaint was abdominal/pelvic pain (142 patients; 45.2%). A total of 123 women (39.1%) grew different microorganisms in vaginal cultures. The most common causative microorganism was *Escherichia coli* (41.5%). According to the antibiogram results, gentamicin was the most sensitive antibiotic and ampicillin was the most resistant antibiotic in both gram (+) and gram (-) groups.

Conclusion: Rapid and accurate identification of etiological microorganisms for proper treatment will significantly reduce the incidence of both sexually transmitted diseases and neonatal infections.

Keywords: Bacterial infection, Candidiosis, Gentamicin, Vaginitis

ÖZ

Amaç: Bakteriyel vajinoz, dünya çapında vajinitin yaygın bir nedenidir ve erken doğum riskinin artması, cinsel yolla bulaşan enfeksiyonlar ve pelvik inflamatuvar hastalık gibi ciddi hastalıklarla ilişkilidir. Bu çalışmada, vajinit ön tanısıyla vajinal sürüntü kültürü alınan kadınların demografik verilerinin, üreyen patojen mikroorganizmaların ve antibiyotik duyarlılık sonuçlarının değerlendirilmesi amaçlanmıştır.

Method: Kafkas Üniversitesi Kadın Hastalıkları ve Doğum Anabilim Dalına başvuran 314 kadının vajinal sürüntü örnekleri çalışmaya dahil edildi. İmmünyüpresif hastalığı olanlar, jinekolojik malignitesi olanlar ve RİA kullanılanlar çalışmaya dışı bırakıldı. Vajinal sürüntü örnekleri transport swab ile alındıktan hemen sonra Tıbbi Mikrobiyoloji laboratuvarına gönderildi. Örnekler laboratuvara ulaştığında bekletilmeden Gram boyamaları yapıldı ve %5 koyun kanlı, Eosin Methylen Blue, Sabouraud Dekstroze agar ve Çukulatamsi besiyerlerine ekimleri yapıldı ve 37° C'de 24-48 saat inkübe edildi. Mikroorganizmalar konvansiyonel ve biyokimyasal yöntemler kullanılarak tanımlandı ve antibiyotik duyarlılık testleri EUCAST kılavuzlarına göre Kirby-Bauer disk difüzyon yöntemi kullanılarak gerçekleştirildi.

Bulgular: Bu çalışmaya dahil edilen 314 kadının ortanca yaşı 39 (20-69) idi. Toplamda %73,6'sı (n=231) premenopozal, %24,5'i (n=77) postmenopozal ve %1,9'u (n=6) gebeydi. En yaygın şikayet karın/pelvik ağrıydı (142, %45,2). 123 kadından (%39,1) alınan vajinal kültürlerde farklı mikroorganizmalar üremiştir. En yaygın etken mikroorganizma *Escherichia coli* (%41,5) idi. Antibiyogram sonuçlarına göre hem gram (+) hem de gram (-) grupta en duyarlı antibiyotik gentamisin, en dirençli antibiyotik ise ampicilin idi.

Sonuç: Doğru tedavi için etiolojide yer alan mikroorganizmaların hızlı ve doğru bir şekilde tanımlanması hem cinsel yolla bulaşan hastalıkların hem de yenidoğan enfeksiyonlarının görülme sıklığını önemli derecede düşürecektir.

Anahtar Kelimeler: Bakteriyel enfeksiyon, Candidiosis, Gentamisin, Vajinit

Introduction

The main problem in vaginitis, inflammation of the mucosa lining the inner surface of the vagina, is a disorder of the vaginal flora (1, 2). Intrauterine devices (IUD), smoking, multipartners, cosmetics (use of soap, vaginal deodorant-perfume, etc.), vaginal cleaning with antiseptics, vaginal douching, textile materials,

detergents used in underwear, antibiotic use, hormonal changes occurring during menopause, pregnancy, and adolescence are risk factors that disrupt the vaginal flora (1, 3, 4, 5).

The vaginal flora is an aerobic environment with different characteristics depending on the secretions,

enzymes, and microorganisms it contains. The normal flora includes Lactobacilli, Bacteroides, *S. epidermidis*, group B and D streptococci, *Chorinobacteria*, *Peptostreptococci*, *E. coli*, *Gardnerella vaginalis*, *Trichomonas vaginalis* and a small amount of *Candida albicans* (6,7). Only aerobic lactobacilli were detected in the vagina during the first few weeks of the neonatal period. During puberty, Streptococci, Staphylococci, Diphtheroids, and *E. coli* bacteria are added to the flora, and the pH acidifies. During the sexually active period, *Candida* species and *Trichomonas vaginalis* can be considered causative agents. After menopause, lactobacilli decrease again and mixed flora emerge (6,7).

In healthy women of reproductive age, the flora has an acidic pH in the range of 3.8-4.2 due to the effect of estrogen. Estrogen stores glycogen in vaginal epithelial cells. Lactic acid is formed as a result of the destruction of glycogen by enzymatic reactions and lactobacilli create an acidic environment (1, 2). Epithelial proliferation decreases, epithelium becomes thinner, and pH rises to 6-8 with a decrease in estrogen levels in the pre-puberty and menopausal periods. Decreased epithelial proliferation makes the vagina more sensitive to trauma and infections, and lactobacilli are replaced by a mixed flora composed of pathogenic cocci (6-8).

The first presenting complaint was itching due to irritation caused by increased vaginal discharge. Other complaints include changes in the odor, color, and consistency of the discharge, feeling of restlessness, burning during urination, pain during sexual intercourse, redness, and edema in the vulvar region (1,9,10). This study aimed to evaluate the culture and antibiogram results of women who underwent vaginal cultures for vaginitis.

Materials and Methods

Patients and Data Collection

This study included 314 women who underwent vaginal culture at the Department of Obstetrics and Gynecology of the Kafkas University Application and Research Center between January 2023 and December 2023. Patients with IUD use, gynecologic malignancies, and immunosuppressive diseases were excluded from the study.

Demographic data such as age, pregnancy, parity, abortion, smoking history, comorbidity, drug use, history of surgery, presenting complaint, and premenopausal/pregnancy/postmenopausal period were obtained retrospectively from medical records. Microorganism growth in vaginal cultures and antibiogram results were recorded.

Vaginal discharge or vaginal swab samples sent with sterile swabs were Gram stained, sown on 5% sheep blood agar, eosin methylene blue agar, Sabouraud dextrose agar, and Chukulatamsi agar, and incubated at 37°C for 24-48 hours. Leukocyte and clue cell status was evaluated by Gram staining. The microorganisms produced after the incubation period were identified using conventional (oxidase, catalase, and coagulase) and biochemical methods (IMVIC test). Antibiotic susceptibility tests of the identified isolates were performed using the Kirby-Bauer disk diffusion method as recommended by the EUCAST guidelines, and the results were evaluated according to the most recent EUCAST guidelines.

Ethics Committee Approval

This study was approved by the Non-Interventional Clinical Research Ethics Committee of the Kafkas University Faculty of Medicine (26/06/2024, 80576354-050-99/ 498). The study complied with the recommendations of the Declaration of Helsinki for human biomedical research.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) for Windows, version 24.0 (SPSS Inc., Chicago, IL, USA). Categorical variables are shown as numbers with corresponding percentages. Continuous variables are shown as mean \pm standard deviation or median (minimum-maximum), depending on their distribution, as determined using the Kolmogorov-Smirnov test. Categorical variables were assessed using the chi-square test or Fisher's exact test when appropriate, whereas continuous variables were assessed using the Mann-Whitney U test. The level used to determine statistical significance was set at $p < 0.05$.

Results

Demographic Data of the Patient

The median age of the 314 women included in this study was 39 (20-69) years. The median gravida was two (0-14), median parity was one (0-10), and median abortion was zero (0-8). A total of 1.3% ($n=4$) of women were smokers. Different comorbidities were present in 17.2% ($n=54$) of the patients, most commonly diabetes mellitus (DM) ($n = 21$, 6.7%). Among women, 11.1% ($n = 35$) were taking medications. Overall, 73.6% ($n=231$) were premenopausal, 24.5% ($n=77$) were postmenopausal, and 1.9% ($n=6$) were pregnant. The most common complaint was abdominal/pelvic pain (142 patients; 45.2%). Vaginal cultures from 123 women (39.1%) showed the growth of different microorganisms. The detailed data are presented in

Table 1.

Table 1. Demographic Data of the Patient

	Total Cohort n=314 (%)
Age (Median (Min-Max))	39 (20-69)
Gravida (Median (Min-Max))	2 (0-14)
Parity (Median (Min-Max))	1 (0-10)
Abortion (Median (Min-Max))	0 (0-8)
Smoking (n, %)	4 (1.3)
Presenting Complaint (n, %)	
Abdominal/Pelvic Pain	142 (45.2)
Vaginal Discharge	97 (30.9)
Vaginal Itching	39 (12.4)
Vaginal Bleeding	30 (9.6)
Dysuria	37 (11.8)
Dyspareunia	7 (2.2)
Prolapse	3 (1.0)
Comorbidity (n, %)	54 (17.2)
Asthma	2 (0.6)
Crohn's disease	1 (0.3)
Diabetes Mellitus	21 (6.7)
Epilepsy	2 (0.6)
Hypertension	18 (5.7)
Hypothyroidism	13 (4.1)
Congenital Heart Disease	5 (1.6)
Migraine	1 (0.3)
Polycystic ovary syndrome	2 (0.6)
Systemic lupus erythematosus	1 (0.3)
Thrombophilia	1 (0.3)
Medication (n, %)	35 (11.1)
Period (n, %)	
Premenopausal	231 (73.6)
Pregnancy	6 (1.9)
Postmenopausal	77 (24.5)
Microorganism (n, %)	
No Growth	191 (60.9)
Reproduction	123 (39.1)

Comparison of Patients with and without Vaginal Culture Growth

The median age of patients with vaginal culture growth was 36 (21-69) years, and the median age of those without vaginal culture growth was 43 (20-66) years. Abdominal/pelvic pain (53.7% vs. 39.8%) was significantly higher in patients with culture growth (p=0.016). Other presenting complaints, gravida, parity, number of abortions, comorbidities, drug use, smoking, and premenopausal, pregnancy, and postmenopausal periods were similar between the two groups. DM (11.4% vs. 3.7%) was significantly higher in patients with culture growth (0.015). The detailed data are presented in Table 2.

Table 2. Comparison of Patients with and without Vaginal Culture Growth

	Vaginal Culture Growth (+) n=123 (%)	Vaginal Culture Growth (-) n=191 (%)	p
Age (Median (Min-Max))	36 (21-69)	43 (20-66)	0.051
Presenting Complaint (n, %)			
Abdominal/Pelvic Pain	66 (53.7)	76 (39.8)	0.016
Vaginal Discharge	38 (30.9)	59 (30.9)	0.999
Vaginal Itching	17 (13.8)	22 (11.5)	0.668
Dysuria	14 (11.4)	23 (12)	1.0
Vaginal Bleeding	4 (3.3)	26 (13.6)	0.004
Dyspareunia	2 (1.6)	5 (2.6)	0.709
Gravida (Median (Min-Max))	1 (0-10)	2 (0-14)	0.248
Parity (Median (Min-Max))	0 (0-10)	1 (0-10)	0.103
Abortion (Median (Min-Max))	0 (0-3)	0 (0-8)	0.103
Smoking (n, %)	3 (2.4)	1 (0.5)	0.304
Comorbidity (n, %)	19 (15.4)	35 (18.3)	0.613
Diabetes Mellitus	14 (11.4)	7 (3.7)	0.015
Hypertension	3 (2.4)	15 (7.9)	0.077
Polycystic ovary syndrome	2 (1.6)	0 (0)	0.077
Hypothyroidism	2 (1.6)	11 (5.8)	0.132
Crohn's disease	1 (0.8)	0 (0)	0.212
Migraine	1 (0.8)	0 (0)	0.212
Epilepsy	1 (0.8)	1 (0.5)	0.753
Thrombophilia	0 (0)	1 (0.5)	0.422
Systemic Lupus Erythematosus	0 (0)	1 (0.5)	0.422
Asthma	0 (0)	2 (1)	0.255
Congestive Heart Disease	0 (0)	5 (2.6)	0.161
Medication (n, %)	10 (8.1)	25 (13.1)	0.238
Period (n, %)			
Premenopausal	92 (74.8)	139 (72.8)	0.744
Pregnancy	28 (22.8)	3 (1.6)	0.744
Postmenopausal	3 (2.8)	49 (25.7)	0.744

Results of Microorganism Growth in Vaginal Culture

The most common causative microorganism was Escherichia coli (41.5%). Other pathogens were Candida albicans (24.4%), Klebsiella pneumoniae (10.6%), Candida glabrata (8.9%), and Enterococcus spp. (8.9%), methicillin-resistant Staphylococcus aureus (MRSA) (6.5%), Proteus mirabilis (3.3%), and Enterobacter spp. (2.4%), Acinetobacter (1.6%), Pseudomonas Aureginosa (0.8%), Serratia marcescens (0.8%), Candida kefyr (0.8%), and methicillin-sensitive Staphylococcus aureus (MSSA) (1.1%) (Table 3).

Table 3. Results of Microorganism Growth in Vaginal Culture

Vaginal Culture Growth (+) n=123 (%)	
Microorganism Agent (n, %)	
Escherichia coli	51 (41.5)
Candida albicans	30 (24.4)
Klebsiella Pneumonia	13 (10.6)
Candida glabrata	11 (8.9)
Enterococcus spp.	11 (8.9)
Methicillin-resistant Staphylococcus aureus (MRSA)	8 (6.5)
Proteus Mirabilis	4 (3.3)
Enterobacter spp.	3 (2.4)
Acinetobacter	2 (1.6)
Pseudomonas Aeruginosa	1 (0.8)
Serratia Marcescens	1 (0.8)
Methicillin-sensitive Staphylococcus aureus (MSSA)	1 (0.8)
Candida kefyr	1 (0.8)

Comparison of Gram (+) and Gram (-) Antibiogram Data (Susceptible and Resistant Antibiotics)

Table 4. Comparison of Gram (+) and Gram (-) Antibiogram Data (Susceptible and Resistant to Antibiotics)

	Gram (-) n=75 (%)	Gram (+) n=20 (%)	P
Antibiogram			
Sensitive (n, %)			
Gentamicin	66 (88)	15 (75)	0.194
Trimethoprim-Sulfamethoxazole	39 (52)	9 (45)	0.761
Ampicillin	15 (20)	8 (40)	0.080
Levofloxacin	61 (81.3)	7 (35)	<0.001
Ciprofloxacin	50 (66.7)	6 (30)	0.007
Rifampicin	1 (1.3)	4 (20)	0.007
Penicillin	1 (1.3)	2 (10)	0.049
Tetracycline	1 (1.3)	2 (10)	0.049
Moxifloxacin	0 (0)	1 (5)	0.475
Resistant (n, %)			
Ampicillin	54 (72)	11 (55)	0.237
Penicillin	14 (18.7)	3 (15)	1.0
Gentamicin	3 (4)	3 (15)	0.105
Tetracycline	0 (0)	2 (10)	0.006
Levofloxacin	5 (6.7)	2 (10)	0.636
Ciprofloxacin	6 (8)	2 (10)	0.673
Moxifloxacin	1 (1.3)	0 (0)	1.0
Trimethoprim-Sulfamethoxazole	5 (6.7)	0 (0)	0.580

In both gram-negative and gram-positive groups, the most sensitive antibiotic was gentamicin ((n=66, 88% vs. n=15, 75%); p=0.194)) and the most resistant

antibiotic was ampicillin ((n=54, 72% vs. n=11, 55%); p=0.237)). The detailed data are presented in Table 4.

Discussion

In this study, the demographic data, culture, and antibiogram results of 314 women who underwent vaginal culture between January 2023 and December 2023 in the Department of Obstetrics and Gynecology of the Kafkas University Application and Research Center were analyzed.

In our study, bacteria and/or fungi were isolated as causative agents in 39.1% of vaginal culture samples. Vaginal discharge is the most common cause of hospital admission for vaginitis. Bacterial vaginosis is the most common cause of abnormal vaginal discharges. It was first described in 1955; however, its etiology remains unknown (11-13). With the decrease in lactobacilli, which constitute 90%-95% of healthy vaginal flora, they are mostly replaced by anaerobic opportunistic bacteria such as gram-positive cocci and gram-negative bacilli (11,14-16). Its prevalence was found to be 58.3% in sub-Saharan South Africa, 30.3% in Zimbabwe, 14.2% in Nigeria, 23.2% in Bangladesh, 32% in Chile, 29.2% in the USA, 4.7% in Australia, and 8.6% in Finland (17-23). Low socioeconomic status, poor hygiene, and malnutrition are considered to increase its incidence.

The diagnosis can be made using the Amsel criteria or Gram staining techniques, but different degrees of accuracy occur between the two diagnostic methods (13,14). Vaginal swab culture is the gold standard for diagnosing most bacterial infections. However, since it is difficult to produce microorganisms in bacterial vaginosis, culture is not the gold standard method. Gram staining is an inexpensive, short-term, and readily accessible laboratory method. In previous studies, the sensitivity and specificity of Gram staining for the diagnosis of bacterial vaginosis ranged between 89-93% and 70-83%, respectively (24). Compared with Gram staining, diagnosis can be made with 70-90% sensitivity and 90-94% specificity according to the Amsel criteria (25).

Studies have shown that the number of lifetime sex partners, sex between women, use of sex toys, early coitus, frequency of vaginal sex, recent change of partners, oral sex, anal sex, intravaginal practices such as vaginal cleansing and/or vaginal douching increase the risk of vaginitis, which in turn increases the risk of sexually transmitted agents such as HIV, Neisseria gonorrhoeae, Chlamydia Trachomatis, Trichomonas Vaginalis and Herpes Simplex virus-2 (11,13,26,27). Some studies have shown that the use of an IUD increases risk (28), whereas others have shown that it decreases risk (29). Women using IUDs were excluded

from our study.

The Centers for Disease Control and Prevention (CDC) treatment guidelines recommend treating only symptomatic women (30). This leads to a higher recurrence rate among asymptomatic women. According to previous studies, the recurrence rate in the first year can vary between 15%-80% (16,31). It has been shown that treatment of asymptomatic women significantly reduces sexually transmitted disease agents (32).

75% of adult women experience at least one episode of vulvovaginal candidiasis in their lifetime and 45% experience at least two episodes each year. *Candida albicans* is the causative agent in 85%-90% of cases. Other *Candida* species have also been observed in immunosuppressed, postmenopausal, and diabetic women (33,34). BV is the second most common cause of vaginitis, worldwide. In studies conducted in our country, the rate of *Candida* growth in vaginal swab cultures was found 16-39.8% (35-37). Its frequency increases in pregnant women, obese women, patients with immune dysfunction, and those with a recent history of antibiotic use. As it causes chronic, recurrent, and resistant infections, it may lead to sexual dysfunction and impaired quality of life in women (35). In our study, 24.4% of *Candida albicans*, 8.9% of *Candida glabrata*, and 0.8% of *Candida kefyr* were found to be causative agents. In the premenopausal period, 11.7% (n=27), 33.3% (n=2), and 1.3% (n = 1) of the participants were postmenopausal (p=0.004). This may be explained by the higher frequency of estrogenic and sexual activity.

As the estrogen level decreases, the vaginal epithelium becomes thinner and a condition called atrophic vaginitis occurs. The vagina is dry, there is usually no odor or discharge, and dyspareunia is the most common complaint. It is one of the most common complaints during the postmenopausal period (36,38). In the present study, 22.8% (n=28) of women were postmenopausal, and different microorganisms were found in 36.4% (n = 28) of the cultures.

Although many microorganisms are found in balance in the aerobic vaginal flora, the most important factor contributing to this is lactobacilli, which constitute 90%. In addition, commensal agents include *C. albicans*, *S. aureus*, and *S. agalactiae* (38). Aerobic vaginitis is characterized by a decrease in the predominance of lactobacilli in the flora and a predominance of commensal pathogens, with more inflammatory changes compared to bacterial vaginosis. It was found to be 5-24% in patients presenting with vaginal discharge and 8-11% in pregnant women (38). In a study of 610 vaginal swab cultures, the causative microorganisms isolated were *E. coli* (24.92%), *K. pneumoniae* (23.5%),

S. aureus (16.52%), and *Enterococcus* spp. (8.40%) and coagulase-negative staphylococci (6.44%) in order of frequency. The growth rate of these bacteria was higher in non-pregnant women and in those between 25-40 years of age (39). Among the gram-negative bacteria isolated in the literature, *E. coli* is the most common, with a rate of 69.3%-76% (35,36). The agents isolated in our study were as follows; 41.5% *E. coli*, 10.6% *Klebsiella Pneumoniae*, 8.9% *Enterococcus* spp., 6.5% MRSA, 3.3% *Proteus mirabilis*, 2.4% *Enterobacter* spp., 1.6% *Acinetobacter*, 0.8% *Pseudomonas Aureginosa*, 0.8% *Serratia Marcescens* and 1.1% MSSA.

The limitations of our study include the small number of cases and the retrospective nature of the study. Vaginitis constitutes an important part of admissions to Gynecology and Obstetrics Outpatient Clinics in all age groups. Failure to provide treatment for the causative agent may result in pelvic inflammatory disease, infertility, and serious complications, such as meningitis and sepsis, in newborns. It can cause deterioration in the quality of life of women due to complaints such as vaginal discharge, bad odor, bleeding, and dyspareunia, and in advanced stages, it can cause serious psychological problems. Therefore, it remains an important health problem. Therefore, it is important to treat asymptomatic women in contrast to CDC recommendations to prevent possible complications. Therefore, it is important to quickly and accurately identify microorganisms involved in the etiology of the correct treatment.

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