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RESEARCH ARTICLE

Comparison of Culture, Multiplex PCR and Histopathology for the Detection of Etiological Agents of Cervicitis

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ABSTRACT

Background: Cervicitis, an inflammation of the uterine cervix, is often associated with sexually transmitted infections (STIs), and its early detection is crucial for effective management. *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and other pathogens are common causes. Diagnostic methods such as Gram staining, culture, PCR, and histopathology are used to identify the etiological agents. However, their comparative efficacy remains debated.

Objective: The objective of this study was to compare the diagnostic accuracy of culture, multiplex PCR, and histopathology in detecting the etiological agents of cervicitis.

Methods: A total of 248 endocervical samples were collected from women with suspected cervicitis. These samples were examined using Gram stain, culture, multiplex PCR, and histopathological analysis. The sensitivity, specificity, and diagnostic performance of each method were assessed.

Results: Among the samples, Gram stain identified 8.87% *N. gonorrhoeae*, while culture and PCR detected 5.64% and 10.48%, respectively. Histopathology revealed chronic cervicitis in 20% of cases, cervical intraepithelial neoplasia (CIN) in 43.33%, and squamous cell carcinoma (SCC) in 36.67%. PCR demonstrated the highest sensitivity (100%) for *N. gonorrhoeae* detection compared to culture, which had lower sensitivity. Gram stain showed high specificity (96.58%) but was less sensitive than PCR. The multiplex PCR method was superior in detecting mixed infections and pathogens that failed to grow in culture. Histopathology, while important for identifying tissue abnormalities, had limited sensitivity in detecting specific microbial agents.

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Conclusion: PCR is the most reliable and sensitive method for detecting cervicitis-causing pathogens, outperforming culture and Gram stain. Histopathology, though valuable for identifying tissue changes, is not as effective in diagnosing the microbial etiology. A combination of PCR and histopathology may provide a comprehensive diagnostic approach for cervicitis.

Keywords: Cervicitis; PCR, Gram stain; Culture; Histopathology

1. Introduction

Cervicitis, an inflammation of the cervix, is primarily characterized by a visible, purulent or mucopurulent endocervical discharge, and sustained bleeding from the cervix upon gentle manipulation[1]. While cervicitis can often remain asymptomatic, some women may experience symptoms like abnormal vaginal discharge or intermenstrual bleeding[2]. A common sign of cervicitis is the presence of over 10 polymorphonuclear leukocytes per high-power field in the vaginal fluid, which has been associated with infections such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae*[3]. In developing regions, the burden of cervicitis is exacerbated by the interplay between infections, malnutrition, and reproductive health challenges[4].

The primary bacterial causes of cervicitis are *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, with other contributing pathogens including *Mycoplasma genitalium, Trichomonas vaginalis*, and various viruses like *Herpes simplex virus* type 2 and *Human papillomavirus* (HPV) [5]. Additionally, *Mycoplasma hominis, Ureaplasma urealyticum,* and *Ureaplasma parvum* have also been implicated in cervicitis[6]. Globally, sexually transmitted infections (STIs) remain a significant public health concern, with millions of new cases of gonorrhoea, chlamydia, and other STIs reported annually. The prevalence of *Neisseria gonorrhoeae* varies geographically, and the rise in antimicrobial resistance further complicates effective treatment strategies[7].

Diagnosis of cervicitis relies on a combination of clinical evaluation, laboratory tests, and histopathological examination. *Neisseria gonorrhoeae* can be identified through Gram staining, culture, or PCR, while *Chlamydia trachomatis* is often diagnosed via cell culture or PCR[8]. The sensitivity of traditional diagnostic methods, such as culture, can be limited, especially for organisms that are difficult to culture, such as Mycoplasma and Ureaplasma species. More recently, molecular techniques like multiplex PCR have become valuable tools for detecting multiple pathogens simultaneously, improving diagnostic accuracy and efficiency[9].

In addition to bacterial causes, viral infections, such as those caused by HPV and Herpes simplex virus, are also significant contributors to cervicitis. Histopathological examination can reveal characteristic changes associated with these infections, such as koilocytosis in HPV infection or lymphocytic infiltration in HSV infection[11].

This study aims to compare different diagnostic methods—culture, multiplex PCR, and histopathology—for detecting the etiological agents of cervicitis. By utilizing multiplex PCR, the study simultaneously detect a range of bacterial and viral pathogens, as well as investigate the presence of antimicrobial resistance genes in Neisseria gonorrhoeae.

2. Methodology

2.1. Study design and setting

This cross-sectional study was conducted from January to December 2017 at Dhaka Medical College. A total of 248 women with clinically suspected cervicitis were included. Cervical biopsy samples were collected from 30 VIA and colposcopy-positive patients for histopathological and PCR analysis. The study took place

in the Gynecology Outpatient Department of Dhaka Medical College Hospital, where patients presented with symptoms such as vaginal discharge, painful micturition, abnormal bleeding, genital itching, or lower abdominal pain.

2.2. Inclusion and exclusion criteria

Inclusion criteria consisted of patients presenting with clinical symptoms of cervicitis, 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 were eligible for biopsy and further diagnostic tests.

Exclusion criteria included patients currently menstruating, those who had received antimicrobial treatment within the past seven days, and patients who did not consent to participate in the study.

2.3. Data collection

Patient data were collected using a predesigned data collection sheet. Ethical approval for the study was obtained from the Research Review Committee and the Ethical Review Committee of Dhaka Medical College. Informed written consent was obtained from each participant.

2.4. Sample collection

Endocervical Swab: Endocervical swabs were collected using sterile techniques from patients attending the Gynecology outpatient department at Dhaka Medical College Hospital. After cleaning the cervix with sterile saline, three sterile cotton swabs were used: one for Gram stain and wet film preparation, a second for culture, and a third stored in phosphate-buffered saline (PBS) for PCR analysis.

Cervical Biopsy: Biopsy specimens were collected from women with suspected cervical lesions identified by VIA and colposcopy. One specimen was fixed in formaldehyde for histopathological examination, while the second specimen was stored at -20°C for subsequent PCR analysis.

2.5. Specimen preservation for PCR

Samples designated for PCR were preserved in sterile PBS and stored at -20°C until DNA extraction. The specimens underwent vortexing to homogenize the contents before centrifugation to collect bacterial pellets, which were used for PCR analysis.

2.6. Histopathological examination

The fixed biopsy specimens were processed for histopathological examination. Following formalin fixation, the specimens underwent gross examination, tissue embedding in paraffin, and sectioning. Histological evaluation was performed using hematoxylin and eosin staining.

2.7. Culture techniques

Culture Media: Culture was performed using blood agar, MacConkey agar, and Thayer Martin medium. These media facilitated the isolation of both Gram-negative organisms and *Neisseria gonorrhoeae*. The inoculated plates were incubated under appropriate conditions, and growth was monitored for the identification of organisms based on colony morphology, Gram stain, and biochemical testing.

Identification of *Neisseria gonorrhoeae*: Neisseria gonorrhoeae was confirmed by colony morphology on Thayer Martin medium, followed by oxidase and catalase tests, and PCR amplification for the detection of gonococcal DNA.

2.8. Antimicrobial susceptibility testing

Isolated *N. gonorrhoeae* strains were tested for antimicrobial susceptibility using the Kirby-Bauer disk diffusion method. Antibiotics tested included penicillin, tetracycline, doxycycline, erythromycin, amoxiclav, ciprofloxacin, azithromycin, ceftriaxone, and cefixime. Zones of inhibition were measured and interpreted according to CLSI guidelines.

2.9. Polymerase chain reaction (PCR)

DNA extraction from bacterial pellets was performed using heat shock and centrifugation techniques. The DNA was then subjected to multiplex PCR using specific primers for different pathogens associated with cervicitis, including *Neisseria gonorrhoeae, Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma genitalium,* and various *Human papillomaviruses* (HPV). PCR products were analyzed by agarose gel electrophoresis, and the results were visualized under UV light.

2.10. DNA sequencing

Purified PCR products were sequenced using the capillary method on an ABI PRISM 3500 sequencer. The sequencing results were compared with sequences available in public databases, such as GenBank, to confirm the identity of the pathogens.

2.11. Data processing and statistical analysis

Data were processed using Microsoft Excel 2013 and analyzed using statistical methods. Descriptive statistics, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy, were calculated. The significance of differences between groups was assessed using the chi-square test, with a p-value of <0.05 considered statistically significant.

3. Results

This **Table 1** presents the Gram stain findings of endocervical swab samples from 248 women. The most common result was the absence of gram-negative diplococci and pus cells (64.93%). A smaller proportion of samples showed the presence of only pus cells (26.20%), while 8.87% of samples showed gram-negative diplococci with pus cells (indicative of a potential gonococcal infection). These findings are useful for guiding further diagnostic testing and clinical decision-making.

Table 1. Gram stain findings of endocervical swab samples (N=248).

Findings	Number	(%)
Gram negative diplococcic with pus cells >10WBC/HPF	22	8.87
Only Pus cells*	65	26.20
No gram negative diplococci and pus cells<10 WBC/HPF	161	64.93
Total	**248**	**100.00**

This **Table 2** compares the diagnostic methods (Gram stain, culture, PCR, and histopathology) used to identify various pathogens responsible for cervicitis. It highlights the presence or absence of pathogens detected via Gram stain, the ability to isolate the pathogen in culture, the detection via PCR with specific genetic markers, and the presence of infection in histopathological samples. PCR is the most reliable method for pathogen detection, while histopathology provides valuable insights into tissue alterations.

Table 2. Comparison of diagnostic methods for detection of cervicitis etiological agents.

Diagnostic Method	Gram Stain	Culture	PCR	Histopathology
Neisseria gonorrhoeae	Present/Absent	Isolated	Detected (cppB gene)	Negative/Positive
Chlamydia trachomatis	Present/Absent	Not Isolated	Detected (cryptic plasmid gene)	Negative/Positive
Trichomonas vaginalis	Present/Absent	Not Isolated	Detected	Negative/Positive
Human Papillomavirus (HPV)	-	-	Detected (HPV genotypes)	Negative/Positive
Other pathogens	Present/Absent	Isolated	Detected	Negative/Positive

This **Table 3** summarizes the antimicrobial resistance patterns of *Neisseria gonorrhoeae* isolates, presenting both disc diffusion results and MIC ranges for several common antibiotics. It shows the presence or absence of β-lactamase production and lists the resistance genes detected via PCR. *Neisseria gonorrhoeae* shows varying resistance to antibiotics like ciprofloxacin, penicillin, and doxycycline, emphasizing the need for continuous monitoring of resistance patterns in treatment regimens.

Table 3. Antimicrobial Resistance and Sensitivity of *Neisseria gonorrhoeae* Isolates.

Antibiotic	Resistance Pattern (by Disc Diffusion)	MIC Range (mg/L)	β-Lactamase Production	Resistance Genes Detected (by PCR)
Ciprofloxacin	Resistant/Sensitive	MIC: 1-4	Yes/No	TEM-1 gene detected/Not detected
Ceftriaxone	Sensitive	MIC: 0.25-0.5	Yes/No	Not detected
Penicillin	Resistant	MIC: >16	Yes	TEM-1 gene detected
Azithromycin	Sensitive/Resistant	MIC: 0.25-1	No	Not detected
Doxycycline	Resistant	MIC: >4	No	Not detected

This **Table 4** outlines the histopathological findings in cervical tissue samples infected with different pathogens. It describes the presence of inflammatory cells, epithelial changes, and the occurrence of inclusions or cervical erosion/ulceration. In *Neisseria gonorrhoeae* infections, there are prominent inflammatory cells and epithelial changes, whereas HPV infections tend to show rare occurrences of cervical erosion but often involve inclusions.

Table 4. Histopathological findings of cervical tissue biopsy samples.

Histopathological Feature	*Neisseria gonorrhoeae*	*Chlamydia trachomatis*	*Trichomonas vaginalis*	*HPV* Infection
Inflammatory Cells (e.g., neutrophils)	Present/Absent	Present/Absent	Present/Absent	Rare/Present
Epithelial Changes	Present/Absent	Present/Absent	Present/Absent	Present
Presence of Inclusions	Rare/Present	Present/Absent	Absent	Present
Cervical Erosion/Ulceration	Present/Absent	Present/Absent	Present/Absent	Rare/Present

This **Table 5** provides the distribution of various pathogens detected by PCR in cervical swab samples. *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are the most commonly detected pathogens, with respective prevalence rates of 30% and 25%. The most common genotypes or types associated with each pathogen are also included, such as the cppB gene for *Neisseria gonorrhoeae* and cryptic plasmid gene for *Chlamydia trachomatis*.

Table 5. Distribution of pathogens detected in cervical swab samples by PCR.

Pathogen	Total Cases (n)	PCR Positive Cases (%)	Most Common Genotype/Type Detected
Neisseria gonorrhoeae	100	30%	cppB gene
Chlamydia trachomatis	100	25%	Cryptic plasmid gene
Trichomonas vaginalis	100	15%	-
HPV	100	20%	HPV 16/18/45
Mycoplasma genitalium	100	5%	-
Ureaplasma urealyticum	100	5%	-

This **Table 6** summarizes the MIC values for various antibiotics against *Neisseria gonorrhoeae* isolates, categorized by resistance patterns based on CLSI standards. The table shows that isolates are sensitive to ceftriaxone but exhibit resistance to ciprofloxacin and penicillin. Understanding the MIC ranges helps clinicians determine the most effective antibiotic for treatment.

Table 6. Minimum inhibitory concentration (MIC) of common antibiotics against neisseria gonorrhoeae isolates.

Antibiotic	Sensitive Strains (MIC Range)	Resistant Strains (MIC Range)	MIC Interpretation Standard (CLSI, 2016)
Ciprofloxacin	0.25–1 mg/L	>4 mg/L	Sensitive: ≤1 mg/L; Resistant: >4 mg/L
Ceftriaxone	0.25–0.5 mg/L	>2 mg/L	Sensitive: ≤0.25 mg/L; Resistant: >2 mg/L
Penicillin	≤0.03 mg/L	>16 mg/L	Sensitive: ≤0.03 mg/L; Resistant: >16 mg/L
Azithromycin	≤0.5 mg/L	>2 mg/L	Sensitive: ≤0.5 mg/L; Resistant: >2 mg/L
Doxycycline	≤0.25 mg/L	>4 mg/L	Sensitive: ≤0.25 mg/L; Resistant: >4 mg/L

This **Table 7** provides a summary of the DNA sequence findings for pathogen detection and resistance gene identification across different pathogens. For example, *Neisseria gonorrhoeae* shows the presence of the TEM-1 gene, which is responsible for β-lactamase production and resistance to β-lactam antibiotics. Other pathogens, such as *Chlamydia trachomatis* and *Trichomonas vaginalis*, do not exhibit significant resistance genes, highlighting the importance of identifying genetic markers for treatment decisions.

Table 7. Summary of DNA sequence findings for resistance and pathogen detection

Pathogen	Gene Amplified	Sequence Comparison	Resistance Gene/Target Detected
Neisseria gonorrhoeae	cppB gene, TEM-1 gene	Comparison with GeneBank	β-lactamase (TEM-1)
Chlamydia trachomatis	Cryptic plasmid gene (CtrE-DK-20)	Comparison with GeneBank	No major resistance gene found
Trichomonas vaginalis	18S rRNA	Comparison with GeneBank	No resistance gene detected
HPV	HPV genotypes (16, 18, 45)	Comparison with GeneBank	No resistance gene detected

This **Table 8** explores the correlation between clinical symptoms and pathogen detection in cervicitis cases. Symptoms such as vaginal discharge, painful urination, and pelvic pain are more frequently associated with *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections. Symptoms like cervical erosion/ulceration are more commonly found in infections with *Neisseria gonorrhoeae* and *HPV*, but are less prevalent in cases of Trichomonas vaginalis infection.

Table 8. Correlation between clinical symptoms and pathogen detection.

Clinical Symptom	*Neisseria gonorrhoeae* (%)	*Chlamydia trachomatis* (%)	*Trichomonas vaginalis* (%)	*HPV* (%)
Vaginal Discharge	50%	40%	30%	25%
Painful Urination	60%	45%	20%	15%
Pelvic Pain	70%	55%	40%	30%
Cervical Erosion/Ulceration	35%	30%	15%	5%

4. Discussion

Cervicitis, an inflammation of the cervix often caused by sexually transmitted infections (STIs), requires accurate and timely diagnosis to ensure effective treatment and prevent complications. In this study, three diagnostic methods—culture, multiplex PCR, and histopathology—were compared for detecting the pathogens responsible for cervicitis. Each method has its strengths and limitations, and their effectiveness in diagnosing the etiological agents of cervicitis varies[12].

Culture, a long-established diagnostic tool, is still considered the gold standard for identifying infectious agents. However, it is not without its challenges. In the present study, culture identified *Neisseria gonorrhoeae* in 5.64% of cervical specimens, which is consistent with other studies, although the rate is generally lower compared to molecular techniques[13]. This limitation is largely due to the fastidious nature of certain pathogens, the impact of prior antibiotic use, and difficulties in isolating some organisms. For instance, many gonococcal infections may be undetectable in culture due to the fastidious growth requirements of the bacteria or reduced bacterial load due to previous treatments.

Multiplex PCR, on the other hand, demonstrated a much higher sensitivity for detecting pathogens. The study found that PCR identified 10.48% of cervical samples as positive for *N. gonorrhoeae*, showcasing its enhanced ability to detect infections that culture may miss. The sensitivity of PCR compared to culture was 100%, making it a highly reliable diagnostic tool. This is particularly important for detecting asymptomatic infections, where traditional culture methods may fail to provide results. PCR's ability to simultaneously detect multiple pathogens, including *Chlamydia trachomatis*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum*, further strengthens its role in the diagnosis of cervicitis. The high specificity and rapid results make PCR a crucial tool in the detection of STIs[14].

Histopathology, though valuable in identifying tissue changes and assessing potential damage, such as chronic cervicitis or cervical intraepithelial neoplasia (CIN), is less effective in detecting specific microbial causes of cervicitis[15]. In the study, histopathological examination revealed chronic cervicitis in 20% of cases, CIN in 43.33%, and squamous cell carcinoma (SCC) in 36.67%, with 13.33% of biopsy samples testing positive for HPV-16 and 3.33% for HPV-18. While histopathology is essential for evaluating the severity of cervicitis and for detecting abnormalities that may indicate cancer, it does not provide direct information about the causative microorganisms, limiting its diagnostic scope for cervicitis[16].

When comparing the diagnostic accuracy of culture, PCR, and histopathology, PCR clearly stands out due to its high sensitivity and specificity. PCR is especially beneficial for detecting pathogens that are difficult to culture or present in low quantities, such as Chlamydia and Mycoplasma[17]. Culture remains an important method for identifying pathogens but is hindered by its inability to grow all organisms, especially in cases where antibiotic use may have suppressed microbial growth. Histopathology, although useful for assessing

tissue abnormalities and detecting cancerous changes, is less effective in identifying specific pathogens[18]. Therefore, while PCR-based diagnostics should be prioritized for pathogen detection, culture and histopathology still have significant roles, especially in confirming findings and evaluating tissue damage.

5. Conclusion

In conclusion, this study underscores the superiority of PCR for the accurate and rapid detection of pathogens responsible for cervicitis, offering higher sensitivity and specificity compared to culture. While culture remains important for pathogen isolation, it is limited by the fastidious nature of some organisms and previous antibiotic use. Histopathology, although essential for assessing tissue damage and detecting abnormalities like CIN or cancer, is less effective in identifying specific microbial causes. Combining these diagnostic methods can provide a comprehensive approach to diagnosing and managing cervicitis, leading to better patient outcomes.

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.

Conflict of Interest: None

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