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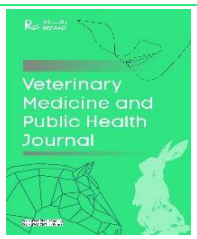

Detection of the Virulence Factors of Bacteria that Cause Bacterial Vaginosis in Women

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Abstract:

Objectives: Bacterial vaginosis (BV) is a vaginal microbiota ecological imbalance that primarily affects women of reproductive age. The study's objectives were to evaluate the risk variables' associations with BV and examine the particular bacteria responsible for BV.

Methods: From May to August 2023, 65 samples obtained from pregnant and non-pregnant women who were registered at the Outpatient Albatool Teaching Hospital participated in this study. In this study, the Vitek 2 compact device was utilized to diagnose BV.

Results: 15 samples showed no clear growth and only 50 samples showed positive bacterial growth. These samples included 15 (30%) samples from pregnant women and 35 (70%) from non-pregnant women, who showed clinical symptoms associated with bacterial vaginosis (BV). Depending on the color of the homogeneous vaginal secretions, showed that bacterial isolation was from the yellow secretion, and it was at a rate of 19 (38 %), then it was followed by the yellow-green secretion, the percentage of isolation was It contained 18 (36 %), while the percentage of bacterial isolation from white vaginal secretions was 13 (26%).

Conclusions: They were diagnosed using the Vitec device to diagnose bacterial isolates, isolated from vaginal secretions and determine their types. the results show that the 20 bacterial isolates were distributed to S. haemolyticus 20 (40%), S. aureus 8 (16%) S. epidermidis 5(10%) E. coli 8 (16 %) Klebsiella pneumonia 6(12%) Gardnerella Vaginalis 3(6%).

Keywords: *Bacterial vaginosis; Virulence Factors; Gardnerella Vaginalis.*

1 Introduction

Vaginitis is an inflammation of the vagina and is typically indicated by any of the following symptoms: vulvar itching, vulvar irritation, vaginal odor, vaginal erythema, dyspareunia, and dysuria (Schwab, 2014). Vaginal discharge frequently contains white blood cells (WBCs) is another sign of vaginitis. Bacterial vaginosis (BV), which is the most common cause of vulvovaginitis, is followed by candidiasis and trichomoniasis (Fiorilli *et al*., 2005). BV is a typical vaginal infection that usually affects pregnant women (Shvartsman et al., 2023). A thin, gray or off-white, homogeneous, foul-smelling vaginal discharge with a pH level greater than 4.5 is a clinical sign of BV. This discharge is more noticeable after menstruation and after sexual activity. Whiff test results reveal a fishy smell when 10% potassium hydroxide is added to the vaginal fluid. Clue cells, a lack of lactobacilli, and a limited number of polymorphonuclear leukocytes (PMNs) (1/hpf) are other signs of BV (Ma et al., 2024). Many BV instances are asymptomatic or only manifest as offensive vaginal discharge without any inflammatory symptoms as a result, BV is referred to be "vaginosis" rather than "vaginitis". The normal flora produces lactic acid, and Lactobacillus is responsible for the vagina's acidic environment by producing hydrogen peroxide (H2O2). By preventing the growth of other species, this acts as a local defense mechanism. (Huynh et al. 2017) Changes in pH brought on by changes in the typical vaginal flora allow a range of facultative and anaerobe bacteria to overgrow, leading to persistent infection and aberrant vaginal discharge (Deese et al., 2018). To support a balanced environment in the vagina and prevent the formation of pathogens, lactobacilli also produce antimicrobial compounds such lactic acid, H2O2, and Bacteriocin (Muzny et al., 2022). In addition to Lactobacillus spp., other bacteria are commonly discovered in healthy women's vaginal microbiome.

The organisms discovered include Gram-positive cocci and Gram-negative rods, specifically Streptococcus spp., Staphylococcus spp., and members of Enterobacteriaceae, primarily E. coli, in addition to, Gardnerella vaginalis (Afzal, 2017). These opportunistic bacteria may overgrow and become the dominant species in the environment if the population level of lactobacilli falls below a crucial level. Vaginal infections during pregnancy can cause serious problems for the mother and the unborn child, as well as gynecologic and obstetric issues (Ahmed et al., 2014). BV also raises the possibility of contracting STDs and HIV, two sexually transmitted diseases. Instead of being an STD, BV may be thought of as a sexually enhanced disease (SED), in which the frequency of sexual activity is important. Therefore, it is important to place more attention on investigating primary preventative methods. (Alahmadi et al., 2021) Targeting risk factors or behaviors for a disease is the goal of preventive measures. Numerous risk factors and behaviors have been linked to BV, according to studies. These include age, marital status, employment status, occupation, recent antibiotic use, douching, sexual activity, younger age of first intercourse, STDs, working as a sex worker, drinking alcohol, smoking, stress, using contraceptives, frequency of vaginal intercourse, and race/ethnicity. Women who use hormonal contraceptives have a lower risk of developing BV again, according to a number of observational studies (Al-Haik and Al-Haddad, 2020). Sexual activity and vaginal douching are two particularly notable risk variables that are changeable. However, just a few research have examined the incidence of BV and its risk factors among Nepalese women. As a result, this study was conducted to evaluate the risk variables' associations with BV and to identify the specific bacteria that cause BV. (Aljamali et al., 2021).

2 Materials and Methods

2.1 Sample Collection and Processing

The gathering and processing of samples. A nonlubricated speculum was placed into the vagina to collect discharge, and its shape, color, consistency, and odor were all physically assessed. collect two vaginal samples using sterile cotton-tipped swabs. The number of women under study was (65), a sample of pregnant and non-pregnant women suffering from clinical symptoms of vaginal infections in Diyala for the period from July to September 2023.

They are between 16-60 years old. The number included three groups non-pregnant women with vaginitis, pregnant women in different months of pregnancy, and women in the postpartum period. The swabs taken from the patients were cultured on the media and incubated in the plates (Chocolate agar, Blood agar, MacConkey agar, Mannitol salt agar, Columbia agar, and Brain heart infusion broth. Growth was examined after that period in the incubator at (37°C) for (24-48) hours. Then a secondary culture was made - from the dishes that

showed Positive result. Isolates were identified according to source based on bacteriological tests using a Vitek 2 compact device. Antibiotic susceptibility testing was performed on Mueller-Hinton agar medium, Penicillin, Ampicillin, Gentamycin, Lincomycin, Metronidazole, Erythromycin, Tetracycline, Cephalothin to conduct susceptibility testing for the bacterial isolates under study. The results were read by observing the zones of inhibition around the antibiotic tablets and the results were interpreted accordingly (Muzny et al., 2022). One of the swabs was taken to the Microbiology Laboratory in a sterile-capped test tube for aerobic and anaerobic cultures in MacConkey agar, Blood agar, and Chocolate agar for bacterial isolate identification. The first two agars were inoculated aerobically for 24 hours at 37°C and then anaerobically for 24 hours in a candle jar.

A different swab was used to create a smear for direct wet mount microscopy and Gram staining. The Gram-stained slides were viewed with a 1000x oil immersion objective. Wet mounting was done to examine the presence of clue cells, white blood cells, Trichomonas vaginalis, and motile oval flagellated protozoa. Gram staining and germ tube testing were conducted on yeasts. When treated in serum for two hours at 37° C, the capacity of Candida albicans to generate germ tubes served as a means of identification.

2.2 Statistical Analysis

It was determined the prevalence of BV and bacteria using a descriptive analysis. Using the Statistical Package for Social Sciences (SPSS-16), chi square values were obtained at the 5% (= 0.05) level of significance

3 Results and Discussion

In this study, 50 samples were collected from pregnant and non-pregnant females from Al-Batoul Teaching Hospital in Diyala Governorate for the period of time (January to March 2023). These samples included 15 (30%) samples from pregnant women and 35 (70%) from non-pregnant women, who showed clinical symptoms associated with bacterial vaginosis (BV). Information about the sick women was recorded, and then phenotypic, microscopic, biochemical and molecular examinations were performed as shown in (Table 1).

Table 1: Distribution of female infection rates with bacterial vaginosis (BV)

Age group	**%**	**No**	
Less than 50 years old	90%	45	P<0.001***
More than 50 years	10%	5	
Pregnancy	%	No	
Pregnant women	30%	15	P<0.001***
Non-pregnant women	70%	35	
Other diseases	%	No	
Inflammation of the urinary tract	90%	45	P<0.001***
And reproductive diseases	10%	5	
Diabetes	%	No	
marital status	90%	45	P<0.001***
married	10%	5	

3.1 Diagnosis by color of secretion

Depending on the color of the homogeneous vaginal secretions, which are white, yellow, or yellow-green, the results of our study showed that bacterial isolation was from the yellow secretion, and it was at a rate of 19 (38 %), then it was followed by the yellow-green secretion, so the percentage of isolation was It contained 18 (36 %), while the percentage of bacterial isolation in white vaginal secretions was 13 (26%).These results agreed with Machado & Cerca, (2015), where the bacteria causing bacterial vaginosis showed the highest percentage in the greenish-yellow secretion, 6.7%, followed by the yellow-colored secretion. (5.3%). A lower percentage was found in white feces (4.3%). The reason for diagnosing bacterial vaginosis is based on the color of the secretion, the unpleasant odor, and the uniformity of the secretions, which confirm the presence of infection. This result of our current study was in agreement with Arabyan et al., (2017) and Arroyo et al., (2021), which indicated that vaginitis may be without, Symptoms But the color of vaginal discharge is one of the common clinical signs of bacterial vaginosis. It is a white or granular homogeneous discharge with or without an unpleasant odor. It also agrees with the study of Asmita and Sobel (2019) as well as 18. Aunkor et al. (2021) which confirmed that vaginal symptoms of

itching and burning in addition to vaginal discharge: in terms of its consistency, viscosity, color and smell, are one of the most important signs of vaginal inflammation. Also, our results showed that there are significant differences between the color Secretion and among the types of isolates. These results are consistent with the study. Barros et al., (2012), which showed that the presence of abnormal vaginal secretions is part of the diagnosis of bacterial vaginosis.

Table 2: Distribution of bacterial isolates depending on the color of vaginal secretions

Color of secretions		Isolates	No.	%
White	%26	*S. haemolyticus*	5	10%
		S. aureus	2	4%
		E. coli	1	2%
		Klebsiella pneumonia	5	10%
Yellow	% 38	*S. haemolyticus*	10	20%
		S. aureus	3	6%
		E. coli	2	4%
		S. epidermidis	3	6%
		Klebsiella pneumonia	1	2%
Greenish-yellow	%36	*S. haemolyticus*	5	10%
		S. aureus	3	6%
		E. coli	5	10%
		S. epidermidis	2	4%
		Gardnerella Vaginalis	3	6%
Total	**%100**		**50**	**100%**
P. Value			**P<0.05***	

3.2 Morphological identification

Clinical samples were examined and classified according to color, odor, and pH of vaginal secretions. The results showed that the colors of vaginal secretions ranged from white to greenish-yellow (20%, 35%, 45%, respectively). The pH of vaginal secretions was in the range (5.0 - 7.0). Then, the bacterial species causing bacterial vaginosis (BV) were initially diagnosed based on the phenotypic characteristics of the bacteria, after the samples were planted and grown directly on culture media on blood agar, chocolate agar, MacConkey agar, and incubated warmly ° 37°C and for 24 hours, the results showed that 20 (40%) bacterial isolates showed positive growth, while 30 (60%) showed negative growth on the culture media. The samples were diagnosed phenotypically and according to biochemical tests, and then confirmed using the Vitek device. Some isolates appeared in pink on MacConkey agar and others in yellow depending on their ability to ferment or not ferment the sugar lactose, while some isolates appeared white, gray, or cream in color on blood agar and chocolate agar media microscopic examination: The bacteria were visible under an optical microscope at X100 magnification after staining with Gram's stain was colored red for Gram-negative bacteria and purple for Gram-positive bacteria. The percentage of Gram stain positive bacteria was 33 (66 %) higher than the percentage of Gram-negative bacteria 17 (34%). As shown in the (Table 3).

Table 3: Distribution of bacterial isolates depending on the Gram stain.

Gram stain	isolates	NO.	%
Gram positive	*S. haemolyticus*		
	S. aureus	33	66%
	S. epidermidis		
Gram negative	*E. coli*		
	Klebsiella pneumonia	17	
	Gardnerella Vaginalis		34%
Total		**50**	**100%**
P value	**P<0.01****		

The results of this study agreed with the results of Hussein and Issa (2018), as well as with Razzak and others (2011), who found that the percentage of Gram stain -positive bacteria was higher (63%) and (66%), respectively, while the Gram stain -negative bacteria had the lowest percentage (36%). (34%), respectively, while it did not agree with what was stated by Aljamal et al., (2021), as their results showed that the incidence of negative bacteria was higher than positive ones.

3.3 Diagnosis by Vitek

For the purpose of ensuring the accuracy of the diagnosis of bacterial isolates isolated from vaginal secretions and determining their types, they were diagnosed using the Vitec device. There was agreement in the results obtained from the Vitec device (Table 4), with the results obtained with traditional diagnostic methods, showing the results of our study. Through the diagnosis of Vitek that the species causing vaginitis included vaginal and intestinal pathogenic bacteria Boyanova et al., (2018). The results show that the 50 bacterial isolates were distributed to Gram positive, and the S. haemolyticus bacteria included the highest percentage of growth in vaginal secretions 20 (40%), S. aureus 8(16%), S. epidermidis 5(10%). whereas Gram negative isolates include E. coli 8 (16%), Klebsiella pneumonia 6(12%), Gardnerella Vaginalis 3(6%).

Table (4): Distribution of clinically isolated bacterial isolates from patients with vaginitis using the Vitek device

Isolates	No.	%
S. haemolyticus	20	40%
S. aureus	8	16%
S. epidermidis	5	10%
E. coli	8	16%
Klebsiella pneumonia	6	12%
Gardnerella Vaginalis	3	6%
Total	**50**	**100%**
P value	**P<0.01****	

4 Conclusions

The study suggests that using contraceptives on anatomical sites may confer a higher risk for BV. Some factors, especially vaginal douching, may increase the risk of BV. Lactobacillus spp. were the predominant isolates found in the vaginal sample followed by a number of Enterobacteriaceae members and Gram-positive bacteria. This finding suggests that the colonization of facultative anaerobes is also more likely a consequence in vaginal ecology. Limited studies on BV have been performed in Nepal. So, similar studies must be carried out in order to improve the health status of women, thereby preventing the risk posed towards BV.

Recommendations:

- Investigating the use of nanoparticles as an alternative to antibiotics in the treatment of bacterial species that cause BV.
- Conducting numerous molecular studies to identify the causes of the disease and the resistance genes of the bacterial isolates causing the disease, as well as understanding the mechanism of antibiotic resistance.

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