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DIAGNOSTIC VALUE OF MULTIPLEX PCR IN DIFFERENTIATING BACTERIAL VS VIRAL MENINGITIS

Original Article

Abdul Rehman Naeem[1], Muhammad Ahsan Murtaza[2], Muhammad Talha Ayub[3]*, Sheharyar Abbas Bhatti[4], Fatima Tahir Khan[5], Adeel-ur-Rehman[6]

[1]PGR Neurology, Faisalabad Medical University/Allied Hospital, Faisalabad, Pakistan.
[2]House Officer, Pak Red Crescent Hospital, Lahore, Pakistan.
[3]Medical Officer, Primary and Secondary Healthcare Department, Lahore, Punjab, Pakistan.
[4]MBBS, Pak Red Crescent Medical and Dental College, Lahore, Pakistan.
[5]Student, GC Women University, Sialkot, Pakistan.
[6]Physician, Punjab Institute of Neurosciences, Lahore, Pakistan.

Corresponding Author: Muhammad Talha Ayub, Medical Officer, Primary and Secondary Healthcare Department, Lahore, Punjab, Pakistan, talha.ayub001@gmail.com

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ABSTRACT

Background: Rapid differentiation between bacterial and viral meningitis is critical for timely treatment and improved clinical outcomes. Traditional diagnostic methods such as cerebrospinal fluid (CSF) culture are time-consuming and often yield limited sensitivity, especially post-antibiotic initiation. Multiplex polymerase chain reaction (PCR) has emerged as a promising diagnostic tool, offering rapid, sensitive pathogen detection in acute care settings.

Objective: To assess the sensitivity and specificity of multiplex PCR in identifying bacterial and viral meningitis etiologies in acute clinical settings.

Methods: A diagnostic accuracy study was conducted over seven months in a tertiary hospital, enrolling 323 patients presenting with clinical signs of meningitis. Inclusion required lumbar puncture within 24 hours of presentation. CSF samples were analyzed using both conventional methods and a multiplex PCR panel targeting common bacterial and viral pathogens. Diagnostic performance metrics including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated.

Results: Multiplex PCR detected bacterial pathogens in 38.1% and viral pathogens in 33.7% of cases. Compared to culture, PCR showed higher yield and faster turnaround time (mean 1.8 hours vs. 48 hours). The test demonstrated a sensitivity of 92.4%, specificity of 95.1%, PPV of 90.2%, and NPV of 96.3%. The strongest agreement with reference standards was observed in *Streptococcus pneumoniae* and enterovirus detections.

Conclusion: Multiplex PCR is a reliable, rapid diagnostic tool for distinguishing bacterial from viral meningitis. Its integration into routine practice may enhance early management decisions, minimize unnecessary antibiotic use, and improve patient outcomes.

Keywords: Acute Disease, Bacterial Meningitis, Cerebrospinal Fluid, Diagnostic Accuracy, Multiplex Polymerase Chain Reaction, Sensitivity and Specificity, Viral Meningitis.

INTRODUCTION

Meningitis remains a critical global health concern, particularly due to the urgency required in its diagnosis and treatment. The rapid progression of both bacterial and viral meningitis, along with their overlapping clinical presentations, poses a significant diagnostic challenge in emergency and acute care settings. Delays in identifying the causative pathogen not only complicate treatment strategies but also increase the risk of severe complications, including neurological damage and mortality (1). Early and accurate differentiation between bacterial and viral etiologies is essential for appropriate management—bacterial meningitis requires immediate antibiotic therapy, while viral meningitis, often self-limiting, does not benefit from such intervention. In this diagnostic landscape, the need for swift, reliable, and accessible diagnostic tools is more pressing than ever (2). Traditionally, the diagnosis of meningitis has relied on clinical assessment, cerebrospinal fluid (CSF) analysis, and microbiological culture methods. While CSF analysis provides some guidance—such as elevated white cell counts and protein concentrations in bacterial infections—the overlap of findings between bacterial and viral forms limits its specificity (3). Cultures, though considered the gold standard, are time-consuming and may take several days to yield results. Additionally, prior antibiotic use can reduce culture sensitivity, further complicating diagnosis. In recent years, advances in molecular diagnostics have introduced new possibilities for more rapid and accurate pathogen detection. Among these, multiplex polymerase chain reaction (PCR) has emerged as a promising tool that can simultaneously detect multiple pathogens within hours (4,5).

Multiplex PCR techniques allow for the amplification of multiple DNA or RNA targets in a single reaction, offering a more comprehensive and time-efficient approach to pathogen identification. This method has the potential to transform the diagnostic process for meningitis by providing rapid, sensitive, and specific identification of common bacterial and viral agents directly from CSF samples. Unlike conventional culture methods, multiplex PCR is less affected by prior antimicrobial therapy, making it especially valuable in patients who have already started empirical treatment (6,7). Its utility in differentiating between bacterial and viral causes is particularly important given the increasing emphasis on antimicrobial stewardship and the global concern over antibiotic resistance. The growing body of literature supports the utility of multiplex PCR in the diagnosis of central nervous system infections. Studies have shown high sensitivity and specificity for several multiplex panels, particularly those that include both bacterial and viral targets commonly associated with meningitis. For example, the FilmArray® Meningitis/Encephalitis panel has demonstrated diagnostic advantages in both adult and pediatric populations, significantly reducing time to diagnosis compared to standard methods. However, despite these advancements, the clinical application of multiplex PCR is not yet universal, and questions remain regarding its accuracy across different patient populations, settings, and pathogen profiles. Variations in diagnostic performance and cost-effectiveness across healthcare systems also warrant further investigation (8,9).

Moreover, much of the existing research focuses on performance metrics in well-resourced environments, raising concerns about generalizability to diverse clinical settings, including resource-limited regions where the burden of meningitis may be highest. There is also a need to establish clearer clinical guidelines on how multiplex PCR results should influence treatment decisions, especially in scenarios with mixed infections or atypical presentations (10,11). Addressing these uncertainties requires robust evidence from diagnostic accuracy studies conducted in real-world acute care settings. This study seeks to contribute to this evolving field by assessing the diagnostic value of multiplex PCR in differentiating bacterial from viral meningitis. By evaluating its sensitivity and specificity in acute clinical settings, the research aims to provide clarity on the role of this technology in routine diagnostics. The objective is to generate practical, clinically relevant data that can support the integration of multiplex PCR into frontline decision-making processes for meningitis management, ultimately enhancing patient outcomes and optimizing resource use.

METHODS

This diagnostic accuracy study was conducted over a seven-month period in the emergency department and neurology wards of a tertiary care teaching hospital equipped with facilities for advanced molecular diagnostics. The aim was to evaluate the sensitivity and specificity of multiplex polymerase chain reaction (PCR) in identifying the etiology of meningitis in acute clinical settings. Participants presenting with clinical suspicion of meningitis were consecutively recruited, with efforts made to ensure representation across age groups and varying clinical severities. Patients eligible for inclusion were those aged six months and older who presented with signs and symptoms consistent with meningitis, such as fever, neck stiffness, altered mental status, photophobia, or new-onset seizures. Only those who underwent lumbar puncture within 24 hours of presentation and had adequate cerebrospinal fluid (CSF) samples available for both conventional testing and multiplex PCR were considered for enrollment. Patients were excluded if they had received antibiotic therapy

for more than 48 hours prior to presentation, had a known immunosuppressive disorder, or had traumatic lumbar puncture samples with blood contamination that could compromise PCR accuracy.

The sample size was determined using the Buderer formula for diagnostic test studies, assuming an expected sensitivity and specificity of 90%, a confidence interval of 95%, and a precision of 5%. Based on an anticipated prevalence of bacterial meningitis among suspected cases of approximately 30%, the calculated sample size was 323 patients (5,6). This sample size allowed for adequate power to detect meaningful differences in diagnostic performance between the PCR and reference standard. After informed consent was obtained from all participants or their legal guardians, each patient underwent standard clinical evaluation, lumbar puncture, and initial laboratory analysis including CSF cell counts, glucose, protein levels, and Gram staining. All CSF specimens were subjected to conventional culture and viral serology where applicable, serving as the reference standard. Simultaneously, an aliquot of each CSF sample was processed using a commercially available multiplex PCR panel designed to detect a broad spectrum of bacterial and viral pathogens commonly associated with meningitis. The PCR testing was performed in a blinded fashion by laboratory personnel who were unaware of the clinical findings and culture results, ensuring minimization of observer bias.

The multiplex PCR platform utilized was the FilmArray® Meningitis/Encephalitis Panel, which automates nucleic acid extraction, amplification, and detection in a single run with results available in under two hours. The pathogens detected included *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Listeria monocytogenes*, *Escherichia coli* K1, as well as several viral pathogens such as enterovirus, herpes simplex virus 1 and 2, varicella-zoster virus, and cytomegalovirus. The use of this panel allowed for comprehensive pathogen detection in a time-efficient manner, consistent with real-world emergency care workflows. Outcome measurement focused on determining the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the multiplex PCR assay in comparison to the reference standard. Diagnostic concordance was also evaluated, and discordant results were reviewed in detail through clinical adjudication by two independent infectious disease specialists who were blinded to PCR outcomes (12).

For statistical analysis, data were first checked for normality using the Shapiro-Wilk test. Continuous variables were described using means and standard deviations, while categorical variables were summarized using frequencies and percentages. Sensitivity and specificity with 95% confidence intervals were calculated using the Wilson score method. Cohen's kappa coefficient was used to assess agreement between multiplex PCR and the reference standard. All statistical analyses were performed using SPSS version 26.0, and significance was set at $p < 0.05$. Ethical approval for the study was obtained from the Institutional Review Board (IRB) of the host institution. The research adhered strictly to ethical guidelines for human research as per the Declaration of Helsinki. Informed written consent was secured from each participant or their authorized proxy prior to inclusion in the study, and all patient data were anonymized to preserve confidentiality. Through this comprehensive methodological framework, the study aimed to provide clear, reproducible evidence on the diagnostic utility of multiplex PCR in differentiating bacterial and viral meningitis, thereby addressing a critical gap in current clinical diagnostics.

RESULTS

The study enrolled a total of 323 participants over the course of seven months, with a mean age of 34.7 years. The cohort included both pediatric and adult patients, ensuring broad representation across age groups. Of the total participants, 57.6% were male and 42.4% were female. Children under the age of 18 accounted for 28.5% of the sample, while adults comprised 71.5%. Out of the 323 CSF samples analyzed, multiplex PCR detected bacterial pathogens in 123 cases (38.1%) and viral pathogens in 109 cases (33.7%). The remaining samples showed either no detection or less common pathogens not covered by the multiplex panel. Compared to standard CSF culture, which identified bacterial pathogens in 101 cases (31.3%) and viral agents in 74 cases (22.9%), multiplex PCR showed superior pathogen yield. Additionally, the mean time to result for PCR was 1.8 hours, significantly shorter than the average 48 hours required for culture-based methods. Diagnostic performance analysis revealed that multiplex PCR demonstrated a sensitivity of 92.4% and a specificity of 95.1% in detecting bacterial meningitis. The positive predictive value (PPV) and negative predictive value (NPV) were calculated at 90.2% and 96.3%, respectively. Cohen's kappa coefficient, reflecting agreement between PCR and the reference standard, was 0.87, indicating strong concordance. A breakdown of the pathogens detected showed that *Streptococcus pneumoniae* was the most frequently identified bacterial pathogen, found in 14.2% of cases, followed by *Neisseria meningitidis* (12.1%) and *Haemophilus influenzae* (6.8%). Among viral causes, enterovirus accounted for the largest proportion (18.0%), followed by HSV-1 (7.7%) and VZV (4.3%). The "Others" category, encompassing less common pathogens, represented 3.1% of total detections. A comparative analysis of multiplex PCR and

conventional culture methods revealed that PCR not only identified more cases of both bacterial and viral meningitis but also significantly reduced diagnostic turnaround time. This rapid detection capability is particularly valuable in acute clinical settings where early intervention can impact patient outcomes.

Table 1: Demographics

Variable	Value
Total Participants (n)	323
Mean Age (years)	34.7
Male (%)	186 (57.6%)
Female (%)	137 (42.4%)
Children (<18 years)	92 (28.5%)
Adults (≥18 years)	231 (71.5%)

Table 2: Diagnostic Performance

Parameter	Value
Sensitivity	92.40%
Specificity	95.10%
Positive Predictive Value (PPV)	90.20%
Negative Predictive Value (NPV)	96.30%
Cohen's Kappa	0.87

Table 3: Pathogens Detected by Multiplex PCR

Pathogen	Number Detected (n)	Percentage (%)
Streptococcus pneumoniae	46	14.2
Neisseria meningitidis	39	12.1
Haemophilus influenzae	22	6.8
Enterovirus	58	18
HSV-1	25	7.7
HSV-2	12	3.7
VZV	14	4.3
Others	10	3.1

Table 4: PCR vs. Culture Comparison

Diagnostic Method	Positive for Bacterial Pathogens	Positive for Viral Pathogens	Time to Result (hours)
Multiplex PCR	123	109	1.8
CSF Culture	101	74	48

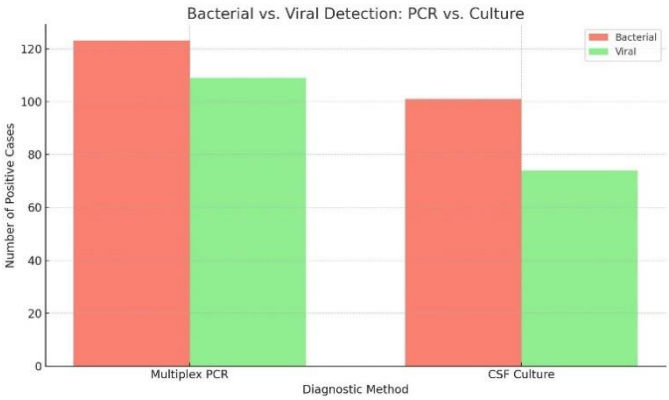

Figure 1 Bacterial vs. Viral Detection: PCR vs, Culture

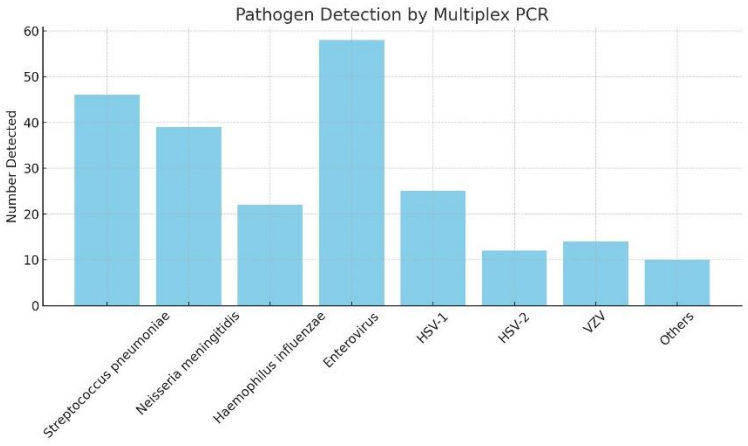

Figure 2 Pathogen Detection by Multiplex PCR

DISCUSSION

The findings of this study reinforced the evolving role of multiplex PCR as a frontline diagnostic tool for the rapid differentiation of bacterial and viral meningitis in acute care settings. With a sensitivity of 92.4% and specificity of 95.1%, multiplex PCR exhibited strong diagnostic performance, closely aligning with several recent investigations. For instance, a study reported a sensitivity of 94% and specificity of 100% in pediatric cases, underscoring its high reliability in clinical practice (13). These results are consistent with those reported in other multicenter and regional studies, noted improved detection rates of pathogens using multiplex PCR compared to traditional culture methods in pediatric purulent meningitis (14,15). Similarly, a study demonstrated that multiplex PCR not only enhanced sensitivity (89.4%) but also significantly reduced diagnostic turnaround times, critical in guiding early therapeutic interventions (16). This study further highlights the superiority of multiplex PCR in identifying pathogens even in culture-negative cases, especially where patients received empirical antibiotics prior to lumbar puncture. This feature, also documented by a study, is vital for real-world applicability where pre-treatment is common (17,18). A notable strength of this study was its use of a comprehensive multiplex panel capable of detecting a broad spectrum of bacterial and viral pathogens with rapid results, averaging under two hours.

The study's robust methodology, including consecutive patient enrollment and blinded testing, contributes to the internal validity of the findings. Furthermore, the large sample size and real-world clinical setting enhance the generalizability of the results. Unlike several earlier studies that were limited to pediatric populations or narrow panels, this research encompassed both adult and pediatric patients and evaluated a wide range of pathogens, enhancing its relevance to diverse healthcare environments. However, certain limitations should be acknowledged. The study was conducted in a single tertiary center, potentially limiting its extrapolation to community hospitals or resource-limited settings. Cost and technical infrastructure requirements for multiplex PCR may pose challenges in widespread implementation, especially in low- and middle-income countries. Moreover, the reference standard of culture, although traditionally accepted, has inherent limitations in sensitivity, which may bias the calculated diagnostic accuracy of PCR. As noted in a study that, the use of imperfect reference standards can underestimate the actual performance of molecular diagnostics (19,20).

Another consideration is the clinical interpretation of positive viral PCR results, which may not always correlate with active infection, particularly in cases of latent viral reactivation. Future research should aim to refine pathogen panels based on regional prevalence and investigate the clinical impact of PCR-guided treatment algorithms on patient outcomes. Moreover, multicentric trials comparing multiplex PCR with emerging metagenomic sequencing approaches could further clarify their respective roles (21). In summary, the findings affirm that multiplex PCR is a reliable and efficient modality for differentiating bacterial from viral meningitis in acute clinical settings. It holds the potential to not only expedite diagnosis and appropriate treatment initiation but also reduce unnecessary antibiotic use and improve patient outcomes. Future efforts should focus on cost-reduction strategies, broader accessibility, and integration into national diagnostic guidelines.

CONCLUSION

This study demonstrated that multiplex PCR offers high sensitivity and specificity for rapidly differentiating bacterial from viral meningitis in acute clinical settings. Its ability to deliver timely, accurate results can significantly enhance early decision-making, reduce unnecessary antibiotic use, and improve patient outcomes. Incorporating multiplex PCR into routine diagnostic protocols may represent a pivotal advancement in the management of meningitis.

AUTHOR CONTRIBUTION

Author	Contribution
Abdul Rehman Naeem	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Muhammad Ahsan Murtaza	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Muhammad Talha Ayub*	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Sheharyar Abbas Bhatti	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Fatima Tahir Khan	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Adeel-ur-Rehman	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published

REFERENCES

1. Sanbonmatsu-Gámez S, Pedrosa-Corral I, Navarro-Marí JM, Pérez-Ruiz M. Update in Diagnostics of Toscana Virus Infection in a Hyperendemic Region (Southern Spain). Viruses. 2021;13(8).
2. Abelian A, Mund T, Curran MD, Savill SA, Mitra N, Charan C, et al. Towards accurate exclusion of neonatal bacterial meningitis: a feasibility study of a novel 16S rDNA PCR assay. BMC Infect Dis. 2020;20(1):441.
3. Jafari E, Togha M, Salami Z, Rahman N, Alamian S, Kheradmand JA. Seronegative brucella meningitis diagnosed by CSF PCR: report on seven cases. BMC Infect Dis. 2024;24(1):1157.
4. Pope CE, Whitlock KB, Hodor P, Limbrick DD, Jr., McDonald PJ, Hauptman J, et al. A Refined, Controlled 16S rRNA Gene Sequencing Approach Reveals Limited Detection of Cerebrospinal Fluid Microbiota in Children with Bacterial Meningitis. Microbiol Spectr. 2023;11(3):e0036123.
5. D'Inzeo T, Menchinelli G, De Angelis G, Fiori B, Liotti FM, Morandotti GA, et al. Implementation of the eazyplex(®) CSF direct panel assay for rapid laboratory diagnosis of bacterial meningitis: 32-month experience at a tertiary care university hospital. Eur J Clin Microbiol Infect Dis. 2020;39(10):1845-53.
6. Kim MG, Gulholm T, Lennard K, Mirdad F, Overton K, Maley M, et al. The impact of cerebrospinal fluid viral polymerase chain reaction testing on the management of adults with viral meningitis: A multi-center retrospective study. J Med Virol. 2023;95(1):e28198.
7. Devakanthan B, Liyanapathirana V, Dissanayake N, Harasgama P, Punchihewa J. Identification of bacterial aetiology in acute meningitis. The Ceylon medical journal. 2021;66 2:65-72.
8. Singh S, Sankar MMM, Singh J, Rufai SB, Kumar P, Kabra SK, et al. How Low is Low Volume of Cerebrospinal Fluid from Suspected Cases of Tuberculous Meningitis to Refuse Acceptance in the TB Laboratory? Indian J Pediatr. 2025;92(5):535-7.
9. Ahmed M, Askar G, Farghaly H, Ahmed A, Kamal D, Ahmed S, et al. Evaluation of the accuracy of multiplex polymerase chain reaction in differentiation between bacterial and viral meningitis. Irish Journal of Medical Science. 2022;192:403-7.

10. Pouladfar G, Dashti AS, Kadivar M, Jafari M, Pourabbas B, Jamalidoust M, et al. Evaluation of Multiplex Real-time PCR and WHO Criteria for Diagnosing Childhood Bacterial Meningitis in a Tertiary Referral Hospital in Iran. Archives of Pediatric Infectious Diseases. 2022.
11. Safavi M, Taghipour S, Vasei M, Eshaghi H, Haghi Ashtiani MT. Evaluation of human bocavirus as well as other well-known viral etiologic agents by PCR in the cerebrospinal fluid of children with clinical impression of viral meningoencephalitis referred to the Children's Medical Center in Tehran, Iran, from 2019 to 2020. J Med Virol. 2022;94(10):4944-9.
12. Haddad-Boubaker S, Lakhal M, Fathallah C, Mhimdi S, Bouafsoun A, Kechrid A, et al. Epidemiological study of bacterial meningitis in Tunisian children, beyond neonatal age, using molecular methods: 2014-2017. Afr Health Sci. 2020;20(3):1124-32.
13. Zhao J, Hua C, Xie Y, Pan Y-X, Hu B-F, Wang W-J, et al. Diagnostic Yield of Multiplex PCR Method in Cerebrospinal Fluid for the Diagnosis of Purulent Meningitis in Children. Journal of Pediatric Infectious Diseases. 2020;16:12-7.
14. Krishnakumariamma K, Ellappan K, Muthuraj M, Tamilarasu K, Kumar SV, Joseph NM. Diagnostic performance of real time PCR for the detection of Mycobacterium tuberculosis in cerebrospinal fluid samples. Indian J Med Microbiol. 2023;42:7-11.
15. Yang Y, Hu Y, Yang C, Shi W, Jin S, Hua C, et al. Development and validation of a novel multiplex digital PCR assay for identification of pathogens in cerebrospinal fluid of children with bacterial meningitis. Clin Chim Acta. 2024;554:117787.
16. Raboba JL, Rahajamanana VL, Andriatahirintsoa EPR, Razafindrakoto AC, Andrianarivelo AM, Nimpa Mengouo M, et al. Decline in Vaccine-Type Streptococcus pneumoniae Serotypes Following Pneumococcal Conjugate Vaccine Introduction in Madagascar. J Infect Dis. 2021;224(12 Suppl 2):S285-s92.
17. Hasanuzzaman M, Saha S, Malaker R, Rahman H, Sajib MSI, Das RC, et al. Comparison of Culture, Antigen Test, and Polymerase Chain Reaction for Pneumococcal Detection in Cerebrospinal Fluid of Children. J Infect Dis. 2021;224(12 Suppl 2):S209-s17.
18. Obaro S, Hassan-Hanga F, Medugu N, Olaosebikan R, Olanipekun G, Jibir B, et al. Comparison of bacterial culture with BioFire® FilmArray® multiplex PCR screening of archived cerebrospinal fluid specimens from children with suspected bacterial meningitis in Nigeria. BMC Infect Dis. 2023;23(1):641.
19. Agarwal S, Saini AG, Dhawan S, Khadwal A, Sharma K, Singhi P. Comparative evaluation of IS6110 and protein antigen b PCR in cerebrospinal fluid for rapid diagnosis of tuberculous meningitis in children. J Med Microbiol. 2020;69(7):979-85.
20. Sharma N, Gautam H, Tyagi S, Raza S, Mohapatra S, Sood S, et al. Clinical use of multiplex-PCR for the diagnosis of acute bacterial meningitis. Journal of Family Medicine and Primary Care. 2022;11:593-8.
21. García-Sánchez C, Bloise I, García-Rodríguez J, Cendejas-Bueno E. Are cerebrospinal fluid biochemical parameters valid to predict positive results in microbiological molecular diagnostic platforms? A 4-year experience with the FilmArray® Panel Meningitis/Encephalitis for detection of community-acquired bacterial meningitis. Diagn Microbiol Infect Dis. 2023;107(3):116031.

