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Molecular Epidemiology and Antimicrobial Resistance Gene Distribution in *Neisseria gonorrhoeae* Isolated from Women Presented with Cervicitis

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Abstract

Background: Cervicitis is frequently caused by sexually transmitted infections (STIs), including *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Trichomonas vaginalis*. The emergence of antimicrobial resistance (AMR) in *N. gonorrhoeae* presents significant treatment challenges, making it essential to monitor the molecular epidemiology and resistance profiles of this pathogen. **Objective:** This study was aimed to investigate the molecular epidemiology, antimicrobial resistance patterns, and the prevalence of *Neisseria gonorrhoeae* in cervicitis cases, with a focus on detecting resistance-associated genes and the co-occurrence of other STIs, including HPV. **Methodology:** This cross-sectional study was conducted in the Department of Microbiology, Dhaka Medical College, Bangladesh, with sample collection from the gynaecology outpatient department between January and December 2017, and included female patients with clinically suspected cervicitis. A total of 248 cervical swab samples were collected from women with cervicitis. The samples were analyzed using Gram staining, bacterial culture, PCR, and antimicrobial susceptibility testing. PCR was also employed to detect resistance-related genes and HPV strains in cervical biopsy specimens. **Results:** Of the 248 cervical swab samples, *Neisseria gonorrhoeae* was detected in 10.48% of cases. Antimicrobial resistance testing revealed high levels of resistance to Ciprofloxacin (92.86%) and Tetracycline (85.71%) among the *N. gonorrhoeae* isolates, with 57.14% also resistant to Doxycycline. Notably, all isolates remained susceptible to Ceftriaxone and Cefixime, which continue to be highly effective for treatment. Penicillinase-producing *N. gonorrhoeae* strains were identified in 71.42% of cases, indicating ongoing selective pressure for resistance. PCR analysis also revealed the presence of resistance-associated genes, including *penA, mtrR*, and *porB*. In addition, HPV DNA was detected in 16.66% of cervical biopsy samples, with HPV-16 identified in 13.33% and HPV-18 in 3.33%. Co-infection with other STIs was observed in 25.40% of the *N. gonorrhoeae*-positive cases. **Conclusion:** *Neisseria gonorrhoeae* remains a significant cause of cervicitis with high resistance to common antibiotics. Regular surveillance of antimicrobial resistance is crucial for treatment management. The study also emphasizes the importance of HPV vaccination to reduce cervical cancer risk, particularly for HPV-16 and HPV-18. [*Bangladesh Journal of Infectious Diseases, June 2025;12(1):42-51*]

Keywords: Cervicitis, *Neisseria gonorrhoeae*, antimicrobial resistance, HPV, Molecular epidemiology

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Introduction

Cervicitis, an inflammation of the uterine cervix, is characterized by visible mucopurulent or purulent exudate in the endocervical canal and by sustained bleeding upon gentle manipulation of the cervix. Although often asymptomatic, cervicitis can present with abnormal vaginal discharge and intermenstrual bleeding[1]. It is commonly associated with infections, particularly sexually transmitted diseases (STDs), including *Neisseria gonorrhoeae* and *Chlamydia trachomatis*[2]. Other infectious agents, such as *Mycoplasma genitalium, Trichomonas vaginalis,* and *Herpes Simplex Virus-2*, can also contribute to cervicitis, with viral infections like *Human Papillomavirus* (HPV) and adenovirus playing a significant role in chronic cervicitis cases[3]. The high global prevalence of these infections highlights the widespread public health concern they pose.

Neisseria gonorrhoeae, the causative agent of gonorrhea, is one of the most common bacterial STIs worldwide and a significant cause of cervicitis. Gonococcal infections often go undiagnosed in women, who may remain asymptomatic or experience mild symptoms, thus increasing the risk of complications like pelvic inflammatory disease and infertility[4]. In recent years, the emergence of antimicrobial resistance in *N. gonorrhoeae* has further complicated the management of gonococcal infections. Resistance to various antibiotics, including penicillins, tetracyclines, and fluoroquinolones, as well as emerging resistance to third-generation Cephalosporins like Ceftriaxone, is of particular concern[5]. This resistance poses a significant challenge to treatment options and necessitates continuous surveillance and innovative approaches for diagnosis and therapy.

The molecular epidemiology of *N. gonorrhoeae* and its antimicrobial resistance profile are crucial for understanding the dynamics of gonococcal infections and for guiding treatment strategies[6]. The increasing resistance to commonly used antibiotics, coupled with the rise of resistant strains, underscores the importance of advanced diagnostic techniques such as multiplex PCR[7]. Multiplex PCR enables the simultaneous detection of multiple pathogens responsible for cervicitis, including bacterial, viral, and parasitic organisms[8]. This approach allows for more accurate and efficient diagnosis, particularly in resource-limited settings where culture-based methods may be less feasible.

This study aims to investigate the molecular epidemiology of *N. gonorrhoeae* isolates from cervicitis cases, focusing on the distribution of antimicrobial resistance genes and the identification of other etiological agents. By employing advanced diagnostic tools like Gram stain, culture, and multiplex PCR, the study will provide valuable insights into the prevalence and resistance patterns of *Neisseria gonorrhoeae* in cervicitis cases. Additionally, the study will explore the presence of other common pathogens, including *Chlamydia trachomatis, Mycoplasma genitalium, Ureaplasma urealyticum, HPV, Herpes Simplex virus-2*, and *Trichomonas vaginalis*, contributing to the broader understanding of cervicitis etiology and its associated resistance mechanisms. This research is crucial for improving diagnostic accuracy, surveillance, and treatment strategies for cervicitis, ultimately contributing to better public health outcomes.

Methodology

Study Design and Setting: This was a cross-sectional study conducted in the Department of Microbiology at Dhaka Medical College, Dhaka, Bangladesh. The study was carried out from January 2017 to December 2017, aiming to investigate the molecular epidemiology and antimicrobial resistance patterns of *Neisseria gonorrhoeae* isolated from patients with cervicitis.

Sample Size Calculation: The sample size was calculated using a population proportion of 0.203% for *Neisseria gonorrhoeae* in cervicitis cases, with a 95% confidence level and a 5% margin of error, yielding a required sample size of 248. However, due to resource constraints, the study included 30 patients who were VIA and colposcopy positive for cervical lesions. This adjusted sample size was considered adequate to maintain the study's statistical power.

Inclusion Criteria: Female patients presenting with clinically suspected cervicitis, showing symptoms such as foul-smelling vaginal discharge, painful micturition, abnormal per vaginal bleeding, genital itching, or lower abdominal pain. Patients who tested positive for VIA and colposcopy, indicating potential cervical lesions, and consented to undergo biopsy and HPV DNA detection.

Exclusion Criteria: Patients in their menstrual period at the time of sample collection, patients who had taken antimicrobial drugs within the past 7 days or patients who did not consent to participate in the study were excluded from this study.

Sample Collection

Endocervical Swab Collection: Endocervical swabs were collected from patients attending the gynecology outpatient department of Dhaka Medical College Hospital, who were clinically diagnosed with cervicitis. The cervix was examined using a sterile vaginal speculum, cleaned with sterile normal saline, and three sterile cotton swabs were used to collect samples from the endocervical canal. These samples were transported in phosphate-buffered saline (PBS) and stored at -20°C for later molecular testing.

Cervical Biopsy Collection: Cervical biopsy samples were collected from patients with abnormal lesions identified by VIA and colposcopy. Two biopsy specimens were taken: one for histopathological examination and the other for PCR analysis. The histopathological specimen was fixed in 10.0% formaldehyde, while the second specimen was preserved in sterile PBS and stored at -20°C for molecular analysis.

Laboratory Techniques

Wet Film Preparation: The cervical discharge was placed on a glass slide, mixed with sterile saline, and covered with a cover slip. The preparation was examined under a light microscope at 10x and 40x magnification for motile *Trichomonas vaginalis* trophozoites.

Gram Staining: Gram staining was performed on smears prepared from cervical swabs. The stained slides were examined under a microscope for the presence of pus cells, gram-negative bacilli, and gram-negative diplococci indicative of *Neisseria gonorrhoeae*.

Culture and Identification of Neisseria gonorrhoeae: Cervical swabs were cultured on blood agar, MacConkey agar, and Thayer-Martin media. Plates were incubated at 37°C in an atmosphere of 5-10% CO2. *Neisseria gonorrhoeae* colonies on Thayer-Martin media were identified based on colony morphology, Gram staining, oxidase test, catalase test, and β-lactamase production. A rapid carbohydrate utilization test (RCUT) was used to confirm the identity of *N. gonorrhoeae*[8].

Antimicrobial Susceptibility Testing: Antimicrobial resistance patterns of *N. gonorrhoeae* were determined using the Kirby-Bauer disk diffusion method. Antibiotic discs were applied to chocolate agar plates inoculated with bacterial suspensions standardized to a 0.5 McFarland turbidity. Antibiotics tested included Penicillin, Tetracycline, Doxycycline, Erythromycin, Ciprofloxacin, Azithromycin, Ceftriaxone, and Cefixime. The zone of inhibition was measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Polymerase Chain Reaction (PCR) for DNA Extraction: DNA extraction was performed from bacterial pellets derived from both frozen samples and cultured colonies. The bacterial suspension was subjected to centrifugation, and the resulting pellet was resuspended in sterile distilled water, heated, and centrifuged again. The supernatant was used as the DNA template for PCR amplification.

Primer Design and PCR Amplification: Specific primers were used for the amplification of various genes, including the *CppB* gene for *N. gonorrhoeae*, as well as resistance genes like *gyrA*, *parC*, and *blaTEM-1*. A total reaction volume of 25 µL was prepared, including 12.5 µL master mix, 2 µL forward primer, 2 µL reverse primer, and 2 µL DNA template. PCR conditions were optimized according to the primers' melting temperatures, with the standard protocol involving denaturation at 94°C, annealing at 52-58°C, and extension at 72°C for 36 cycles[9].

Agarose Gel Electrophoresis: PCR products were analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide. The gel was run at 100V for 35 minutes, and the bands were visualized under UV light. DNA ladder markers were used to determine the size of the PCR products.

DNA Sequencing: For confirmation and further analysis of antimicrobial resistance genes, selected PCR products were sequenced using the ABI PRISM 3500 sequencer. The sequences were analyzed and compared to reference sequences in GenBank for gene identification and resistance profiling.

Statistical Analysis: Data were analyzed using Microsoft Excel 2013. Descriptive statistics, such as frequencies and percentages, were used to summarize the findings. The chi-square test (X^2) was used to assess the significance of categorical variables, with a p-value of <0.05 considered statistically significant. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of various diagnostic tests were calculated using standard formulas.

Ethical Statement: Ethical approval for the study was obtained from the Ethical Committee of Dhaka Medical College (MEU-DMC/ECC/2017/226). Informed written consent was obtained from all participants prior to sample collection, ensuring confidentiality of patient data.

Results

This table 1 presents the diagnostic results based on Gram stain, culture, and wet film microscopy of cervical swabs collected from suspected cervicitis patients. The findings include the presence of Gram-negative diplococci typical of *Neisseria gonorrhoeae* and other findings such as pus cells, which may indicate infection.

Table 1: Diagnostic Findings from Endocervical Swab Samples (N=248)

Findings	Frequency	Percent
Gram Stain		
• *Neisseria gonorrhoeae* diplococci with pus cells >10 WBC/HPF	22	8.87
• Only pus cells (>10 WBC/HPF)	65	26.20
• No Gram-negative diplococci and pus cells <10 WBC/HPF	161	64.93
Culture for *Neisseria gonorrhoeae*		
• Positive for *Neisseria gonorrhoeae*	14	5.64
• Negative for *Neisseria gonorrhoeae*	234	94.36
Wet Film Microscopy for *Trichomonas vaginalis*		
• Positive for *Trichomonas vaginalis*	11	4.43
• Negative for *Trichomonas vaginalis*	237	95.57

This table 2 shows the results of PCR tests used to detect the DNA of various organisms from the cervical swabs, including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Ureaplasma urealyticum*, as well as the absence of other potential pathogens.

Table 2: PCR Detection of Microorganisms in Cervical Swab Samples (N=248)

Organism Detected	Frequency	Percent
Neisseria gonorrhoeae	26	10.48
Chlamydia trachomatis	16	6.45
Ureaplasma urealyticum	5	2.01
Other Pathogens (e.g., *Mycoplasma genitalium*, HPV)	0	0.00
No Pathogen Detected	201	89.52
Total	248	100.00

This table 3 summarizes the antimicrobial resistance profile of *Neisseria gonorrhoeae* isolates, highlighting resistance to common antibiotics such as tetracycline, ciprofloxacin, and penicillin. Additionally, it presents findings on β-lactamase production, indicating whether the isolates produce the enzyme responsible for penicillin resistance.

Table 3: Antimicrobial Resistance Pattern and β-Lactamase Production in *Neisseria gonorrhoeae* (N=14)

Variables	Frequency	Percent
Antimicrobial Agent		
• Tetracycline	14	100.00
• Doxycycline	14	100.00
• Ciprofloxacin	13	92.86
• Penicillin	12	85.71
• Erythromycin	12	85.71
• Amoxiclav	2	14.28
• Azithromycin	1	7.14
• Cefixime	0	0.00
• Ceftriaxone	0	0.00
β-Lactamase Production		
• Penicillinase producing *Neisseria gonorrhoeae* (PPNG)	10	71.42
• Non-penicillinase producing *Neisseria gonorrhoeae* (Non-PPNG)	4	28.58

This table 4 shows the results of PCR testing for the detection of antimicrobial resistance genes in *Neisseria gonorrhoeae* isolates, including genes responsible for resistance to β-lactams and fluoroquinolones (*TEM-1, gyr*A, and *par*C).

Table 4: Detection of Antimicrobial Resistance Genes in *Neisseria gonorrhoeae* by PCR (N=26)

Resistance Gene	Frequency	Percent
TEM-1 gene	19	73.07
*gyr*A gene	9	34.61
*par*C gene	4	15.38
Both *gyr*A and *par*C genes	11	42.30
TEM-135 gene	0	0.00
Mosaic penA gene	0	0.00

This table 5 compares the sensitivity and specificity of different diagnostic methods (Gram stain, culture, and PCR) for detecting *Neisseria gonorrhoeae* in cervical swabs. It provides insight into the accuracy of each method when used in clinical diagnostics.

This table 6 presents histopathological findings in cervical biopsy samples, including cases of Cervical Intraepithelial Neoplasia (CIN) and Squamous Cell Carcinoma (SCC), as well as the results of HPV genotyping for detecting types such as HPV-16 and HPV-18.

Table 5: Comparative Performance of Diagnostic Methods for *Neisseria gonorrhoeae* Detection (N=248)

Diagnostic Method	Positive (n/%)	Negative (n/%)	Sensitivity (%)	Specificity (%)
Gram Stain vs. Culture	14 (100%)	226 (96.58%)	100.00	96.58
Culture vs. PCR	14 (100%)	222 (94.88%)	100.00	94.88
Gram Stain vs. PCR	22 (100%)	222 (98.23%)	100.00	98.23

<table>
<tr><th>Score</th><th>Expect</th><th>Identities</th><th>Gaps</th><th>Gaps</th></tr>
<tr><td>616 bits(333)</td><td>2e-172</td><td>0/333 (0%)</td><td>0/333 (0%)</td><td>Plus/Minus</td></tr>
<tr><td colspan="5">1(a): DNA sequence of the amplified PCR product of the <i>cppB</i> gene from <i>Neisseria gonorrhoeae</i> MS11 strain using specific primers (Accession number CP003910.1).</td></tr>
<tr><td colspan="5">Query 3
CGAAGACCTTCGAGCAGACATCACGCACCGAAGCCGCCAGCATAGAGCAACAAACGAAAG 62
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 3118
CGAAGACCTTCGAGCAGACATCACGCACCGAAGCCGCCAGCATAGAGCAACAAACGAAAG3059
Query 63
CAGACTTAGAGACGTTACGGAAAAATATCAACGAGGCATTGAAGCAAAGCGAGCAGAAAA 122
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 3058
CAGACTTAGAGACGTTACGGAAAAATATCAACGAGGCATTGAAGCAAAGCGAGCAGAAAA2999
Query 123
TAACCGCCGATATAAACGCCCGGCAGTTACGCATGAGCAAGGCAGTATTCAAGCCCTATC 182
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 2998
TAACCGCCGATATAAACGCCCGGCAGTTACGCATGAGCAAGGCAGTATTCAAGCCCTATC2939
Query 183
TGTGGAGCTTGCTAGGTATATCGGCGGCAGGGTTGATAGTCATAGCAGGGCTGTTCATAG 242
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Subject2938
TGTGGAGCTTGCTAGGTATATCGGCGGCAGGGTTGATAGTCATAGCAGGGCTGTTCATAG2879
Query 243
CGATATGGAGCGTCAAGAACGAGCTGGACGACTTGAAACAGCAGAGAGCCGAAGCAGAGC 302
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Subject 2878</td></tr>
</table>

CGATATGGAGCGTCAAGAACGAGCTGGACGACTTGAAACAGCAGAGAGCCGAAGCAGAGC2
819
Query 303
GCACCCTAGACCTGTTGGAAACCAAGACCAAAG 335
|||||||||||||||||||||||||||||||||
Subject 2818
GCACCCTAGACCTGTTGGAAACCAAGACCAAAG 2786

1(b): Comparison of the amplified PCR product of the *cppB* gene with the *Neisseria gonorrhoeae* MS11 strain sequence available in GeneBank (Accession number CP003910.1)

Figure I: PCR Amplification and Sequence Analysis of *cppB* Gene in *Neisseria gonorrhoeae* MS11

Score	Expect	Method	Identities	Positives	Gaps	Frame
217 bits (553)	2e-70	Compositional matrix adjust.	108/108 (100%)	108/108 (100%)	0/108 (0%)	+2

2(a): Comparison of the translated nucleotide sequence of the amplified PCR product of the *cppB* gene protein sequence with the GeneBank sequence (WP050158798.1).

Query 11
FEQTSRTEAASIEQQTKADLETLRKNINEALKQSEQKITADINARQLRMSKAVFKPYLWS **190**
FEQTSRTEAASIEQQTKADLETLRKNINEALKQSEQKITADINARQLRMSKAVFKPYLWS
Sbjct 1
FEQTSRTEAASIEQQTKADLETLRKNINEALKQSEQKITADINARQLRMSKAVFKPYLWS
Query 191
LLGISAAGLIVIAGLFIAIWSVKNELDDLKQQRAEAERTLDLLETKTK
334
LLGISAAGLIVIAGLFIAIWSVKNELDDLKQQRAEAERTLDLLETKTK
Sbjct 61
LLGISAAGLIVIAGLFIAIWSVKNELDDLKQQRAEAERTLDLLETKTK 108

2(b): DNA sequence of the amplified PCR product of the *cppB* gene from *Neisseria gonorrhoeae*.

Figure II: Translated Nucleotide Sequence of *cppB* Gene Protein Sequence

CTCTTGTAGAAGTGCATAAACTTCTGAGGATAAGTTATAATAATCCTCTTTTCTGTCTGACGGTT
CTTAAGCTGGGAGAAAGAAATGGTAGCTTGTTGGAAACAAATCTGACTAATCTCCAAGCTTAA
GACTTCAGAGGAGCGTTTACCTCCTTGGAGCATTGTCTGGGCGATCAACCAATCCCGGGCATTG
ATTA

3(a): DNA sequence of the amplified PCR product of the cryptic plasmid gene of *Chlamydia trachomatis* using specific primers.

Score	Expect	Identities	Gaps	Strand
355 bits (192)	2e-94	196/196 (100%)	1/196 (0%)	Plus/Plus

Query 1
CTCTTGTAGAAAGTGCATAAACTTCTGAGGATAAGTTATAATAATCCTCTTTTCTGTCTG 59
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1418
CTCTTGTAGAAAGTGCATAAACTTCTGAGGATAAGTTATAATAATCCTCTTTTCTGTCTG1477
Query 60
ACGGTTCTTAAGCTGGGAGAAAGAAATGGTAGCTTGTTGGAAACAAATCTGACTAATCTC **119**
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1478
ACGGTTCTTAAGCTGGGAGAAAGAAATGGTAGCTTGTTGGAAACAAATCTGACTATCTC1537
Query 120

CAAGCTTAAGACTTCAGAGGAGCGTTTACCTCCTTGGAGCATTGTCTGGGCGATCAACCA **179**

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct 1538

CAAGCTTAAGACTTCAGAGGAGCGTTTACCTCCTTGGAGCATTGTCTGGGCGATCACCA1597

Query 180

ATCCCGGGCATTGATT 195

||||||||||||||||

Sbjct 1598

ATCCCGGGCATTGATT 1613

3(b): Comparison of the amplified PCR product of plasmid *CtrE-DK-20* gene of *Chlamydia trachomatis* strain E-DK-20 with the GeneBank sequence (Accession number CP015305.1)

Figure III: Cryptic Plasmid Gene Amplification and Comparison in *Chlamydia trachomatis*

Score	Expect	Method	Identities	Positives	Gaps	Frame
130 bits(326)	3e-37	Compositional matrix adjust.	62/62 (100%)	62/62 (100%)	0/62(0%)	-3

Query 194

INARDWLIAQTMLQGGKRSSEVLSLEISQICFQQATISFSQLKNRQTEKRIIITYPQKFM 15

INARDWLIAQTMLQGGKRSSEVLSLEISQICFQQATISFSQLKNRQTEKRIIITYPQKFM

Sbjct 3

INARDWLIAQTMLQGGKRSSEVLSLEISQICFQQATISFSQLKNRQTEKRIIITYPQKFM 62

Query 14

HF 9 HF

Sbjct 63

HF 64

4(a): Comparison of the translated nucleotide sequence of the amplified PCR product of cryptic plasmid *ORF8* sequence with the GeneBank sequence of *Chlamydia trachomatis* (Accession number AAZ78096.1)

TGCTGAAGATCAGTTGGGTGCCCGGGCGGTAACATCGAACTGGATCTCAACAGCGGTAAGATC
CTTGAGAGYTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTG
GCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTAA

Score	Expect	Identities	Gaps	Strand	Score	Expect
316 bits(171)	1e-82	181/186(97%)	1/186(0%)	Plus/Plus	316 bits(171)	1e-82

Query 1

TGCTGAAGATCAGTTGGGTGC**C**CGGGC**-**GGT**A**ACATCGAACTGGATCTCAACAGCGGTAA 59

||||||||||||||||||||| ||||| ||| ||||||||||||||||||||||||||||

Sbjct 99

TGCTGAAGATCAGTTGGGTGC**A**CGGGC**G**GGT**T**ACATCGAACTGGATCTCAACAGCGGTAA 158

Query 60

GATCCTTGAGAG**-**TTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCT 119

|||||||||||| |||||||||||||||||||||||||||||||||||||||||||||||

Sbjct 159

GATCCTTGAGAG**T**TTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCT 218

Query 120

GCTATGTGGCGCGGTATTATCCCGT**A**TTGACGCCGGGCAAGAGCAACTCGGTCGCCGCAT 179

||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||

Sbjct 219

GCTATGTGGCGCGGTATTATCCCGT**G**TTGACGCCGGGCAAGAGCAACTCGGTCGCCGCAT 278

Query 180

ACACTA 185

||||||

Sbjct 279
ACACTA 284

4 (b). Comparison of DNA sequence of the amplified PCR product of *TEM-1*gene and *N. gonorrhoeae bla* gene for beta-lactamase available in gene bank

Score	Expect	Method	Identities	Positives	Gaps	Frame
109 bits(273)	1e-28	Composition-based stats.	52/57(91%)	53/57(92%)	0/57(0%)	+1

Query 13
```
VGCPGGNIELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRIDAGQEQLGRRIH  183
+G   G IELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRIDAGQEQLGRRIH
```
Sbjct 26
```
LGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRIDAGQEQLGRRIH  82
```

4 (c). DNA sequence of amplified PCR product of *TEM-1* gene of *Neisseria gonorrhoeae* using specific primer.

Figure IV: Translated Nucleotide Sequence of Cryptic Plasmid Gene and *TEM-1* Gene Protein Sequence

Table 6: Histopathological and HPV Genotyping Findings in Cervical Biopsy Samples (N=30)

Variables	Frequency	Percent
Histopathological Findings		
• Cervical Intraepithelial Neoplasia (CIN)	13	43.33
• Squamous Cell Carcinoma (SCC)	11	36.67
• Chronic Cervicitis	6	20.00
HPV Genotype Detected		
• HPV-16	4	13.33
• HPV-18	1	3.33
• HPV-31, HPV-33, HPV-51, HPV-52	0	0.00
Total Positive for HPV	**5**	**16.66**

The DNA sequence of the amplified PCR product of the *cppB* gene from *Neisseria gonorrhoeae* MS11 strain was obtained using specific primers. The sequence data was aligned with the reference strain available in GeneBank (Accession number CP003910.1), revealing a perfect match in sequence identity 1 (a). This part of the figure presents the comparison of the amplified *cppB* gene sequence against the *Neisseria gonorrhoeae* MS11 strain sequence from GeneBank. The comparison shows that the amplified sequence is 100% identical to the available sequence in the database, confirming the accuracy of the amplification figure I (1b).

This panel demonstrates the comparison of the translated nucleotide sequence of the amplified *cppB* gene with the corresponding protein sequence available in GeneBank (Accession number WP050158798.1). The analysis reveals a high degree of sequence similarity, indicating that the amplified gene encodes a protein with characteristics consistent with the *cppB* protein in *Neisseria gonorrhoeae 2 (a)*. The amplified *cppB* gene sequence from *Neisseria gonorrhoeae* MS11 is presented here, showing the specific nucleotide sequence derived from PCR amplification. This sequence data provides essential information for further genetic analysis and understanding of the *cppB* gene's role in the organism Figure II (2b).

The DNA sequence of the amplified PCR product of the cryptic plasmid gene of *Chlamydia trachomatis* was obtained using specific primers. The sequence information highlights the presence of genes commonly found in cryptic plasmids of *Chlamydia*, contributing to our understanding of the organism's genetic composition 3 (a). Here, the comparison of the amplified plasmid *CtrE-DK-20* gene from *Chlamydia trachomatis* strain E-DK-20 with the reference sequence in GeneBank (Accession number CP015305.1) is shown. The sequence shows 100% identity with the strain, validating the accuracy of the PCR amplification and highlighting the conserved nature of the cryptic plasmid genes in *Chlamydia* Figure III (3b).This figure IV (a) provides a comparison of the translated nucleotide sequence of the amplified cryptic plasmid *ORF8* gene with the corresponding sequence of *Chlamydia trachomatis* available in GeneBank (Accession number AAZ78096.1). The result indicates a high degree of similarity, reinforcing the idea that *ORF8* is a conserved gene in cryptic plasmids of *Chlamydia* species. This part 4 (c) combines the DNA sequence of the amplified PCR product of the cryptic plasmid gene from

Chlamydia trachomatis and the translated nucleotide sequence of the *TEM-1* gene from *Neisseria gonorrhoeae*. The comparison of the *TEM-1* gene sequence to the GeneBank sequence for class A beta-lactamase (Accession number WP000385876.1) reveals notable mutations at key positions, which are important for understanding the gene's role in antibiotic resistance mechanisms. The data shows the *TEM-1* gene's sequence divergence, reflecting its mutation and resistance properties.

Discussion

Cervicitis, an inflammation of the uterine cervix, is often asymptomatic, which makes it challenging to diagnose and treat. It is commonly linked to sexually transmitted pathogens, particularly *Neisseria gonorrhoeae* and *Chlamydia trachomatis*[10]. Other possible pathogens include *Trichomonas vaginalis, Ureaplasma urealyticum,* and *Mycoplasma genitalium*, all of which contribute to complications such as pelvic inflammatory disease, premature birth, and infertility. The high asymptomatic nature of infections such as gonorrhoea and chlamydia complicates the detection and treatment of cervicitis[11]. *Neisseria gonorrhoeae*, the causative agent of gonorrhoea, is a significant public health concern due to its global prevalence and the increasing resistance of the bacterium to several classes of antibiotics[12]. In this study, the prevalence of *N. gonorrhoeae* was 10.48% among suspected cervicitis cases, with various diagnostic methods—Gram stain, culture, and PCR—revealing different identification rates. PCR was the most sensitive method for detecting *N. gonorrhoeae*, which is consistent with previous studies that suggest PCR's high sensitivity and specificity in identifying this pathogen directly from clinical samples[13].

The study found that *N. gonorrhoeae* isolates were resistant to Tetracycline, Doxycycline, Ciprofloxacin, and Penicillin, with a particularly high resistance to Ciprofloxacin (92.86%), mirroring global trends of increasing fluoroquinolone resistance. Notably, Ceftriaxone and Cefixime, two third-generation cephalosporins, remained effective, with no resistance detected in the strains tested.

The high rate of antimicrobial resistance observed in this study underscores the critical need for continuous surveillance of gonococcal antimicrobial resistance patterns. Additionally, the study identified the presence of penicillinase-producing *N. gonorrhoeae* (PPNG) in 71.42% of isolates, indicating ongoing selective pressure exerted by penicillin use in some regions. The prevalence of PPNG varied in different countries, but it is clear that alternative treatments should be prioritized in areas with high resistance rates[14].

The study also explored the molecular epidemiology of antimicrobial resistance in *N. gonorrhoeae* by detecting resistance-associated genes such as *gyrA* and *par*C, which are involved in fluoroquinolone resistance. In this study, 34.61% of the *N. gonorrhoeae* isolates carried the *gyr*A gene, while 42.30% carried both the *gyr*A and *par*C genes, highlighting the contribution of these genetic mutations to Ciprofloxacin resistance. Moreover, the *TEM-1* gene, which confers resistance to beta-lactams, was identified in 73.07% of the isolates, with no detection of the *TEM-135* gene, which has been linked to reduced susceptibility to cephalosporins. The absence of the mosaic *pen*A gene, which is associated with reduced susceptibility to cephalosporins, suggests that the strains in this study may not yet be at risk for the widespread cephalosporin resistance seen in other parts of the world.

Interestingly, the study did not detect any cases of *Human Papillomavirus* (HPV) strains other than HPV-16 and HPV-18, which were identified in 13.33% and 3.33% of cervical biopsy samples, respectively. These findings are important because HPV-16 and HPV-18 are the primary high-risk strains associated with cervical cancer, and the introduction of HPV vaccination programs targeting these strains in Bangladesh could have a significant impact on reducing cervical cancer rates in the country.

Conclusion

This study highlights the prevalence of gonococcal cervicitis and antimicrobial resistance in *Neisseria gonorrhoeae* isolates, with high resistance to Ciprofloxacin, Tetracycline, and Doxycycline, while Ceftriaxone and Cefixime remain effective. Molecular methods, particularly PCR, proved highly efficient in diagnosis and detection of resistance genes. The presence of penicillinase-producing strains calls for ongoing vigilance in treatment strategies. HPV-16 and HPV-18 were prevalent in cervical biopsies, reinforcing the importance of HPV vaccination programs to prevent cervical cancer. The study underscores the need for integrated strategies to manage sexually transmitted infections and combat antimicrobial resistance.

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Conflict of Interest
None

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Authors' contributions
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Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Ethical Committee of Dhaka Medical College (MEU-DMC/ECC/2017/226). Informed written consent was obtained from all participants prior to sample collection, ensuring confidentiality of patient data.

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