

Association Of Gardnerella Vaginalis Virulence Genes With Human Papillomavirus Infection In Women With Bacterial Vaginosis

Shatha Ahmed Khalaf¹, Asma Sumiea Karomi²

¹Department of Biology, Collage of Science, Kirkuk university, Kirkuk, Iraq, sh7t53@gmail.com

²Department of Biology, Collage of Science, Kirkuk university, Kirkuk, Iraq, dr.asmakaromi@uokirkuk.edu.iq

Abstract

Introduction: Gardnerella vaginalis, is a major pathogen of bacterial vaginosis in reproductive age women, produces virulence factors like vaginolysin and sialidase enzyme which change the vaginal mucosal barrier, increasing the probability of STI Infection. The aim of this study was to identify the association between Gardnerella vaginalis virulence factors and HR-HPV infection. **Methods:** A total of 224 vaginal and cervical specimens were collected from symptomatic women. Amsel's criteria was used for the diagnosis of bacterial vaginosis, G. vaginalis, Vly, NanH1, and NanH3 genes were detected by polymerase chain reaction (PCR) while HR-HPV infection and viral load were detected by real time PCR. **Results:** Out of 224 vaginal specimens, bacterial vaginosis was diagnosed in (25%). G. vaginalis was detected in (81.48%) of the BV positive samples. Vly, NanH1, and NanH3 genes were identified in (76.19%), (95.23%), and (59.09%) of the isolates, respectively. HR-HPV was observed in (25%) of G. vaginalis positive samples. Genotype 51 was the most prevalent (25%) followed by Genotype 56 (23.21%), genotypes 31, 45, and 59 had the same prevalence rate (1.78%). A statistically significant association was observed between bacterial vaginosis and age ($P \leq 0.05$), with the highest prevalence in women aged 21-30yr, also between HPV infection and age ($P \leq 0.01$), with highest prevalence observed in women aged 31-40yr. A significant association ($P \leq 0.05$) was found between the presence of Vly, NanH1, and NanH3 and HR-HPV infection. **Conclusion:** Our results suggested that G. vaginalis virulence genes were significantly associated with HR-HPV infection, and may act as co-factors in the development of infection.

Introduction

Bacterial vaginosis (BV) is a vaginal condition that more common in women of reproductive age, in this condition the beneficial bacteria primarily Lactobacilli spp. are diminished whereas facultative and strict anaerobes including Gardnerella vaginalis will grow and thrive (1). Changes in microbiota composition are accompanied with several health issues such as an increased incidence of sexually transmitted infections like HPV (2). Gardnerella vaginalis produces sialidase enzyme, which disrupts of vaginal mucosa so contributing to the exfoliation and detachment of vaginal epithelial cells facilitating bacterial adhesion to the epithelium (3). Another virulence factor is vaginolysin, it makes pores in the epithelial cell membranes leading to cell damage so vaginal mucosal barrier will destroy (4). HPVs able to infect mucosal epithelial tissues in different areas of the body, comprising the vagina, vulva, and penis (5). Despite of most HR-HPV infections are transient, a small percentage of them persist and progress to cancer. There are about 14 high-risk HPV genotypes including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 (6). A close correlation between BV infection and HPV persistence is found. Women with BV are more susceptible to HPV infections, BV infection prolongs the duration and the regression time of HPV infections (7).

Aim: To evaluate the relationship between the presence of Gardnerella vaginalis virulence genes and HPV infection in women diagnosed with bacterial vaginosis.

Materials & Methods

Design of The Study and Collection of Vaginal and Endocervical Samples

This study was conducted from April to December 2025. Vaginal and cervical swabs were collected from 224 symptomatic married women at reproductive age group who attended to hospital. Vaginal swabs was collected from the posterior vaginal fornix.(8). From each patient, two swabs were collected, one transported in Amie's transport medium for Amstel's criteria.

order to obtain a sufficient amount of cervical epithelial cells. Further, the swab was transported into 2ml of Viral Transport Medium (VTM). Cervical samples were stored at -20°C until the laboratory testing (10).

Diagnosis of Bacterial Vaginosis by Amsel's Criteria

Bacterial vaginosis was diagnosed according to Amsel's Criteria. A diagnosis of BV was established when at least three of the following criteria were present : homogenous vaginal discharge, vaginal pH>4.5, presence of clue cells, and a positive whiff test (fishy amine odor) (11).

Molecular Detection of *Gardnerella vaginalis*

DNA Extraction: Genomic DNA was extracted from bacterial samples using the (ABIO pure, Taiwan) based on the manufacture's protocol. The purified DNA was eluted in 60µl of buffer AE and stored at -20°C until use in subsequent PCR tests. Primers used for the amplification of *Gardnerella vaginalis* genes were synthesized by Macrogen Company (Seoul, Korea). Lyophilized primers were reconstituted with nuclease-free water to get a stock concentration of 100pmol/µl and diluted to a working concentration of 10pmol/µl. For *Gardnerella vaginalis* and its virulence genes specific primers were chosen from previous published studies. Primer sequences, annealing temperature, and amplicon sizes and references are listed in Table (1).

Polymerase Chain Reaction (PCR): PCR amplification was performed using specific primers. The composition of the reaction mixture and thermal cycling parameters were optimized based on the manufacturer's instructions and previously published protocol.

Agarose Gel electrophoresis: PCR products were analyzed by electrophoresis on 1.5% agarose gel prepared in 1x TAE buffer and stained with ethidium bromide (10mg/ml). Electrophoresis was performed at 100V for approximately 60min. DNA fragments were visualized using a gel documentation system.

Table (1): Primer Sequences and PCR Conditions Used in This Study

Primer Name	Sequence (5'-3')	Annealing Temp. (C°)	Product Size (pb)	Reference NO.
G. vaginalis-F	GGGCGGGCTAGAGTGCA	52	210	12
G.vaginalis-R	GAACCCGTGGAATGGGCC			
NanH1-F	GACGACGGCGAATGGCACGA	58	252	13
NanH1-R	TACAAGCGGCTTTA CTCTTG			
NanH3-F	CAGTTCCAA TGGAAGTGTGC	51	322	14
NanH3-R	AGCATCTGGAATGCTCTTG			
Vaginolysin-F	GCACCAGATAGCCCAGCAG	55	450	12
Vaginolysin-R	TTCGGTGCCGTACTCATCCC			

Detection of HPV by Real Time PCR

Genomic DNA was extracted from cervical swab samples using the ReliaPrep™ gDNA Miniprep System (Promega, USA) according to the manufacturer's instructions. High Risk-Human Papillomavirus (HR-HPV) DNA was subsequently identified by real-time PCR following the manufacturer's protocol. Amplification reactions were performed in a final volume of 25µl of template DNA and 15µl of reaction mixture. Positive and negative controls were included in each run to validate the results.

Results and Discussion

Table (2): Characteristics of Vaginal Discharge Among Women with Bacterial Vaginosis

Discharge	Number	Percentage (%)
Thin, grey, homogenous	14	25.0%
Thick white	39	69.64%
No discharge	3	5.35%
Total	56	100%
χ^2 : (P-value)	---	36.838 ** (0.0001)
** (P≤0.01).		

** Highly Significant

The result was suggested that the majority of women with BV infection exhibited thick, white discharge (69.64%), only (25%) of patients showed the typical thin, grey, homogenous discharge. A minority of cases (5.35%) revealed no visible discharge Table (2). Although thin, grey, homogenous discharge considered the classical clinical feature of BV, the predominance of thick white discharge in this study indicates that clinical appearance alone may be misleading. Statistical analysis revealed a highly significant difference among the observed discharge patterns ($P \leq 0.01$), indicating that the distribution of vaginal discharge characteristics was not random within the studied population. In our study the majority of these women produced characteristic vaginal discharge (94.64%), in contrast to the study by (15) in Malaysia who was reported lower rate (78.7%).

Table (3): Vaginal pH levels Among Study Participants

Vaginal PH	Number of patients	Percentage (%)
≤4.5	168	75%
>4.5	56	25%
Total	224	100%
χ^2 : (P-value)	---	56.00 ** (0.0001)
** (P≤0.01).		

** Highly Significant

Vaginal pH, was evaluated, in our study (25%) of patients revealed elevated pH>4.5 while (75%) showed ≤4.5 Table (3). Statistical analysis demonstrated a highly significant difference between the two categories ($P \leq 0.01$) indicating that the distribution of vaginal pH values within the study population was not random. This finding was lower than that reported by (16,17) who observed elevated pH in (67.6%, 64%) of cases. A pH of ≤4.5 signifies the absence of vaginitis, whereas a pH of >4.5 is classified as vaginitis as the presence of BV leads to high pH of vaginal secretion (18).

Table (4): Whiff Test Findings Among the Study Sample

Whiff test	Number	Percentage (%)
Positive	16	7.14%
Negative	208	92.85%
Total	224	100%
χ^2 : (P-value)	---	164.57 ** (0.0001)
** (P≤0.01).		

** Highly Significant

In Table (4) only (7.14%) of the samples revealed a positive Whiff test, whereas (92.85%) of specimens gave negative result, statistical analysis demonstrated highly significant difference between positive and negative Whiff test findings ($P \leq 0.01$), so the distribution was unlikely to be due to random variation. This result was close to result that reported by (19) who reported (15.5%). The odor was due to the activity of anaerobic bacteria that produce amines such as trimethylamine, putrescine, and cadaverine, as products of anaerobic metabolism (20).

Table (5): Clue Cells Findings Among The Study Sample

Clue cell	Number	Percentage (%)
Presence	56	25%
Absence	168	75%
Total	224	100%
χ^2 : (P-value)	---	56.00 ** (0.0001)
** (P≤0.01).		

** Highly Significant

Out of 224 samples, clue cells were detected in (56) samples (25%) as shown in **Table (5)**. The highly significant ($P \leq 0.01$) confirms that the variation in clue cell findings among the examined specimens was not due to random variation. Clue cells were found in all BV women (100%) in our study. This result consistent with (21) who also revealed the presence of clue cells in all BV positive cases (100%). The presence of clue cells in BV positive cases results from the heavy colonization of vaginal epithelial surfaces by *Gardnerella vaginalis* and the concurrent depletion of protective *Lactobacilli* spp. (22).

Table(6): Prevalence of Bacterial Vaginosis Among Women

Total Number of Samples	Positive BV No. (%)	Negative BV No. (%)	Chi-Square (P-value)
224	56 (25.00%)	168 (75.00%)	56.001 ** (0.0001)
** (P≤0.01).			

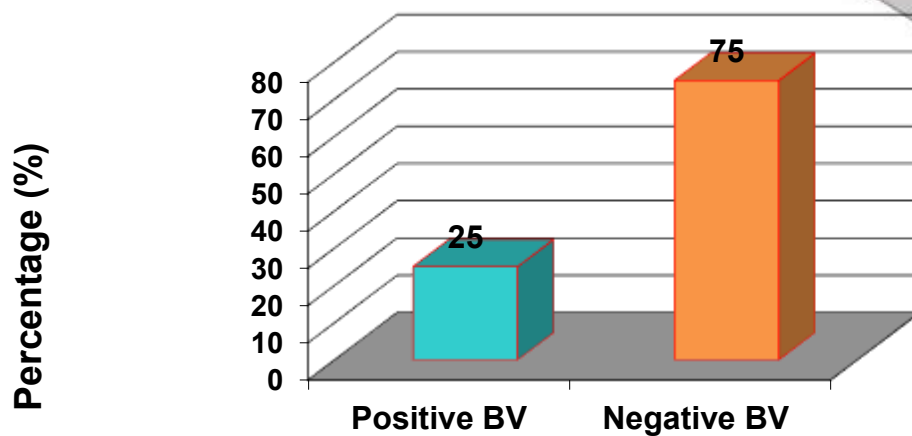


Figure (1) Prevalence of BV among Women

In this study bacterial vaginosis (BV) was detected in 56(25%) of the examined women **Table(6)** according to Amsel's criteria, they met three or four of Amsel's criteria whereas 168(75%) cases were BV negative. Statistically there was highly significant difference ($P \leq 0.01$), suggesting that the observed findings was unlikely to have occurred by chance. Our finding was close to the result of (23) in Zakho City/ Kurdistan /Iraq. who demonstrated (27.33%). Also close to the result of the study in Cameroon and in Egypt by (24, 25) who reported (26.2% and 24%) respectively, in Brazil (26) was recorded higher rate (70%), in Vietnam (9) was suggested lower percentage (11.1%). Variations in BV prevalence rates are primarily due to differences in sexual behaviors, genetic/ethnic factors, hygiene practices (such as vaginal douching), socioeconomic conditions, and local healthcare policies.

Table(7): Detection of Gardnerella vaginalis by PCR Among Selected BV Samples.

** Highly Significant

Vaginal samples that were diagnosed as positive for bacterial vaginosis according to Amsel's criteria were further analyzed by polymerase chain reaction (PCR). PCR amplification of the target DNA fragment produced the expected amplicon size of 210pb following agarose gel electrophoresis Figure (2). The gel image showed clear DNA ladder, distinct bands at 210pb for all tested samples. Out of the 56 samples diagnosed as positive using Amsel's criteria, a subset of 27samples was further tested using PCR for molecular confirmation. 22samples gave positive (81.48%) PCR results, while a five samples (18.51%) gave negative result Table (7) statistically there was a highly significant association in the detection of Gardnerella vaginalis between the studied groups ($P \leq 0.01$), suggesting that the distribution of detected and non-detected cases differed significantly and was not due to chance. Our result was close to the result of (27) in Tikrit city, who found that the prevalence of G. vaginalis was (87.7%). A study from the United State by (28) was reported a lower prevalence rate (65%).

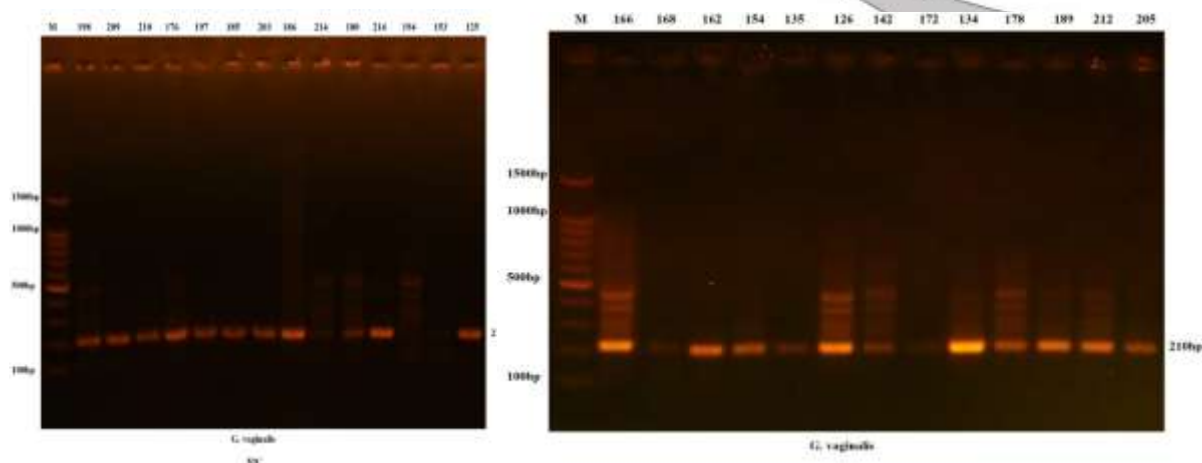


Figure (2) Agarose gel electrophoresis PCR amplification products of *G. vaginalis* showing the expected 210bp bands. M:100 ladder

Detection of *Gardnerella vaginalis* virulence Genes

Detection of vly gene Molecular detection of vaginolysin gene (Vly) was performed for *G. vaginalis* isolates as shown in Table (8). The results revealed that 16(76.19%) of the isolates were positive for this gene. The Vly gene was detected with a significant frequency ($P \leq 0.05$), indicating a moderate distribution among samples. The positive results were detected by the confirmed of 540bp band when compared with ladder as illustrated in figure (3). Our result was close to the study reported by (12) which recorded a rate of (66.65%), However our finding disagreed with the studies of (26, 29) who reported higher rates (98.3%, 90%), respectively. In contrast (30) revealed a lower rate of (36.7%).

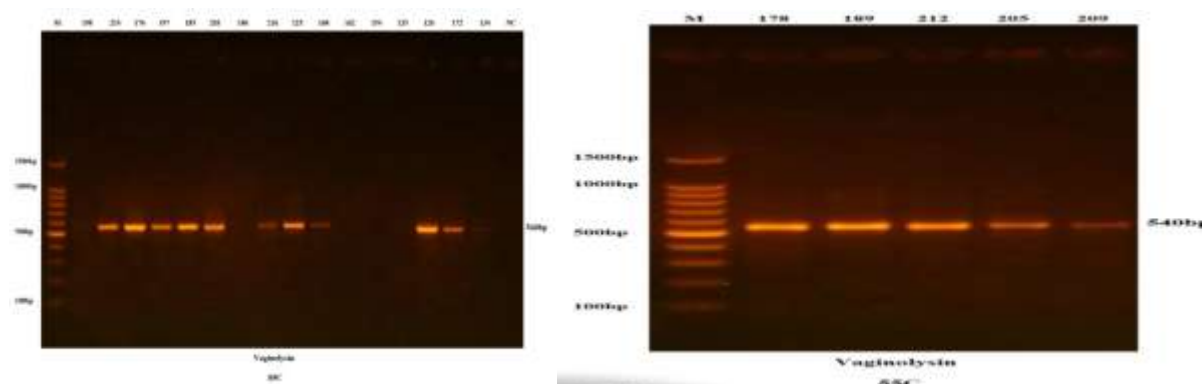
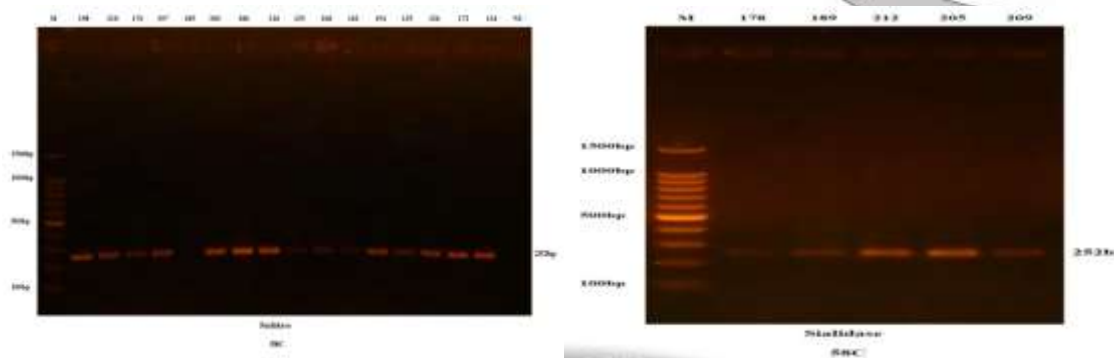


Figure (3) Vaginolysin gene of *Gardnerella vaginalis* were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker.

Detection of NanH1 gene (sld) It was identified in 20(95.23%) of the isolates, NanH1 showed a highly significant prevalence ($P \leq 0.01$) among samples as observed in Table (8) suggesting it was the most commonly detected gene among examined isolates. The positive results were detected by the confirmed of 252bp band when compared with ladder as illustrated in figure (4). Our finding was higher than that revealed by (31, 32) who reported rates (75%) and (80.3%) respectively. Also higher than that documented by (33, 34) who demonstrated (70%) and (67.8%) respectively.



Figure(4) amplification of NanH1gene of *Gardnerella vaginalis* were fractionated on 1.5% agarose gel electrophoresis stained with Eth. Br. M: 100bp ladder marker.

Detection of NanH3 gene Among *G. vaginalis* isolates the prevalence of the NanH3 gene was 13(59.09%) it was detected without statistical significance (P NS) as shown in **Table (8)**. The positive results were detected by the confirmed of 322bp band when compared with ladder as observed in **figure (5)**. This finding was close with previous study by (14) who reported a prevalence rate (65%), whereas a study by (35) revealed lower rate (44.1%), another study by (36) was detected NanH3 gene in higher prevalence rate (93.7%).

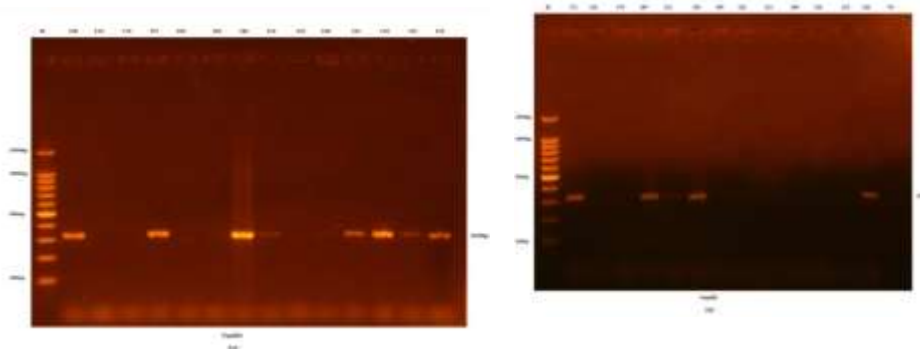


Figure (5) Results of the amplification of NanH3 gene of *Gardnerella vaginalis* were fractionated on 1.5% agarose gel electrophoresis stained with Eth. Br. M: 100bp ladder marker.

Table (8) Prevalence of Virulence Genes Among *G. vaginalis* Isolates

Virulence gene	Positive No. (%)	Negative No. (%)	P-value	Total
Vly gene	16 (76.19%)	5 (23.80%)	0.0164 *	21(100%)
NanH1 gene	20 (95.23%)	1 (4.76%)	0.0001 **	21(100%)
NanH3 gene	13 (59.09%)	9 (40.90%)	0.3938 NS	22(100%)
* (P≤0.05), ** (P≤0.01).				

NS= Non Significant, *, Significant, **, Highly Significant

The Distribution of Virulence Genes Among The Same *G. vaginalis* Isolate

Our result revealed variation in the distribution of virulence genes among the same isolates of *G. vaginalis*. It suggested a significant variation in the distribution of virulence gene combinations among *Gardnerella vaginalis* ($P \leq 0.05$). Table (9) showed that 7(31.81%) of the isolates were positive for Vly, NanH1 and NanH3 genes, 5(22.72%) isolates showed positivity for NanH1 and NanH3 genes, whereas 8(36.36%) isolates were positive for Vly and NanH1 genes. Only one isolate 1(4.54%) was positive for Vly alone and another isolate was positive to NanH3 alone 1(4.54%). These results demonstrate that virulence genes of *Gardnerella vaginalis* tend to coexist rather than occur individually, meaning that the combined action of several virulence factors may improve bacterial pathogenesis (37).

Table(9): Distribution of virulence gene combinations among the same *Gardnerella vaginalis* isolates

Virulence Gene	Number of isolates	Percentage
Vly, NanH1, NanH3	7	31.81%
NanH1, NanH3	5	22.72%
Vly, NanH1	8	36.36%
Vly	1	4.54%
NanH3	1	4.54%
Total	22	100%
χ^2 :	---	9.518 *
* ($P \leq 0.05$).		

Identification and Genotyping of HR-HPV Using Real -Time PCR

The amplification results was illustrated in Figure (7, 8). In our study, fourteen (14) HR-HPV positive cases were detected, their frequencies were summarized in Table (10). Among women diagnosed with bacterial vaginosis the prevalence of HR-HPV was (25%), while (75%) of the samples showed no evidence of HR-HPV infection. HPV-51 was the most prevalence (25%), followed by HPV-56 (23.21%). Genotypes 31, 45 and 59 were identified at equal frequencies (1.78%). Genotypes 16, 18, 33, 35, 39, 52, 58, 66 and 68 were not detected in all examined samples. A highly significant difference in the distribution of HPV genotypes among women ($P \leq 0.01$) were confirmed by Statistical analysis which means that the observed differences were unlikely to have occurred by chance. Multiple infections were observed in all HR-HPV positive cases as found in Table (11) 12 cases had double infection, out of them 11(78.57%) cases showed co-infection with genotypes 51 and 56, 1(7.14%) case had co-infection with 31, 51. Two cases had triple infection, one case (7.14%) had co-infection with genotype 45, 51, and 56 the second case (7.14%) had co-infection with 51, 56, and 59. The observed differences in the distribution of multiple HPV infections were statistically highly significant ($P \leq 0.01$). The distinction between single and multiple infections is important in understanding the carcinogenic role of HPV in cervical cancer.

Table (10): Distribution of High-Risk Human Papillomavirus Genotypes Among women with Bacterial Vaginosis

Genotype	Frequency	Percentage %
HPV-16	0	0
HPV-18	0	0
HPV-31	1	1.78%
HPV-33	0	0
HPV-35	0	0
HPV-39	0	0
HPV-45	1	1.78%
HPV-51	14	25%
HPV-52	0	0

HPV-56	13	23.21%
HPV-58	0	0
HPV-59	1	1.78%
HPV-66	0	0
HPV-68	0	0
χ^2 : (P-value)	---	12.982 ** (0.0001)
** (P≤0.01).		

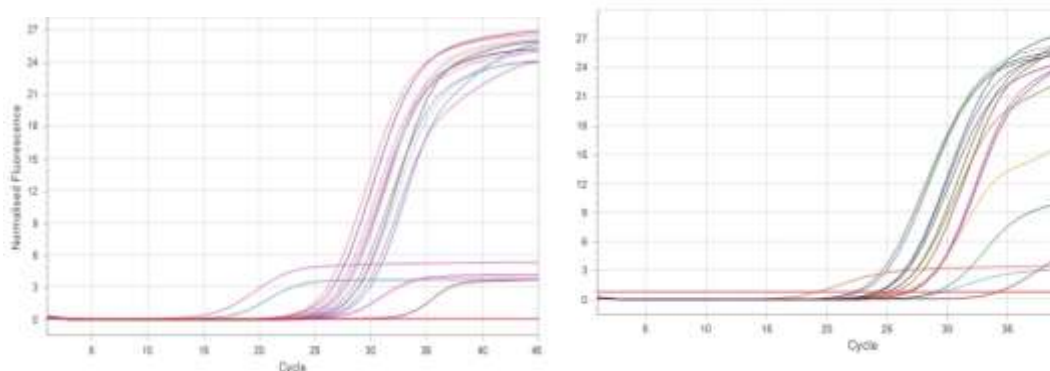
Table

** Highly Significant

(11): The
Prevalence of
Multiple HPV

Genotype Infections Among HPV Positive Women

Type of infection	Frequency	Genotypes	Percentage
Double infection	11	HPV-51, HPV56	78.57%
	1	HPV-31, HPV51	7.14%
Triple infection	1	HPV-51, HPV-56, HPV-59	7.14%
	1	HPV-45, HPV51, HPV-56	7.14%
Total	14	---	100%
P-value	---	---	0.0001 **
** (P≤0.01).			



Figure(7): qPCR amplification of HPV-51 on Cy5 fluorescence channel

Figure (8.):qPCR amplification of HPV-56 on Cy5 fluorescence channel

These results revealed a distinct distribution pattern of HR-HPV genotypes compared with several studies, a study by (38) in Shanxi /China was found (16.66%) which was close to our result, but he reported that the most common genotype was HPV-16 accounting (6.48%), it was not in agreement with our finding. In Jinan, China (39) was demonstrated a higher HR-HPV prevalence accounted for (84.32%) which was higher than our result and the most

frequently detected types were HPV-52 (19.97%), HPV-16 (12.43%), HPV-58 (11.05%), HPV-51 (7.20%), HPV-53 (7.03%), and HPV-66 (6.26%). Among the HPV-positive cases, (67.42%) had a single genotype infection, while (32.58%) manifested multiple genotype infections, including (22.11%) with double genotype infections, (6.62%) with triple genotype infections, and (3.85%) with over three genotype infections. Four cases exhibited infection with up to nine genotypes. These results contrast with our findings where the genotype pattern and distribution differed, particularly in the predominance of HPV-51 and HPV-56. (40) was identified HR-HPV in (12.4%) of the participants. The three most prevalent HR-HPV types were HPV-16 (15.05%), HPV-52 (13.65%), and HPV-56 (10.33%) which was partially align with our result, particularly regarding HPV-56. Unlike the majority of global reports where HPV-16 was the predominant genotype, our study identified HPV-51 and HPV-56 were the most frequent genotypes, highlighting a distinctive regional genotype distribution.

Prevalence of Bacterial Vaginosis and HR-HPV in different age groups

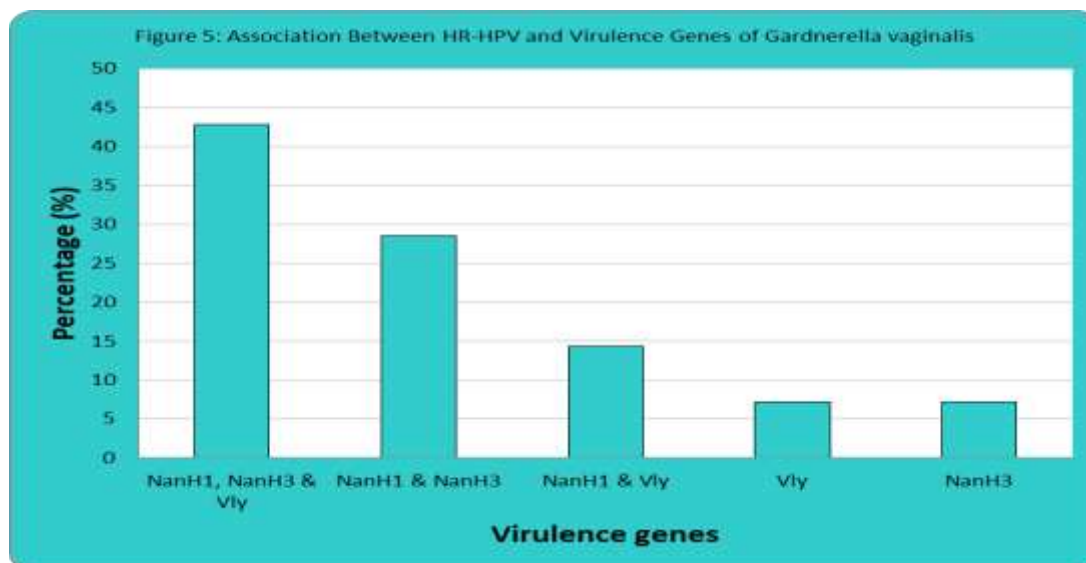
The highest BV prevalence (46.43%) was present in women aged 21-30years, followed by those aged 31-40 (32.14)%. Lower rates were reported in women aged ≤ 20 years (10.71%) and 41-50years (8.93%), While the lowest prevalence was found in women older than 50years (1.79%). The highest prevalence of HR-HPV was found in the 31-40years age group (42.86%), followed by 21-30 group (35.71%). The age groups ≤ 20 , 41-50 and >50 years showed the same lowest prevalence (7.14%) Table (12). A significant association was observed between the prevalence of BV and age ($P \leq 0.05$). Also a highly significant association was observed between HR-HPV infection and age ($P \leq 0.01$) suggesting that the distribution of both infections varied among different age groups. These results were consistent with the finding of (41) who found that the high BV prevalence (42%) in women aged ≤ 30 years, and the lowest BV prevalence (16%) was reported in the 30-40years age group, in contrast to our result in which the lowest BV prevalence among women aged >50 years age group and the highest prevalence of HR-HPV (43.7%) within the age group ≥ 40 and the lowest HR-HPV prevalence (26.5%) in women aged ≤ 30 years which disagreement with our results. A study by (42) was documented high BV prevalence (42.9%) among women aged ≤ 30 years which was close to our finding, and the lowest BV prevalence (28.%) was reported in 30-40years age group, which contrasts with our finding. and the highest prevalence of HR-HPV (44.9%) among women aged group ≤ 30 years whereas the lowest prevalence (21%) was observed in the 30-40years group which was in disagreement with our results.

Table (12): Prevalence of Bacterial Vaginosis and HR-HPV in Different Age Groups

Age Group	Number (Percentage)	
	Bacterial Vaginosis	HR-HPV
≤ 20 yr.	6 (10.71%)	1 (7.14 %)
21-30 yr.	26 (46.43%)	5 (35.71%)
31-40 yr.	18 (32.14 %)	6 (42.86 %)
41-50 yr.	5 (8.93%)	1 (7.14 %)
>50 yr.	1 (1.79 %)	1 (7.14 %)
Total	56 (100%)	14 (100%)
χ^2 :	38.821 **	8.857 *
* ($P \leq 0.05$), ** ($P \leq 0.01$).		

Table (13): Distribution of Virulence Genes of Gardnerella vaginalis Among HR-HPV Positive Samples

NanH1 & Vly	2	14.29%
Vly	1	7.14%
NanH3	1	7.14%
Total	14	100%
χ^2 : (P-value)	---	6.714 * (0.0499)
* (P≤0.05).		



Figure(9) Association between HR-HPV and virulence genes of *G. vaginalis*

Among HPV positive samples, our study revealed a heterogeneous distribution of *G. vaginalis* virulence genes. The presence of NanH1, NanH3 and Vly constituted the predominant virulence pattern, accounting for (42.86%) of the positive samples. This was followed by the combined detection of NanH1 and NanH3 in (28.57%), the combinations of NanH1 and Vly were found in lower frequencies (14.29%). Both Vly or NanH3 were present individually and in the same rate (7.14%) Table (13). The majority of HR-HPV-positive samples harbored *G. vaginalis* strains carrying multiple virulence genes instead of single gene. Statistical analysis demonstrated a significant variation in the distribution of the virulence patterns ($P \leq 0.05$), indicating that the frequencies were not occurred by chance. Furthermore, the significant predominance of multi-virulence gene profiles support the hypothesis that highly virulent *G. vaginalis* strains may play a role in maintaining vaginal dysbiosis, which act as an important cofactor in HPV persistence and progression toward cervical lesion (43).

Measurement of Viral Load in Multiple HR-HPV Genotypes Co-infections

The viral load values among women harboring multiple high risk HPV genotypes present in Table (14). The values ranged from several thousand copies/ μ l, to values exceeding one hundred thousand copy/ μ l, whereas one sample exhibited a markedly higher viral load (10.150.000)copy/ μ l demonstrated a broad spectrum of viral burden among infected women. this variation may be due to differences in the immune response, viral persistence, stage of infection, and the influence of multiple HPV genotype interactions. Samples with higher viral loads suggest increased viral replication and may indicate a greater risk of persistent infection and progression to cervical epithelial abnormalities (44).

Table (14): Viral load of HR-HPV Genotypes in Women With Multiple Infections

Sample NO.	HPV Genotype	Viral Load		
		Copies/ μ l		
1.	HPV-51 & HPV-56	832.4	39.510	
2.	HPV-51 & HPV-56	10.930	6355	
3.	HPV-51 & HPV-56	1331	102600	
4.	HPV-51 & HPV-56	5419	35.500	
5.	HPV-51 & HPV-56	5642	21.910	
6.	HPV-51 & HPV-56	1568	105.000	
7.	HPV-51, HPV-56, HPV-59	3762	6950	1908
8.	HPV-51 & HPV-56	6926	9167	
9.	HPV-51 & HPV-56	7088	21.570	
10.	HPV-51 & HPV-56	14.160	14.490	
11.	HPV-51 & HPV-56	4175	136.900	
12.	HPV-51 & HPV-56	2267	38.570	
13.	HPV-31 & HPV-51	63.830	52.27	
14.	HPV-45, HPV-51 & HPV-56	31.250	10.150000	1493

Conclusions

Gardnerella vaginalis was detected in women suffered from bacterial vaginosis. The most prevalent HPV genotypes in women infected with G. vaginalis were HPV-51 and HPV-56. A significant association was demonstrated between age and both bacterial vaginosis and HPV infection. A significant association was observed between the presence of G. vaginalis virulence genes and HPV infection, suggesting that virulent G. vaginalis may contribute in increasing susceptibility to HPV infection.

References

1. Riaz, R. ; Khan, K. ; Aghayeva, S. and Reaz Uddin, R. (2025). Combatting antibiotic resistance in Gardnerella vaginalis: A comparative in silico investigation for drug target identification. Plos One.Vol.20. N.3. P.2.
2. Sourazur, G. (2023). Evaluating Vitamin C Vaginal Tablets Effect on Gardnerella vaginalis Infection. Microbiology and Infectious Diseases. Vol.11. N.3. P.168.

3. Bodaszewska-Lubaś, M. ; Karolina Kot, K. and Brzychczy-Włoch, M. (2026). The Role of Sialidase in The Development of Bacterial Vaginosis and their Use as Potential Markers in The Diagnosis of Vaginal Infections. *Advancements of Microbiology*. Vol.65. N. 1. P.24.
4. Pleckaityte, M. (2020). Cholesterol-Dependent Cytolysins Produced by Vaginal Bacteria: Certainties and Controversies. *Frontiers in cellular Microbiology*. Vol.9. P.1.
5. Baruah, F. K. ; Sharma, A. ; Das, Ch. ; Hazarika, N. K. ,and Hussain, J. H. (2014). Role of Gardnerella vaginalis as an etiological agent of bacterial vaginosis. Vol.6. N.(6). *Iranian journal of Microbiology*.
6. Pisani, T. and Cenci, M. (2024).Prevalence of Multiple High Risk Human Papilloma Virus (HR-HPV) Infections in Cervical Cancer Screening in Lazio Region, Italy. *Cancer Diagnosis & Prognosis*. Vol.4. P.42.
7. Lin, W. ; Zhang, Q. ; Chen, Y. ; Dong, B. Xue, H. Lei, H. ; Lu, Y. ; Wei, X. and Sun, P. (2022). Changes of the vaginal microbiota in HPV infection and cervical intraepithelial neoplasia: a cross-sectional analysis. *Scientific Reports*. Vol.18. P.2.
8. Gomaa, F. A. M. ; Hafez, H. M. ; Al Anwar, A. and El-Metwally, N. (2017). Diagnosis of Bacterial Vaginosis among Symptomatic Egyptian Women: Value of Assessing Bacterial Sialidase Activity. *International Journal of Current Microbiology and Applied Sciences*. Vol. 6. N.4. P.908.
9. Nguyen, A. T. ; Nguyen, N. T. ; Tran, T. T. ; Nguyen, T. T. ; Hoang, T.T. ; Tran, L. M. ; Tran, K. T. ; Phan, T. D. ; Nguyen, X. T. ; Le, T.M. ; Le, A. V. ; Salumets, A. and Mändar, R. (2024). Prevalence and associated factors of bacterial vaginosis among pregnant women in Hue, Vietnam. *The journal of infection in developing countries*. Vol.18. N.6. P.925.
10. Mohammed, W. J. ; Hussein, T. A. ; Tawfeeq, N. T. ; Dina S. Ibrahim, D.S. (2024). Distribution of human papillomavirus in women by genotype, age, education, and geography in Baghdad, 2021-2022. *Journal of Biotechnology Research Center*. Vol.13. N.2 P. 180.
11. Mashkoo, K. T. ; Khalil, S. A. and Mahdi, A. N. (2010). Comparison Between Modified Amsel's Criteria and The Old Standard Methods in Diagnosis of Bacterial Vaginosis. *Kufa Med. Journal*. Vol.13. N.1. P.136.
12. Tripathi, D. ; Saxena, R. K. and Agarwal, S. (2023). The pattern of antimicrobial resistance and the presence of the vaginolysin gene in Gardnerella vaginalis isolates from pregnant women. *Current Medicine Research and Practice*. Vol.13. N.1. P.173.
13. Pillay, K. ; Durga, T. ; Mabaso, N. and Abbai, M. (2024). Diversity of the virulence genes of Gardnerella vaginalis isolates across the different bacterial vaginosis states in a South African pregnant cohort. *he Journal of Medical Laboratory Science & Technology South Africa*. Vol.6. N.1. P.7.
14. Novak, J. ; Belleti, R. ; Pinto, G. V. D. ; Bolpetti, A. D. N. ; da Silva, M. G. and Marconi, C. (2023). Cervicovaginal Gardnerella sialidase-encoding gene in persistent human papillomavirus infection. *Scientific Reports*. Vol.13. P.2.
15. Younus, N. K. ; Gopinath, R. ; Jegasothy, R. ; Nordin, S. A. ; Belkum, A. V. ; Mary, N. and Neela, V. K. (2017). An update on Gardnerella vaginalis associated bacterial vaginosis in Malaysia. *Asian Pacific Journal of Tropical Biomedicine*. Vol.7. N.9. P.833.
16. Rao, S. R. ; GirishaPindi, K. ; Rani, U. ; Sasikala, G. and Kawle, V. (2016). Diagnosis of Bacterial Vaginosis: Amsel's Criteria vs Nugent's scoring. *Scholars Journal of Applied Medical Sciences (SJAMS)*. Vol.4. N.6. P.2029.
17. Strugała, I. C. ;Iwona Gołębiewska, I. G. ; Banaszewska, B.

22. Agarwal, K. ; Choudhury, B. ; S. Robinson, L. S. ; Morril, S. R. ; Bouchibiti, Y. ; Chilin-Fuentes, D. ; Rosenthal, S. B. ; Fisch, K. M. ; Peipert, J. F. ; Lebrilla, C. B. ; Allsworth, J. E. ; Lewis, A. L. and Lewis, W. G. (2024). Resident microbes shape the vaginal epithelial glycan landscape. HHS Public Access. Vol. 15. P. 2.
23. Haydar, S. O. and Naqid, I. A. (2022). A Study of Bacterial Vaginosis and Associated Risk Factors among Married Women in Zakho City, Kurdistan Region, Iraq. Journal of Life and Bio-sciences Research Vol. 03, No. 02. P.33.
24. Kamga, Y. M. ; Ngunde, J. P. and Akoachere, J. K. T.. (2019). Prevalence of bacterial vaginosis and associated risk factors in pregnant women receiving antenatal care at the Kumba Health District (KHD), Cameroon. BMC Pregnancy and Childbirth. Vol.19. N.166. p.1.
25. Shawaky, S. M. ; Al Shammari, M. M. ; Sewelliam, M. S. ; GhazaL, A. A. and Amer, A. N. (2022). A study on vaginitis among pregnant and non-pregnant females in Alexandria, Egypt: An unexpected high rate of mixed vaginal infection. AIMS Microbiology. Vol. 8. N.2. P. 167.
- Hassan, M. A. and Suliman, Z. (2024). Evaluation of RT-PCR in molecular diagnosis of Gardnerella vaginalis and Trichomonas vaginalis infection in comparison with other conventional method in Tikrit province. The Medical Journal of Tikrit University. Vol.30. N.2. P.137.
26. De Souza, D. M. K. ; Diniz, C. G. ; Filho, D. S. C. ; de Oliveira, L. M. A. ; Coelho, D. M. ; Talha, L. D. ; Nascimento, T. C. ; Machado, A. B. F. and da Silva, V. L. (2016). Antimicrobial susceptibility and vaginolysin in Gardnerella vaginalis from healthy and bacterial vaginosis diagnosed women. Journal of infection in developing countries. Vol.10 N. 9. P.914.
27. Hassan, M. A. and Suliman, Z. (2024). Evaluation of RT-PCR in molecular diagnosis of Gardnerella vaginalis and Trichomonas vaginalis infection in comparison with other conventional method in Tikrit province. The Medical Journal of Tikrit University. Vol.30. N.2. P.137.
28. Oyenih, A. B. ; Haines, R. ; Trama, J. ; Faro, S. ; Mordechai, E. ; Adelson, M. E. and Sekyere, O. J. (2024). Molecular characterization of vaginal microbiota using a new 22-species qRT-PCR test to achieve a relative-abundance and species-based diagnosis of bacterial vaginosis. Front. Cell. Infect. Microbiol. Vol.14. P.5.
29. Najim, S. A. ; Hussain, S.S. ; Abdulrazaq, R.A. (2024). Detection, Optimization, Characterization, and Cytotoxic Effect of Vaginolysin Extracted from Gardnerella vaginalis Isolated From Iraqi Women Who had Abortions. J. Contemp Med Sci. Vol. 10, No. 3 P.201.
30. Bunyan, I. A. ; Abdul-Lateef, L. A. and Al-Rubaeae, A. A. (2018). Genotyping of Vaginolysin Gene of Gardnerella vaginalis Isolated From Preterm Labor Patient in Hilla City. Pak. J. Biotechnol. Vol.15. N.2. P.379.
31. Hardy, L. ; Jepers, V. ; Van Den Bluck, M. ; Buyze, J. ; Musengamana, L. ; Vanechoutte, M. and Crucitti, T. (2017). The presence of the putative Gardnerella vaginalis sialidase A gene in vaginal specimens is associated with bacterial vaginosis biofilm. Journal poneone. Plos one. Vol. 12. P.2.
32. Vasse, M. ; Mayrhofer, N. ; Masete, V. ; Passmore, J. S. ; Pierre, H. J. ; Godreuil, S. ; Froissart, R. and Bravo, I. G. (2026). Sialidase variability in Gardnerella : genetics, taxonomy, function, and clinical presentation. Hal open science. P.4.
33. Shipitsyna, E. ; Krysanova, A. ; Khayrullina, G. ; Shalepo, K. ; Savicheva, A. ; Guschin, A. and Unemo, M. (2019). Quantitation of all Four Gardnerella vaginalis Clades Detects Abnormal Vaginal Microbiota Characteristic of Bacterial Vaginosis More Accurately than Putative G. vaginalis Sialidase A Gene Count. Molecular Diagnosis & Therapy. Vol.23. P.145.
34. Mohammad zadeh, R. ; Kalani, B. S. ; Kashanian, M. ; Oshaghi, M.

38. Wang, W. ; Zhang, X. ; Li, M. ; Hao, C. and Liang, H. (2020). Association between Vaginal Infections and the Types and Viral Loads of Human Papillomavirus: A Clinical Study Based on 4,449 Cases of Gynecologic Outpatients. *Canadian Journal of Infectious Diseases and Medical Microbiology*. Vol.2020. P. 2.
39. Chen, X. ; Zhou, H. ; Li, R. ; Li, R. and Liu, P. (2026). Clinical epidemiology and associations between HPV infection and vaginal infections in Jinan, China: a cross-sectional analysis. *Frontiers in Cellular and Infection Microbiology*. Vol.16. P. 3.
40. Cheng, X. ; Lue, H.; Ma, J. ; Wang, Y. and Su, J. (2024). Correlation between indicators of vaginal microbiota and Human Papilloma virus infection.:A retrospective study. *Clinical and Experimental obstetrics & Gynecology*. Vol.51. N.4. P.
41. Lu, H. ; Jiang, P. ; Zhang, X. ; Hou, W. ; Wei, Z. ; Lu, J. ; Zhang, H. ; Xu, G. ; Chen, Y. ; Ren, Y. ; Wang, L. ; Zhang, R. and Han, y. (2015). Characteristics of bacterial vaginosis infection in cervical lesions with high risk human papilloma virus infection. *Int J Clin Exp Med* Vol.8.N.11. P.21082.
42. Li, H. ; Xiao, Z. ; Xing, B. ; Wu, S. ; Wang, Y ; Liu,Z. ; Zeng, Y. ; Mushi, J. C. ; Sun, H. and Li, P. (2020). Association between common vaginal and HPV infections and results of cytology test in the Zhoupu District, Shanghai City, China, from 2014 to 2019. *Virology Journal*.Vol.19. N.127. P.4.
43. Segui-Perez, C. ; Smoorenburg, M. Y. ; Maranus, A. E. ; Geijtenbeek, T. B. H. and Strijbis, K. (2025). Impact of bacterial vaginosis on sexually transmitted viral infections: a bacterial point of view. *FEMS Microbiology Reviews*. VOL.49. P.2.
44. Zhou, F. ; Zhou, Q. ; Zhu, Z. ; Hua, K. ; Chen, L. and Ding, J. (2020). Types and viral load of human papillomavirus, and vaginal microbiota in vaginal intraepithelial neoplasia: a cross-sectional study. *Annals of Translational Medicine*. Vol.. 8. N. 21. P.8.