DOI: https://doi.org/10.53350/pjmhs22166392

ORIGINAL ARTICLE

Diagnosis of Gardnerella Vaginalis Isolated from Vaginal Discharge of Women in Babylon Province

SAWSAN TALAL. ABDULLAH[1], AZHAR O. ALTHAHAB[2], SURA I.A. JABUK[3]
[1,2,3]Department of Biology, College of Science, University of Babylon, Iraq
Correspondence to: Sawsan T.Abdalla, Email: saousentalal@gmail.com

ABSTRACT

Bacterial vaginitis is a prevalent condition that affects women all over the world and is the most common cause of vaginitis that has been recognized. Gardnerella vaginalis was found to be the most often identified pathogen in the vaginal discharge samples taken from patients diagnosed with vaginal infections. Two hundred (100 for Amsel test, 100 for culture) vaginal samples were taken from 100 women (two swaps from each woman) by the gynecologist to study the identification of G. vaginalis by Wet smear, Direct staining, Amsel test, Culture identification, and Molecular identification by 16sRNA. The result of Amsel test presents 10(18%) samples positive to four Amsels criteria and other samples positive to three of Amsel's criteria from the 56 samples for positive Gardnerella vaginalis samples. The result of culture, biochemical, and Gram stain present the percentage of samples positive for G. vaginalis was 56 (56%). Electrophoresis of the PCR results of G. vaginalis DNA extracted from vaginal samples revealed the presence of a band with a size of 300 base pairs in only 12 of the 56 positive culture samples. As a result, it is imperative that diagnostic techniques for the detection of this illness be improved, particularly in terms of differentiating BV from other potential causes of vaginal infections

Keywords: Bacterial vaginitis, Amsel test, Polymerase chain reaction.

INTRODUCTION

Bacterial vaginosis (BV) is the most prevalent cause of vaginal discharge in women of reproductive age. BV has been associated with an increased risk of sexual diseases, urinary incontinence, post-surgical problems, fertility problems, pregnancy failures, premature delivery, and cancer. (Brusselaers, Shrestha, Van De Wijgert, Verstraelen, & gynecology, 2019; van de Wijgert, 2017).

Lactobacilli levels are lower in BV, and a polymicrobial consortia, often containing a significant number of G. vaginalis, is overexpressed. Most women don't tell their doctors about their BV symptoms, even though they have clinical symptoms and/or inflammation mediators. (Balashov, Mordechai, Adelson, & Gygax, 2014; Masson et al., 2019).

G. vaginalis, a facultative anaerobic bacterium, is the most common cause of bacterial vaginosis. Virulence factors of G. vaginalis include attachment to vaginal mucosal epithelial cells, biofilm formation, and cytotoxicity. These findings provide further support for the particular function that G. vaginalis plays in the pathogenesis of bacterial vaginosis. (Machado & Cerca, 2015; Schwebke, Muzny, & Josey, 2014)The study aims to isolate and identification of G. Vaginalis in vaginitis, the identification of Gardnerella vaginalis, based on: Colonial morphology, β-hemolysis, Gram-stained smear from a colony, Biochemical tests, Molecular detection.

MATERIALS AND METHODS

Samples Collection: Two hundred (100 for Amsel test, 100 for culture) vaginal samples were taken from 100 women (two swaps from each woman) by the gynecologist were collected from hospitals in Babylon Province /Iraq: A-l Zahraa hospital for maternity and Al- exandria general hospital during the period from (December 2021 to April 2022), in the vaginal fornix, using sterile cotton-tipped swabs. After inserting a Swap into the vagina, an analysis of the discharge was carried out to determine its form, color, viscosity and smell.

Bacterial Identification

1 Wet smear: This method was used to observe the clue cells that are covered with bacteria (Catlin, 1992).

2 Direct staining: The direct staining conducted by using Gram stain of vaginal secretion to differentiate bacteria that was surrounded in clue cells. Positive result is observed clue cells. (Catlin, 1992)

3 Amsel Test: One hundred swabs were utilized in the process of developing the Amsel criteria in order to notice three of the following characteristics, which are usually believed to be supportive of the diagnosis of BV: (1) a discharge that is homogenous and thin; (2) a pH that is higher than the usual range (>4.5); and (3) the detection of a fishy odor, with or without the treatment of the sample with 10% potassium hydroxide. (Forsum, Larsson, & Spiegel, 2008).

4 Culture Identification: One hundred swabs were transported in a sterile test tube with a cap to the microbiology laboratory for aerobic and anaerobic cultures in MacConkey agar, Blood agar, and Columbia blood agar supplemented with 5% fresh blood with the addition of Nalidixic acid, Gentamycin, and Amphotericin B for the identification of bacterial isolates. Inoculation of the MacConkey agar and Blood agar was performed aerobically at 37 ◦ C for 24hours, while inoculation of the Columbia blood agar was performed anaerobically at 37◦ C in a candle jar for 36-72. (Ranjit, Raghubanshi, Maskey, & Parajuli, 2018).

5 Gram's stain: Single pure colonies of G. vaginalis bacteria stained with Gram stain to observe the shape and reaction of cells were determined under a light microscope.

6 Molecular Identification: Kit for the extraction of DNA (G-SpinTM Total DNA extraction kit (iNtRON/ Korea)) was used in DNA extraction from grown bacterial colonies. The forward primer for detection of 16SrRNA gene was GCTCAACCAGGCACA AAAA C A, while the reverse primer was TCCACGCCTAGTTGGGTCTA. Gene detection in G. vaginalis required 5 minutes of 95°C denaturation, 50 seconds of 59°C annealing, and an additional 45 seconds of 72°C extension during the course of the PCR process. PCR Reaction Mixture were performed in a 25 µl contained 2µl of forward primer, 2µl of reverse primer, 5µl of extracted DNA, and 12.5µl of Taq PCR Master Mix, and 3.5µl nuclease-free water. PCR PreMix is a substance that has been lyophilized and contains all of the other components that are required for a PCR reaction. These components include: (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, MgCl2, stabilizer, and tracking dye). Successful PCR amplification was confirmed by gel electrophoresis on 1.5% agarose gels for 50 min at 70 V (Mohammadzadeh, Kalani, Kashanian, Oshaghi, & Amirmozafari, 2019).

RESULTS AND DISCUSSIONS

The study included the collection of 200 samples from women infected with bacterial vaginosis, for the period from December 2021 to April 2022 for pregnant and non-pregnant women. The wet swabs prepared directly from the vaginal secretions and with staining showed the presence of clue cells Figure 1.

Gardnerella vaginalis grow on Columbia agar supplemented with 5% fresh blood with the addition of Nalidixic acid, Gentamycin, and amphotericin B and the colonies tend to be smooth, small colonies (hear pin) circular entire, glistening, and opaque colonies variable-Blood hemolysis the addition of Antibiotics were allowed to selective isolation of G. vaginalis that are described by Figure 2.

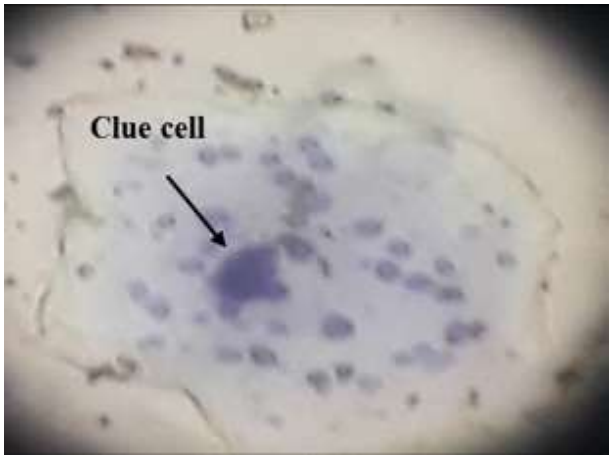

Figure 1: Direct staining of vaginal secretion clue cell appears covered with Gardnerella Vaginalis

Figure 2: Gardnerella Vaginalis growth on Columbia blood agar. The colonies appear smooth, small colonies (hear pin)

The smears of G. vaginalis bacteria stained with Gram stain showed the presence of small and heterogeneous bacilli of Gram-positive to heterogeneous forms of Gram stain, and they do not elongate into filamentous shapes and spread in the microscopic field, it was noted that increasing the incubation period of these bacteria leads to the transformation of bacteria from heterozygous from Gram-positive to Gram-negative (Vieira-Baptista & Bornstein, 2019).

The bacteria have catalase variable, oxidase negative, Hippurate hydrolysis - variable, β-hemolysis variable, Urea hemolysis negative, glucose fermentation positive, and lactose fermentation positive Table 1.

Table 1: Biochemical test of Gardnerella vaginalis

Test	Result
Oxidase	-
Catalase	±
Hippurate hydrolysis	±
β-hemolysis	±
Urea hemolysis	-
Methyl red test	+
Glucose fermentation	+
Lactose fermentation	+

The result of culture present of only G. vaginalis isolates was 0%, while G. vaginalis and gram-positive, G. vaginalis and gram-negative were 19%, 37%, respectively. Also present the percentage of only gram-positive or only gram-negative was12%, 21%, respectively, and only 11% of the samples was No growth Table 2.

Table 2: Distribution Gardenalla vaginalis isolate among cause agents

Type of Causes agent	Number	Percentage
Only G. vaginalis	0	0%
Only Gram-positive	12	12%
Only Gram-negative	21	21%
G. vaginalis and Gram-positive	19	19%
G. vaginalis and Gram-negative	37	37%
No growth	11	11%
Total	100	100%

The results of Amsel's criteria shown in Table 3. present 10(18%) samples positive to four criteria and other samples positive to three of Amsel's criteria from the 56 samples. The gold standard method for diagnosing BV is Amsel's criteria, especially in developing countries where numerous criteria are employed for BV confirmation. Amsel's criteria are based on a clinical diagnosis and a few simple lab tests (Bhujel, Mishra, Yadav, Bista, & Parajuli, 2021).

The Amsels criteria have a poor diagnostic performance for bacterial vaginosis, although they are widely employed and should be the first step for the diagnosis of vaginitis. (Vieira-Baptista et al., 2021)

Table 3: Amsel criteria for patient

character	Discharge	Whiff test	PH	Clue cell	No. (%)
1	+	+	+	+	10(18%)
2	+	-	+	+	21(38%)
3	+	+	-	+	11 (20%)
4	+	+	+	-	8 (14%)
5	-	+	+	+	6 (11%)
Total					56(100%)

The result of culture, biochemical, and Gram stain present the percentage of samples positive for G. vaginalis was 56 (56%), including 7%, 26%,13%,8%, and 2% isolated from < 20 ,20-30, 31-40, 41-50, and >50 age, respectively Table 4.

Table 4: Distribution of Gardnerella vaginalis isolates according to the age

Age	NO. of positive samples (%)	NO.of negative samples(%)	Total (%)
< 20	7(7%)	3(3%)	10(10%)
20-30	26(26%)	19(19%)	45(45%)
31-40	13(13%)	14(14%)	27(27%)
41-50	8(8%)	6(6%)	14(14%)
>50	2(2%)	2(2%)	4(4%)
Total	56(56%)	44(44%)	100(100%)

BV is the most prevalent cause of vaginitis and is a widespread problem for women around the world. G. vaginalis is the most frequently found pathogen in samples taken from patients who have had vaginal infections. However, there is no reliable diagnostic tool for detecting this bacterium. For this reason, new and improved diagnostic tests are needed to better differentiate between this infection and other causes of vaginal infections. (Hashemi et al., 2021).

Table 5: Distribution of Gardnerella vaginalis isolates according to the age diagnosis by 16sRNA

Age	NO. of positive samples (%)	NO. of negative samples (%)	Total (%)
< 20	4(7%)	3(5%)	7(13%)
20-30	5(9%)	21(38%)	26(46%)
31-40	1(2%)	12(21%)	13(23%)
41-50	1(2%)	7(13%)	8(14%)
>50	1(2%)	1(2%)	2(4%)
Total	12(21%)	44(79%)	56(100%)

For the electrophoresis of PCR products of G. vaginalis DNA, only 12 of the 56 positive culture samples showed a 300 bp band including 7%,9%,2%,2%, and 2% isolated from < 20, 20-30,

31-40, 41-50, and >50 age, respectively (Figure 3, Table 5). In addition to this, it has been demonstrated that the PCR method for diagnosing BV is more sensitive than the culture method.

Molecular techniques such as polymerase chain reaction (PCR) are utilized in the diagnostic process of a variety of disorders. However, in order to develop a simpler method that is applicable in clinical laboratories, particularly in developing countries, we opted to select a simple PCR method for the diagnosis of G. vaginalis in vaginal samples. (Hashemi et al., 2021).

Different studies have employed molecular techniques to diagnose vaginal infections. (Cartwright et al., 2013) employed nucleic acid amplification-based assays for the identification of vaginitis in 323 symptomatic women with vaginal infection.

According to (Sha et al., 2005), the Amsel criteria had a low predictive value for the diagnosis of BV. They also said that the PCR approach was substantially more accurate than Amsel criteria in determining the presence of this disease.

According to research conducted by (Menard, Fenollar, Henry, Bretelle, & Raoult, 2008), molecular quantification of G. vaginalis has a sensitivity of 96 percent and a specificity of 99 percent. (Obata-Yasuoka, Ba-Thein, Hamada, Hayashi, & Gynecology, 2002) employed the PCR technique to identify BV.

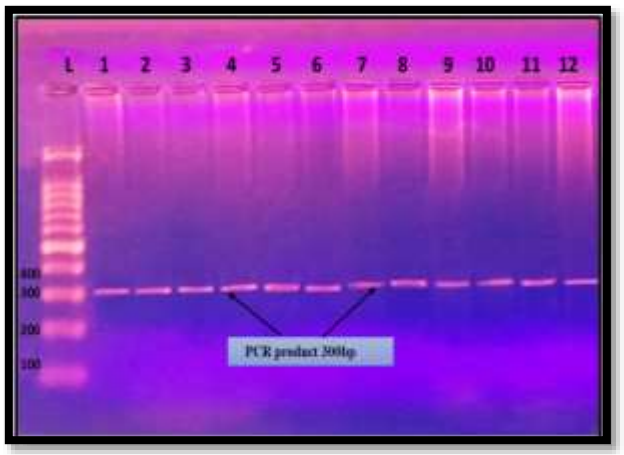

Figure 3: Agarose gel electrophoresis staining with Ethidium bromide stains (1.5% agarose, 70volt for 60 min) for Gardnerella Vaginalis 16 s RNA gene product (amplified size 300 bp) using DNA template of Gardnerella Vaginalis isolates. Lane (L) Molecular size marker for DNA molecules (100-bp ladder). Lanes (1-12) show positive results.

(Makarova et al., 2000) examined G. vaginalis diagnosis approaches based on the morphology, microbiology, serology, and genetics of the organism. For the diagnosis of G. vaginalis, they found that the PCR method was more accurate than other methods.

According to a study conducted by Menard et al. (2008), the sensitivity of the molecular diagnosis of G. vaginalis was found to be 95%, while the specificity was found to be 99%. The findings of these studies agree our results, demonstrating that molecular methods for detecting BV are more sensitive and specific than microscopy methods.

REFERENCES

1 Balashov, S. V., Mordechai, E., Adelson, M. E., & Gygax, S. E. J. J. o. m. m. (2014). Identification, quantification and subtyping of Gardnerella vaginalis in noncultured clinical vaginal samples by quantitative PCR. 63(2), 162-175.
2 Bhujel, R., Mishra, S. K., Yadav, S. K., Bista, K. D., & Parajuli, K. J. B. i. d. (2021). Comparative study of Amsel's criteria and Nugent scoring for diagnosis of bacterial vaginosis in a tertiary care hospital, Nepal. 21(1), 1-6.
3 Brusselaers, N., Shrestha, S., Van De Wijgert, J., Verstraelen, H. J. A. j. o. o., & gynecology. (2019). Vaginal dysbiosis and the risk of human papillomavirus and cervical cancer: systematic review and meta-analysis. 221(1), 9-18. e18.
4 Cartwright, C. P., Lembke, B. D., Ramachandran, K., Body, B. A., Nye, M. B., Rivers, C. A., & Schwebke, J. R. J. J. o. C. M. (2013). Comparison of nucleic acid amplification assays with BD affirm VPIII for diagnosis of vaginitis in symptomatic women. 51(11), 3694-3699.
5 Catlin, B. W. J. C. M. R. (1992). Gardnerella vaginalis: characteristics, clinical considerations, and controversies. 5(3), 213-237.
6 Forsum, U., Larsson, P. G., & Spiegel, C. J. A. (2008). Scoring vaginal fluid smears for diagnosis of bacterial vaginosis: need for quality specifications. Letter to the Editor. 116(2), 156-159.
7 Hashemi, H., Shakibapour, M., Fazeli, H., Varshowsaz, J., Aliyari, Z., & Yousofi Darani, H. J. A. o. C. I. D. (2021). Molecular Study of Gardnerella vaginalis Isolated from Vaginal Discharge of Women Referring to Gynecology Clinics in Chaharmahal and Bakhtiari, Iran, in 2017. 16(2).
8 Machado, A., & Cerca, N. J. T. J. o. i. d. (2015). Influence of biofilm formation by Gardnerella vaginalis and other anaerobes on bacterial vaginosis. 212(12), 1856-1861.
9 Makarova, L., Kravtsov, E., Vasil'eva, E., Dmitriev, G., Medvedeva, E., Kirillov, M. Y., . . . Medicine. (2000). Modern methods for diagnosis ofGardnerella infection. 130(2), 780-782.
10 Masson, L., Barnabas, S., Deese, J., Lennard, K., Dabee, S., Gamieldien, H., . . . Van Damme, L. J. S. t. i. (2019). Inflammatory cytokine biomarkers of asymptomatic sexually transmitted infections and vaginal dysbiosis: a multicentre validation study. 95(1), 5-12.
11 Menard, J.-P., Fenollar, F., Henry, M., Bretelle, F., & Raoult, D. J. C. I. D. (2008). Molecular quantification of Gardnerella vaginalis and Atopobium vaginae loads to predict bacterial vaginosis. 47(1), 33-43.
12 , R., Kalani, B. S., Kashanian, M., Oshaghi, M., & Amirmozafari, N. J. M. J. o. t. I. R. o. I. (2019). Prevalence of vaginolysin, sialidase and phospholipase genes in Gardnerella vaginalis isolates between bacterial vaginosis and healthy individuals. 33, 85.
13 Obata-Yasuoka, M., Ba-Thein, W., Hamada, H., Hayashi, H. J. O., & Gynecology. (2002). A multiplex polymerase chain reaction-based diagnostic method for bacterial vaginosis. 100(4), 759-764.
14 Ranjit, E., Raghubanshi, B. R., Maskey, S., & Parajuli, P. J. I. j. o. m. (2018). Prevalence of bacterial vaginosis and its association with risk factors among nonpregnant women: A hospital based study. 2018.
15 Schwebke, J. R., Muzny, C. A., & Josey, W. E. J. T. J. o. i. d. (2014). Role of Gardnerella vaginalis in the pathogenesis of bacterial vaginosis: a conceptual model. 210(3), 338-343.
16 Sha, B. E., Chen, H. Y., Wang, Q. J., Zariffard, M. R., Cohen, M. H., & Spear, G. T. J. J. o. c. m. (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. 43(9), 4607-4612.
17 van de Wijgert, J. H. J. P. m. (2017). The vaginal microbiome and sexually transmitted infections are interlinked: consequences for treatment and prevention. 14(12), e1002478.
18 Vieira-Baptista, P., & Bornstein, J. (2019). Candidiasis, bacterial vaginosis, trichomoniasis and other vaginal conditions affecting the vulva. In Vulvar Disease (pp. 167-205): Springer.
19 Vieira-Baptista, P., Silva, A. R., Costa, M., Figueiredo, R., Saldanha, C., Sousa, C. J. I. J. o. G., & Obstetrics. (2021). Diagnosis of bacterial vaginosis: Clinical or microscopic? A cross-sectional study.