Comparison of Microscopy, Culture and Molecular Methods for Diagnosing Gonorrhea

Abstract:

Gonorrhea, a sexually transmitted infection caused by *Neisseria gonorrhoeae*, continues to be a significant global public health concern. The timely and accurate diagnosis of this infectious disease is crucial for its effective management. Traditional methods, especially culture, were historically considered the gold standard for diagnosing gonorrhea. However, the introduction of nucleic acid amplification tests (NAATs), such as Real-Time PCR, has revolutionized diagnostic approaches. Currently, the Centers for Disease Control and Prevention (CDC) recommend NAAT as the primary diagnostic method, with culture reserved for specific cases, particularly for testing antibiotic susceptibility in instances of suspected treatment failure. The International Union against Sexually Transmitted Infections (IUSTI) provides guidelines for the use of NAAT or culture, depending on clinical scenarios.

This study conducted a retrospective comparative analysis of various diagnostic methods at the Apex Regional STD Centre in New Delhi, India, spanning from January 1, 2022, to December 31, 2022. Culture, Real-Time PCR, and smear examination were compared for the diagnosis of gonorrhea. A total of 33 samples were included in the analysis, with the following percentages: culture (92.02%), PCR (100%), and smear examination (100%). An intriguing finding was that 7.98% of samples were culture-negative but PCR-positive, highlighting a significant disparity between the two methods. This observation underscores the limitations of relying solely on culture for gonorrhea diagnosis and the potential consequences, including treatment delays, disease transmission, and the development of antibiotic-resistant strains.

In summary, this study underscores the critical need for accurate and reliable diagnostic methods for gonorrhea. It emphasizes the evolving diagnostic landscape, with NAATs emerging as essential tools. The findings from multiple studies stress the complementary roles of different diagnostic methods and the necessity of adapting to evolving diagnostic techniques. This research highlights the importance of collaborative approaches to enhance accuracy and address the evolving challenges of gonorrhea diagnosis. Ultimately, the significance of laboratory testing extends beyond individual patient care to broader public health goals and the prevention of sexually transmitted infections.

Keywords: Gonorrhea, *Neisseria gonorrhoeae*, nucleic acid amplification tests (NAAT), Real-Time PCR, culture, smear examination, antibiotic susceptibility testing, confirmatory testing, diagnostic methods, public health.

Introduction:

Gonorrhea, caused by *Neisseria gonorrhoeae*, is a significant global public health concern, as reported by the World Health Organization (WHO) in 2020. Timely and accurate diagnosis is crucial for managing this sexually transmitted infection. Traditionally, culture was the gold standard for gonorrhea diagnosis. However, the advent of nucleic acid amplification tests (NAATs), such as Real-Time PCR, has revolutionized diagnostics. Currently, the Centers for Disease Control and Prevention (CDC) recommends NAAT as the primary method, with culture used, when necessary, especially for antibiotic susceptibility testing in suspected treatment failure cases [1]. The International Union against Sexually Transmitted Infections (IUSTI) advocates for NAAT or culture use, depending on clinical scenarios. Culture is preferred for antibiotic susceptibility testing, while NAAT is recommended for asymptomatic cases and sample transportation. A confirmatory test is advised when the positive predictive value falls below 90%, often due to test specificity, case scarcity, and sample source issues [2].

Methods:

In a retrospective comparative study conducted at the Apex Regional STD Centre in New Delhi, India, from January 1, 2022, to December 31, 2022, various diagnostic methods for gonorrhea were assessed, including culture, Real-Time PCR, and Gram smear examination, all in adherence to ethical protocols. Samples were obtained from individuals attending the male and female STI clinics within the gynecology out-patient department and the STD department. These samples were subsequently processed and analyzed in the laboratory. Gram staining was employed to detect the presence of intracellular, gram-negative diplococci. The culture identification method involved an evaluation of colony morphology and the performance of positive oxidase tests. The Rapid Carbohydrate Utilization test (API NH, bioMérieux) was also utilized in this process.

Samples intended for Real-Time PCR underwent DNA isolation using the Genomic DNA isolation kit provided by Norgen biotek. The RT-PCR cycle was carried out using the Applied

Biosystems 7500 Fast Dx Real-Time PCR equipment in conjunction with kits from Bioron. The PCR method utilized Tran system transport swabs from Copan Italia SpA.

The results obtained from PCR demonstrated a perfect match with those obtained through smear examination, with 100% consistency (as illustrated in Graph 1).

Results

A total of 33 samples were analyzed in this study. The gonorrhea detection percentage breakdown for each diagnostic method is as follows: culture (92.02%), PCR (100%), and smear examination (100%). Notably, 7.98% of the samples were culture-negative but PCR-positive, revealing a significant discrepancy between the two methods.

The finding of 7.98% of culture-negative but PCR-positive results stands out as a critical observation in this study. This disparity underscores the limitations of relying solely on culture for the diagnosis of gonorrhea. In these cases, if culture testing were the exclusive method employed, these individuals would have been erroneously categorized as negative for the infection. Consequently, this misclassification could lead to untreated infections and potential health complications.

Implications of Delay in Treatment:

The delay in initiating treatment due to false negatives in culture testing can have serious consequences. Gonorrhea is a sexually transmitted infection associated with various complications, including pelvic inflammatory disease, infertility, and an elevated risk of HIV transmission if left untreated. The postponement of treatment stemming from false-negative culture results can exacerbate these risks, impacting individual health outcomes and increasing the burden on healthcare systems.

Transmission Risk:

Individuals with undiagnosed gonorrhea who continue to engage in sexual activity may unknowingly transmit the infection to their partners, contributing to the ongoing spread of the disease. The accuracy and timeliness of diagnosis are pivotal in curtailing the transmission of sexually transmitted infections like gonorrhea. The 7.98% of culture-negative but PCR-positive

cases highlight the potential for unchecked transmission if culture-based testing is exclusively relied upon.

Antibiotic Resistance Concerns:

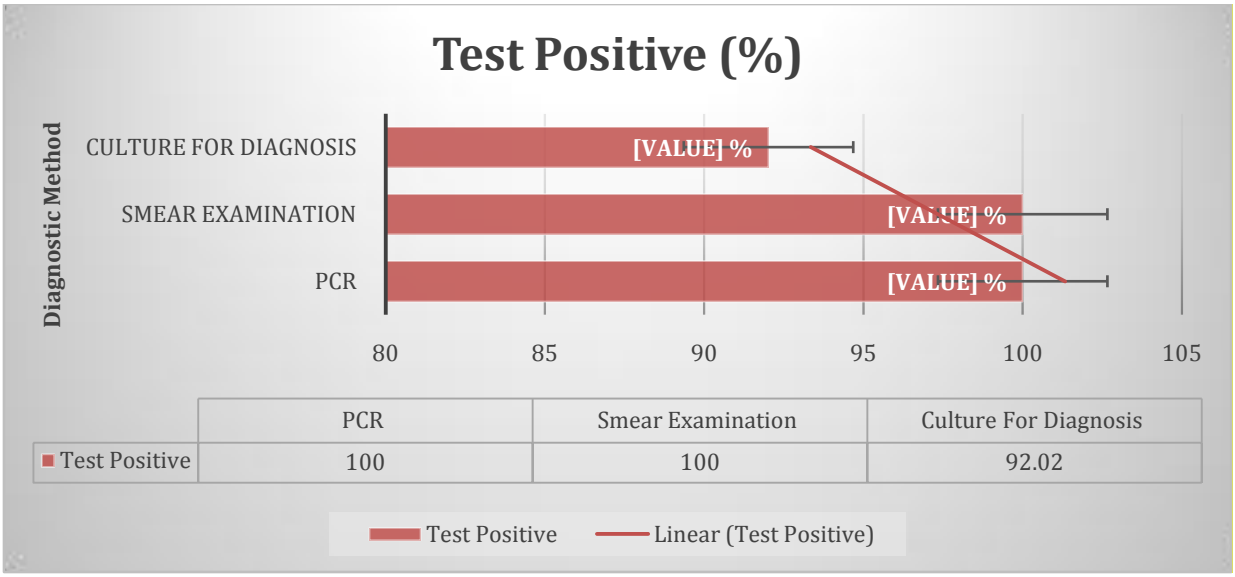
The delayed or inadequate treatment resulting from false negatives in culture testing can also foster the development of antibiotic-resistant strains of gonorrhea. When the infection is not completely eradicated, surviving bacteria may acquire resistance to common antibiotics, rendering future treatment more challenging. This finding underscores the broader implications of diagnostic accuracy for public health, as the emergence of antibiotic-resistant strains poses a serious threat.

Economic and Resource Implications:

The economic burden of false-negative culture results should not be underestimated. Patients presenting with gonorrhea-like symptoms may undergo additional tests, consultations, and healthcare resources when the initial culture results are negative. This places a strain on healthcare systems and leads to unnecessary costs. Accurate and efficient diagnostic methods are essential not only for patient care but also for the efficient allocation of healthcare resources.

In summary, the results of this study emphasize the critical need for accurate and reliable diagnostic methods for gonorrhea. The observed discrepancies between culture and PCR, with culture failing to detect a substantial proportion of positive cases, underscore the potential health consequences and economic burdens associated with misdiagnosis. Timely and accurate diagnosis is vital in preventing the spread of gonorrhea, mitigating treatment delays, and addressing the emerging threat of antibiotic resistance.

Graph 1: Comparison of PCR, smear examination, and culture for diagnosis of gonorrhea



Discussion:

Summary of Findings:

The results obtained from the three different diagnostic methods offer valuable insights into the challenges and complexities of diagnosing gonorrhea, shedding light on the changing landscape of diagnostic techniques. In a study conducted in Bangladesh, which involved 183 patients presenting with symptoms of urethritis, a comparison of multiple diagnostic methods revealed significant variations in sensitivity. Microscopic examination identified gonorrhea in 27.57% of cases, while culture detected the infection in 26.49% of instances. However, the Multiplex PCR Ng test outperformed both traditional methods, achieving a detection rate of 30.27% [3]. This finding underscores the enhanced sensitivity of molecular methods, especially Multiplex PCR Ng, in accurately identifying cases of gonorrhea when compared to conventional culture techniques.This result highlights the potential benefits of adopting molecular diagnostic methods like Multiplex PCR Ng, which can lead to more accurate and timely diagnoses of gonorrhea, ultimately contributing to better patient care and more effective disease control. The second study, conducted in India with 250 women exhibiting symptoms of vaginal discharge or lower abdominal pain, highlighted a significant discrepancy in diagnostic sensitivity between culture and PCR for *N. gonorrhoeae* infection. Culture identified gonorrhea in only one case, while PCR effectively detected the infection in 17 cases. This substantial difference underscores the

heightened sensitivity of PCR in diagnosing *N. gonorrhoeae* infection compared to traditional culture methods [4].

In the third study conducted in Bulgaria, which analyzed 617 samples from symptomatic and asymptomatic patients, including 96 men and 521 women, both in-house PCR and gonorrhea culture were employed as diagnostic methods. The results demonstrated a notable concordance between PCR and culture for 12 samples (6 from male patients and 8 from female patients), emphasizing the reliability of both PCR and culture methods in specific diagnostic scenarios [5].

Factors Affecting Diagnostic Accuracy:

While these studies offer important findings, it is essential to recognize the factors that can influence diagnostic accuracy in the realm of gonorrhea testing. These factors include decreased sensitivity in culture-based methods and decreased specificity in nucleic acid amplification tests (NAATs) [6]. The decreased sensitivity of *N. gonorrhoeae* culture methods is attributed to issues such as inadequate sample collection, inappropriate sampling techniques, and challenges in material transportation. These issues collectively contribute to the potential for false-negative results, underscoring the need for more sensitive diagnostic methods [7].

On the other hand, decreased specificity in NAATs can result from factors such as cross-reactivity with other microorganisms, contamination, and the persistence of *N. gonorrhoeae* DNA post-treatment, introducing the potential for both false-positive and false-negative results. To address these specificity concerns, utilizing NAATs that target multiple *N. gonorrhoeae* genes has shown promise in enhancing test specificity and reducing the likelihood of misdiagnosis and its associated clinical and psychological consequences [8].

The global trend observed in these studies leans significantly towards molecular methods, particularly NAAT, for gonorrhea diagnosis. The advantages of speed, accuracy, and reduced labor requirements align with the endorsement of NAAT as the primary diagnostic method by the Centers for Disease Control and Prevention (CDC) [9]. The complementarity of diagnostic methods, as highlighted by the studies, underscores the value of combining PCR, smear examination, and culture for comprehensive insights, particularly in the context of antibiotic susceptibility testing and addressing the limitations of smear examination [10].

The role of culture methods in gonorrhea diagnosis remains crucial, primarily for antibiotic susceptibility testing, which is of growing importance given the rising concerns related to antibiotic resistance. Recommendations for confirmatory testing when the predictive values of diagnostic methods fall below the 90% threshold emphasize the need for reliable diagnostics to reduce false positives and the associated clinical and psychological repercussions. In the constantly evolving diagnostic landscape, laboratories must remain adaptable and vigilant. Continuous quality control, proficiency testing, and the regular updating of protocols are essential to ensure that diagnostic techniques keep pace with advancements in the field [10].

The significance of laboratory testing extends beyond individual patient care to screening asymptomatic populations, aiding in disease control, and facilitating epidemiological surveillance. Adaptability in response to evolving diagnostics is vital not only for addressing the challenges of gonorrhea but also for tackling other sexually transmitted infections [11]. In conclusion, the findings from these studies, along with the challenges and nuances discussed, highlight the dynamic landscape of gonorrhea diagnosis. The significant differences observed between culture and molecular methods underscore the importance of adopting sensitive and accurate diagnostic approaches. As the field of diagnostics continues to evolve, the pursuit of reliable, efficient, and adaptable methods remains paramount in the quest to control gonorrhea and its associated public health implications. These findings collectively inform the need for ongoing advancements in diagnostic methodologies to enhance accuracy and address the evolving landscape of gonorrhea diagnosis.

Conclusion:

In conclusion, this study sheds light on the dynamic and evolving nature of gonorrhea diagnosis. It underscores the growing importance of molecular methods, particularly NAAT, in the accurate detection of *Neisseria gonorrhoeae*, while also acknowledging the enduring significance of culture and smear examination. The findings from various studies highlight the diverse landscape of diagnostic techniques, emphasizing their complementary roles. Adapting to the evolving diagnostic landscape is crucial in the effective management of gonorrhea. This adaptation involves proactive measures such as stringent quality control, proficiency testing, and the continuous updating of protocols. These efforts not only enhance the accuracy of diagnosis but

also contribute to broader public health goals, including disease control and epidemiological surveillance.

As the field of gonorrhea diagnostics continues to advance, the pursuit of reliable, efficient, and adaptable methods remains paramount. The collaborative potential of diagnostic approaches, including PCR, smear examination, and culture, must be harnessed to provide comprehensive insights and address the limitations of each method. Moreover, the role of culture methods in antibiotic susceptibility testing is of growing importance in the face of rising antibiotic resistance. In a constantly changing diagnostic landscape, laboratories must remain vigilant and flexible to keep pace with advancements. This adaptability is not only crucial for individual patient care but also for the broader context of screening asymptomatic populations and contributing to disease control. The significance of laboratory testing extends beyond diagnosis and treatment, ultimately serving the greater public health objectives and the prevention of gonorrhea and other sexually transmitted infections.

Authors' contributions

The research was designed and performed by SM SR and AL. AL, NV and PV gathered the information.AL analyzed the data. The entire manuscript was written collaboratively by SM, AL, PG, PV, SR and DS. SM and NK reviewed the manuscript. The final manuscript was read and approved by all authors.

Ethics approval

This study was approved by the Institutional Ethics Committee.

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