

Genetic Study of *Gardnerella vaginalis* Virulence Factors Isolated from Vaginal Discharge

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Abstracts

Background: Vaginitis is the most prevalent infectious condition affecting the female genital system. Prevalent vaginal infections, namely, bacterial vaginosis. *Gardnerella vaginalis* is one bacterial genus almost always associated with bacterial vaginosis. **Objective:** Isolation and identification of *G. vaginalis*. and Diagnosis some virulence such as Vaginolysin, Sialidase, Phospholipase. **Materials and Methods:** Collected 100 vaginal samples from women then cultures in Columbia blood agar to isolated *G. vaginalis* and genetic detection some virulence factors. **Results:** The findings revealed that 12 (12%) of the samples tested positive for *G. vaginalis*., including (7%, 26%, 13%, 8%, and 2% isolated from 20 to 50 years age, respectively. The results of PCR showed that out of 12 isolates only 4 (33%), 9 (75%) and, 6 (50%) of *G. vaginalis* isolates gave positive results to *vly*, *sld* and, *pho* gene respectively gave positive results from 12 *G. vaginalis* isolates. **Conclusions:** A healthy vaginal microbiota is considered to be significant for maintaining vaginal health and preventing infections. Bacterial vaginosis is the condition that occurs when the balance of the vaginal flora is dysregulated, and replaced by pathogenic bacteria in the vagina like *G. vaginalis*.

Keywords: *Gardnerella vaginalis*, Columbia blood agar, vaginal infections

INTRODUCTION

The most prevalent infectious condition affecting the female genital tract in women of childbearing age is vaginitis. *G. vaginalis* is an anaerobic facultative organism. The cells are small, nonmotile pleomorphic rods associated with vaginosis.^[1-3]

Vaginolysin (VLY), is one of the virulence factors of *G. vaginalis*.^[4] Sialidase (or neuraminidase), an enzyme that catalytically eliminates terminal sialic acid residues from a variety of glycoconjugates, improves the ability of bacteria to evade the host immune system and to interact with cells while also supplying them with nutrition. Several bacterial vaginosis -associated species are capable of producing sialidases. In the cervicovaginal environment, *Gardnerella spp.* are thought to be the primary source of this enzyme.^[5]

The enzyme phospholipase belongs to a large family of enzymes and is regarded as a lipolysis enzyme. These enzymes play a crucial part in increased bacterial persistence and pathogenicity. Phospholipase plays a direct role in infection and bacterial establishment

through the hydrolysis of phospholipids, which depletes structural lipids, or through the production of lytic reaction products, which both cause cytotoxicity and a loss of membrane integrity.^[6,7]

A number of diseases are diagnosed using molecular methods like polymerase chain reaction (PCR) to identify particular virulence determinants of *G. vaginalis* including sialidase, phospholipase, and vaginolysine.^[8] This study aimed to investigate the role *G. vaginalis* in vaginitis, and the genetic detection of some virulence factors.

MATERIALS AND METHODS

- Samples Collection

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Submission: 07-Nov-2022 **Accepted:** 13-Nov-2022 **Published:** 27-Apr-2023

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How to cite this article: Abdullah ST, Althahab AO, A. Jabuk SI. Genetic study of *Gardnerella vaginalis* virulence factors isolated from vaginal discharge. Med J Babylon 2023;20:85-9.

Access this article online

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Website:
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DOI:
10.4103/MJBL.MJBL_267_22

Table 1: The list of primers used in this study

Genes	Primer sequence (5'-3')	Size (bp)	Reference
Vaginolysin (<i>vly</i>)	F-5' GCACCAGATAGCCCAGCAGA3' R-5' TTCGGTGCCGTACTCATCCC 3'	540	Mohammad-Zadeh <i>et al.</i> , 2019
Sialidase (<i>sld</i>)	F-5' AGCCCGCATATCCCGTATCG 3' R-5' GGACCTGGCCAACATGGAGT 3'	454	
Phospholipase C (<i>Pho</i>)	F-5' GCGTGCTCCGCTTCGATTAG 3' R-5' TCCGCGGTAACGTTCTCTT 3'	421	

Table 2: Master mix reaction content

Contents of reaction mixture	Volume (μl)
Master Mix	12.5
Forward primer	2
Reverse primer	2
Template DNA	5
Nuclease free water	Up to 25
Total	25

By using sterile cotton-tipped swabs, a gynecologist collected 100 vaginal samples from women at two hospitals in Babylon Province, Iraq) A-I Zahraa hospital for maternity and Al-exandria general hospital(.

- Culture Identification

One hundred swabs culturing on Columbia blood agar.^[9]

- Molecular Identification

DNA was extracted from grown bacterial colonies. The primer sequence, PCR content, mentioned in [Tables 1 and 2] and condition according to the Mohammadzadeh *et al.*^[10]

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 7/17/7003 (including the number and the date in 21/11/2021) to get this approval.

RESULTS

G. vaginalis typically stains as a Gram-variable coccobacillus, exhibiting both Gram-positive and Gram-negative cells. Several studies have shown a relationship between these *G. vaginalis* strains and symptoms of vaginosis. The colonies of *G. vaginalis* on Columbia agar form smooth, small colonies with blood hemolysis.^[6,7]

[Table 3] showed the percentage of samples positive for *G. vaginalis* was 12 (12%) from different women age.

The results show that out of 12 isolates only 4 (33%) of *G. vaginalis* isolates gave positive results to *vly* gene

Table 3: *Gardnerella vaginalis* isolates according to the age

Age	NO. of positive samples(%)	NO. of negative samples(%)	Total(%)
< 20	4(4%)	6(6%)	10(10%)
20–30	5(5%)	40(40%)	45(45%)
31–40	1(1%)	26(26%)	27(27%)
41–50	1(1%)	13(13%)	14(14%)
>50	1(1%)	3(3%)	4(4%)
Total	12(12%)	88(88%)	100(100%)

with 540 bp when compared with the ladder as shown in [Figure 1].

A total of 12 *G. vaginalis* isolates were 9 (75%). isolates gave positive results for *sld* gene with molecular weight 454 bp in PCR amplification when compared isolates gave with ladder, as shown in [Figure 2].

The results show that out of 12 isolates only 6 (50%) of *G. vaginalis* isolates gave positive results for *pho* gene with molecular weight 421 bp PCR amplification when compared with ladder as shown in [Figure 3].

The result of this study appeared that out of 12 isolates(3) of *G. vaginalis* isolates gave positive results for *Vly* gene, *Sld* gene and *pho* gene, (2) of *G. vaginalis* isolates gave positive results for *Sld* gene and *pho* gene, (1) of *G. vaginalis* isolates gave positive results for *Sld* gene and *Vly* gene, (3) of *G. vaginalis* isolates gave positive results only for *Sld* gene, (1) of *G. vaginalis* isolates gave positive results only for *pho* gene, (2) of *G. vaginalis* isolates gave negative results for *Vly* gene, *Sld* gene and *pho* gene [Figure 4].

DISCUSSIONS

G. vaginalis is a facultative anaerobe and uses carbohydrates as its major energy source, this microorganism has a fermentative metabolism and acetic acid is a major product. The percentage of samples positive for *G. vaginalis* was 56 (56%). The colonies of *G. vaginalis* on Columbia agar form smooth, small colonies with blood hemolysis.^[8]

The results show that out of 12 isolates only 4 (33%) of *G. vaginalis* isolates gave positive results to *vly* gene. The result of this study similar to the result abstained by other researchers^[6] who used molecular detection of vaginolysin

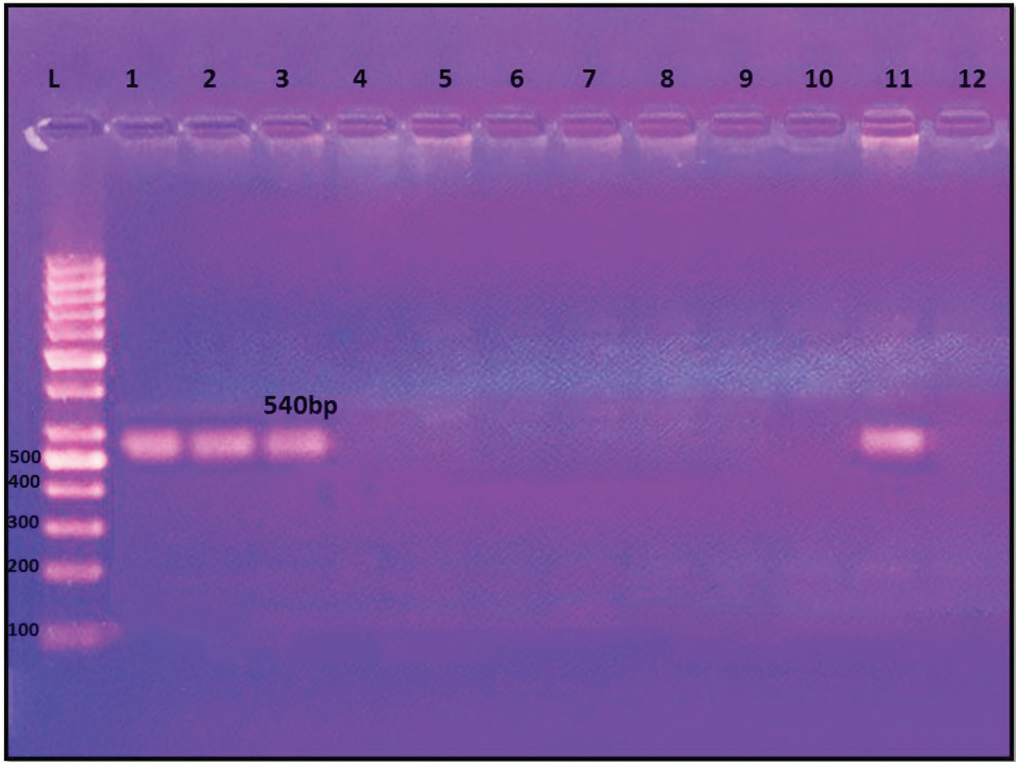


Figure 1: 1.5% Agarose gel electrophoresis for Vly gene showed positive and negative results

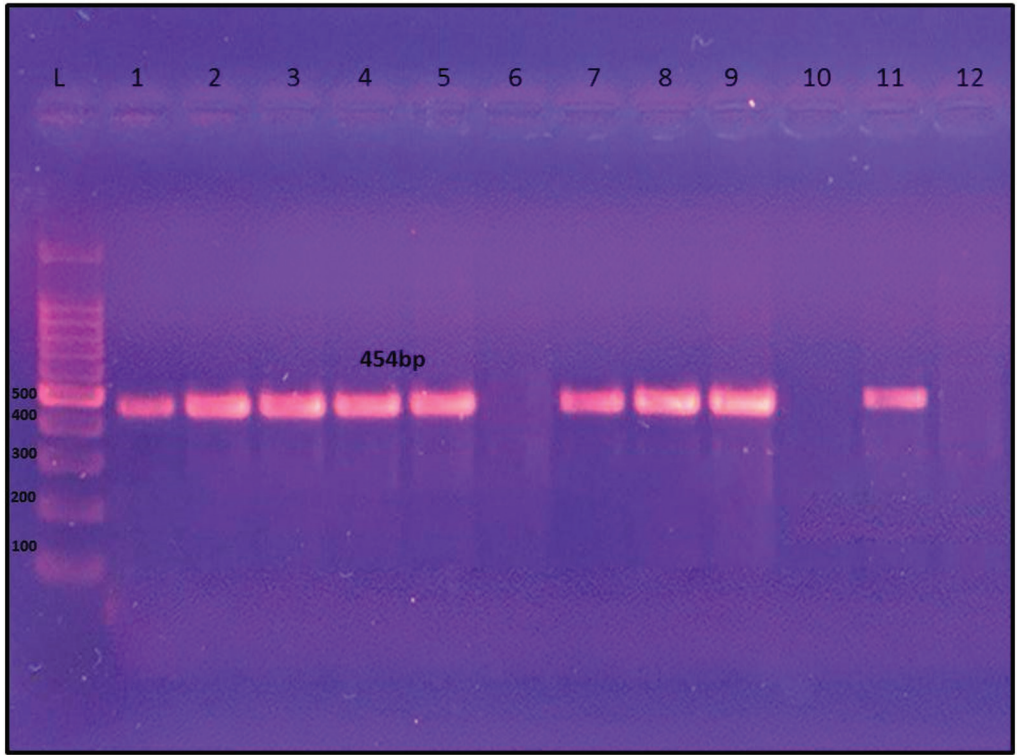


Figure 2: 1.5% Agarose gel electrophoresis at 70 volt for 60 min for sld gene showed positive and negative results

gene was showed that 12 samples (36.7%) were positive results and, according to,^[10] there were 10 (35.7%) of vly genes discovered in BV-associated *G. vaginalis*. But less than the result found by de Souza *et al.*^[11] who found the

vly gene in 98.3% of the bacteria from BV patients and in all *G. vaginalis* isolates from non-BV women and de Souza *et al.*^[11] who found PCR results, (86.8%) of the *G. vaginalis* isolates had vly genes.

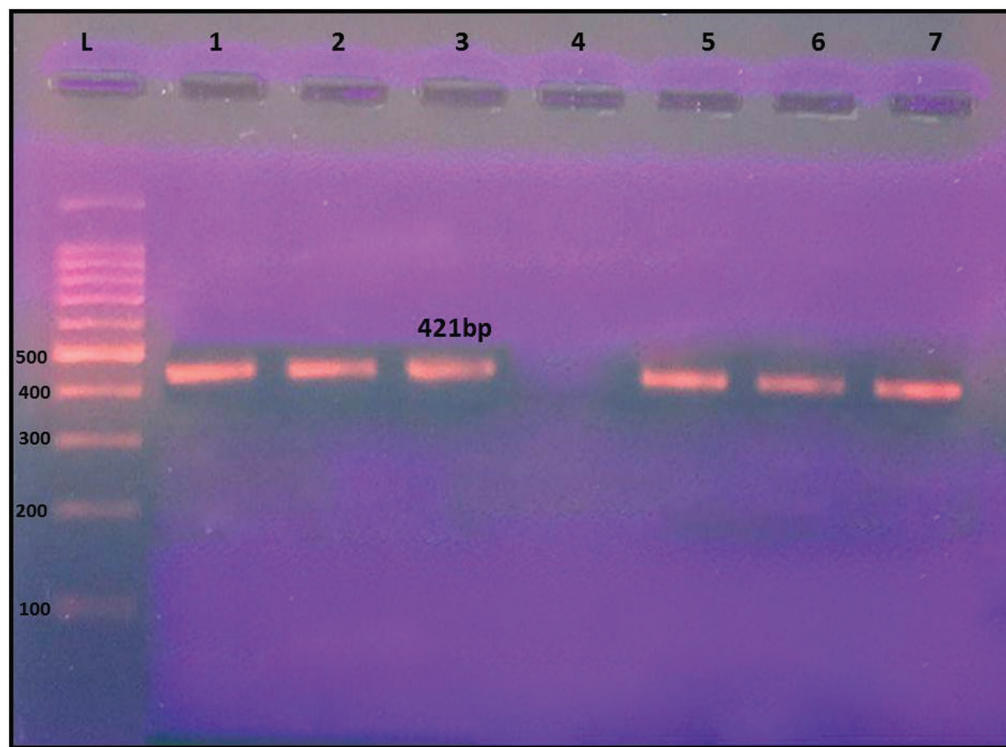


Figure 3: 1.5% Agarose gel electrophoresis at 70 volt for 60min after staining with ethidium bromide for *pho* gene showed positive and negative results

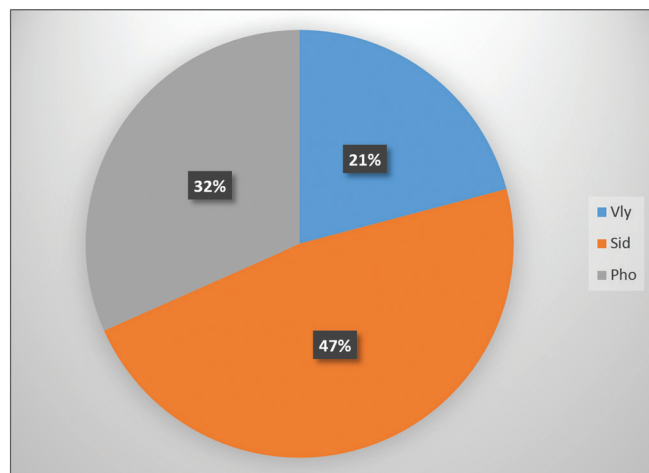


Figure 4: Distribution of virulence factors of *Gardnerella vaginalis*

G. vaginalis possesses some virulence factors that are already well recognized. One of these is the cytolytic toxin that functions as a hemolysin and is known as vaginolysin. This toxin is implicated in the pathogenesis of BV, which ultimately results in cell death.^[12]

Out of 12 *G. vaginalis* isolates were 9 (75%), isolates gave positive results for *sld* gene. The result is agreement to the result of,^[6] the putative sialidase A gene was found in 75% of the *G. vaginalis*-positive vaginal specimens and,^[13] who found the *G. vaginalis* sialidase gene was discovered in (70%) Russian women.

Also, 10. Mohammadzadeh *et al.*^[10] found the prevalence of *sld* genes in BV-associated *G. vaginalis* was 19 (67.8%). *G. vaginalis* contains a virulence factor called sialidase, which is encoded by the *sld* gene and contributes to the establishment of BV.^[14]

G. vaginalis an anaerobic pathogen, is responsible for the production of the enzyme sialidase, which cleaves terminal sialic acid residues off human glycans. Notably, a high sialidase activity level has been linked to premature birth as well as a low birth weight.^[15]

The results show that out of 12 isolates only 6 (50%) of *G. vaginalis* isolates gave positive results for *pho* gene.^[10] found the prevalence of *pho* genes in BV-associated *G. vaginalis* was 6 (21.4%).^[16] was detected the phospholipase gene in(50%) of the *G. vaginalis*-positive vaginal samples.

Phospholipases have the ability to cleave phospholipids found in eukaryotic membranes, which makes them an important factors of virulence. In addition, the significance that these enzymes play in evading the immune response of the host makes them very necessary for the pathogenicity of certain bacteria.^[17] Phospholipase C may facilitate the release of arachidonic acid and prostaglandins, which contributes to the progression of the processes that result in premature delivery.^[18] A number of researchers present relationships between urinary tract infections and vaginitis.^[19,20]

CONCLUSIONS

Vaginitis is the most prevalent infectious condition affecting the female genital system. Prevalent vaginal infections, namely, bacterial vaginosis. *G. vaginalis* is one bacterial genus almost always associated with bacterial vaginosis. The results showed the PCR standard method used to detection *G. vaginalis* virulence factor.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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