Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 02 July 2026
Revised 23 Aug 2026
Accepted 06 Sep 2026

Natural Resources for Human Health 2026, Vol. 6 (19s): 1005–1015

KEYWORDS:
Lorem ipsum; dolor sit amet; consectetur; adipiscing elit; natural resources; phytochemistry.

Molecular Detection, Antimicrobial Resistance Genes, and Phylogenetic analysis of Gardnerella vaginalis in Women with Bacterial Vaginosis

[1]Shatha Ahmed Khalaf, [2]Asma Sumiea Karomi

[1]Department of Biology, Collage of Science, Kirkuk university, Kirkuk, Iraq, sh7t53@gmail.com
[2]Department of Biology, Collage of Science, Kirkuk university, Kirkuk, Iraq, dr.asmakaromi@uokirkuk.edu.iq

ABSTRACT: Gardnerella vaginalis is gram-negative to gram-variable, small, pleomorphic rods that is non motile and does not have endospores, or capsules. It is a part of the female vaginal microflora. This bacterium is associated with a condition called bacterial vaginosis (BV). The treatment of bacterial vaginosis is by metronidazole and/or clindamycin according to the recommendations of centers for disease control and prevention (CDC).

Amis: To determine the prevalence of Gardnerella vaginalis among women with bacterial vaginosis, assess the association between intrauterine device (IUD) use and bacterial vaginosis, identify the prevalence of metronidazole and clindamycin resistance genes among the isolates and to investigate the genetic diversity using 16S rRNA gene sequencing and phylogenetic analysis.

Methods: 224 high vaginal swabs were collected from symptomatic women. Amsel´s criteria were used to diagnose bacterial vaginosis. PCR was performed in order to detect Gardnerella vaginalis, also metronidazole and clindamycin resistance genes were detected. 16SrRNA gene sequencing was done to investigate genetic diversity and phylogenetic analysis.

Results: Out of 224 vaginal specimens, bacterial vaginosis was diagnosed in (25%). G. vaginalis was detected in (81.48%) of the BV positive samples. Erm a gene was detected in (50%) of the isolates while Rdx genes was not detected in all bacterial isolates (100%). 16 S rRNA gene sequencing confirmed all G. vaginalis isolates (98.18%-100%) identity, and phylogenetic tree analysis showed close genetic relatedness with minor sequence variation.

Conclusions: Gardnerella vaginalis was prevalent in women with bacterial vaginosis. No significant relationship was found between the use of intrauterine device (IUD) and the occurrence of bacterial vaginosis. G. vaginalis isolates harbored Erm a gene which suggest a potential risk of clindamycin resistance among the isolates, Rdx gene was absent in all isolates. 16S rRNA gene sequencing and phylogenetic tree confirmed the identity of the isolates and revealed minor genetic variation.

INTRODUCTION:

Gardnerella vaginalis is gram-negative to gram-variable, small, pleomorphic rods that is non motile and does not have endospores, or capsules (1). It can grow in microaerophilic conditions. It is a part of the female vaginal microflora. This bacterium is associated with a condition called bacterial vaginosis (BV) (2). Bacterial vaginosis is a type of gynecological diseases, associated with a depletion in *Lactobacillus* species, and an increase of anaerobic bacteria, and abundant microbial

imbalance. Also, it is related to pelvic inflammation, miscarriage neonatal infection, premature birth, and infertility (3). The treatment of bacterial vaginosis is by metronidazole and/or clindamycin according to the recommendations of centers for disease control and prevention (CDC). Metronidazole, a first-generation nitroimidazole, is effective against anaerobes, but it has little or no activity against aerobes. *G. vaginalis* is also sensitive to the hydroxymetabolite of metronidazole. Clindamycin, as a lincosamide, has anti-inflammatory properties with a broader range of activity against BV than metronidazole (4). Metronidazole and clindamycin are available as oral capsules and vaginal ointments (5).

AMIS:

The purpose of this study was to determine the prevalence of *Gardnerella vaginalis* among women with bacterial vaginosis, evaluate the association between intrauterine device (IUD) use and bacterial vaginosis, identify the prevalence of metronidazole and clindamycin resistance genes among the isolates, to investigate the genetic deversity using 16S rRNA gene sequencing and phylogenetic analysis.

MATERIALS & METHODS

Sample Collection: The study was conducted from April to December, 2025. 224 sample were collected from patients who attended to hospital in Kirkuk city, high vaginal samples were collected by using sterile cotton swab from posterior vaginal fornix. Two swabs were collected from each patient, one was transported in to Amies transported medium for Amsel´s criteria and the second swab was collected in to Tris–Buffer (TE-Buffer) for further molecular test (6). Information such as name, age, using of intrauterine device (IUD) were collected.

Amsel´s criteria: The Amsel´s criteria comprise elevation of pH (pH >4.5), presence of gray, homogeneous, vaginal discharge, release of a fishy odor after adding of 10% potassium hydroxide (KOH)to the secretion, and presence of clue cells. The presence of at least three of the above criteria is considered as BV (7).

DNA Extraction: Genomic DNA was extracted from the suspended samples using the ABIOpure Extraction Kit according to the manufacturer's instructions. The samples were lysed with proteinase K and buffer BL, followed by the addition of ethanol. The lysates were transferred to spin columns, washed sequentially with buffers BW and TW, and the DNA was finally eluted with 60µl of buffer AE and stored for further molecular tests. Lyophilized primers were reconstituted in nuclease-free water to prepare stock solutions of 100pmol/µl based on the manufacturer´s instructions. Primers and their sequences were summarized in **Table(1).**

Table(1): Primer Sequences and PCR Amplification Conditions

Primer Name	Sequence (5`-3`)	Annealing Temp. (C°)	Product Size (pb)	Reference No.
G. vaginalis-F	GGGCGGGCTAGAGTGCA	52	210	8
G. vaginalis-R	GAACCCGTGGAATGGGCC			
Erma-F	AACACCCTGAACCCAAGGGACG	55	405	9
Erma-R	CTTCACATCCGGATTCGCTCGA			
Rdx-F	GCAGGAGCATCAGATAGTTCT	62	169	9
Rdx-R	GGGATTTTATTGTATGCTACAA			

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

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 100Vfor approximately 60min. DNA fragments were visualized using a gel documentation system.

Standard Sequencing: The products of PCR were sent for Sanger sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation–Korea. The results were received by email then analyzed using geneious software.

RESULTS AND DISCUSSION

Table (2): Pattern of Vaginal Discharge Among Patients with Bacterial Vaginosis

Discharge	Number	Percentage (%)
Thin, grey, homogenous	14	25.%
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

Table (3): Distribution of Positive and Negative Results According to Amsel´s Criteria

Criteria	Positive No.	Negative No.	χ^2	P-value
pH	56	168	56.00 **	** (P≤0.01
Whiff Test	16	208	164.57 **	** P≤0.01
Clue Cell	56	168	56.00 **	** P≤0.01

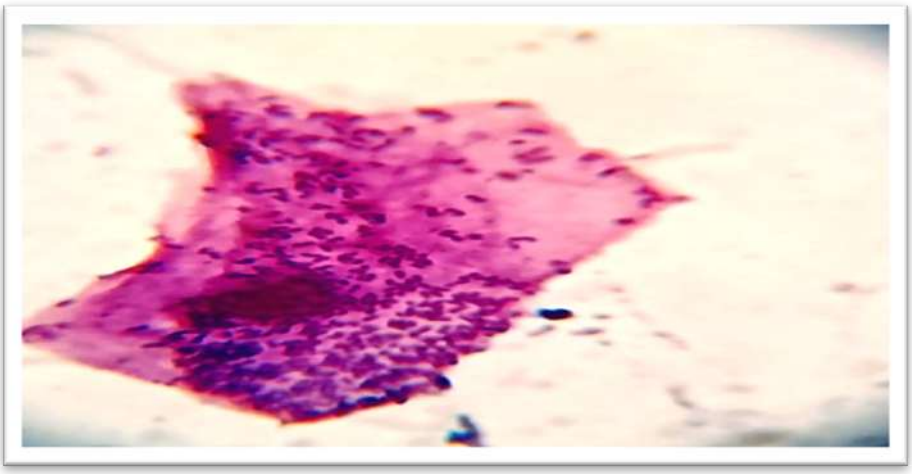


Figure (1): Microscopic Appearance of Clue Cell in a vaginal Smear

As in **Table (2)** out of 224 patients, 14(25%) had Thin, grey, homogenous, 39(69.64%) showed white, thick discharge whereas 3(5.35%) had no discharge. Statistical analysis demonstrated a highly significant difference among the observed discharge patterns (P≤0.01), suggesting that the distribution of vaginal discharge characteristics was not random within the

studied population. the majority of these women produced characteristic vaginal discharge (94.64%), in contrast to the study by (10) in Malaysia who was reported lower rate (78.7%). In our study 56(25%) of patients showed elevated pH>4.5 while 168(75%) showed ≤4.5 **Table(3).** Statistical analysis revealed a highly significant difference between the two categories (P≤ 0.01) indicating that the distribution of vaginal pH values in the study population was not random. This finding was lower than that reported by (11, 12)) who observed elevated pH in (67.6%, 64%) of cases. O Also among the 224 samples only 16(7.1%) gave positive Whiff test, while 208(92.85%) of specimens gave negative result, statistical analysis revealed highly significant difference between positive and negative Whiff test findings (P≤0.01), so the distribution was unlikely to be due to random variation. This finding was close to result that reported by (13) who documented (15.5%) whereas lower than that revealed by (14) who reported (93%). Clue cells were identified in 56(25%) of the specimens. The highly significant P-value (P≤0.01) confirms that the variation in clue cell results among the examined specimens was not due to random variation. They were observed under microscopic examination using Gram stain as illustrated in **figure (1**). In our study clue cells were found in all BV women (100%). This finding consistent with (15) who also reported the presence of clue cells in all BV positive cases (100%).

Table(4) showed that bacterial vaginosis (BV) was identified in 56(25%) of the examined cases according to Amsel´s criteria. 168(75%) of women were BV negative. Statistically there was highly significant difference (P≤0.01), revealed that the observed results was unlikely to have occurred by chance. Our finding was close to the result by (16) in Zakho City/ Kurdistan /Iraq. who demonstrated (27.33%). in Brazil (17) was reported higher rate (70%).

Table(4): Prevalence of Bacterial Vaginosis Among Study Population

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).			

Intrauterine Device (IUD) Use and Its relationship to Bacterial Vaginosis: Intrauterine device (IUD) a type of contraceptive option to prevent pregnancy. The relationship between intrauterine device (IUD) use and bacterial vaginosis was assessed. Our result showed although there were differences in the distribution between of BV cases between IUD users and non-users, statistical analysis revealed no significant association between IUD and BV (P>0.05) **Table (5).** IUD wasn't the risk factor for the development of bacterial vaginosis. This finding was align with the previous studies conducted by (18), in contrast to the study by (19) who revealed that IUD have been related to higher rates of microbial dysbiosis.

Table (4.2): Association Between IUD and Bacterial Vaginosis Among the Studied Women

IUD	BV Positive Number (%)	BV Negative Number (%)
IUD user	15(26.78%)	43(25.59%)
IUD Non User	41(73.21%)	125(74.40%)
Total	56(100%)	168(100%)
χ^2: (P- Value)		0.031 (0.860)
(P>0.05)		

Natural Resources for Human Health
eISSN: 2583-1194

Identification of *Gardnerella vaginalis* by PCR: Vaginal samples that were detected as positive for bacterial vaginosis were further tested 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. Of the 56 samples identified as positive according to Amsel's criteria, 27samples was further analyzed using PCR for molecular confirmation. 22samples gave positive (81.48%) results, whereas a five samples (18.51%) gave negative result, statistically there was a highly significant association in the detection of *Gardnerella vaginalis* between the study groups (P≤0.01), confirming that the distribution of detected and non-detected cases varied significantly and was not due to chance **Table (5)** Our result was close to the finding of (1) in Tikrit city, who identified that the prevalence of *G. vaginalis* was (87.7%). A study by (20) was reported a lower prevalence rate (65%) than that observed to our study.

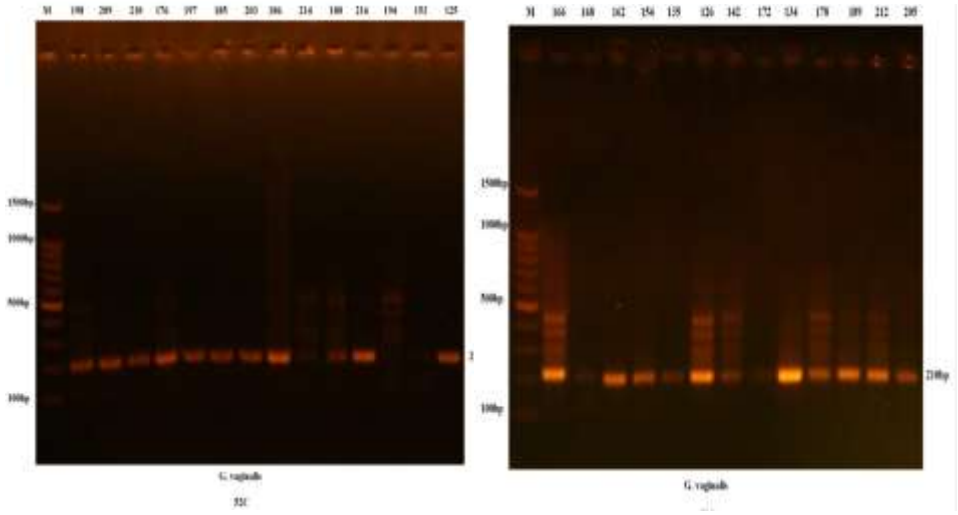


Figure (2) Agarose gel electrophoresis PCR amplification products of *G. vaginalis* showing the expected 210pb bands. M:100 ladder

Table(5): Distribution of *G. vaginalis* Among BV women

Bacterial Type	Positive NO. (%)	Negative NO. (%)	Total
Gardnerella vaginalis	22 (81.48%)	5 (18.51%)	27(100%)
χ^2:	10.703 **		---
(P-value)	(0.0001)		
** (P≤0.01).			

Detection of Antibiotic Resistance Genes

Detection of *Erm* a gene: A Polymerase chain reaction test was performed to examine if *G. vaginalis* isolates carried the *erm* a gene which is known to be linked to clindamycin resistance, agarose gel electrophoresis of the PCR products revealed distinct DNA bands at 405pb **Figure (3)** The result demonstrated that 10(50%) of the examined isolates carried this gene with no statistically significance (P NS) as showed in **Table (6**). in Hilla city a study by (9) reported a much lower prevalence (10%) among the isolates. In contrast, (21) documented a prevalence of (61.8%) which was closely aligned with the finding of our study. The resistance mechanism mediated by *erm* a includes methylation of 23S rRNA binding of ribosomes. This methylation changes the antibiotic binding site, so decreasing the affinity of macrolides, lincosamides and strepogramin B antibiotics (22).

Natural Resources for Human Health
eISSN: 2583-1194

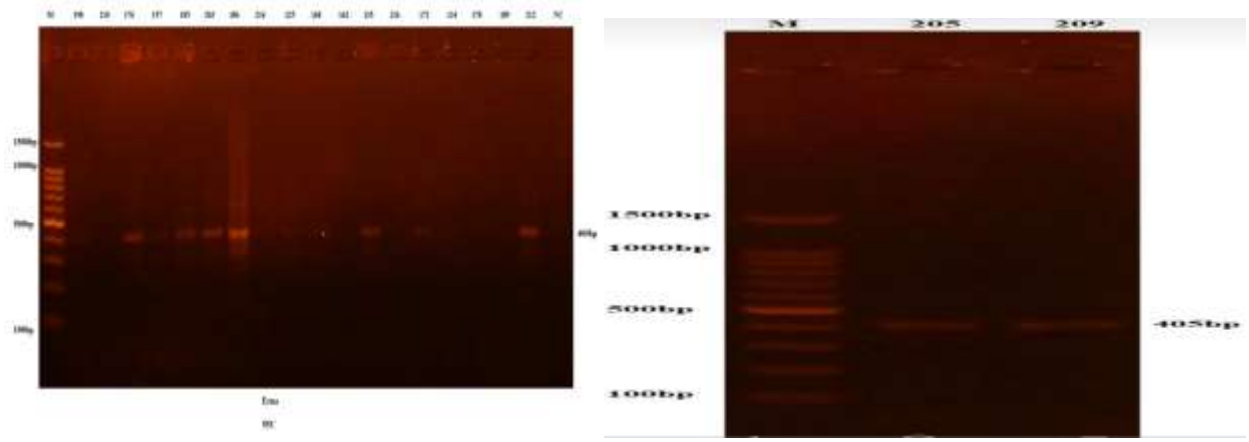


Figure (3) Results of the amplification of *Erm* a gene of *Gardnerella vaginalis* were fractionated on 1.5% agarose gel electrophoresis stained with Eth. Br. M: 100bp ladder marker. Lanes 198- 212, lanes 205, and 209 resemble 405bp PCR products.

Detection of *Rdx* Gene: The *Rdx* gene was not identified in any of the isolates and all isolates were negative (100%), showing a highly significant difference (P≤0.01) **Table (6),** as demonstrated by the absence of the expected 169pb PCR band on agarose gel electrophoresis **Figure(4)**. This finding was align with that reported by (9). The *Rdx* A gene encodes an oxygen-insensitive NADPH nitroreductase enzyme necessary to activate metronidazole into the toxic, antimicrobial form. When the *rdx*A gene is inactivated-typically through deletions, mutations, or frame shifts, the bacteria become unable to activate the drug, leading to metronidazole resistance (23).

Table (6): Distribution of Antibiotic Resistance Genes in *Gardnerella vaginalis* isolates

Gene	Positive No. (%)	Negative No. (%)	P-value	Total
erm A	10 (50%)	10 (50%)	1.00 NS	20(100%)
Rdx	0 (0%)	20 (100%)	0.0001 **	20 (100%)
** (P≤0.01).				

16srRNA Gene sequencing for Identification of *Gardnerella vaginalis:* The 16srRNA sequencing results of the 21 *G. vaginalis* isolates were examined using BLAST. The description of strains, Accession number, query number, percentage identity and E-value presented in **Table (7).** In this study 16srRNA gene sequencing was conducted to confirm identification and assess genetic relatedness with reference strains in the NCBI database. The sequencing data for the positive *G. vaginalis* isolates (total number=21) were submitted to the GenBank-NCBI database and designated as (SHA-1, to SHA-21). BLAST results showed high sequence identity (98.18%-100%) with registered strains, confirming the accuracy of PCR identification. In our study eighteen (18) isolates showed 100% sequence identity with reference strains deposited in GenBank, indicating strong genetic conservation. Two isolates showed 99.39% sequence identity and one isolate showed 98.18% suggesting minor genetic variation, they may represent locally distinct strains within the same species. These findings indicate that 16srRNA gene sequencing served both as a confirmatory tool and as a method to reveal genetic diversity among the isolates. Previous studies in Kurdistan/Iraq by (24) identified *G. vaginalis* with 100% sequence identity. Another study in Al-Diwaniyah city/Iraq by (25) was detected the presence of identity ranging from 99.44% to 99.86%. In Durban, South Africa a study by (26) was identified *G. vaginalis* with 97% sequence identity.

Natural Resources for Human Health
eISSN: 2583-1194

Table(7): BLAST Alignment Results of 16rRNA Gene Sequences Confirming PCR-Identified *Gardnerella vaginalis* Isolates

Isolate NO.	Description	Reference Accession NO.	Isolate Accession NO.	Query NO.	Percent Identity	E-value	New Assess
SHA-1	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX906031.1	100%	100%	1e-83	_
SHA-2	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX906540.1	100%	98.18%	1e-79	Yes
SHA-3	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX911526.1	100%	100%	1e-83	_
SHA-4	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX911524.1	100%	100%	1e-83	_
SHA-5	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX911525.1	100%	100%	1e-83	_
SHA-6	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX911527.1	100%	100%	1e-83	_
SHA-7	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX906600.1	100%	99.39%	6e-83	Yes
SHA-8	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX919716.1	100%	100%	1e-83	_
SHA-9	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX919717.1	100%	100%	1e-83	_
SHA-10	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX919718.1	100%	100%	1e-83	_
SHA-11	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX919713.1	100%	100%	1e-83	_
SHA-12	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX919711.1	100%	100%	1e-83	_
SHA-13	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX919710.1	100%	100%	1e-83	_
SHA-14	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX919709.1	100%	100%	1e-83	_
SHA-15	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX915621.1	100%	99.39%	6e-83	Yes
SHA-16	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX919708.1	100%	100%	1e-83	_

SHA-17	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX919707.1	100%	100%	1e-83	_
SHA-18	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX919706.1	100%	100%	1e-83	_
SHA-19	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX919705.1	100%	100%	1e-83	_
SHA-20	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX919703.1	100%	100%	1e-83	_
SHA-21	*Gardnerella vaginalis* ATCC 14019	CP002104.1	PX919704.1	100%	100%	1e-83	_

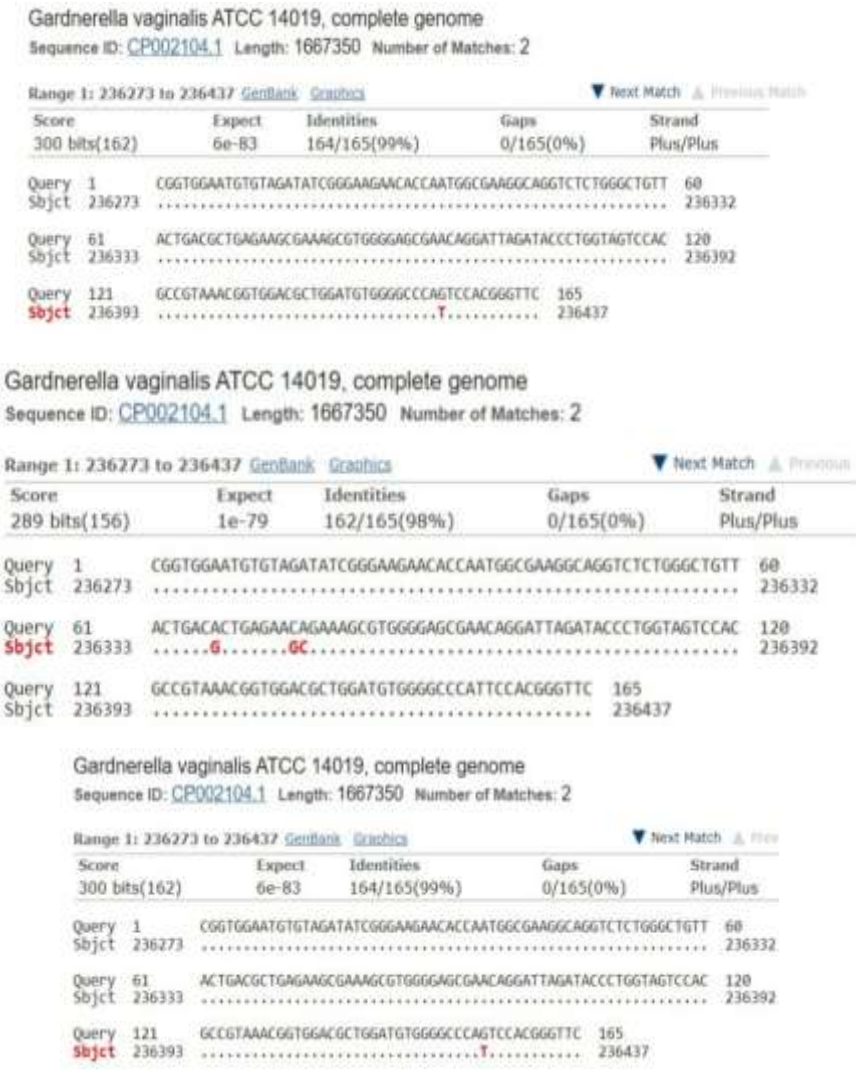


Figure (4) Blast alignment of 16s rRNA gene sequence of *Gardnerella vaginalis* isolates against the reference strain ATCC 14019.

Natural Resources for Human Health
eISSN: 2583-1194

Sequence alignment analysis of the *Gardnerella vaginalis* isolates against the reference strain ATCC 14019 showed high sequence identities ranging from 98.18% to 99.39% **Figure ( ).** Insertion or deletion were not detected, there were no gaps (0%), revealed a high degree of sequence conservation within the analyzed 16sr RNA gene region. However, several nucleotide substitutions were recorded, representing minor genetic diversity among the isolates. Two isolates displayed 99.39% sequence identity (164/165bp) and shared an identical cytosine to thymine (C to T) substitution, demonstrating the presence of a point mutation. Another isolate exhibited 98.18% identity (162/165bp), suggesting the presence of additional nucleotide substitutions. Although these sequence differences, the high identity values and the absence of gaps confirm that the isolates were highly conserved and fell within the same species, whereas the detected substitutions was represented natural occurring intraspecies genetic polymorphisms.

Phylogenetic Analysis Based on 16srRNA Gene Sequencing of *Gaardnerella vaginalis* Isolates

In this study 16SrRNA gene sequencing based approach conformed the taxonomic identity of the isolates as *Gardnerella vaginalis*, also it revealed interaspecies variation among the local isolates. The phylogenetic tree **Figure(4)** suggested very small genetic distance (0.015-0.0175), which confirmed that all isolates within the same species. The topology of the tree separated the isolates in two genetic clusters. The isolates SHA-2, SHA-7 and SHA-15 formed a sub cluster with almost negligible divergence, indicating nearly identical 16s rRNA sequences. Whereas the remaining isolates were distributed along a distinct branch, indicating minor but consistent sequence variation. The pattern strongly consistent with the BLAST identity percentages obtained against reference sequences in GenBank, were some isolates showed 100% whereas others ranged between 99.39% and 98.18%. Our results were in agree with the findings of a study in Thi-Qar, Iraq by (27) on *G. vaginalis*, where 16s rRNA gene sequence analysis exhibited nucleotides diversity and the distribution of isolates across multiple phylogenetic branches. The findings of the present phylogenetic were align with the study by (9) in AL-Hilla city, Iraq who revealed that local isolates of *Gardnerella vaginalis* clustered closely with reference strains while forming a slightly distinct branch as a result of a minor nucleotide variations in the 16s rRNA gene. Their isolates showed 98.5% sequence identity with the reference strain, and small genetic variation (0.5-1.5%) influenced the tree.

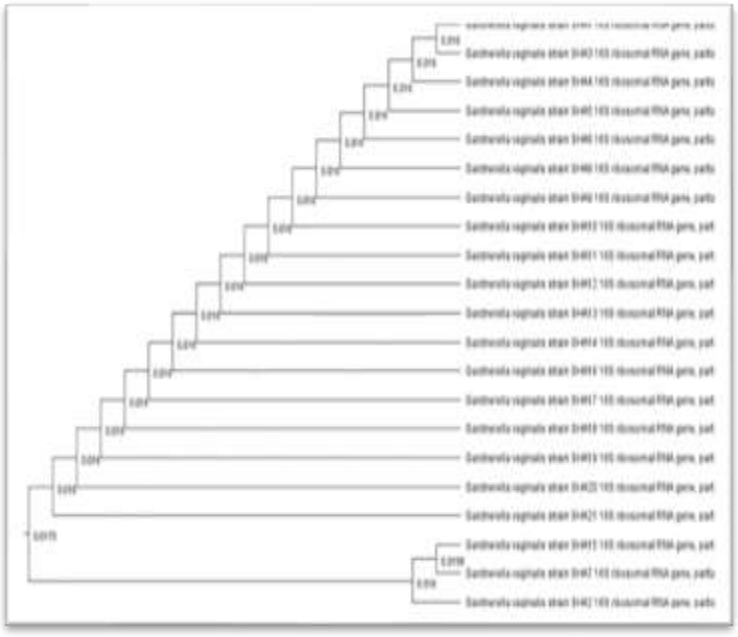


Figure (4) Phylogenetic Tree Constructed from 16S rRNA Gene Sequences Showing The Evolutionary relationship Between the Studied Isolates and Reference Strains of *Gardnerella vaginalis*

CONCLUSIONS:

Gardnerella vaginalis was detected in women with bacterial vaginosis. No significant association was found between intrauterine device (IUD) use and bacterial vaginosis. Presence of *Erm* a gene suggests a potential risk of clindamycin resistance among

the studied isolates, whereas *Rdx* gene not detected in any isolate. 16S rRNA gene sequencing and phylogenetic tree revealed the identity of the isolates and minor genetic diversity.

REFERENCES

[1] 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.

[2] Mohammadi, N. ; Mohammadi-Sichani, M. and Allahdadian, M. (2021). Identification of *Gardnerella vaginalis* by Molecular Methods in Women Diagnosed With Bacterial Vaginosis in Isfahan, Iran. Hormozgan Medical Journal. Vol.25. N.4. P.176.

[3] Deng, T. ; Song, X. ; Liao, Q. ; Zheng, Y. ; Sun, H. ; Zhang, L. and Chen, X. (2025). Best among the key molecular diagnostic markers of bacterial vaginosis. AMB Express. Vol.15. N.35. P.2.

[4] Zhang, T. L. Z. ; Wang, F. ; He, Y. ; Zong, X. ; Bai, H. and Liu, Z. (2020). Antimicrobial Susceptibility Testing of Metronidazole and Clindamycin against *Gardnerella vaginalis* in Planktonic and Biofilm Formation. Canadian Journal of Infectious Diseases and Medical Microbiology. Vol.2020. P.2.

[5] Rashidifar, S. ; Harzandi, N. ; Jahromi, S. H. and Gharavi, M. J. (2023). Prevalence of *Gardnerella vaginalis* infection and antibiotic resistance pattern of isolates of gynecology clinic patients at Shahriar Noor Hospital from January to June 2023 by PCR and culture methods. Iranian journal of Microbiology. Vol.15. N.4. P.514.

[6] Mishra, G. ; Gupta, K. ; Mohanty, S. ; Mitra, S. ; Singh, A. K. (2025). Original Article Evaluation of diagnostic methods for detection of trichomoniasis in symptomatic women with vaginal discharge. The journal of infection in developing countries. Vol. 19. N.1. P.156.

[7] González-Mustri, K. V. ; Guerra-Infante, F. M. and Villeda-Range, G. (2023). Frequency and molecular detection of *Gardnerella vaginalis* in a third level institute. Perinatología y Reproducción Humana. Vol.37. N.3. P.2.

[8] 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.

[9] Bunyan, I. A. ; Abdul-Lateef, L. A. and Al-Rubaeaee, 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.

[10] Younus, N. K. ; Gopinath, R. ; Jegasothy, R. ; Nordin, S. A. ; Belkum, A. V. ; Mary, N. and Neela, V. K. (2017). An update on *Gardneralla vaginalis* associated bacterial vaginosis in Malaysia. Asian Pacific Journal of Tropical Biomedicine. Vol.7. N.9. P.833.

[11] Rao, S. R. ; Girisha Pindi, 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.

[12] Strugała, I. C. ; Iwona Gołębiewska, I. G. ; Banaszewska, B. ; Mateusz, M. T. ; Brudecki, G. ; Elamin, W. and zdziak, B. Z. (2024). Bacterial Vaginosis (BV) and Vaginal Microbiome Disorders in Women Suffering from Polycystic Ovary Syndrome (PCOS). Diagnostics. Vol. 14. P.1.

[13] Mengistie, Z. ; Woldeamanue, Y. ; Asrat, D. and Yigeremu, M, (2013). Comparison of Clinical and Gram Stain Diagnosis Methods of Bacterial Vaginosis Among Pregnant Women in Ethiopia. Journal of Clinical and Diagnostic Research. Vol.7. N.7. P.2702.

[14] Mahajan, G.; Mahajan, A. ; Chopra, S. and Chand, K. (2017). Comparison of Different Diagnostic Methods of Bacterial Vaginosis-Amsel's vs Neugent. International Journal of Current Microbiology and Applied Sciences. Vol.6. N.5. P. 1444-1446.

[15] 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. BMC Infectious Diseases. Vol.21. N.1. P.1- 6.

[16] 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.

[17] de Souza, D. M. K. ; Diniz, C. G. ; Filho, D. S. C. ; de Oliveira, L. M. A. ; Coelho, D. M. ; Talha, L. D. ; do Nascimento, T. C. ; Ferreira-Machado, A. B. and da Silva, V. L. (2016). Antimicrobial susceptibility and vaginolysin in

Gardnerella vaginalis from healthy and bacterial vaginosis diagnosed women. The journal of infection in developing countries. Vol.9. N.10. P.

[18] Biutifasari, V. ; Hafel, E. F. ; Prasetyadi, F. O. H. ; Adiwinoto, R. P. and Hardjono, E. A. (2025). The Intrauterine Device (IUD) Use And Bacterial Vaginosis Incidence, Insight From 2023 Study In A Surabaya, Indonesia. Indonesian Midwifery and Health Sciences Journal. Vol.9. N.2 P.247.

[19] Kuciel, J. ; Tomczak, D. ; Mroczka, M. ; Erazmus, K. ; Hrapkowicz, R. ; Czernecka, A. ; Świtała, K. ; Pysz, P. ; Borecki, M. ; Jalocha, K. (2025). Effects of contraceptive choice on vaginal microbiome: current evidence and perspectives. Medical Science. Vol.29. P.5.

[20] Oyenihi, 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.

[21] Muzny, C. A. ; Elnaggar, J. H. ; Sousa, L.G.V. ; Lima, Â. ; Aaron, K.J. ; Eastlund, I. C. ; Graves, K. J. ; Dong, C. ; Gerwen, O. T. V. ; Luo, M. ; Tamhane, A. ; Long, D. ; Cerca, N. ; Taylor, C. M.. (2024). *Gardnerella, Prevotella* and *Fannyhessea* prior to incident bacterial vaginosis: protocol for a prospective, observational study. BMJ Open.Vol.14. P.2.

[22] Abbas, A. ; Srivastava, P. and Nirwan, p. S (2015). Prevalence of Mlsb Resistance and Observation of *erm* A & *erm* C Genes At A Tertiary Care Hospital. Journal of Clinical and Diagnostic Research. Vol.9. N.6. P.8.

[23] El Maghraby, H. M. and Mohaseb, S. (2020).Frequency of rdx A Deletion Mutation Conferring Metronidazole Resistance in *Helicobacter pylori.* Egyptian Journal of Medical Microbiology. Vol.29. N.3. P.59.

[24] Abdullah, S. T. ; Althahab, A. O. and Jabuk, S. I. A. (2023). Genetic Study of *Gardnerella vaginalis* Virulence Factors Isolated from Vaginal Discharge. 3 Medical Journal of Babylon. Vol.20. Issue.1. P.85.

[25] Alkhuzaie, M. M. (2025). Molecular Characterization and Sequencing of *Gardnerella vaginalis* in pregnant Women with Recurrent Vaginosis. Journal of Medical Case Reports and Reviews. Vol.8. N.8. P. 1572.

[26] Pillay, K. ; Nzimande, S. ; Naicker, M. ; Ramsuran, V. ; Tinarwo, P. and Abbai, N. (2020). Prevalence of Genotypes and Subtypes of *Gardnerella vaginalis* in South African Pregnant Women. Infectious Diseases in Obstetrics and Gynecology. Vol. 2020. P.3.

[27] Mutar, A. D. (2024). PCR And Sequencing Technique Detection Of *Gardnerella* Spp. Associated With Urinary Tract Vaginosis. South Eastern European Journal of Public Health**.** Special Volume XXIV. No.S2. P.481.