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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2024.404.412

Research Article
Microbial Vaginosis and its Relation to Single or Multi-Species Biofilm in Iraqi Women: Clinical and Microbiological Study

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Abstract

Background and Objective: Bacterial vaginosis (BV) is the primary aetiology of vaginal discharge causing significant public health consequences. The study aims to detect the frequency of bacterial vaginosis and to assess the effectiveness of the Amsel's criteria and Nugent's score system as diagnostic tests for BV. **Materials and Methods:** A total of 135 high vaginal swab samples were obtained and analyzed microbiologically to detect the presence of Amsel clinical criteria and to determine the Nugent's score using gram staining. The microbiological culture, antimicrobial susceptibility test and bacterial biofilm generation were conducted using standardized laboratory conditions. The study data were analyzed using SPSS version 16.0 and Microsoft Excel. The Chi-square test was used to ascertain any significant differences with a p-value of less than 0.05. **Results:** Out of 135 HVS, 60 (44.4 %) specimens revealed bacterial vaginosis and 30 (22.2%) represent *Candida albicans* vaginitis. In comparing Amsel's criteria with Nugent's score, the sensitivity, specificity, positive and negative predictive values were 94.7, 92.3, 90 and 96%, respectively. Also, 26 (50%) of the study isolates were produced biofilm strongly. Further, *Gardnerella vaginalis* was the study isolate that produced biofilm strongly (66.6%) followed by *Pseudomonas aeruginosa* (57.1%). **Conclusion:** The study highlights the significance of Amsel's clinical criteria and the Nugent's score system as diagnostic tests for bacterial vaginosis in outpatient settings. Additionally, there is an association between recurrent bacterial vaginosis and vulvovaginal candidiasis. Moreover, addressing vaginal disorders caused by single-species or multi-species biofilms create the researcher to be focused on studying multi-species biofilms.

Key words: Bacterial vaginosis, biofilm, Nugent's score, Amsel's criteria, vulvovaginal candidiasis, *Gardnerella vaginalis*

Citation: Murshid, R.M., M.T.S. Al-Ouqaili and B.A.J. Kanaan, 2024. Microbial vaginosis and its relation to single or multi-species biofilm in Iraqi women: Clinical and microbiological study. Pak. J. Biol. Sci., 27: 404-412.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The BV is a clinical condition characterized by a significant imbalance in the vaginal microbiota, where the beneficial *Lactobacillus* species are reduced or absent and there is an overgrowth of various anaerobic and facultative species. This leads to a greater diversity in the bacterial population in the vagina[1]. Lactobacilli are the primary microorganisms found in the vaginal microbiota of healthy women. They play a crucial role in maintaining the ecological balance and preventing infections. Their protective function is carried out by many processes, including adherence and permanence in the epithelium or mucosa, competitive exclusion of pathogens, formation of antimicrobial chemicals such as hydrogen peroxide, lactic acid (LA) and bacteriocin-like substances, as well as modification of the host's immune response. Hence, including probiotic products containing vaginal lactobacilli presents a new approach to replenishing the vaginal microbiota[2].

Different reasons including both infectious and non-infectious agents, have the potential to disturb the vaginal environment. Bacterial vaginosis (BV) is the primary pathological factor responsible for vaginitis in women who are in their reproductive years. The condition is distinguished by the existence of vaginal discharge and a disagreeable scent[3]. The BV is a condition where the usual vaginal bacterial Lactobacillus is replaced by a combination of bacteria including *Gardnerella vaginalis*, *Mobiluncus* species, *Mycoplasma hominis*, *Bacteroides* species and other anaerobic bacteria[4]. Developing countries exhibit a greater prevalence of BV in comparison to other countries. The prevalence of BV ranges from 20 to 47% in non-pregnant women of developing countries and this variation is driven by factors such as geographic location, socioeconomic status and ethnicity. While BV is more frequently observed in African countries and less common in Asian and European countries, it is important to highlight that specific regions in Africa have lower BV rates, whereas Asia and Europe demonstrate greater rates[5]. Bacterial vaginosis (BV) can result in several adverse outcomes, such as chorioamnionitis (infection of the amniotic fluid), low weight of birth, premature rupture of the membranes, preterm birth, elevated risk of pelvic infection following abortion, vaginal cuff cellulitis, a conditions known as cervicitis, urinary tract infection, cervical intraepithelial neoplasia, higher likelihood of ectopic pregnancy, chronic pelvic pain and infertility[6,7].

The Nugent's score and Amsel's criteria are commonly utilized by doctors to identify bacterial vaginosis[8,9]. In 1991, this scoring system was introduced by Nugent *et al.*[10] which is the method of assessing the presence of *Lactobacillus* and other species like *Gardnerella vaginalis*, *Prevotella* spp. and *Mobiluncus* in various forms) in high vaginal swabs. It is indicated by numerical score which ranges from 0 to 10 with scores of 7 to 10 referring to the presence of BV. Due to high sensitivity, this approach has been considered the golden standard for BV[9,10]. In 1938, Amsel *et al.*[11] established a standard for diagnosing bacterial vaginosis. They suggested four criteria and the presence of at least three of them was enough to establish a definite diagnosis of bacterial vaginosis: (1) Vaginal discharge becomes more uniform, has a strong odour and appears white or brown, (2) Acidity of the vagina changes to become more alkaline, with a pH value higher than 4.5, (3) When 10% potassium hydroxide is added to the vaginal secretions, an amine odour is released and (4) Clue cells are observed in the wet mount examination of the vaginal discharge[12].

It is well documented that bacterial biofilm is consists of clusters of *Gardnerella vaginalis*, which are thick, organized and polymicrobial. The strong adherence of BV-associated anaerobes which includes *Atopobium vaginae*, *Prevotella bivia*, *Mobiluncus mulieris*, *Fusobacterium nucleatum* and *Peptoniphilus* spp., to vaginal epithelial cells is a crucial factor in the development of BV[13]. The biofilm possesses a complex internal structure, comprising an extracellular matrix that represents the physical barrier against antimicrobial agents and human immune responses. Also, it will enhance tolerance to undesired conditions. This matrix contains channels that facilitate nutrient movement[13,14]. Vaginal biofilms are important not only in the development of BV but also in the treatment failure leading to chronicity. The biofilm's architecture hinders the antimicrobial agents infiltration into the matrix and restricts their interaction with the microorganisms. Concurrently, there is a noticeable rise in antibiotic resistance, leading to the ineffectiveness of treatments[15,16]. Therefore, a study has been conducted to identify the occurrence of BV and ascertain their susceptibility to antimicrobial agents. Further, to evaluate Amsel's diagnostic criteria sensitivity and specificity when applied to patients with both low and high Nugent's scores with positive results. It will provide a clearer understanding of the usefulness of clinical criteria, which are commonly utilized in outpatient settings. Furthermore, is to detect the biofilm formation ability of certain medically significant microbial agents responsible for BV.

MATERIALS AND METHODS

Study patients: A descriptive cross-sectional was achieved from March to June, 2024 and the detailed information about a specific population at a certain point in time has been considered. High Vaginal Samples (HVS) were obtained from

women who were referred to the Department of Obstetrics and Gynecology at Ramadi Teaching Hospital for Child and Maternity in addition to the Private Clinics in Al Anbar Governorate. These samples were then examined in the laboratory for clinical microbiology for further investigations. A total of 135 High Vaginal Swab (HVS) samples were obtained from individuals who had aberrant per vaginal (PV) discharge. This study included a group of the reproductive-age women who had a history of abnormal vaginal discharge as the inclusion criterion. The exclusion criteria for this study include patients experiencing vaginal bleeding, individuals with the malignant genital tract such as endometrial and cervical carcinomas and vulval cancers, patients currently undergoing therapy and pregnant women.

Ethics statement: This study obtained approval from the Medical Ethics Committee at the University of Anbar, Iraq, on 6 March, 2024, in accordance with the Declaration of Helsinki, 1979 (approval number 39). All patients participating in the trial provided verbal informed consent and the researcher asked each possible participant if they would like to participate in the study. If the participant consents to participate, their agreement is documented either by spoken consent recorded on an audio recorder or through written notes made by the researcher.

Collection of specimen: Two HVS have been taken from each female patient by an expert Gynecologist. After collection of HVS, consistency, color and specimen homogeneity were observed. The swabs were placed in a sterile container containing sterile normal saline for further processing in the laboratory for microbiological examinations.

Laboratory investigation

Microbiology culture: The gynecologist obtained specimens from each female by swabbing the upper part of the vagina using two sterile cotton swabs. After HVS had been collected, the color, consistency and homogeneity were detected. The specimens were placed in a sterile container and processed in the microbiological laboratory for further processing.

Biofilm production: The isolates' ability to produce biofilms was initially detected using the microtiter plate technique. This technique was started by adding the standardized bacterial sample into the wells of a microtiter plate. After an overnight incubation, the methanol was added to each well for 15 min. After that, the plates were drained and left to dry in the open air. Crystal violet was added to each well for 5 min and rinsed to remove it from the wells. Each well was treated with 160 µL of acetic acid and the O.D. for each well was measured at wavelengths of 570 and 630 nm. The O.D. at 630 nm was used to define the ability of an isolate to develop a biofilm either as weak (OD<1.078), intermediate (OD = 1.078-2.156) or strong biofilm producer (OD>2.156)[17].

Antimicrobial susceptibility surveillance: The antimicrobial agents activity has been detected using the standardized Kirby-Bauer method CLSI, 2023. The antimicrobial susceptibility surveillance was conducted under aseptic conditions. A total of 2 to 5 colonies were placed in 2 mL of nutritional broth and incubated at 37°C for overnight incubation. This process was achieved to create a bacterial suspension that was adjusted to a turbidity level equivalent to 0.5 McFarland, which corresponds to a concentration of 10^8 CFU/mL. Also, a sterile swab was inserted into a bacterial suspension. The plate was inoculated using a swab, which was used to streak the whole surface of the plate. The plate was then rotated at a 45° and streaked across the plate entire surface. After that, the plates were allowed to undergo the drying process. The selected antimicrobial agents disks were gently applied to the agar surface to ensure full contact using sterile forceps. Further, the plates were placed in an incubator of 37°C for 24 hrs. The data were interpreted according to the standards established by CLSI, 2023, at which the diameter of the inhibition zone for each antibiotic disk was reported and measured[18].

Amsel's criteria: The second high vaginal swab has been utilized for pH assessment and submitted for wet mount preparation, as well as the smell test. Further, the other swab underwent processing for gram's staining. The vaginal pH was assessed by employing litmus paper, with the alteration in the colour of the paper being observed and recorded. The wet mount preparation was conducted to examine clue cells. The sniffing technique was conducted by introducing a small amount of 10% Potassium Hydroxide (KOH) solution to the discharge on a pristine glass slide and observing for a noticeable fishy scent to confirm positive outcomes. Amsel's criteria were utilized for the diagnosis of BV. According to Amsel's criteria[11], a diagnosis of BV has been established if at least three out of the following four criteria were met: The symptoms of the condition include a vaginal discharge that is greyish-white, thin and uniform consistency. The pH level of the vagina is greater than 4.5. When 10% Potassium Hydroxide (KOH) is added, there is a fishy or amine odour. Additionally, microscopic examination reveals the presence of clue cells, which account for more than 20% of the cells observed.

Table 1: Distribution of Nugent's scores among *Lactobacillus*, *Gardnerella vaginalis* and curved gram variable bacilli after staining with gram stain

Score	*Lactobacillus* morphotypes-average per high powered (1000×oil immersion) field	*Gardnerella* morphotypes-average per high powered (1000×oil immersion) field	Curved gram variable rods-average per high powered (1000×oil immersion) field
0	4+	0	0
1	3+	1+	1+/2+
2	2+	2+	3+/4+
3	1+	3+	-
4	0	4+	-

0 = /Oil immersion field = 0, ≤1 = /Oil immersion field = 1 plus, 1-4 = /Oil immersion field = 2 plus, 5-30 = /Oil immersion field = 3 plus and >30 = /Oil immersion field = 4 plus

Nugent's score: A highly qualified microbiologist performed gram staining to ascertain the Nugent's score. Nugent's scoring system relied on quantifying the various bacteria morphology, particularly *Lactobacillus*-like which appears as large, uniform, gram-positive bacilli, *Gardnerella vaginalis* as small, pleomorphic, gram-variable bacilli, *Mobiluncus*-like (curved, gram-variable bacilli) and *Prevotella*/*Bacteroides*-like (small, gram-negative bacilli). According to Nugent *et al.*[10], a Nugent's score of 7-10 indicates the presence of BV, a score of 4-6 suggests an intermediate condition and a score of 0-3 indicates the absence of BV. These interpretations were presented in Table 1.

Statistical analysis: The study data were analyzed using SPSS version 16.0 in addition to Microsoft Excel. The analysis was based on the distribution of frequencies and percentage interpretation. The Chi-square test was used to ascertain any significant interactions, using a p-value of less than 0.05 as indicative of significance.

RESULTS

Isolation and identification of bacterial vaginosis: In total, 135 vaginal swab samples were collected from women with symptoms of vaginal discharge. Isolated and identified using culture and phenotypic methods. All bacterial isolates were analyzed under a light microscope and gram-stained smears were prepared from fresh cultures and biochemical tests such as biochemical test like IMVIC, catalase, oxidase, urease and TSI were used to confirm the prevalence of 60 (44.4 %) with bacterial vaginosis, 30 (22.2%) with *Candida albicans* vaginitis and 45 (33.3%) no growth of pathogenic aerobic bacteria, as shown in Table 2.

Amsel's versus Nugent's score: Out of a total of 135 cases, the prevalence of BV has been determined to be 60 (44.4%) using Amsel's criterion and 57 (42.2%) using Nugent's scoring criteria. Among the 57 recorded cases that tested positive according to Nugent's score method, 54 cases also tested positive according to Amsel's criteria. An additional six cases met the requirements set by Amsel for a positive result but were determined to be negative based on the Nugent's score system. When the study compared Amsel's criteria with Nugent's score, which is a very reliable approach for diagnosing BV, the sensitivity, specificity, positive predictive value and negative prediction value of Amsel's criteria were determined to be 94.7, 92.3, 90 and 96%, respectively (Table 3). The instances that were falsely identified as being positive were classified as intermediate flora according to Nugent's grading system. However, all the instances that were identified as false negatives based on clinical criteria had a low positive Nugent's score (NS = 7-8). In addition, Amsel's criteria were individually compared with Nugent's score, specifically the low positive range (NS = 7-8) and the high positive range (NS = 9-10). This was shown in Table 3 and Fig. 1.

In the antimicrobial susceptibility surveillance section, it was noticed that *Pseudomonas aeruginosa* demonstrated an elevated level of resistance (>57%) to specific antimicrobial agents, including cephalexin, ceftazidime, ceftriaxone, nalidixic acid, ampicillin, gentamicin and tetracycline. *Klebsiella pneumoniae* also exhibited a high level of resistance (>41%). In addition, *E. coli* has shown resistance rates above 30% to ampicillin, trimethoprim-sulfamethoxazole and tetracycline. *Citrobacter* spp. exhibited resistance rates above 47% to some drugs. *Staphylococcus aureus* has resistance rates of over 45% against ampicillin, trimethoprim-sulfamethoxazole, tetracycline, oxacillin, vancomycin and penicillin G. On the other hand, *Streptococcus* spp. demonstrates complete resistance (100%) to rifampicin and vancomycin. Multiple species, such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Citrobacter* spp. and *S. aureus*, displayed both multiple-drug resistance and prevalent drug resistance.

Biofilm production: The study results demonstrate that 50% (26 isolates) had a strong ability to create biofilm, whereas 15.4% (8 isolates) showed a moderate ability and 9.6% (5 isolates) displayed a weak ability. However, 13 out of the total sample (25%) did not form biofilms, as indicated in Table 4.

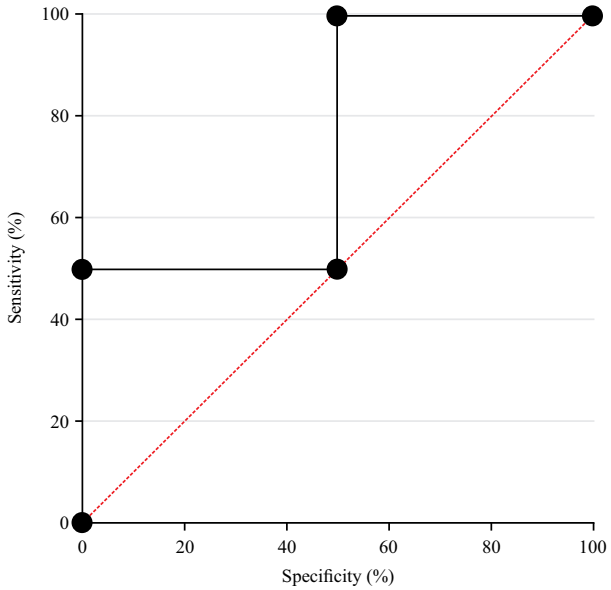

Fig. 1: ROC curve represent the relation between Amsel's criteria and Nugent's score system

Table 2: Prevalence bacterial vaginosis, *Candida albicans* vaginitis and no growth of pathogenic aerobic bacteria isolated from high vaginal swab cultured

Microbial infection	Percentage (%)	Total number
Bacterial vaginosis		
One pathogen		
Escherichia coli	12 (20.0)	60 (100%)
Klebsiella pneumoniae	10 (16.7)	
Staphylococcus aureus	9 (15.0)	
Pseudomonas aeruginosa	7 (11.7)	
Gardnerella vaginalis	6 (10.0)	
Streptococcus spp.	5 (8.3)	
Citrobacter freundii	3 (5.0)	
Mix pathogens		
Streptococcus spp. and *Candida albicans*	1 (1.6)	
Streptococcus spp. and *Escherichia coli*	1 (1.6)	
Streptococcus spp. and *Klebsiella pneumoniae*	1 (1.6)	
Streptococcus spp. and *Gardnerella vaginalis*	1 (1.6)	
Gardnerella vaginalis and *Klebsiella pneumoniae*	1 (1.6)	
Gardnerella vaginalis and *Candida albicans*	1 (1.6)	
Enterococcus faecalis and *Candida albicans*	1 (1.6)	
Pseudomonas aeruginosa and *Candida albicans*	1 (1.6)	
Candida albicans vaginitis		30 (22.2%)
No growth of pathogenic aerobic bacteria		45 (33.3%)

Table 3: Sensitivity, specificity, positive predictive value and negative prediction value of Amsel's criteria and Nugent's score for the diagnosis of BV

	Nugent's criteria						
Diagnostic methods	Positive	Negative	Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Amsel's criteria							
Positive	54	6	60	94.7	92.3	90.0	96
Negative	3	72	75				
Total	57	78	135				

The bacteria that were most frequently associated with biofilm production were *Escherichia coli*, with 8 isolates (20.5%) and *Klebsiella pneumoniae*, also with 8 isolates (20.5%). Following closely behind were *Staphylococcus aureus* and *Pseudomonas aeruginosa*, both with 7 isolates (17.9%) each. *Gardnerella vaginalis* had 5 isolates (12.8%) associated with biofilm production. Both *Streptococcus pyogenes* and *Citrobacter freundii* exhibited the lowest levels of biofilm

Table 4: Frequency and percentage of biofilm production for the study isolates using microtiter plate assay

Bacterial biofilm	Frequency	Percentage
None	13	25.0
Weak	5	9.6
Moderate	8	15.4
Strong	26	50.0

Table 5: Frequency and percentage of biofilm production for the study isolates using microtiter plate assay

	Bacterial biofilm production no. (%)				
Bacterial isolates	None	Weak	Moderate	Strong	Total
Escherichia coli	4 (33.3)	1 (8.3)	2 (16.7)	5 (41.7)	12 (20.0%)
Klebsiella pneumoniae	2 (20)	1 (10)	1 (10)	6 (40)	10 (16.7%)
Staphylococcus aureus	2 (22.2)	1 (11.1)	1 (11.1)	5 (55.6)	9 (15.0%)
Pseudomonas aeruginosa	0 (0.0)	1 (14.3)	2 (28.6)	4 (57.1)	7 (11.7%)
Gardnerella vaginalis	1 (16.7)	0 (0.0)	1 (16.7)	4 (66.6)	6 (10.0%)
Streptococcus spp.	3 (60)	1 (20)	1 (20)	0 (0.0)	5 (8.3%)
Citrobacter freundii	1 (33.3)	0 (0.0)	0 (0.0)	2 (66.7)	3 (5.0%)
Total	13	5	8	26	52

generation in the research, with only 2 (5.1%) of each isolate producing bacterial biofilm. According to the strength of biofilm production, it was observed that *Gardnerella vaginalis* was the most study isolate produced biofilm strongly (66.6%) followed by *Pseudomonas aeruginosa* (57.1%) and *Staphylococcus aureus* (55.6%), as show in Table 5.

DISCUSSION

The BV is the primary aetiology of vaginitis and a prevalent condition among women globally. While the diagnosis of BV using the Amsel's criteria is straightforward, it is generally lacking in sensitivity. Based on Amsel's criteria, the prevalence of BV was determined to be 60 cases, accounting for 44.4% of the total. According to Nugent's scoring criteria, the prevalence was 57 cases, representing 42.2% of the total. Among the 57 cases that tested positive according to Nugent's score methodology, 54 cases also tested positive based on Amsel's criterion. The positive six cases according to Amsel's criteria and the negative according to Nugent's score. In comparing Amsel's criteria with Nugent's score, the sensitivity, specificity, positive predictive value and negative predictive value of Amsel's criteria were found to be 94.7, 92.3, 90 and 96%, respectively. Further, half of the study isolates produced biofilm strongly. Furthermore, *Gardnerella vaginalis* produced biofilm strongly followed by *Pseudomonas aeruginosa*. The formation of lactic acid (LA) is the cause of and hence closely linked to, the acidic nature of the vagina (pH 3.5-4.5). Hydrogen peroxide is a substance that causes oxidation and is poisonous to bacteria that lack the enzyme catalase, such as the majority of microorganisms that thrive in the absence of oxygen. The *Lactobacillus* spp., that produce H_2O_2 also helps maintain the balance of the microbiota by promoting the release of antimicrobial substances from epithelial cells and enhancing the effectiveness of existing factors such as muramidase and lactoferrin[19]. The vagina of females before they start menstruating is covered by a single layer of cube-shaped cells called simple cuboidal epithelium. The pH level is neutral and the epithelium is inhabited by skin commensals. During puberty, the presence of estrogen leads to the development of stratified squamous epithelium and Lactobacilli become the most prevalent organism. The pH decreases to a range of 3.5 to 4.5. Following menopause, atrophic alterations take place, resulting in a restoration of the skin's flora that closely resembles its original state. The pH subsequently increases to 7.0[20]. In this study cases, also identified mixed microbiological aetiology of bacterial vaginosis. Two basic hypotheses have been presented to interpret this: The solitary agent theory and the polymicrobial consortia hypothesis[21]. There is evidence suggesting that some bacterial species, such as *Gardnerella* spp., play a crucial role in initiating BV. However, it is also recognized that certain secondary anaerobic bacteria contribute to the development of BV[22].

In a study laid down by Schwebke *et al.*[23], the sensitivity and specificity of the Amsel's criteria were found to be 70 and 94%, respectively in comparison with the Nugent's score and the study focused on non-pregnant women population. Despite the Nugent gram stain has its advantages, it is unable to detect *M. hominis* because it lacks a cell wall. Further, it does not provide quantitative data regarding the specific bacteria associated with BV[24]. Further, Redelinghuys *et al.*[25] documented that the diagnosis of BV is typically made using either the Amsel's criteria or the Nugent's score. The Amsel's criteria have assessed the clinical symptoms linked to BV and a positive diagnosis is made when three out of the four

established criteria are present[11]. It is well documented that the Nugent's score includes testing a vaginal smear under a microscope and quantitatively classifying the different morphotypes present in the sample using a specific scale. A score greater than 7 indicates a positive case of BV[26].

The present research reached an important finding: *Candida albicans* has been detected significantly in clinical cases, specifically in 30 cases (22.2%). Out of the four, 13.3% were found to coexist with the bacterial isolates. The main bacterial agents in vulvovaginal candidiasis are *Candida* spp., which most likely originated from the gastrointestinal system and initially establish asymptomatic colonization in the vagina as commensal organisms. *Candida* spp., thrive in a balanced and non-hostile environment established by protective vaginal microbiota, living as saprophytes. The presence of organic acids, such as acetic and lactic acid, enhances the ability of the vagina to tolerate *Candida* spp.[27]. The estrogen's local effect is the primary factor in facilitating *Candida* commensalism in the healthy lower genital tract microbiome. The colonization of the vaginal area by yeast can persist for a significant period and is controlled by both genetic elements of the host and external variables that promote colonization, including behavioral and biological factors[28]. The result of symptomless colonization as well as *Candida vaginitis* is influenced by three elements: Yeast, vaginal microbiota and host mucosal immune components. Several investigators have carefully investigated the human vaginal mucosa, host immunity and the function of vaginal microbiota in the development of VVC, as reflected by Sobel and Vempati[1].

Mixed bacterial infection of *Candida albicans* with bacteria is highly observed in our work which may indicate a question highlighted by a recent research group on whether vulvovaginal candidiasis is a result of bacterial vaginosis. Sobel and Vempati[1] reported an association between bacterial vaginosis and vulvovaginal candidiasis. Further, they proposed that healthcare providers may not fully recognize the significant effect of recurrent bacterial vaginosis (RBV) on the Recurrence of Vulvovaginal Candidiasis (RVVC) in specific groups. Administering preventive fluconazole alongside antibiotic therapy for RBV is an effective strategy for women with a history of RVVC.

The most common microorganisms in a healthy vaginal microbiome are *Lactobacillus* species, which yield lactic acid and hydrogen peroxide, resulting in an acidic environment. The BV is characterized by a high presence of anaerobic species in the vaginal microbiome. These species interact and form a polymicrobial biofilm on the vaginal epithelium. The predominant indications of infection are the presence of vaginal discharge and a pungent odour, along with an increase in vaginal pH[26].

In this work, the robust bacterial biofilm formation in 50% of the bacterial isolates studied (26 out of 52) was observed with 15.4 and 9.6% exhibiting moderate and weak biofilm production, respectively. The main factor that plays a contributing role in the reoccurrence of bacterial vaginosis is the presence of a biofilm in the infection, which poses challenges in effectively the infection elimination[29]. On the other hand, our study suggested that *Gardnerella vaginalis* was the most prominent isolate in producing a robust biofilm, followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. When considering the polymicrobial character of BV, certain studies suggest that the presence of two or more species associated with BV results in increased resistance to antimicrobial therapies[26]. In their 2008 study, Swidsinski *et al*.[30] documented that the presence of biofilm posed a challenge in treating BV. They documented that a biofilm primarily consisting of *Gardnerella* spp. and *Fannyhessea vaginae* was temporarily suppressed during a seven-day treatment with metronidazole. The biofilm regained its activity once the treatment had stopped. Machado *et al*.[31] proposed that the existing treatments for bacterial vaginosis are inadequate in addressing the vaginal dysfunction caused by multi-species biofilms. By effectively addressing the intricate interactions established in multi-species biofilms, one can tackle bacterial vaginosis.

CONCLUSION

The study found that gram-negative bacteria and *Gardnerella vaginalis* are the main pathogens associated with BV. The Amsel's clinical criteria and Nugent's score system are crucial diagnostic tests for BV. Recurrent BV significantly contributes to vulvovaginal candidiasis recurrence. Gram-negative bacteria exhibit greater resistance and studying multidrug resistance mechanisms could help understand these mechanisms. Current treatments for BV are inadequate for vaginal disorders caused by single-species or multi-species biofilms, so researchers should focus on addressing multi-species biofilms.

SIGNIFICANCE STATEMENT

The BV is a significant risk factor for gynecological and obstetric outcomes such as pregnancy and STIs. It is characterized by an increase in vaginal pH, gray discharge

color with a fishy smell. The study aims to detect the frequency of BV and to assess the effectiveness of the Amsel's criteria and Nugent's score system as diagnostic tests for BV. In the future, research should prioritize the prevention of transmission and the development of better treatments for the bacterial biofilm induced by this pathogen and anaerobic organisms in the host.

REFERENCES

1. Sobel, J.D. and Y.S. Vempati, 2024. Bacterial vaginosis and vulvovaginal candidiasis pathophysiologic interrelationship. Microorganisms, Vol. 12. 10.3390/microorganisms12010108.
2. Nader-Macías, M.E.F. and P.R. de Gregorio, 2022. Probiotics and Urogenital Health. In: Probiotics: Advanced Food and Health Applications, Brandelli, A. (Ed.), Academic Press, Cambridge, Massachusetts, ISBN: 9780323851701, pp: 355-388.
3. Abbe, C. and C.M. Mitchell, 2023. Bacterial vaginosis: A review of approaches to treatment and prevention. Front. Reprod. Health, Vol. 5. 10.3389/frph.2023.1100029.
4. Chen, X., Y. Lu, T. Chen and R. Li, 2021. The female vaginal microbiome in health and bacterial vaginosis. Front. Cell. Infect. Microbiol., Vol. 11. 10.3389/fcimb.2021.631972.
5. Bhat, S., S. Muthunatarajan, S.S. Mulki, K.A. Bhat and K.H. Kotian, 2021. Bacterial infection among cancer patients: Analysis of isolates and antibiotic sensitivity pattern. Int. J. Microbiol., Vol. 2021. 10.1155/2021/8883700.
6. Hay, P., 2018. Vaginal discharge. Medicine, 46: 319-324.
7. Ng, B.K., J.N. Chuah, F.C. Cheah, N.A.M. Ismail, G.C. Tan, K.K. Wong and P.S. Lim, 2023. Maternal and fetal outcomes of pregnant women with bacterial vaginosis. Front. Surg., Vol. 10. 10.3389/fsurg.2023.1084867.
8. Naicker, D., V. Ramsuran, M. Naicker, F. Dessai, J. Giandhari, P. Tinarwo and N. Abbai, 2021. Strong correlation between urine and vaginal swab samples for bacterial vaginosis. South. Afr. J. Infect. Dis., Vol. 36. 10.4102/sajid.v36i1.199.
9. Sheervalilou, R., F. Haidari and M. Razavi, 2022. Diagnostic value of Amsel clinical criteria compared to Nugent scoring system for the diagnosis of bacterial vaginosis in pregnant women. Curr. Appl. Sci., 2: 49-58.
10. Nugent, R.P., M.A. Krohn and S.L. Hillier, 1991. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J. Clin. Microbiol., 29: 297-301.
11. Amsel, R., P.A. Totten, C.A. Spiegel, K.C. Chen, D. Eschenbach and K.K. Holmes, 1983. Nonspecific vaginitis: Diagnostic criteria and microbial and epidemiologic associations. Am. J. Med., 74: 14-22.
12. Gupta, N., N. Kaur, H. Kumar, S.P.K. Sidhu, J. Chauhan, R. Bala and S. Chauhan, 2023. Diagnostic accuracy of Nugent scoring in comparison to Amsel's criteria for diagnosis of bacterial vaginosis in women of reproductive age group at a tertiary care hospital. JK Sci.: J. Med. Educ. Res., 25: 13-18.
13. Leyva-Gómez, G., M.L.D. Prado-Audelo, S. Ortega-Peña, N. Mendoza-Muñoz and Z. Urbán-Morlán *et al*., 2019. Modifications in vaginal microbiota and their influence on drug release: Challenges and opportunities. Pharmaceutics, Vol. 11. 10.3390/pharmaceutics11050217.
14. Jwair, N.A., M.T.S. Al-Ouqaili and F. Al-Marzooq, 2023. Inverse association between the existence of CRISPR/Cas systems with antibiotic resistance, extended spectrum β-lactamase and carbapenemase production in multidrug, extensive drug and pandrug-resistant *Klebsiella pneumoniae*. Antibiotics, Vol. 12. 10.3390/antibiotics12060980.
15. Verstraelen, H. and A. Swidsinski, 2019. The biofilm in bacterial vaginosis: Implications for epidemiology, diagnosis and treatment: 2018 update. Curr. Opin. Infect. Dis., 32: 38-42.
16. Al-Ouqaili, M.T.S., R.O. Saleh, H.I.M. Amin, Z.H. Jawhar and M.R. Akbarizadeh *et al*., 2023. Synthesize of pluronic-based nanovesicular formulation loaded with *Pistacia atlantica* extract for improved antimicrobial efficiency. Arab. J. Chem., Vol. 16. 10.1016/j.arabjc.2023.104704.
17. Al-Ouqaili, M.T.S., R.A. Hussein, Y.H. Majeed and F. Al-Marzooq, 2023. Study of vacuolating cytotoxin A (vacA) genotypes of ulcerogenic and non-ulcerogenic strains of *Helicobacter pylori* and its association with gastric disease. Saudi J. Biol. Sci., Vol. 30. 10.1016/j.sjbs.2023.103867.
18. Al-Kubaisy, S.H., R.A. Hussein and M.T.S. Al-Ouqaili, 2021. Molecular screening of ambler class C and extended-spectrum ß-lactamases in multi-drug resistant *Pseudomonas aeruginosa* and selected species of Enterobacteriaceae. Int. J. Pharm. Res., 12: 3605-3613.
19. Tomás, M., A. Palmeira-de-Oliveira, S. Simões, J. Martinez-de-Oliveira and R. Palmeira-de-Oliveira, 2020. Bacterial vaginosis: Standard treatments and alternative strategies. Int. J. Pharm., Vol. 587. 10.1016/j.ijpharm.2020.119659.
20. Richardson, A. and A.J. Robinson, 2022. Vaginal discharge. Medicine, 50: 254-258.
21. Jung, H.S., M.M. Ehlers, H. Lombaard, M.J. Redelinghuys and M.M. Kock, 2017. Etiology of bacterial vaginosis and polymicrobial biofilm formation. Crit. Rev. Microbiol., 43: 651-667.
22. Cerca, N., 2019. Could targeting neighboring bacterial populations help combat bacterial vaginosis? Future Microbiol., 14: 365-368.
23. Schwebke, J.R., S.L. Hillier, J.D. Sobel, C.M.J.A. McGregor and R.L. Sweet, 1996. Validity of the vaginal gram stain for the diagnosis of bacterial vaginosis. Obstet. Gynecol., 88: 573-576.

24. Sha, B.E., H.Y. Chen, Q.J. Wang, M.R. Zariffard, M.H. Cohen and G.T. Spear, 2005. Utility of Amsel criteria, nugent score and quantitative PCR for *Gardnerella vaginalis*, *Mycoplasma hominis* and *Lactobacillus* spp. for diagnosis of bacterial vaginosis in human immunodeficiency virus-infected women. J. Clin. Microbiol., 43: 4607-4612.
25. Redelinghuys, M.J., J. Geldenhuys, H. Jung and M.M. Kock, 2020. Bacterial vaginosis: Current diagnostic avenues and future opportunities. Front. Cell. Infect. Microbiol., Vol. 10. 10.3389/fcimb.2020.00354.
26. Sousa, L.G.V., S.A. Pereira and N. Cerca, 2023. Fighting polymicrobial biofilms in bacterial vaginosis. Microb. Biotechnol., 16: 1423-1437.
27. Lourenço, A., N.A. Pedro, S.B. Salazar and N.P. Mira, 2019. Effect of acetic acid and lactic acid at low pH in growth and azole resistance of *Candida albicans* and *Candida glabrata*. Front. Microbiol., Vol. 9. 10.3389/fmicb.2018.03265.
28. Jafarzadeh, L., M. Ranjbar, T. Nazari, M.N. Eshkaleti, S.A. Gharehbolagh, J.D. Sobel and S. Mahmoudi, 2022. Vulvovaginal candidiasis: An overview of mycological, clinical, and immunological aspects. J. Obstet. Gynaecol., 48: 1546-1560.
29. Li, T., Z. Zhang, F. Wang, Y. He, X. Zong, H. Bai and Z. Liu, 2020. Antimicrobial susceptibility testing of metronidazole and clindamycin against *Gardnerella vaginalis* in planktonic and biofilm formation. Can. J. Infect. Dis. Med. Microbiol., Vol. 2020. 10.1155/2020/1361825.
30. Swidsinski, A., W. Mendling, V. Loening-Baucke, S. Swidsinski and Y. Dörffel *et al*., 2008. An adherent *Gardnerella vaginalis* biofilm persists on the vaginal epithelium after standard therapy with oral metronidazole. Am. J. Obstet. Gynecology, 198: 97.E1-97.E6.
31. Machado, D., J. Castro, A. Palmeira-de-Oliveira, J. Martinez-de-Oliveira and N. Cerca, 2015. Bacterial vaginosis biofilms: Challenges to current therapies and emerging solutions. Front. Microbiol., Vol. 6. 10.3389/fmicb.2015.01528.