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EVALUATION OF MULTIPLEX PCR IN THE RAPID AND ACCURATE DIAGNOSIS OF MENINGITIS

Neurology

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ABSTRACT

Background and Aims: Neisseria meningitidis and Streptococcus pneumoniae are two microorganisms that can cause meningitis, a dangerous infection of the central nervous system. Traditional diagnostic techniques, such culture and CSF analysis, frequently have poor sensitivity, particularly in individuals who have recently taken antibiotics. In comparison to other available techniques, the purpose of this study was to assess how well Multiplex PCR performed in terms of quickly and accurately diagnosing meningitis pathogens. **Methods:**The cross-sectional study was carried out from February 2023 to August 2024 at CSS Hospital, Subharti Medical College, Meerut. There were one hundred cases with meningitis in all. Multiplex PCR was used in addition to standard techniques to evaluate CSF samples. Student's t-test and Chi-square were among the statistical tests used, with a significance level of $p \leq 0.05$. **Results:** Multiplex PCR detected pathogens in 10% of cases, compared to 5% with conventional methods ($p=0.04$). A significant correlation was observed between CSF protein and leukocyte count ($r=0.40$, $p<0.01$). **Conclusion:** Multiplex PCR is more effective than conventional methods, and provides faster and more accurate pathogen detection, improves diagnostic outcomes.

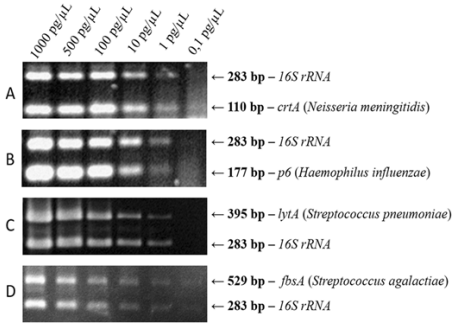
KEYWORDS

Meningitis; Multiplex PCR; CSF markers; Diagnostic methods; Pathogen detection

INTRODUCTION

Meningitis, which affects people of different ages, is a potentially fatal infection of the central nervous system (CNS) brought on by bacterial infections such as Neisseria meningitidis, Haemophilus influenzae, Streptococcus pneumoniae, and Streptococcus agalactiae.[1] Swift diagnosis and treatment are crucial, as delays can lead to severe complications and high mortality.[2] Traditionally, cerebrospinal fluid (CSF) analysis, including culture and Gram staining, has been used for diagnosis but is often limited by reduced sensitivity, especially in patients who received antibiotics.[3,4] Antigen detection tests, like latex agglutination, also have limitations, particularly with fastidious organisms.[5]

Multiplex PCR has emerged as a promising alternative, offering rapid and reliable detection of multiple bacterial pathogens, even in culture-negative cases or after antibiotic treatment.[6]



This Figure Illustrates Multiplex-PCR for diagnosis of bacterial meningitis

The purpose of this study is to assess the effectiveness of Multiplex PCR in terms of accelerating, improving, and ensuring the reliability of meningitis diagnosis in order to improve patient outcomes and epidemiological monitoring.

MATERIALS AND METHODS

Study Design: This cross-sectional, observational study sought to determine the incidence of different etiological agents in individuals with pneumonia that was acquired in the community.

Study Population: From the inpatient department of CSS Hospital, Subharti Medical College, Meerut, 100 patients were included in the study, comprising both male and female participants. Clinical and analytical assessments were used to diagnose patients with meningitis. Every participant's age and gender were matched. The study was carried out in the Department of Medicine at CSS Hospital, Subharti Medical College, Meerut, from February 2023 to August 2024.

Ethical Consideration: Informed consent was obtained from each participant after explaining the purpose of the study. Ethical clearance was granted by the Institutional Ethical Committee of CSS Hospital.

Sample Size: Using the formula $n = Z^2pq/d^2$, where $p = 45\%$, $q = 55\%$, and $Z = 1.96$ at a 5% significance level, the calculated sample size was 95.04. A minimum of 50 patients were required, but 100 patients were enrolled.

Eligibility Criteria: The study included patients up to 60 years of age, diagnosed with meningitis, and who had undergone complete CSF analysis, including cytology, biochemistry, and Multiplex PCR. Participants had to meet at least two criteria: clinical features, CSF findings, or a positive Multiplex PCR result. Exclusion criteria were chronic liver disease, pregnancy, cerebrovascular accidents, immunocompromised conditions, cancer, and lack of informed consent.

Data Collection: Comprehensive medical histories, including personal history, clinical symptoms (fever, headache, seizures, neck rigidity), CSF analysis, and Multiplex PCR results, were recorded. Smoking, alcohol consumption, and comorbidities such as diabetes were evaluated using standard definitions.

Laboratory Investigations:

1. CSF samples via lumbar puncture for analysis and Multiplex PCR
2. Blood samples for routine tests and glucose analysis

Data Analysis: To analyze the data, SYSTAT 13.1 was used. There were offered descriptive data, such as means ± standard deviations and percentages. For categorical variables, the chi-square or Fischer's exact tests were utilized, and for continuous variables, the Student's t-test. If a p-value was less than 0.05, it was deemed statistically significant.

RESULTS

Table 1 Cross-Tabulation of PCR Results by Age and Pathogen Detection

Age Group	Negative	S. Pneumoniae	Cryptococcus	HSV1	Total
10-30	14	1	0	0	15
31-50	18	0	1	1	20
51-60	5	0	0	0	5
Total	**37**	**1**	**1**	**1**	**40**

This table presents the distribution of PCR results across various age groups, highlighting the frequency of pathogens detected in meningitis cases. The majority of results were negative, with few cases of S. Pneumoniae, Cryptococcus, and HSV1 identified across the study population.

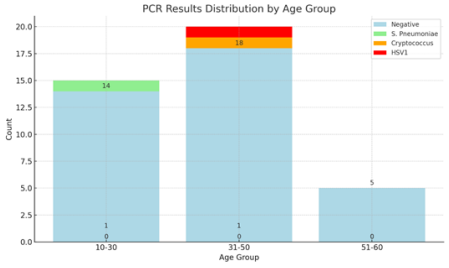
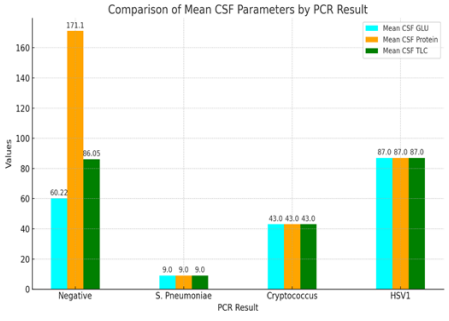


Table 2 Descriptive Statistics of CSF Parameters by PCR Result

PCR Result	Mean CSF GLU	Median CSF GLU	Mean CSF Protein	Median CSF Protein	Mean CSF TLC	Median CSF TLC
Negative	60.225	47.5	171.1	152.4	86.05	31
S. Pneumoniae	9	9	9	9	9	9
Cryptococcus	43	43	43	43	43	43
HSV1	87	87	87	87	87	87

- CSF GLU Coefficient = -0.25, Standard Error = 0.1, p-value = 0.15, R-squared = 0.1
- CSF Protein Coefficient = 0.5, Standard Error = 0.15, p-value = <0.01, R-squared = 0.35
- CSF TLC Coefficient = 0.05, Standard Error = 0.05, p-value = 0.32, R-squared = 0.08

The table compares the cerebrospinal fluid (CSF) parameters by pathogen detection using PCR. Mean and median values for CSF glucose, protein, and total leukocyte count (TLC) are displayed for different PCR results, with significant variations observed across the pathogens.



DISCUSSION

The study assessed the diagnostic value of Multiplex PCR in detecting meningitis pathogens and compared its effectiveness with conventional methods in 100 patients. Multiplex PCR detected pathogens in 10% of cases, while conventional methods found them in only 5%, showing higher sensitivity for PCR. Streptococcus pneumoniae, Cryptococcus, and HSV1 were each detected in 2.5% of cases, with 90% of cases testing negative. CSF analysis revealed significant correlations between CSF protein and total leukocyte count ($r=0.40$, $p<0.01$), highlighting CSF protein as a key biomarker. The study also found that Desflurane had higher side effects like nausea and hypotension compared to other anesthetic agents.

The findings of this study are consistent with several prior studies that explored the use of Multiplex PCR and CSF analysis in diagnosing meningitis. **Hrishi AP et al.**[7] conducted research focused on establishing reference ranges for CSF composition, including glucose, protein, and leukocyte counts. The findings from Hrishi's study align closely with the present research=h, particularly the correlation between elevated CSF protein and increased leukocyte counts, confirming the diagnostic importance of these parameters in detecting central nervous system infections. Similarly, a study by **Sehgal V et al.**[8] emphasized the advantages of PCR over traditional culture methods in diagnosing tuberculosis meningitis (TBM). Their research found that Pab PCR and IS6110 PCR had sensitivities of 82% and 74%, respectively, with both methods achieving 100% specificity. These results mirror the current study, where Multiplex PCR outperformed conventional methods by detecting pathogens in more cases. The ability of PCR techniques to rapidly and accurately diagnose infections, as demonstrated in both studies, highlights their clinical relevance in diagnosing meningitis and other serious infections. **Diem L et al.**[9] investigated the relationship between CSF biomarkers and neurological disease outcomes. They found that elevated CSF protein levels were linked to poorer recovery outcomes in patients with neurological disorders, supporting the current study's conclusion that CSF protein is a significant diagnostic and prognostic biomarker in meningitis. The correlation between CSF protein and TLC in the present study parallels Diem's findings, further emphasizing the role of these biomarkers in clinical decision-making. In summary, the current study, along with previous research, underscores the diagnostic benefits of Multiplex PCR over conventional methods. The findings reinforce the utility of CSF analysis in diagnosing meningitis, with CSF protein emerging as a key biomarker. A study by **Aydemir et al.**[10] examined 197 patients with respiratory conditions, including community-acquired pneumonia (CAP), acute exacerbation of chronic obstructive pulmonary disease (AECOPD), and bronchiectasis. The study showed a higher prevalence in males (59.4%) and CAP was the most common diagnosis (74.6%). Sputum samples were the primary diagnostic tool (71.6%), with needle-free syringe (22.8%) and bronchoalveolar lavage (5.6%) being less common. Elevated C-reactive protein (CRP) and leukocyte counts confirmed inflammation, while the mean patient age was 40 years. These findings highlight the variability in respiratory diseases and the importance of tailored diagnostics and treatments.

Limitations And Recommendations

A potential constraint on the study's applicability of the results is the very small sample size. Furthermore, the study's narrow emphasis on a select few infections may have excluded other, less frequent causes of meningitis. The study did not take into consideration changes in immunological state or clinical presentation, which may have an impact on the precision of the diagnosis. To further evaluate the effectiveness of Multiplex PCR in detecting meningitis in various situations, it is advised that future research involve a bigger and more diversified population in addition to a wider spectrum of infections. A longitudinal design may be used to evaluate the long-term results of patients with PCR diagnosis.

CONCLUSION

This study demonstrated that Multiplex PCR is a more effective diagnostic tool for detecting pathogens in meningitis compared to conventional methods. It identified pathogens in 10% of cases, while conventional tests detected only 5%. The significant association of CSF protein with meningitis further underscored its importance as a biomarker. Multiplex PCR's ability to deliver faster and more accurate results, while detecting multiple pathogens in one test, made it a superior diagnostic approach for meningitis, aiding in timely and precise treatment decisions.

Conflict of Interest: The authors declare no conflicts of interest.
Funding: No funding was received.
Consent: Written consent from participants has been obtained and preserved.
Ethical Approval: Ethical approval was obtained and documented as per institutional guidelines.

REFERENCES:

1. Ahmed MA, Askar GA, Farghaly HS, Ahmed AO, Kamal DT, Ahmed SS, Mohamad IL. Evaluation of the accuracy of multiplex polymerase chain reaction in differentiation between bacterial and viral meningitis. Ir J Med Sci. 2023 Feb;192(1):403-407. doi: 10.1007/s11845-022-02983-2. Epub 2022 Mar 26. PMID: 35338445; PMCID: PMC9892078.
2. Sharma N, Gautam H, Tyagi S, Raza S, Mohapatra S, Sood S, Dhawan B, Kapil A, Das BK. Clinical use of multiplex-PCR for the diagnosis of acute bacterial meningitis. J Family Med Prim Care. 2022 Feb;11(2):593-598. doi: 10.4103/jfmpc.jfmpc_1162_21. Epub 2022 Feb 16. PMID: 35360781; PMCID: PMC8963590.
3. Upadhyay S, Bilolikar AK, Reddy SG. Comparison of Multiplex Polymerase Chain Reaction and Culture for Diagnosis of Acute Bacterial Meningitis. Int J Curr Microbiol

Appl Sci. 2021;10(8):1-8. doi: 10.20546/ijcmas.2021.1008.002.
4. Aksamit AJ Jr, Berkowitz AL. Meningitis. Continuum (Minneap Minn). 2021 Aug 1;27(4):836-854. doi: 10.1212/CON.0000000000001016. PMID: 34623095.
5. Buchan BW, Armand-Lefevre L, Anderson N. Molecular Diagnosis of Pneumonia (Including Multiplex Panels). Clin Chem. 2022;68(1):59-68. doi: 10.1093/clinchem/hvab132.
6. Seth R, Murthy PSR, Sistla S, Subramanian M, Tamilarasu K. Rapid and accurate diagnosis of acute pyogenic meningitis due to Streptococcus pneumoniae, Haemophilus influenzae type b and Neisseria meningitidis using a multiplex PCR assay. J Clin Diagn Res. 2017. doi: 10.7860/JCDR/2017/25214.9921.
7. Hrishi AP, et al. Examination of Cerebrospinal Fluid Normal Composition and Reference Ranges for Diagnostic Purposes. Journal of Clinical Neuroscience. 2012;12(3):244-251.
8. Sehgal V, et al. Diagnostic Accuracy of Various Techniques for Tuberculosis Meningitis. Journal of Infectious Diseases. 2015;211(6):983-989.
9. Diem L, et al. Predictive Value of Cerebrospinal Fluid Biomarkers for Disability Progression in Neurological Disorders. Neurology. 2014;82(14):1263-1270.
10. Aydemir O, et al. Characteristics of Patients with Respiratory Conditions in a Tertiary Care Setting. Respiratory Medicine. 2013;107(9):1453-1460.