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Research Article

Identification of *Gardnerella vaginalis* by Molecular Methods in Women Diagnosed With Bacterial Vaginosis in Isfahan, Iran

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Abstract

Background: Bacterial vaginosis (BV) is one of the most common causes of abnormal vaginal discharge in women. The disease is characterized by an imbalance in the vaginal bacterial flora. We aimed to determine the frequency of *Gardnerella vaginosis* by cultivation and molecular method.

Methods: In summer 2019, the vaginal secretion of 110 women with BV were collected and isolated for *G. vaginalis*, in Isfahan. The isolates were identified by the biochemical test. Polymerase chain reaction (PCR) was performed to detect *G. vaginalis* in vaginal secretions. Antibiotic susceptibility of the isolates was evaluated by disc diffusion method.

Results: *Gardnerella vaginalis* was isolated from five samples among the 110 patients with symptoms of BV by cultivation. Based on molecular identification, *G. vaginalis* was shown in 32 (29.1%) samples. *G. vaginalis* isolates were resistant to clindamycin (20%) and amoxicillin/clavulanic acid (80%). All the isolates were sensitive to Metronidazole. All women with this infection were married and most (43.8%) belonged to the 25-30 year-old age group. A significant difference was found between participants with positive clue cell ($P<0.01$) and pH>4.5 vaginal discharge ($P<0.01$) in the PCR-positive and the PCR-negative women.

Conclusion: High prevalence of *Gardnerella vaginalis* in women with vaginosis confirms the important role of bacteria in the incidence of BV. Identification of pathogenic agents of *G. vaginosis* using molecular methods and determining their antibiotic susceptibility pattern is essential for proper treatment in different societies.

Keywords: *Gardnerella vaginalis*, Bacterial vaginosis, Drug resistant, Amsel criteria

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Background

Gardnerella vaginalis is a gram-variable, non-encapsulated, non-motile, coccobacillus (0.5-1.5 µm) that can grow in micro-aerobic conditions (1). *G. vaginalis* is a part of the lower female vaginal microflora. This bacterium is an important causative agent for bacterial vaginosis (BV). *G. vaginalis* is a single etiological agent for BV. Clinical isolates of *G. vaginalis* are catalase and oxidase negative, blood beta hemolysis and, Glucose and Maltose fermentative (2, 3). BV is a common vaginal disorder characterized by changes of vaginal bacteria from a normal to a heterogeneous state containing a complex population of anaerobic microbial organisms (4).

G. vaginalis either alone or in combination with other microorganisms are the causative agents of BV (5). The Amsel criteria are used as the main clinical method for the diagnosis of BV and include a vaginal pH>4.5, the presence of adherent white vaginal discharge, a positive Whiff test (unpleasant amine odor) and clue cell observation in gram staining smear (2). *G. vaginosis* is treated with both metronidazole, clindamycin, and amoxicillin/clavulanic acid that are available as oral capsules and vaginal ointment (6).

Since *G. vaginalis* is a bacterium in vaginal flora of healthy women, the isolation of this bacterium may not be used for BV diagnosis. But in many BV cases increased number of *G. vaginalis* is associated with the presence of BV (7, 8). The increase in the prevalence of BV and the lack of clinical symptoms in most patients, which have resulted in failure to treat these cases or inadequate treatments, re-admission of patients and failure to control vaginal infections, as well as increased drug resistance bacteria (9), necessitate further studies in this regard.

Objectives

The aim of this study was to evaluate the frequency of *G. vaginalis* in patients with BV referring to Isfahan clinics using cultivation and the molecular method.

Methods

Sampling

In this study, from September to April 2018, 110 women with evident clinical symptoms of vaginal discharge were studied. Women who had symptoms of BV, according to

a gynecologist were included in the study. To diagnose BV, four Amsel criteria were used. These criteria included vaginal pH > 4.5, the presence of clue cells in Gram stain, homogenous white discharge, and fishy odor (10, 11).

An informed consent was obtained from the participants. Women who had received any anti-inflammatory medication or antibiotics for two weeks prior to the study or who were pregnant were excluded.

A questionnaire containing patient characteristics such as age, place of residence, education, occupation, marital status, and having a child was completed by each woman.

Vaginal Sample Collection

Vaginal specimens were collected during clinical examination. A sterile swab was saturated with vaginal secretion and immediately cultured on selective medium. Additional vaginal samples were collected for molecular identification.

Vaginal discharges were cultured on chocolate agar and brain heart infusion agar supplemented by starch. Plates were incubated at 37°C for 48-72 hours in microaerophilic atmosphere with 5-10% CO_2. After incubation, the gram-variable or gram-negative bacilli and small and transparent colonies were presumptively identified as *G. vaginalis* and differential biochemical tests were performed (12, 13).

Kirby Bauer's disc diffusion method was used to determine the antibiotic sensitivity of *G. vaginalis* isolates for metronidazole (50 µg), clindamycin (2 µg) and amoxicillin/clavulanic acid (20/10 µg) according to recommendations by the Clinical Laboratory Standard Institute (CLSI) (14).

Primer Design and Polymerase Chain Reaction Assay

DNA was extracted from the vaginal swaps with the Aron-Gene Pars kit, according to the manufacturer's instructions. For specific molecular identification, the primers GVNM forward (5'-TTGACATGTGCCTGACGACTG-3') and GVNM reverse (5'-GCACCATGTCACCATGAAGCAA-3') were designed based on the conserved region of the 16S rRNA gene fragments. The BLAST analysis of these primers provided an identity value of 100% with the *G. vaginalis* gene.

The polymerase chain reaction (PCR) was done in a final volume of 25 µL containing 2.5 µL PCR buffer (1X), 0.5 µL dNTP mix (200 µM), 0.75 µL $MgCl_2$ (1.5 mM), 0.2 µL Taq DNA polymerase, 1 µL of each primer, 5 µL of DNA template and 14.05 µL nuclease-free water. The PCR amplification included the following steps: an initial denaturation (5 minutes at 94°C), followed by 35 cycles of denaturation (35 seconds at 94°C), annealing (35 seconds at 55°C), and extension (30 seconds at 72°C), with a single final extension (5 minutes at 72°C). The PCR products were analyzed in 1% agarose gel for 50 minutes at 80 V, and detected by gel documentation system (15).

Statistical Analyses

The collected data were analyzed using SPSS software, version 17.0, descriptive statistics, and Chi-square test. $P \leq 0.05$ was considered statistically significant.

Results

We included 110 women with a mean ± SD age of 30.6 ± 6.2 years (range: 21-45 years) diagnosed with vaginosis.

Gardnerella vaginalis was isolated from 5 (4.5%) samples based on cultivation. The colony morphology and gram-stain (for clue cell) are shown in Figure 1. The biochemical test of culture–positive samples were catalase-negative, oxidase-negative, starch/hydrolysis-positive, and glucose and maltose fermentation-positive.

Gardnerella vaginalis was found in 32 (29.1%) samples through the PCR method (Figure 2). All vaginal samples with a positive culture for *G. vaginalis* also showed positive molecular identification.

The results of antibiotic sensitivity pattern of isolates showed that *G. vaginalis* were 20% resistant to clindamycin and 80% to amoxicillin/clavulanic acid. All isolates were sensitive to metronidazole (Table 1).

All the participants were married and most (49.1%) belonged to the age group of 25- 30 years. All women had a normal marriage with their spouses. There was no relationship between age and BV ($P = 0.200$). The

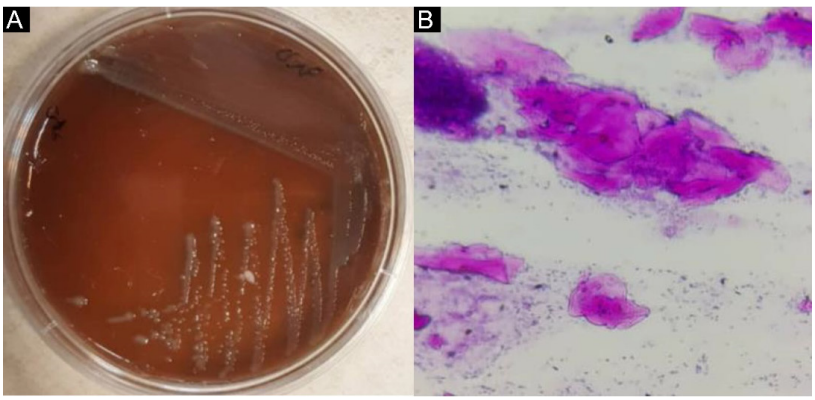

Figure 1. Colony Morphology of *Gardnerella vaginalis* (A) and Microscopic Picture of Vaginal Epithelial Clue Cells (B).

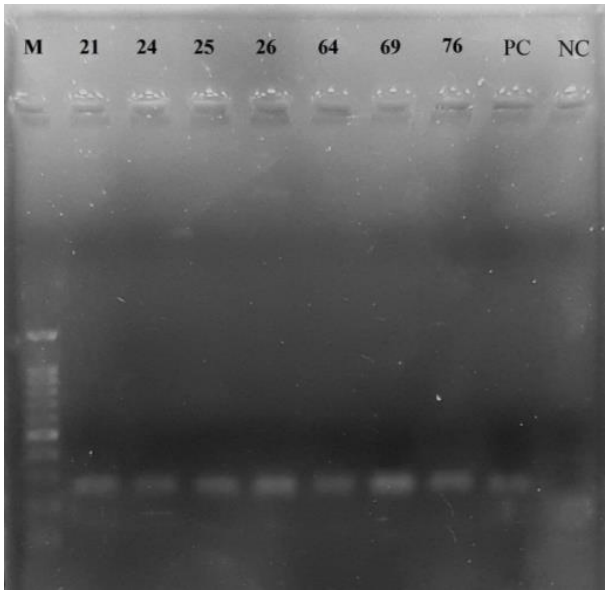

Figure 2. Agarose Gel Electrophoresis of PCR Performed on Vaginal Discharge for the Detection of *Gardnerella vaginalis*. M indicates DNA ladder (100bp), NC: negative control and PC: positive control.

demographic data of 110 precipitants were analyzed (Table 2).

We found no statistically significant association between place of residence, college education, occupation, having a child, and abortion history and PCR-positive and PCR-negative participants. The frequency of clue cell, amine odor and pH > 4.5 in vaginal secretions is presented in Figure 3.

Chi-square test showed a significant difference in pH > 4.5 in PCR-positive and PCR-negative groups ($P = 0.017$). The Whiff test was positive in 87.5% of PCR-positive and 86.92% of PCR-negative samples for *G. vaginalis*. There was no significant difference between Whiff test between the two groups ($P = 0.208$). Clue cells were observed in 87.5% of PCR-positive and 25.64% of PCR-negative groups ($P < 0.001$).

Discussion

BV, caused by *G. vaginalis*, is an infectious disease characterized by increased vaginal discharge, itching, and burning during urination or itching around the outside of the vagina, or both. In addition to annoying symptoms, this bacterium can increase the risk of many upper genital tract infections. Therefore, rapid diagnosis and antimicrobial treatment of this infection are very important.

Based on the Amsel criteria, all the women who participated in this study had BV. The results of bacterial culture showed that the prevalence of *G. vaginalis* among the 110 women with BV symptoms was 4.5%. Since *G. vaginalis* is a choosy bacterium, it is always difficult to isolate it using conventional culture methods. For example, Farajzadeh Sheikh and Hemmati reported that the rate of isolation for this bacterium in a culture medium was 23.8% (16). However, this bacterium was isolated from 29.1% of the samples when PCR was used as a more sensitive and accurate method. There are different

Table 1. Antibiotic Susceptibility Pattern of *Gardnerella vaginalis* Isolates

Number of Samples	Amoxicillin/ Clavulanic Acid	Results	Clindamycin	Results	Metronidazole	Results
5	11.7 ± 1.5	Resistant	-	Resistant	10.3 ± 0.6	Sensitive
24	-	Resistant	27.7 ± 2.5	Sensitive	12.8 ± 0.8	Sensitive
25	-	Resistant	27.0 ± 1.7	Sensitive	13.8 ± 1.0	Sensitive
54	-	Resistant	28.5 ± 1.3	Sensitive	15.3 ± 1.5	Sensitive
76	21.0 ± 3.6	Sensitive	28.8 ± 1.0	Sensitive	13.5 ± 1.5	Sensitive

Table 2. Sociodemographic characteristics of women diagnosed with vaginosis (n = 110)

Characteristics		PCR-Positive (%)	PCR-Negative (%)	Total (%)	*P* Value
Age range (y)		25-30	25-30	25-30	0.200
Mean age(y)		31.3 ± 6.3	30.0 ± 5.8	30.6 ± 6.2	
Place of residence	Town	13 (40.6)	47 (60.3)	60 (54.5)	0.060
	Country	19 (59.4)	31 (39.7)	50 (45.5)	
College education	Yes	4 (12.5)	7 (9.0)	11 (10)	0.987
	No	28 (87.5)	71 (91.0)	99 (90)	
Occupation	Employed	3 (9.4)	8 (10.3)	11 (10)	1.000
	Housewife	29 (90.6)	70 (89.7)	99 (90)	
Having a child	Yes	27 (84.4)	68 (87.2)	95 (86.4)	0.697
	No	5 (15.6)	10 (12.8)	15 (13.6)	
Abortion history	Yes	23 (71.9)	61 (78.2)	84 (76.4)	0.478
	No	9 (28.1)	17 (21.8)	26 (23.6)	

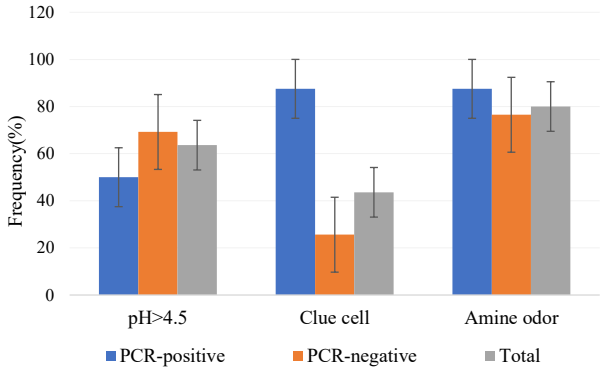

Figure 3. Frequency of Amsel Criteria in PCR-Positive and PCR-Negative Groups.

reports about the prevalence of this bacterium around the world. The prevalence of *G. vaginalis* was reported to be 28.1% in China (17), 26.2% in Cameroon (18), and 24.4% in Lithuania (19). However, Balashov et ak employed PCR to isolate *G. vaginalis* and reported that this bacterium was found in all 60 samples of vaginal discharge taken from women with BV (15). In another study on 27 cases of BV, the prevalence of *G. vaginalis* was reported to be 78% (20).

Most studies have reported a mean age of over 30 years for affliction with BV. Rezaei et al showed that the highest prevalence of BV was among the participants aged over 40 years (16.2%) (21). However, Kalantari et al reported that the highest prevalence of BV was among women aged 20-30 years (22). Although all of these studies concluded that the sexual activity of women of these ages was the main cause of affliction with BV, none of them found a significant relationship between age group and BV. Other studies showed that there was a significant relationship between level of education and affliction with BV, which was attributed to awareness of and compliance with health issues (21, 23). Ramezani Tehrani reported that the prevalence of BV in employed women was lower than unemployed ones (24), which is not consistent with the findings of the present study. We found no significant relationship between the prevalence of BV and the participants' place of residence, college education, employment status, having a child, and history of abortion in the two groups of afflicted with (PCR-positive) and not afflicted with (PCR-negative) *G. vaginalis*.

Previous studies have reported different antibiogram results for isolates of *G. vaginalis*. All strains were sensitive to metronidazole and 80% of them were sensitive to clindamycin (25). Goldstein reported that 28% of the clinical isolates of *G. vaginalis* were resistant to metronidazole, whereas all of the isolates were sensitive to clindamycin and ampicillin/sulbactam (26). Moreover, Maghsoudi showed that 75% of *G. vaginalis* isolates were resistant to clindamycin (27). On the other hand, another study showed that 9% of the isolates of this bacterium were sensitive to ampicillin (25). This discrepancy is probably related to different patterns of antibiotic consumption in different regions and the prevalence of resistant strains of *G. vaginalis*. The results of this study indicated that increased vaginal pH (>4.5) was significantly related to positive whiff test results and infection with *G. vaginalis* in patients with BV. Accordingly, although some studies have shown that *G. vaginalis* is among the vaginal bacterial flora, increased vaginal pH and positive whiff test seem to be appropriate criteria for the diagnosis of BV, which is consistent with our findings.

Given the importance of early diagnosis and specific treatment of *G. vaginalis* to eradicate this organism, gynecologists and obstetricians are recommended to prescribe a test on the stained smear of vaginal discharge (to view the clue cells) and measure its pH in women aged over 25 years. Therefore, the use of molecular methods in the definitive diagnosis of *G. vaginosis* may be more appropriate.

Authors' Contribution

Study concept and design: MMS and NM; Analysis and interpretation of data: MMS and
NM; Drafting of the manuscript: MMS and MA; Critical revision of the manuscript for important intellectual content: MMS and MA; Statistical analysis: MMS.

Conflict of Interests

The authors declare no conflict of interest.

Ethical Approval

This research project was approved by the Ethics Committee of the Islamic Azad University (IR.IAU.SHK.REC.1397.053). In addition, all ethical considerations, including the confidentiality of patient information were observed.

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