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Comparison of Culture and PCR Methods in the Detection of *Neisseria gonorrhoeae* in Patients Suspected of Gonorrhea

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ABSTRACT

Background: This study compares the effectiveness of culture and PCR methods in detecting *Neisseria gonorrhoeae* , a Gram-negative bacterium causing gonorrhea, which can lead to serious health complications, especially in women, and highlights the advantages of nucleic acid amplification tests over traditional culture-based diagnosis.

Methods: This study involved 70 individuals suspected of gonorrhea who referred to Baqiyatallah Hospital in Tehran between September and February 2022, with ethical approval and informed consent obtained from all participants. Two swab samples were collected from each individual—one for culture and one for molecular testing—and stored appropriately for microbiological and genomic analysis.

Results: This study examined 70 women suspected of having gonorrhea, with a mean age of 32.32 ± 8.58 years, and found a prevalence of 30% by vaginal culture and 28.57% by PCR testing. Using PCR as the gold standard, the sensitivity and specificity of culture for diagnosing gonorrhea were determined to be 45% and 76%, respectively.

Conclusion: Of course, the differences could be due to the size of the studied populations, but the important point is the high accuracy and sensitivity of the PCR test in identifying *Neisseria gonorrhoeae*, which has been mentioned in most studies.

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Introduction

Neisseria gonorrhoeae is a gram-negative diplococcus and an obligate human pathogen that belongs to the genus Neisseria within the family *Neisseriaceae* (1). This bacterium is the causative agent of gonorrhea, a prevalent sexually transmitted infection (STI) worldwide (2). In 2020, approximately 82.4 million cases of gonorrhea were reported globally among adults (3). The disease predominantly affects individuals aged 15–49 years, with an estimated incidence rate of 20 cases per 1,000 females and 26 cases per 1,000 males each year (4).

Although comprehensive data on the incidence of *N. gonorrhoeae* infections in Iran are limited, a 2008 World Health Organization (WHO) report indicated that approximately 3.1 million new cases were reported from the Eastern Mediterranean region, including Iran (5). *N. gonorrhoeae* primarily infects mucosal surfaces such as the urethra, endocervix, pharynx, and rectum, leading to localized or disseminated infections (6). If left untreated, gonorrhea can result in severe complications, particularly in women, including pelvic inflammatory disease (PID), ectopic pregnancy, infertility, and increased susceptibility to human immunodeficiency virus (HIV) infection (7, 8).

Effective management and control of gonorrhea rely on accessible antimicrobial therapy, preventive strategies, rapid diagnostic methods, and robust epidemiological surveillance (7, 8). However, over the past 70 to 80 years, treatment options have been significantly reduced due to the emergence and global spread of antimicrobial resistance (AMR) in *N. gonorrhoeae*. Resistance has developed against all previously recommended first-line antibiotics, including sulfonamides, penicillins, tetracyclines, first-generation cephalosporins, macrolides, and fluoroquinolones (9-11).

Various diagnostic methods have been employed for the detection of *N. gonorrhoeae* infection (12). Historically, culture was considered the "gold standard" for diagnosing gonorrhea, particularly in resource-limited settings, due to its relatively low cost, high specificity, and ability to provide isolates for antimicrobial susceptibility testing (13). However, this method has several limitations, including the fastidious growth requirements of *N. gonorrhoeae*, which can lead to low recovery rates if proper transport and culture conditions are not maintained (13). In recent years, nucleic acid amplification tests (NAATs) have largely replaced traditional culture methods as the preferred diagnostic approach. Such assays provide superior specificity and sensitivity, along with the ability to detect *N. gonorrhoeae* directly from clinical samples with no need for viable organisms. Thus, NAATs are now widely regarded as the current gold standard for the diagnosis of gonorrhea (14).
Among the nucleic acid amplification tests (NAATs), several molecular methods are commonly employed for the detection of *Neisseria gonorrhoeae*, including ligase chain reaction (LCR), strand displacement amplification (SDA), nucleic acid sequence-based amplification (NASBA), and polymerase chain reaction (PCR). PCR assays often target specific functional genes, such as the opa gene, which is conserved among *N. gonorrhoeae* strains (15) .

Several studies have directly compared the performance of culture and PCR methods in diagnosing *N. gonorrhoeae* infection in individuals suspected of having gonorrhea. Numerous PCR-based assays have been developed not only for pathogen identification but also to detect molecular markers associated with antimicrobial resistance, enabling prediction of resistance profiles (15-16). Compared to traditional culture methods, NAATs offer significant advantages, including rapid detection, minimal sample manipulation, and a reduced risk of contamination due to the absence of live organism handling (17).

Overall, PCR-based methods consistently demonstrate higher sensitivity than culture.

Reported sensitivities for PCR range from 94.57% to 100%, whereas culture sensitivities vary between 86.2% and 96% (18). In addition, PCR assays generally exhibit superior specificity and overall diagnostic accuracy (19). Notably, the TaqMan quantitative PCR (qPCR) assay has shown particularly promising results, outperforming both conventional PCR and culture methods in some studies (20).

Despite these advantages, culture remains an essential method for antimicrobial susceptibility testing, which is crucial for guiding appropriate treatment, especially in the context of increasing antimicrobial resistance (18). Therefore, while PCR-based methods typically surpass culture in terms of sensitivity and speed, a combined approach—using both molecular and culture techniques—may be optimal in certain clinical and public health settings to ensure comprehensive diagnosis and resistance monitoring (21).

This study aims to compare the sensitivity and specificity of culture and PCR methods in detecting *Neisseria gonorrhoeae* among patients suspected of gonorrhea, in order to evaluate their diagnostic accuracy and clinical utility.

Materials and Methods

This cross-sectional study was conducted on 70 clinical samples collected from individuals suspected of gonorrhea who referred to Baqiyatallah Hospital in Tehran, Iran, between December 2020 and May 2021.

This study included individuals who presented with clinical signs and symptoms suggestive of gonorrhea, such as urethral or vaginal discharge, dysuria, or genital irritation, and were referred to the clinic for diagnostic evaluation. All suspected cases were further assessed by a specialist physician, and only those with a confirmed preliminary diagnosis based on both clinical findings and initial laboratory results—such as Gram staining or rapid diagnostic tests—were enrolled in the study. This ensured that participants had a high likelihood of *Neisseria gonorrhoeae* infection and met the necessary criteria for further microbiological and molecular analysis. Patients were excluded from the study if they were diagnosed with other sexually transmitted infections based on laboratory confirmation, as the presence of co-infections could interfere with the accuracy of diagnostic test results. Additionally, individuals with incomplete sample collection or those who were unwilling or unable to fully cooperate during the study period—for example, due to loss to follow-up or refusal to provide required specimens—were also excluded to maintain the integrity and reliability of the data.

Sample Collection

Two separate swab samples were collected from each participant by an experienced nurse. For male participants, urethral discharge was sampled, while for female participants, vaginal discharge was collected. One swab was used for molecular testing and immediately placed into a sterile tube containing Phosphate Buffered Saline (PBS), then stored at -20 °C until PCR analysis and DNA extraction. The second swab was placed into a transport medium and rapidly transferred to the microbiology laboratory for culture-based detection of *Neisseria gonorrhoeae*.

Microbiological Analysis

Swabs were inoculated onto selective and differential culture media, containing blood agar, Eosin Methylene Blue (EMB) agar, and modified Thayer-Martin (MTM) agar supplemented with antibiotics to inhibit the commensal flora growth. The cultures underwent incubation at 37 °C in a CO_2-enriched atmosphere (5–10% CO_2) for up to 48 hours. Cultures were assessed after 24 and 48 hours for the existence of typical *N. gonorrhoeae* colonies, which were further identified using standard biochemical tests.

Molecular detection

DNA extraction

Genomic DNA was extracted from clinical swab samples collected from urethral or vaginal secretions using the Synagen DNA extraction kit (Catalog No: DN 8115C), according to the manufacturer's instructions. Extracted DNA was quantified using a NanoDrop spectrophotometer and kept at –20 °C until further usage. The purified DNA was then used as a template for real-time polymerase chain reaction (RT-PCR) amplification.

Primer Design

Primers targeting the cppB gene of *Neisseria gonorrhoeae* were designed using conserved regions of the gene sequence retrieved from GenBank. The cppB gene is found both on the bacterial chromosome and on the cryptic 4.2 kb plasmid, making it a reliable target for molecular detection. The forward (F) and reverse (R) primers amplified a 390-bp fragment during PCR. Primer sequences were as follows:

Real-Time PCR

Real-time PCR was performed using the Cepheid Smart Cycler II system (16-well format) with Maxima SYBR Green/ROX qPCR Master Mix (2×) (Catalog No: K0221, Thermo Scientific). Each reaction mixture (total volume: 10 µl) contained 0.5 µl of each primer (10 µM), 2 µl of DNA template (approximately 50 ng/µl), 5 µl of 2× SYBR Green master mix, and nuclease-free water to adjust the final volume. Positive and negative controls were included in each run to ensure assay validity and prevent cross-contamination.

The thermal cycling conditions were as follows: Initial denaturation at 50 °C for 2 minutes (UDG activation), Followed by enzyme activation at 95 °C for 2 minutes, Then 25 cycles of denaturation at 95 °C for 15 seconds, Annealing and extension at 60 °C for 30 seconds. After amplification, a melt curve analysis was performed by increasing the temperature from 60 °C to 95 °C in increments of 0.3 °C to confirm the specificity of the amplified product.

Electrophoretic Analysis

To verify the size of the amplified PCR product, 5 µl of each reaction was subjected to electrophoresis on a 1.5% agarose gel prepared in 1× TAE buffer. The gel was stained with ethidium bromide and visualized under a UV transilluminator. A DNA ladder (100 bp) was used to determine the size of the amplified fragment. A specific band of 390 bp confirmed the presence of *N. gonorrhoeae* DNA in the sample.

Statistical Analysis

To describe the quantitative variables, the mean and standard deviation were reported, and for the qualitative variables, the number (percentage) was reported. Comparison of the frequency of different variables according to PCR results was done using Chi-Square test. t-test was used to compare the mean age according to PCR results. True positive (TP), false positive (FP), true negative (TN) and false negative (FN) values were calculated based on the study data and using PCR results as the gold standard. Then the accuracy indices including sensitivity, specificity, positive predictive value, negative predictive value and LR+ and LR- with a 95% confidence interval were calculated for *Neisseria gonorrhoea* culture. Data were analyzed using Stata software (version 14). For all statistical tests, $p<0.05$ was considered statistically significant.

Results

A total of 70 women suspected of gonorrhea were assessed. The average age of the participants was 32.32 ± 8.58 years, ranging from 16 to 59 years. Of these, 35.71% (n=25) were under 30 years of age, while 64.29% (n=45) were 30 years or older. A history of sexually transmitted infections (STIs) was reported by 32.86% (n=23) of the participants. Additionally, 54.29% (n=38) reported having undergone an annual Pap smear test. The most common clinical symptoms included vaginal discharge, which was present in 31.43% (n=22) of cases, and dysuria, reported by 21.43% (n=15) of the women (Table 2).

Based on vaginal culture results, the prevalence of Neisseria gonorrhoeae infection was 30% (n=21). Similarly, molecular detection using PCR identified gonorrhea in 28.57% (n=20) of the participants.

According to the PCR results, the prevalence of gonorrhea was higher among individuals under 30 years of age (40%) compared to those aged 30 years or older (22.22%). Although a decreasing trend in prevalence was observed with increasing age, this difference was not significant ($p = 0.11$). The average age of people with gonorrhea was significantly lower than compared to that of non-infected people (28.15 years vs. 34 years) (P=0.009).

The vaginal discharge prevalence was significantly higher among women with gonorrhea compared to uninfected cases, with rates of respectively 70% and 16% ($p < 0.001$). Similarly, a history of sexually transmitted diseases (STDs) was notably more common in women diagnosed with gonorrhea (65%) than in those without infection (20%), indicating a significant difference ($p < 0.001$).

Regarding preventive health practices, 65% of women with confirmed gonorrhea reported undergoing annual Pap smear tests, compared to 50% of non-infected women. Additionally, dysuria was significantly more prevalent in the gonorrhea group, with a highly significant p -value (<0.001) (Table 3).

Using PCR as the reference standard, the sensitivity and specificity of vaginal culture in diagnosing Neisseria gonorrhoeae infection were calculated at 45% and 76%, respectively. The negative predictive value (NPV) and positive predictive value (PPV) were respectively 77.55% and 42.86% (Table 4).

Among the 20 PCR-positive cases, 11 (55%) were misclassified as culture-negative (false negatives). These individuals ranged in age from 22 to 33 years. A history of STDs was reported by the majority (n=7, 63.64%). Vaginal discharge was present in 6 (54.55%) of these cases, and dysuria was reported by 4 (36.36%). Additionally, 63.64% (n=7) of the false-negative individuals had undergone an annual Pap smear test.

Table 1. primers sequences.

Primer	Sequence
cppB (Forward)	5’- GCTACGCATACCCGCGTTGC -3’
cppB (Reverse)	3’- CGAAGACCTTCGAGCAGACA -5’

Table 2. Frequency of different variables in the investigated women.

Various variables	**Positive (%)**	**Negative (%)**
Vaginal Discharge	31.43	68.57
History of STIs	32.86	67.14
Annual Pap Smear	54.29	45.71
Pain During Urination	21.43	78.57

Table 3. Comparison of the frequency of *Neisseria gonorrhoea* based on PCR according to different variables in the examined women.

Different variables		Positive N`:20	Negative	P-value*
		N (%)	N (%)	
Vaginal discharge	Positive	14 (70)	8 (16)	0.001<
	Negative	6(30)	42(84)	
History of sexually transmitted diseases	Positive	13(65)	10(20)	0.001<
	Negative	7(35)	40(80)	
Pap smear	Positive	13(65)	25(50)	0.25
	Negative	7(35)	25(50)	
Pain during urination	Positive	7(35)	8 (16)	0.001<
	Negative	13(65)	42(84)	

* Using the Chi2 test

Table 4. Sensitivity and specificity and other accuracy indicators for *Neisseria gonorrhoeae* culture results in the diagnosis of gonorrhea in the examined women.

Accuracy indicators	**estimate**	**95% confidence interval**
Sensitive (%)	45	23.68-0.6.47
Specify (%)	76	61.86-83.94
Positive predictive value (PPV) (%)	42.86	21.65-82.98
Negative Predictive Value (NPV) (%)	77.55	63.88-38.23
LR^+	1.87	-
LR^-	0.72	-

Discussion

Gonorrhea, caused by *Neisseria gonorrhoeae*, is one of the most prevalent STDs worldwide (22). The number of new cases has been rising in many countries, with the World Health Organization estimating over 85 million new gonococcal infections globally in 2016 among individuals aged 15–49 years (23). Diagnosis of gonorrhea can be established by a combination of physical examination, clinical history, and supportive laboratory tests, including Gram staining, culture, and non-culture methods such as PCR and nucleic acid amplification tests (NAATs) (24). Direct

microscopic examination of urethral or cervical secretions, followed by bacterial culture, is still a commonly applied and valuable approach for detecting *Neisseria gonorrhoeae*. In the last twenty years, developments in biomolecular methods have broadened the array of tools for detecting and identifying microorganisms (24, 25).

In our research, which included 70 samples with an average age of 32.32 ± 8.58 years, the prevalence of gonorrhea was determined to be 30% through vaginal culture for *Neisseria gonorrhoeae* and 28.57% via PCR testing. In the study by Abdullahi et al. (2022), which included 379 samples, 6.3% of participants tested positive for gonorrhea using PCR (26). Waseem et al. (2021), in a study involving 60 samples with a mean age of 38 years, reported that 40% of patients were positive for *Neisseria gonorrhoeae* infection based on PCR results (27). Barbee et al. (2021), in a study of 89 vaginal swab samples, found that 3% of samples were positive for *Neisseria gonorrhoeae* (28). Furthermore, Enitan et al. (2022), in a study of 100 samples, reported a 12% prevalence of *Neisseria gonorrhoeae* based on culture results (29).

Gonorrhea can lead to serious complications and long-term sequelae, disproportionately affecting women. These include pelvic inflammatory disease, vaginal discharge, ectopic pregnancy, infertility, and an increased risk of HIV transmission (1, 4, 30). Therefore, recognizing symptoms early is crucial for prognosis.

In our study, the prevalence of vaginal discharge was significantly higher among women with gonorrhea (70%) compared to non-infected women (16%) ($p < 0.001$). Additionally, a history of sexually transmitted infections was significantly more common in women with gonorrhea than in those without ($p < 0.001$). Similarly, the prevalence of pain during urination was significantly higher in individuals with gonorrhea ($p < 0.001$).

In a study by Parsapour et al. (2022), conducted on 348 women with a mean age of 34.93 ± 7.57, 25.6% of participants reported dysuria, and 73.6% exhibited cervical secretions (31). Ayalew et al. (2022), in a study involving 325 samples where 50% tested positive for gonorrhea via culture, confirmed that symptoms such as vaginal and urethral discharge were commonly associated with infection (32). Dela et al. (2019), in a study of 950 samples (58% from women), reported that 28% of cases were proved as gonorrhea, with painful urination and urethral discharge identified as significantly associated and commonly observed factors (33).

Mabonga et al. also highlighted a significant association between *Neisseria gonorrhoeae* infection and other STDs, such as HIV (34). The average age of individuals diagnosed with gonorrhea was significantly lower than that of non-infected individuals ($p = 0.009$), which aligns with the results reported by Chang (32) and Abdullahi (26). The culture test possesses high specificity; however, a major limitation is the relatively long time required to obtain positive results (35).

PCR is a commonly used molecular method for amplifying *Neisseria gonorrhoeae* DNA under controlled laboratory conditions (36). This approach requires several key components: a double-stranded DNA template containing the target sequence, a DNA polymerase enzyme, nucleotide triphosphates, and a pair of specific primers. The fundamental principle of PCR is the selective amplification of specific genetic material present in all living organisms (37). Compared to culture, PCR provides higher speed, accuracy, sensitivity, and efficiency, enabling the detection of even small quantities of bacterial DNA (38).

Here, using PCR findings as the gold standard and considering culture results (positive or negative) for *Neisseria gonorrhoeae*, the specificity and sensitivity of culture in diagnosing gonorrhea were found to be 45% and 76%, respectively. Farooqi and Amini (2020), in a study

involving 60 specimens, identified PCR as a reliable and accurate method for detecting *Neisseria gonorrhoeae* (39). Similarly, Morris et al. (2021), in a large-scale study involving 1,585 samples from individuals aged 14 to 80 years, reported a sensitivity of 97.4% and a specificity of 99.4% for the PCR method in identifying *Neisseria gonorrhoeae* (40).

In the study by Boiko et al. using the culture method to identify *Neisseria gonorrhoeae*, a specificity of 100% was reported, while sensitivity was 82.6% to 47.8% (41). However, Xiu et al. (2023), in a study involving 1,488 samples, reported a sensitivity and specificity of 96.44% and 95.15%, respectively, for the culture method in detecting Neisseria gonorrhoeae (42).

These differences may be attributed to variations in sample size and population characteristics across studies. However, an important and consistent finding across most studies is the high accuracy and sensitivity of the PCR test in identifying *Neisseria gonorrhoeae*, highlighting its value as a reliable diagnostic tool.

Conclusion

Gonorrhea, caused by *Neisseria gonorrhoeae*, represents a significant public health concern, particularly due to its increasing incidence and associated complications such as pelvic inflammatory disease, infertility, and a heightened risk of HIV. In this study involving 70 women (mean age: 32.32 ± 8.58), the prevalence of the infection was found to be 30% through culture and 28.57% via PCR. Symptoms such as vaginal discharge, dysuria, and a history of STIs were notably more prevalent among those infected. Although culture methods exhibit high specificity, their low sensitivity and lengthy processing times limit their effectiveness. In contrast, PCR showed superior performance, with sensitivity and specificity exceeding 95%, as supported by various studies. These results advocate for the adoption of molecular techniques like PCR for precise and prompt diagnosis, highlighting the need for early detection and enhanced screening strategies to mitigate the burden of the disease.

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Ethics approval and consent to participate

Ethical approval for this study was obtained from the Ethics Committee of the University of Tehran, ensuring compliance with ethical standards in research conduct and patient confidentiality (Code no: IR.IAU.CHALUS.REC.1402.001).

Conflict of interest

The authors declare no conflict of interest.

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