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Original Research Article

Prevalence of *Neisseria gonorrhoeae* Infection among Reproductive-Age Women Attending a Tertiary Care Hospital in Uttar Pradesh: A Cross-Sectional Study Using Chip-Based Real-Time PCR

Pratibha Rathaur[1], Anjali Agarwal[2]

[1]Junior Resident, Department of Microbiology, Hind Institute of Medical Sciences, Safedabad, Barabanki, Uttar Pradesh, India

[2]Professor and Head, Department of Microbiology, Hind Institute of Medical Sciences, Safedabad, Barabanki, Uttar Pradesh, India

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Corresponding author: Dr. Anjali Agarwal

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Abstract

Background: *Neisseria gonorrhoeae* remains one of the most common bacterial sexually transmitted pathogens worldwide and is a major cause of cervicitis, pelvic inflammatory disease, infertility, and adverse reproductive outcomes in women. Early diagnosis is often challenging because a substantial proportion of infected women remain asymptomatic, while conventional diagnostic methods demonstrate limited sensitivity. Nucleic acid amplification tests (NAATs) provide rapid and highly sensitive detection and may improve case identification in resource-limited settings. This study aimed to determine the prevalence of *Neisseria gonorrhoeae* infection among reproductive-age women using chip-based real-time PCR at a tertiary care hospital in Uttar Pradesh.

Methods: A hospital-based cross-sectional study was conducted in the Department of Microbiology, Hind Institute of Medical Sciences, Barabanki, from 01 January 2025 to 31 January 2026. A total of 120 women aged 18–49 years attending the Obstetrics and Gynaecology outpatient department were enrolled after obtaining informed consent. Vaginal swabs were processed by wet mount microscopy, Gram staining, and culture, while endocervical swabs were analysed using the Truenat chip-based real-time PCR assay for molecular detection of *Neisseria gonorrhoeae.*

Results: Molecular testing detected *Neisseria gonorrhoeae* in 10 of 120 participants, giving an overall prevalence of 8.3%**.** Conventional culture identified only 2 (1.7%) positive cases, demonstrating markedly lower detection than PCR. The highest positivity was observed among women aged 26–30 years, with age showing a statistically significant association with infection (p = 0.035). Most infected women were married, belonged to rural areas, and were from lower socioeconomic groups; however, these associations were not statistically significant.

Conclusion: The study demonstrated that chip-based real-time PCR detected substantially more *Neisseria gonorrhoeae* infections than conventional diagnostic methods. Molecular screening can facilitate early diagnosis, prompt treatment, and improved control of gonococcal infection among reproductive-age women, particularly in settings where asymptomatic infection is common.

Keywords: *Neisseria gonorrhoeae*; *Gonorrhoea*; Chip Based Real time PCR, Sexually Transmitted Infections.

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Introduction

Sexually transmitted infections (STIs) continue to represent a major global public health challenge, with substantial consequences for reproductive, maternal, and neonatal health. [1] Among the bacterial STIs, *Neisseria gonorrhoeae*, the causative agent of gonorrhoea, remains one of the most prevalent infections worldwide. According to the World Health Organization (WHO), approximately 82 million new gonorrhoea cases occur annually among adults aged 15–49 years, with a disproportionately high burden in low- and middle-income countries. [1,2] Untreated gonococcal infection may result in cervicitis, pelvic inflammatory disease (PID), ectopic pregnancy, tubal infertility, chronic pelvic pain, and adverse pregnancy outcomes. [3] Furthermore, infected mothers can transmit the organism to newborns during delivery, leading to ophthalmia neonatorum and other neonatal complications. [3] A major challenge in controlling gonorrhoea is that infection in women is frequently asymptomatic or presents with nonspecific clinical manifestations.

Consequently, many infected individuals remain undiagnosed and continue to transmit the organism within the community. [4] Syndromic management alone is often inadequate because clinical symptoms overlap with those of other reproductive tract infections, making laboratory confirmation essential for accurate diagnosis and appropriate treatment. [2,4]

The diagnosis of *N. gonorrhoeae* has traditionally relied on direct microscopy and culture. Although Gram stain remains useful in selected clinical settings, particularly in symptomatic males, its sensitivity in women is considerably lower because of the presence of mixed vaginal flora and lower bacterial load. [5] Culture, while regarded as the conventional reference method and essential for antimicrobial susceptibility testing, is technically demanding and requires viable organisms, selective media, prompt transportation, and incubation under strict environmental conditions. These limitations frequently result in reduced diagnostic sensitivity, especially in resource-constrained healthcare settings. [6,7]

Nucleic acid amplification tests (NAATs) have transformed the laboratory diagnosis of gonorrhoea by providing rapid, highly sensitive, and highly specific detection of pathogen-specific nucleic acid directly from clinical specimens.8 Unlike conventional culture, NAATs can detect infection even in patients with a low bacterial burden or those who are asymptomatic. [6,9] In recent years, chip-based real-time PCR platforms such as the Truenat system have emerged as practical molecular diagnostic tools because they combine high analytical performance with shorter turnaround time, simplified workflow, and suitability for decentralized laboratories. These characteristics make them particularly valuable in regions where access to sophisticated molecular laboratories is limited. [10,11]

India continues to experience a considerable burden of sexually transmitted infections; however, reliable epidemiological data on gonorrhoea remain limited because of underdiagnosis, social stigma, limited screening programmes, and dependence on less sensitive conventional diagnostic techniques. [12] Published studies have reported considerable variation in gonorrhoea prevalence across different geographical regions and patient populations, highlighting the need for region-specific surveillance using sensitive molecular methods. [4,13] Data from Eastern Uttar Pradesh are particularly scarce, despite the large population and substantial attendance at tertiary-care gynaecology clinics.

Accurate estimation of the prevalence of *Neisseria gonorrhoeae* is essential for guiding screening strategies, strengthening STI surveillance, facilitating early treatment, and reducing long-term reproductive complications. The adoption of molecular diagnostic techniques may also contribute to improved case detection among asymptomatic women who would otherwise remain undiagnosed. [14]

Therefore, the present study was undertaken to determine the prevalence of *Neisseria gonorrhoeae* infection among reproductive-age women attending the Obstetrics and Gynaecology Outpatient Department of a tertiary care hospital in Eastern Uttar Pradesh using chip-based real-time polymerase chain reaction (PCR). The study also compared molecular detection with conventional microbiological methods to evaluate the added diagnostic value of NAAT in routine clinical practice.

Materials and Methods

Study Design and Setting: A hospital-based cross-sectional analytical study was conducted in the Department of Microbiology (Bacteriology and Molecular Laboratory), Hind Institute of Medical Sciences (HIMS), Safedabad, Barabanki, Uttar Pradesh, India, over a period of one year from 01January 2025 to 31January 2026. The study aimed to determine the prevalence of *Neisseria gonorrhoeae* infection among reproductive-age women using chip-based real-time polymerase chain reaction (PCR) and to compare its diagnostic performance with conventional microbiological methods.

Ethical Approval: The study protocol was approved by the Institutional Ethics Committee (IEC) of Hind Institute of Medical Sciences, Barabanki. Written informed consent was obtained from all participants before enrolment. Confidentiality of patient information was maintained throughout the study

Study Population: Women aged 18–49 years attending the Obstetrics and Gynaecology Outpatient Department (OPD) during the study period were screened for eligibility. A total of 120 consecutive participants fulfilling the inclusion criteria were enrolled

Inclusion Criteria

- Women aged 18–49 years.
- Attending the Obstetrics and Gynaecology OPD during the study period.
- Willing to provide written informed consent.

Exclusion Criteria

- Refusal to participate.
- Menstruation at the time of specimen collection.
- Antibiotic therapy within the preceding two weeks.
- Unmarried women.

Sample Collection: Following aseptic precautions, two high vaginal swabs and one endocervical swab were collected by trained gynaecologists using sterile Dacron or nylon flocked swabs. [15] The first vaginal swab was used for wet mount microscopy. The second vaginal swab was processed for Gram staining and culture. The endocervical swab was collected for molecular detection of *Neisseria gonorrhoeae* using the Truenat chip-based real-time PCR platform.

Transport: All specimens were transported immediately to the Microbiology Laboratory. Whenever immediate processing was not possible, specimens were stored at 2–8°C and processed within 24 hours.

Direct Microscopy & Culture: Wet mount examination was performed using normal saline to detect any inflammatory cells or any Parasite. [16,17]

Gram-stained smears were examined under oil immersion (1000× magnification) for any pus cell, or clue cells, or yeast, bacterial morphology as intracellular Gram-negative diplococci suggestive of *Neisseria gonorrhoeae*. [5]

Culture was performed by inoculating vaginal specimens onto Blood Agar, Chocolate Agar, and Thayer–Martin Agar (Himedia).

Blood Agar and Chocolate Agar plates were incubated at 37°C in a 5–10% CO_2 atmosphere for 24–48 hours. Thayer–Martin Agar was used as the selective medium for isolation of *Neisseria gonorrhoeae*. Suspected colonies were identified using standard microbiological procedures. [6]

Molecular Detection by Chip-Based Real-Time PCR: Endocervical swabs were transferred into the MOLBIO Truenat Transport Medium immediately after collection. Samples were transported under refrigerated conditions (2–8°C) and processed according to the manufacturer's instructions. [11]

DNA extraction, amplification, and real-time detection were performed using the Truenat chip-based real-time PCR platform (Molbio Diagnostics Pvt. Ltd., Goa, India). The assay detects pathogen-specific DNA and provides automated interpretation of test results, thereby reducing operator-dependent variability and improving diagnostic accuracy. [10]

Statistical Analysis: Data were analysed using IBM SPSS Statistics version 22.0. Continuous variables were expressed as mean ± SD and categorical variables as frequencies (%). Associations were assessed using the Chi-square or Fisher's exact test. Statistical significance was set at $p<0.05$.

Results

A total of 120 reproductive-age women attending the Obstetrics and Gynaecology Outpatient Department were enrolled in the study. All participants fulfilled the eligibility criteria and were included in the final analysis. Among the 120 female participants, 10 (8.3%) were positive for *Neisseria gonorrhoeae*. The highest proportion of infected women was found in the 26–30 years age group (50.0%), followed by 41–45 years (30.0%) and 36–40 years (20.0%); the association with age was statistically significant (p=0.035). All infected women were housewives (100%), while 90% were married. Most infected women belonged to the lower socioeconomic group (70%), were illiterate (60%), and were from rural areas (70%). However, occupation (p=0.609), marital status (p=0.092), socioeconomic status (p=0.207), literacy status (p=0.753), and residence (p=0.404) showed no statistically significant association with *Neisseria gonorrhoeae* infection.

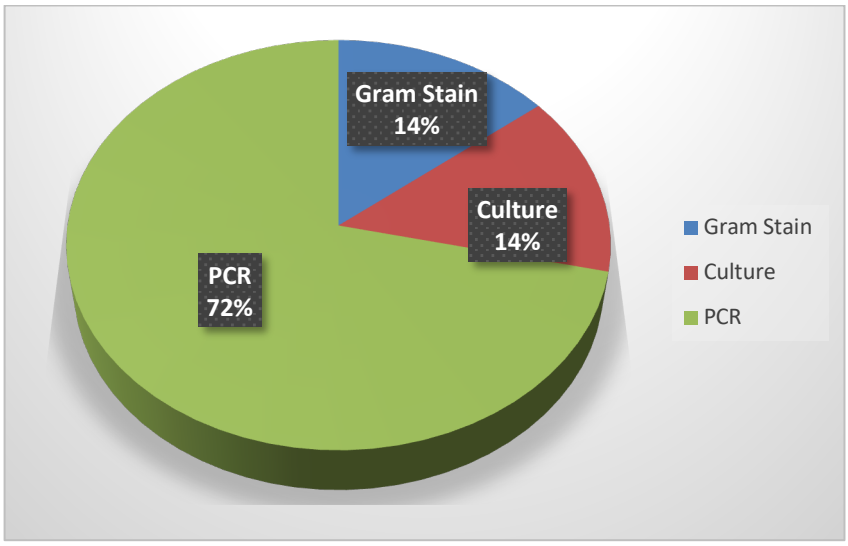

Figure 1: Showing Cases in Culture & rtPCR

Table 1: Demographic Variables of sexually transmitted infections according to patients with questionnaire in the present study.

Variable	Category	*Neisseria gonorrhoeae* Not Detected (n=110)	*Neisseria gonorrhoeae* Detected (n=10)	p-value
Age Group (Years)	20–25	11 (10.0%)	0 (0.0%)	**0.035**
	26–30	22 (20.0%)	5 (50.0%)	
	31–35	44 (40.0%)	0 (0.0%)	
	36–40	18 (16.4%)	2 (20.0%)	
	41–45	15 (13.6%)	3 (30.0%)	
Occupation	Housewife	100 (90.9%)	10 (100.0%)	**0.609**
	Student	1 (0.9%)	0 (0.0%)	
	Working	9 (8.2%)	0 (0.0%)	
Marital Status	Married	107 (97.3%)	9 (90.0%)	**0.092**
	Widowed	1 (0.9%)	1 (10.0%)	
	Divorced	2 (1.8%)	0 (0.0%)	
Socioeconomic Status	Lower	47 (42.7%)	7 (70.0%)	**0.207**
	Middle	51 (46.4%)	3 (30.0%)	
	Upper	12 (10.9%)	0 (0.0%)	
Literacy Status	Illiterate	44 (40.0%)	6 (60.0%)	**0.753**
	Primary	45 (40.9%)	3 (30.0%)	
	Graduate	13 (11.8%)	1 (10.0%)	
	Postgraduate	8 (7.3%)	0 (0.0%)	
Residence	Rural	62 (56.4%)	7 (70.0%)	**0.404**
	Urban	48 (43.6%)	3 (30.0%)	

Out of 120 endocervical swabs, 2 were culture positive for *Neisseria gonorrhoeae* (2/120), with Gram stain findings of intracellular Gram-negative diplococci (GNDC). (Table no 2)

Table 2: Detection of *Neisseria gonorrhoeae* (n = 120) on culture

Culture Result	Number (n)	Percentage (%)
Positive	2	1.7
Negative	118	98.3
Total	120	100

Chip-based real-time PCR detected *Neisseria gonorrhoeae* in 10 of the 120 endocervical swab sample, yielding an overall prevalence of 8.3%, whereas 110 (91.7%) participants tested negative. (Table no 3)

Table 3: Prevalence of *Neisseria gonorrhoeae* detected by chip-based Real-time PCR (rtPCR) among reproductive-age women (n = 120).

rtPCR Result	Number	Percentage
Positive	10	8.3%
Negative	110	91.7 %

Discussion

The present study determined the prevalence of *Neisseria gonorrhoeae* infection among reproductive-age women attending a tertiary care hospital in Eastern Uttar Pradesh using chip-based real-time PCR and compared its diagnostic performance with conventional microbiological methods. Molecular testing detected *N. gonorrhoeae* in 10 of 120 women, corresponding to an overall prevalence of 8.3%. In contrast, selective culture and Gram stain identified only two positive cases each, highlighting the superior diagnostic yield of molecular testing and more sensitive (Table no. 3).

The observed prevalence of 8.3% indicates that gonococcal infection continues to be an important public health concern among reproductive-age women in our setting. Differences in prevalence reported across studies may be attributed to variations in study population, geographical region, healthcare-seeking behaviour, diagnostic methods, and inclusion criteria. [4,13,12] Studies employing NAATs generally report higher detection rates than those relying solely on culture because molecular assays are capable of detecting low bacterial loads and non-viable organisms. [18,7]

Age was the only sociodemographic variable significantly associated with *Neisseria gonorrhoeae* infection in the present study, with the highest positivity observed among women aged 26–30 years. This finding is consistent with the epidemiology of gonorrhoea, where sexually active young adults remain the most affected population. [4,19] Although infection was more common among married women, housewives, rural residents, women

from lower socioeconomic groups, and illiterate participants, these associations were not statistically significant. These observations suggest that gonococcal infection cannot be reliably predicted on the basis of demographic characteristics alone, emphasizing the importance of laboratory-based diagnosis.

A major finding of the present study was the marked difference between molecular and conventional diagnostic methods. Chip-based real-time PCR detected five times more cases than selective culture and Gram stain. The poor sensitivity of culture may be explained by the fastidious nature of *N. gonorrhoeae*, which requires appropriate transport conditions, selective media, viable organisms, and immediate processing. [6,7] Similarly, Gram stain has limited sensitivity in women because cervical specimens often contain mixed bacterial flora and relatively low numbers of gonococci. [5] These findings support the increasing role of NAATs as the preferred diagnostic method for gonococcal infection. [8,18]

Early and accurate diagnosis of *N. gonorrhoeae* infection has important clinical implications. Many infected women remain asymptomatic and therefore do not receive timely treatment. Delayed diagnosis may result in pelvic inflammatory disease, infertility, ectopic pregnancy, chronic pelvic pain, and adverse pregnancy outcomes. [3] Integration of rapid molecular assays into routine reproductive healthcare services may facilitate early detection, prompt treatment, interruption of disease transmission, and reduction of long-term reproductive complications. [11,9]

The strengths of the present study include the use of a highly sensitive chip-based real-time PCR platform, comparison with conventional diagnostic methods, and evaluation of women attending a tertiary care centre in a region where epidemiological data on gonorrhoea are limited. These findings contribute valuable regional evidence regarding the burden of *Neisseria gonorrhoeae* infection in Eastern Uttar Pradesh. [12]

Strengths: The present study has several strengths. It employed chip-based real-time PCR, a highly sensitive molecular diagnostic technique, and directly compared its performance with conventional microbiological methods for the detection of *Neisseria gonorrhoeae*. The inclusion of reproductive-age women attending a tertiary care hospital provided valuable regional epidemiological insights. In addition, the study offers baseline prevalence data on gonococcal infection from Eastern Uttar Pradesh, where published epidemiological evidence is scarce, thereby contributing to the existing literature and providing a foundation for future surveillance, research, and public health interventions.

Limitations: Partner screening and follow-up of infected women were not included, precluding assessment of reinfection and transmission dynamics. Furthermore, the study was conducted at a single tertiary care centre with a relatively small sample size, which may limit the generalizability of the findings. The cross-sectional design also did not permit assessment of incident infections or long-term clinical outcomes.

Challenges: The diagnosis and control of *Neisseria gonorrhoeae* infection remain challenging because many infected women are asymptomatic or present with non-specific clinical features, leading to delayed diagnosis and continued transmission to sexual partners. Social stigma associated with sexually transmitted infections often discourages patients from disclosing their sexual history and seeking timely medical care, resulting in underdiagnosis and delayed treatment.[4] In addition, the increasing emergence of antimicrobial-resistant strains, including penicillinase-producing *Neisseria gonorrhoeae* (PPNG), has limited treatment options and poses a major challenge to effective disease management. Early detection using highly sensitive molecular diagnostic techniques, followed by timely treatment and partner management, is essential to interrupt disease transmission, prevent complications such as pelvic inflammatory disease (PID), infertility, ectopic pregnancy, and chronic pelvic pain, and ultimately reduce disease morbidity and the overall burden of gonococcal infection in society. [2]

Conclusion

Overall, the findings of the present study demonstrate that chip-based real-time PCR substantially improves the detection of *Neisseria gonorrhoeae* compared with conventional microbiological methods. Gonorrhoea is a highly communicable sexually transmitted infection that can be transmitted to sexual partners, thereby facilitating continued community transmission if left undiagnosed and untreated.

Early and accurate diagnosis using sensitive molecular techniques enables prompt initiation of appropriate antimicrobial therapy, partner notification and treatment, and interruption of the chain of transmission. Furthermore, untreated gonococcal infection may lead to serious reproductive health complications, including pelvic inflammatory disease (PID), chronic pelvic pain, tubal factor infertility, ectopic pregnancy, Fitz-Hugh -Curtis Syndrome and adverse pregnancy outcomes.

Therefore, wider implementation of molecular diagnostic techniques, particularly in resource-limited settings, has the potential to strengthen STI

control programmes, reduce disease transmission, and improve reproductive health outcomes.

Larger multicenter studies incorporating antimicrobial resistance surveillance are recommended to better understand the epidemiology of *Neisseria gonorrhoeae* and to guide national STI control strategies.

Integration of molecular diagnostic platforms into routine reproductive health services may improve STI surveillance, facilitate early diagnosis, and strengthen evidence-based public health interventions.

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