Comparative Evaluation of Quantitative PCR and Conventional Diagnostic Techniques for Accurate Diagnosis of Bacterial Vaginosis in Reproductive-Age Women

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Abstract: Reproductive-age women need an accurate diagnosis to treat and prevent Bacterial Vaginosis (BV). Traditional diagnostic methods like Nugent score and Amsel criteria are time-consuming and biassed. Critical BV-associated bacteria may be identified and quantified using quantitative multiplex PCR (qPCR), which may increase sensitivity and reproducibility. Women 18 and older who attended hospitals had 125 vaginal specimens obtained. BV was diagnosed by Nugent scoring and multiplex real-time PCR (ATRiDA, Netherlands). Gardnerella vaginalis, Atopobium vaginae, Lactobacillus spp., and total bacterial load are measured by multiplex real-time PCR. Amsel criteria or BD Affirm were employed to evaluate conflicting data. Nugent score yielded 15.45% intermediate and 36.36% BV-positive. While 12.72% of samples were transitional or undefined BV, qPCR proved 48.18% were BV-positive. The two methods agreed 81.81%. PCR successfully recognized two intermediate Nugent cases as normal, however qPCR could not find any BV-positive patients by Nugent scoring. Overall, qPCR detected 11% more BV infections than other approaches. Traditional BV diagnostic tests are subjective, while quantitative polymerase chain reaction (PCR) makes them more sensitive and standardized for childbearing women. Our findings suggest that qPCR might replace or supplement labour-intensive diagnostic approaches in women's health, improving clinical management and diagnosis.

Keywords: Bacterial Vaginosis (BV), Quantitative PCR (qPCR), Conventional diagnostic techniques, Nugent scoring, Amsel criteria, Reproductive-age women, Molecular diagnosis

INTRODUCTION

Bacterial vaginosis (BV), which is the most common vaginal infection among women of reproductive age across the world, results in a disruption of the normal vaginal flora. In the vagina, Lactobacillus species undergo a decrease while anaerobic bacteria, notably Atopobium vaginae and Gardnerella vaginalis, undergo overgrowth. In order to properly treat and prevent complications such as preterm birth, pelvic inflammatory disease, and increased susceptibility to sexually transmitted diseases, it is of utmost importance to get an accurate diagnosis of bacterial vaginosis (BV) [1].

Epidemiology and Clinical Significance of BV

The prevalence rates of bacterial vaginosis range from twenty to fifty percent among women across the globe. These percentages are further divided according on age, ethnicity, and sexual activity. Despite the fact that it is common, accurate laboratory diagnosis is important since many cases do not display any symptoms. A diagnosis that is overlooked or treatment that is delayed might result in negative effects for a woman's reproductive health and gynaecological well-being.

Conventional Diagnostic Techniques

Conventionally, BV is identified by means of:

- **Amsel Criteria:** A technique to clinical diagnosis that focuses on the existence of three out of the following four clinical indicators: continuous vaginal discharge, a pH level that is elevated, a positive scent test, and microscopically visible clue cells [2].
- **Nugent Scoring:** A method that uses Gramme staining to ascertain the relative abundance of bacterial morphotypes Nugent scoring is considered the gold standard in spite of the fact that it is labor-intensive and prone to inter-observer variability due to its subjective interpretation.

Molecular Diagnostics: Quantitative PCR (qPCR)

The development of quantitative polymerase chain reaction (qPCR) assays for bacterial vaginosis (BV) is a direct consequence of advancements in the field of molecular diagnostics. Some of the capabilities of quantitative polymerase chain reaction include the following:

- The ability to determine the presence of significant bacteria that are linked with BV and quantify them with pinpoint precision.
- Results that are more objective and simpler to duplicate than those obtained using more traditional methods

The ability to discover cases in the subclinical or transitional phases, which may escape detection when using more conventional procedures [3].

Need for Comparative Evaluation

Despite the fact that conventional procedures are still in broad use, the limitations of these approaches highlight the need of the development of more reliable diagnostic techniques. You may compare quantitative polymerase chain reaction (qPCR) to conventional techniques in order to assess the sensitivity and specificity of molecular testing relative to established processes. Investigate the ways in which qPCR may be incorporated into regular diagnostic methods for clinical applications [4]. Cut down on inconsistencies and enhance treatment outcomes by assisting in the standardization of BV diagnosis for women of reproductive age. This study will compare quantitative polymerase chain reaction with conventional diagnostic procedures in women of reproductive age in order to evaluate the accuracy, specificity, and potential advantages of molecular diagnostics in the improvement of therapeutic therapy for bacterial vaginosis.

OBJECTIVES

1. To assess how well quantitative PCR (qPCR) detects bacterial vaginosis in women of reproductive age in comparison to traditional techniques (Nugent score and Amsel criteria).

2. To assess qPCR's sensitivity and dependability as a possible substitute for traditional BV diagnostic methods.

MATERIAL AND METHOD

The study included 125 women from two Indian gynaecology clinics and a university research lab. Women had to get a normal gynaecological checkup between June and October 2023 to participate. Participants have to be 18 or older and had not used vaginal medication or antibiotics for 14 days. After verbal consent, participants provided two additional vaginal swabs for quantitative polymerase chain reaction (qPCR) testing and graded Gramme stain analysis (Nugent score). Demographic and clinical data such age, pregnancy status, history of recurrent BV, and co-infections were collected anonymously to ensure participant privacy. We utilized non-traceable study numbers. After excluding 15 participants owing to incomplete evaluations, 110 were eligible for research.

Traditional diagnostic evaluation

The Amsel criteria were applied by clinicians at each location to evaluate vaginal swabs [5]. Vaginal samples were subjected to additional analysis using Nugent Graded Gramme staining at med fusion, in accordance with the approach that was previously described in detail [6].

Evaluation of molecular diagnostics

DNA was extracted in accordance with the standard protocol for total nucleic acid by using an automated COBAS AmpliPrep (CAP) system and a total nucleic acid isolation kit (TNAI kit, Roche Diagnostics). To summarize, the samples were extracted from the cells by gently agitating them, and then 650 microlitres of the sample was eluted in 75 microlitres. For the purpose of amplification, a reaction volume of 25 microlitres was prepared using a combination of ten microlitres of DNA extract and fifteen microlitres of BV master mix. Before the test may begin, the BV master mix reaction mixture is prepared by whipping together 10 µl of PCR-mix-1-FRT Bacterial vaginosis mix and 5 µl of a combination of PCR-mix-2-FRT and polymerase. A total of six controls and calibrators were included in every single batch [7]. The list includes NTC, DNA calibrator FC1, DNA calibrator FC2, BV-NC, BV-PC, and BV-SPC, among others. The information that was gathered was analyzed using clinical correlation using ratio coefficients (RC1, RC2, and RC3) that were obtained for combinations of targets that were evaluated by PCR. This was done using Excel-Macro (see Table 1). If they were accessible, conflicts between the "Nugent Score" and the polymerase chain reaction (PCR) method were resolved by using the Amsel criteria or other tests, such as the BD Affirm test.

Statistical analysis

The Wilcoxon rank-sum test was used to examine the quantitative distribution of the bacteria in bacterial vaginosis and normal flora. Differential expression was considered to have reached statistical significance when the P value was less than 0.01. When we used a dichotomous primary endpoint that had an error rate of 0.05% and an incidence of 20%, we were able to distinguish venomous infections with more than 80% power. Furthermore, we utilised binary logistic regression analyses (SPSS statistical software, version 22) to contrast the predictive value of specific targets in relation to vaginosis versus normal results. In these analyses, we took into consideration co-infections with other sexually transmitted infections (STIs), including C trachomatis and N gonorrhoea, as well as non-STIs such as yeast. The p-values

are below the threshold of 0.001 in both cases. [8] A binary primary endpoint with a 0.05% error rate and a 20% incidence rate enabled us to distinguish between venomous infection and the normal condition with above 80% power. Furthermore, we utilised binary logistic regression analyses (SPSS statistical software, version 22) to contrast the predictive value of specific targets in relation to vaginosis versus normal results. In these analyses, we took into consideration co-infections with other sexually transmitted infections (STIs), including C trachomatis and N gonorrhoea, as well as non-STIs such as yeast. When confounders were taken into account, P values that were less than 0.05 were considered to be significant findings.

RESULT

Table 1: Interpretation of the results using a BV PCR-based technique

RC Ratio / Condition	Result Interpretation	Clinical Significance
RC1 > 1	Negative	Absence of signs of bacterial vaginosis
RC1 < 0.5	Positive	Typical of Bacterial Vaginosis
$0.5 \leq RC1 \leq 1$	Intermediate	In line with the departure of typical vaginal flora
RC2 > 1 and RC3 > 2, RC1 can take any value	Unspecified	In line with changes in vaginal flora that are not connected to bacterial vaginosis
Bacteria DNA < 10^5 copies/ml	Invalid	There is not enough bacteria for analysis. Bring back a fresh specimen.

Table 2: Study cohort demographics

Variable	N (110)	% N
Race/Ethnicity		
Caucasian	67	60.91
Hispanic	21	25.45
African American	10	9.09
Others	5	4.55
Pregnant	14	12.72
Recurrent BV	16	14.55
Co-infections	25	22.72
C. trachomatis	4	–
N. gonorrhea	1	– *Yeast co-infection*
Yeast	21	

Table 2 displays information on the clinical and demographic characteristics of the study cohort. The ethnic composition of the participants in the research was as follows: 60.91 percent were white, 25.45 percent were Hispanic, and 9.09 percent were African American. The registrants included twelve point seven two percent (12.72%) pregnant women [9]. In 22.72 percent of the subjects, yeast, Chlamydia trachomatis, and Neisseria gonorrhoeae were identified to be the most prevalent co-infections. Recurrence of bacterial vaginosis (BV) was seen in 14.55 percent of the individuals who participated.

Table 3: Comparison between NUGENT graded gramme stain and BV PCR test performance

Graded Gram Stain	Molecular BV Test: Vaginosis	Intermediate / Unspecified	Normal	Total
Vaginosis	40	0	0	40
Intermediate	6	9	2	17
Normal	7	5	41	53
Total	53	14	43	110

The overall agreement between the NUGENT Graded Gramme Stain and the PCR BV test is shown in Table 3. When the Nugent method was used, 36.36 percent of patients were found to have BV, and 15.45 percent of patients were found to have transitional BV. According to the polymerase chain reaction (PCR) method, on the other hand, 48.18 percent of the cases were categorised as BV, whereas 12.72 percent were categorised as either transitional BV or BV of unclear origin [10]. In all, the two techniques exhibited an 81.81 percent degree of agreement with one another. The PCR approach did not overlook any of the instances when the Nugent score was used as the gold standard for designating BV positives. The polymerase chain reaction (PCR) method was used in the determination of the typicality of just two of the Nugent intermediates. The PCR-based BV test was shown to be 10 percent more sensitive than the "Nugent Score" and to identify 10 percent more positive cases. Seven cases of genuine bacterial vaginosis (BV) and five cases of transitional BV were discovered by means of PCR-based BV tests; these cases were considered to be normal according to the NUGENT score.

Table 4: Amsel criterion performance in comparison to NUGENT graded gramme stain.

Graded Gram Stain	Amsel Criteria: Positive	Amsel Criteria: Negative	Total
Vaginosis / Intermediate	29	21	47
Normal	16	44	56*

Total	42	61	110

The Amsel criteria and the NUGENT Graded Gramme stain were found to have a total concordance of 64.08 percent, as can be shown in Table 4 [11]. Amsel was not particularly successful, as it had a sensitivity of just 61.70 percent and a specificity of only 78.57 percent.

Table 5: Importance of individual targets for predicting normal vs vaginosis states after taking co-infections into consideration.

Bacterial Marker	Normal Mean	Normal STDEV	Vaginosis Mean	Vaginosis STDEV	P-value
Gardnerella vaginalis	2.81	2.93	7.20	2.79	<0.001
Atopobium vaginae	3.38	3.12	7.18	1.39	<0.001
Lactobacillus spp.	7.22	0.91	6.52	1.59	≤0.01
Total DNA	7.40	0.46	8.11	0.44	–

The predictive significance of individual targets for the Normal vs Vaginosis condition is shown in Table 5, which takes into account co-infections with C. trachomatis, N. gonorrhoea, and yeast. The average log copies/mL for GV, AV, Lacto, and total DNA in the normal population were 2.81, 3.38, 7.22, and 7.40, respectively. On the other hand, the BV population had values of 7.20 ($p < 0.0001$), 7.18 ($p < 0.0001$), 6.52 ($p \leq 0.01$), and 8.11 ($p < 0.0001$), as can be shown in Figure 1A and Figure 1B. After accounting for any confounding factors, the values remained statistically significant [12].

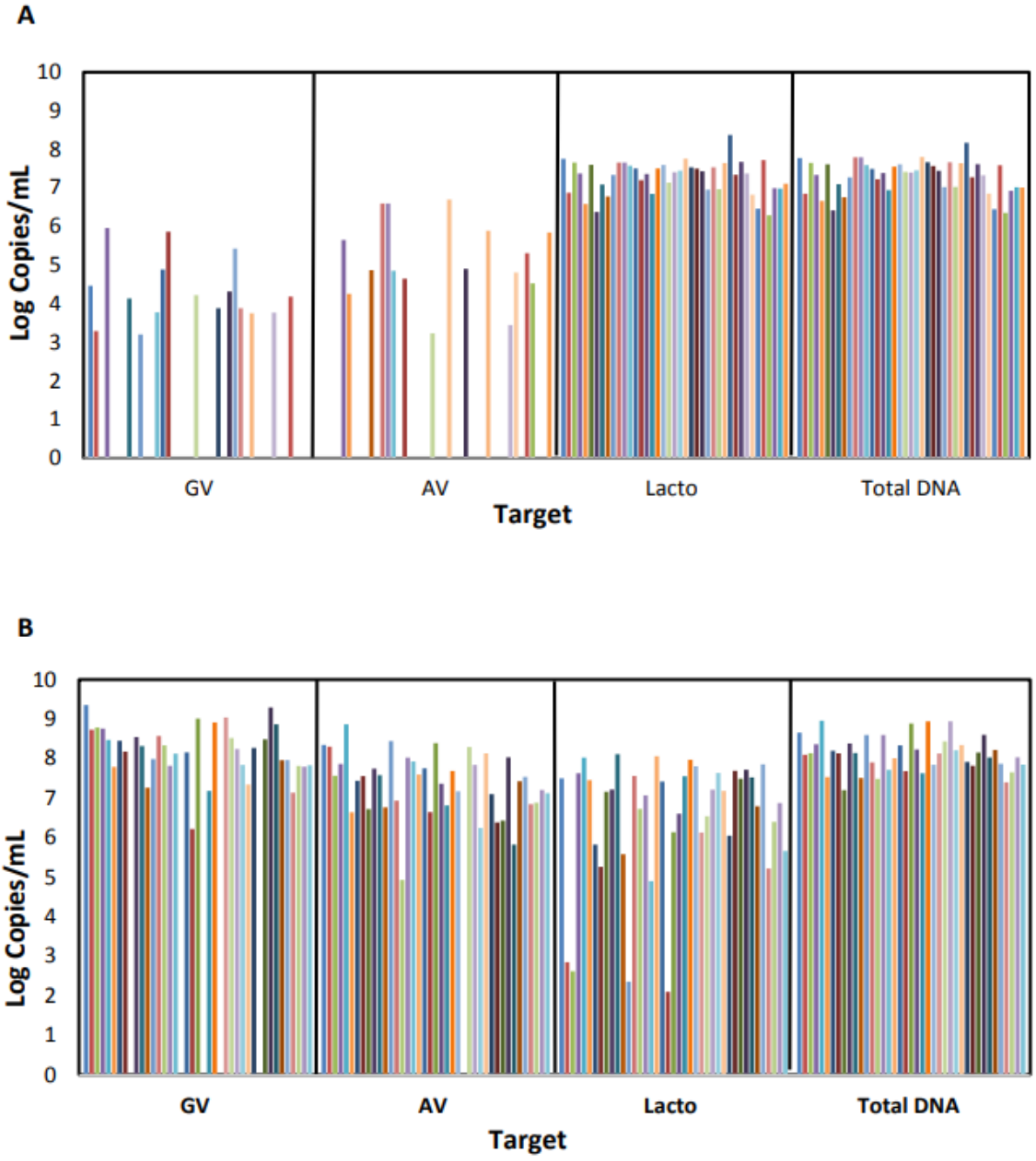

Figure 1: Mean log copies/mL of G. vaginalis, A. vaginae, Lactobacillus spp., and total DNA in normal vs. BV population

DISCUSSION

A polymicrobial infection known as bacterial vaginosis (BV) interferes with the natural flora of the vagina. It is distinguished by an increase in the amount of anaerobic bacteria, such as Gardnerella vaginalis and Atopobium vaginae, and a decrease in Lactobacillus species. Because of the increased likelihood of adverse reproductive outcomes, a precise diagnosis is

particularly critical for the effective treatment of women of childbearing age [13]. For a long time, the Nugent scoring system has been considered an important part of laboratory diagnosis. However, this method is time-consuming and susceptible to subjective interpretation since it relies on the morphological examination of microorganisms. This gets much more complicated in the presence of non-contributory microorganisms that seem to be similar [14].

Quantitative polymerase chain reaction (qPCR) is a dependable and unbiased technique that molecular diagnostics may use to quantify the most prevalent bacterial targets that are linked with bacterial vaginosis (BV) in contrast to the normal vaginal flora. The Lactobacillus spp. ratio has been shown to have a consistently good specificity for BV diagnosis when compared to A. vaginae and G. vaginalis [15]. Our study has shown that quantitative polymerase chain reaction (qPCR) is more sensitive and is capable of overcoming any misclassification that may be caused by morphologically similar non-contributory bacteria. It also identifies a greater proportion of people who have BV and transitional BV than the Nugent scoring system.

Quantitative polymerase chain reaction (qPCR) was able to distinguish between bacterial vaginosis (BV) of unknown origin and transitional BV (as determined by Nugent score), which might be influenced by co-infections, including yeast infections as well as Chlamydia trachomatis and Neisseria gonorrhoeae infections. Despite the fact that our sample size for bacterial vaginosis of unknown origin was modest, our findings indicate that molecular diagnostics may provide us with more information on vaginal microbial imbalances. Our results have shown the dynamic and complex microbial communities that are found in the vagina. The findings also demonstrated that the amount of bacterial DNA in BV-positive samples increased by approximately five times. It was feasible to get a more accurate assessment of the overall change in the vaginal flora by normalising the bacterial variances with the use of ratio coefficients (RC1, RC2, RC3). Additionally, total DNA quantification was used as a quality control tool to verify that there was a enough amount of the samples for precise testing.

A single collection and transport method may be used to test for Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Candida spp., and Herpes simplex virus, in addition to bacterial vaginosis, with the use of quantitative polymerase chain reaction (qPCR). It is challenging to make generalisations about clinical connections across different populations since the vaginal microbiota naturally varies depending on factors such as age, pregnancy

status, race, and ethnicity. QPCR-based molecular diagnostics offers the potential to increase sensitivity and repeatability while simultaneously standardising BV testing in women of reproductive age and reducing the inherent subjectivity of conventional methods. There is evidence from our research that quantitative polymerase chain reaction (qPCR) may be a valuable resource for the diagnosis of women's health, especially in settings that lack microbiological expertise.

CONCLUSION

Quantitative polymerase chain reaction (qPCR) provides a very sensitive and objective method for diagnosing bacterial vaginosis (BV) in women of reproductive age, which is in stark contrast to more conventional procedures such as Nugent score and Amsel criteria. As opposed to traditional morphological assessments, the findings of our research demonstrate that quantitative polymerase chain reaction (qPCR) is more reliable and repeatable when it comes to diagnosing BV and transitional cases. The use of ratio coefficients and total DNA quantification may be of assistance in the standardisation of bacterial variations in order to have a better understanding of the fluctuations that occur in vaginal flora. Furthermore, molecular diagnostics enhance women's health screening by enabling the identification of a variety of illnesses from a single sample all at once. Quantitative polymerase chain reaction (qPCR) is an appealing alternative to conventional bacterial vaginosis (BV) diagnostic techniques due to the fact that it may potentially be standardised, can be more sensitive, and is less subjective. In the management of reproductive health, it may potentially be an asset that is beyond measure in the present day.

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