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BioFire FilmArray Meningitis/Encephalitis Panel for Rapid Diagnosis of Central Nervous System Infections: Retrospective study from a tertiary Care Centre in South India

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ABSTRACT

Introduction: Rapid identification of the underlying cause of meningoencephalitis (ME) is essential for starting appropriate treatment to improve patient outcomes and reduce morbidity and mortality. The Film Array Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid-based diagnostic test capable of simultaneous and rapid detection of 14 pathogens directly from CSF specimens. While this test offers significant benefits in terms of speed and accuracy, studies evaluating its effectiveness in diagnosing ME in Indian patients are limited **Materials and Methods:** We conducted a retrospective analysis of patients admitted to a tertiary care hospital in South India with meningitis/encephalitis syndrome who underwent the Biofire film array test on cerebrospinal fluid (CSF) from January 2023 to August 2024. CSF samples were also subjected to bacterial cultures. The performance of the BioFire Film Array was compared with CSF bacterial culture. **Results:** A total of 251 patients with suspected ME syndrome underwent the BioFire FilmArray® Meningitis/Encephalitis test. Pathogens were detected in 39 (15.5%) of these patients. Bacterial agents were identified in 22 patients, while 16 had viral infections. One patient had Cryptococcal meningitis. Streptococcus pneumoniae was the most common, found in 12 patients. Other notable pathogens included Streptococcus agalactiae (7 patients) and enterovirus (6 patients), and Varicella zoster virus (5 patients). Herpes simplex virus (3 patients) and Escherichia coli K2 was detected in 2 samples. Other pathogens detected were Cryptococcus neoformans (1), Cytomegalovirus (1), Haemophilus influenzae (1), and Human herpesvirus 6 (1).Only 5 (1.9%) samples grew organisms, including Streptococcus pneumoniae (2), Streptococcus agalactiae (2), and Cryptococcus neoformans (1). **Conclusion:** The BioFire Meningitis Panel greatly enhances diagnostic yield over traditional bacterial culture methods. This enables the prompt initiation of targeted antibiotic therapy, leading to better patient outcomes.

1. INTRODUCTION:

Acute central nervous system (CNS) infections are among the most serious clinical conditions in medicine. Bacterial meningitis is associated with morbidity due to neurological complications and also mortality in both children and adults[1,2]. Streptococcus pneumoniae and Neisseria meningitidis are responsible for approximately 80% of the cases of bacterial meningitis globally. Enteroviruses are currently the leading recognizable cause of aseptic meningitis syndrome accounting for 85–95% of all pathogens identified [1].

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Traditional diagnostic workup includes analysis of cerebrospinal fluid (CSF) for white blood cell counts (WBC), glucose and protein levels, Gram staining and CSF culture, for identification of either bacterial or fungal Meningoencephalitis (ME), while molecular methods are usually applied for detection of viral ME [3-5]. The conventional methods have limitations in sensitivity and specificity, while the culture can take several days to result. Due to the potential adverse consequences of delaying the diagnosis or treatment, patients suspected of having ME are often hospitalized and started on empiric antimicrobial treatment while awaiting CSF cultures [3,4].

Polymerase chain reaction (PCR)-based methods can improve the process of identifying viral or bacterial pathogens in the CSF by increasing the diagnostic yield, improve care, optimize antibiotic utilization and providing faster turnaround times. Rapid identification of the underlying cause of meningoencephalitis (ME) is essential for starting appropriate treatment to improve patient outcomes and reduce morbidity and mortality. In recent years, there has been a shift in infectious disease diagnostics from a conventional to a syndromic approach which is based on molecular assays that simultaneously detect the presence of several pathogens (including bacteria, viruses, fungi, and parasites) within a short time [1,4,6] .

The Film Array Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid-based diagnostic test capable of simultaneous and rapid detection of 14 pathogens directly from CSF specimens which include 7 viruses, 6 bacteria, and 1 fungus. The pathogens included in the ME panel are Escherichia coli K1, Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus pneumoniae, cytomegalovirus (CMV), enterovirus (EV), herpes simplex virus 1 (HSV-1), HSV-2, human herpesvirus 6 (HHV-6), human parechovirus (HPeV), varicella-zoster virus (VZV) and Cryptococcus neoformans/Cryptococcus gattii in CSF [7-9] .

This study was undertaken to determine the clinical usefulness of the BioFire® FilmArray® for the simultaneous detection of common pathogens associated with meningitis in CSF samples of suspected cases of meningitis and to compare the yield from standard bacterial culture with the BioFire® FilmArray® Meningitis/Encephalitis Panel

MATERIAL AND METHODS:

This study was reviewed and approved by the Institutional ethics committee (IEC-NI/25/08/107/162)

This retrospective cross-sectional study was carried out at a tertiary care university teaching hospital during the period from January 2023 to August 2024. Medical records were reviewed for both adult and paediatric patients admitted with a meningitis/encephalitis syndrome who had undergone multiplex PCR testing on cerebrospinal fluid (CSF). In total, 251 CSF samples were included for analysis.

The diagnostic performance of the FilmArray Meningitis/Encephalitis (FA-ME) panel was evaluated by comparing its results with routine CSF investigations, including cell count, biochemistry, Gram's stain, bacterial culture, and other relevant tests. Patient information such as age, sex, clinical history, and examination findings were collected from the clinical records department.

CSF parameters were assessed in detail, including total and differential cell counts, protein, sugar, Gram's stain, and special stains such as acid-fast bacilli (AFB) stain, India ink, and KOH stain. Results of CSF culture and antimicrobial susceptibility testing for bacterial and fungal pathogens were also reviewed. The CSF FA-ME panel (BioFire Diagnostics) findings were then noted and compared with standard microbiological methods to determine the diagnostic yield of the FA-ME panel relative to routine testing and to assess its clinical utility.

BioFire® FilmArray® Meningitis/Encephalitis Panel [1,7]. The steps are depicted in figure -1 and the organisms in the panel are listed in Figure-2

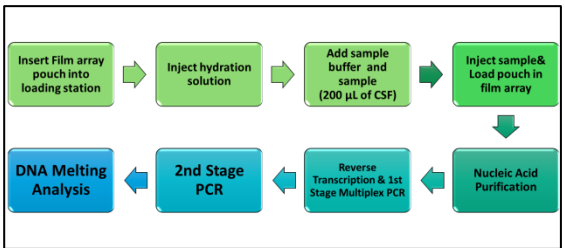

Figure-1 : Steps of the BioFire® FilmArray® Meningitis/Encephalitis Panel

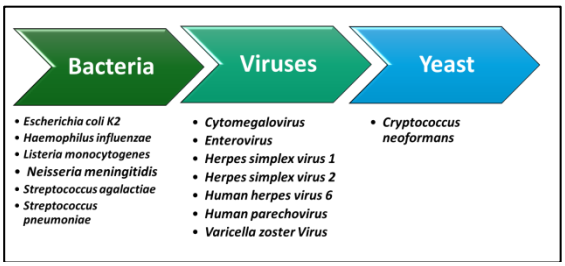

Figure-2 : Organisms include in the Biofire ME panel

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Nucleic Acid Purification - Nucleic acid purification occurs in the first three blisters of the pouch. The sample is lysed by agitation (bead beating) and the liberated nucleic acid is captured, washed and eluted using magnetic bead technology.

Reverse Transcription and 1st Stage Multiplex PCR - Some pathogens identified by the FilmArray ME pouch are RNA viruses, and a reverse transcription (RT) step is performed to convert the viral RNA into cDNA prior to amplification. The purified nucleic acid solution is combined with a preheated master mix to initiate the RT step and subsequent thermocycling for multiplex PCR. The effect of 1st stage PCR is to enrich for the target nucleic acids present in the sample

2nd Stage PCR - The products of 1st stage PCR are diluted and mixed with fresh PCR reagents containing an intercalating fluorescent DNA dye (LCGreen® Plus, BioFire Defense, LLC). This solution is distributed over the 2nd stage PCR array. The individual wells of the array contain primers for different assays (each present in triplicate) that target specific nucleic acid sequences from each of the pathogens detected, as well as control template material. These primers are 'nested' or internal to the specific products of the 1st stage multiplex reaction, which enhances both the sensitivity & specificity of the reactions

DNA Melting Analysis – After 2nd stage PCR, the temperature is slowly increased and fluorescence in each well of the array is monitored and analyzed to generate a melt curve. The temperature at which a specific PCR product melts (melting temperature or Tm) is consistent and predictable and the FilmArray software automatically evaluates the data from replicate wells for each assay to report results.

Statistical analysis: Descriptive statistics of all measured variables and parameters were tabulated.

RESULTS:

During the study period, 251 patients suspected of meningoencephalitis underwent CSF analysis using the BioFire FilmArray. The average turnaround time for the ME panel was approximately 2 hours. The age of patients ranged from neonates (<4 weeks of age) to 70 years.

The FilmArray Meningitis/Encephalitis Panel was positive in 39/251 (15.5%) patients (Figure-3). Among these 39 patients, 14 belonged to the pediatric age group. Bacterial agents were detected in 22 patients, viral pathogens in 16 patients, and one patient had cryptococcal meningitis. (Figure-4)

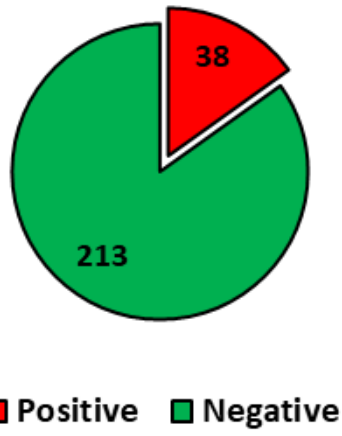

Figure-3 : Biofire Meningitis/Encephalitis Panel Positive

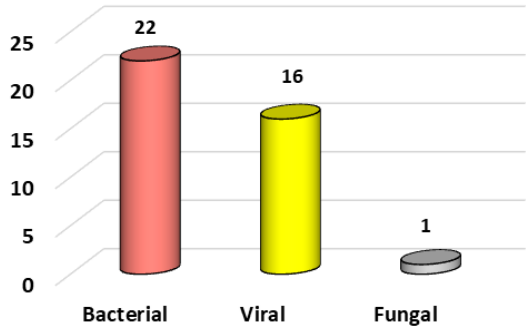

Figure-4: Distribution of Meningitis/Encephalitis Panel positives

Among bacterial pathogens, *Streptococcus pneumoniae* (12/22) was the most common, followed by *Streptococcus agalactiae* (7/22). Other bacterial pathogens included *Haemophilus influenzae* (1/22) and *E. coli* K1 (2/22). Among viral pathogens, enterovirus (6/16) was the most common, followed by varicella-zoster virus (5/16). The other viruses detected were HSV-1 (3/16), CMV (1/16), and HHV-6 (1/16). The distribution of organism detected using Biofire ME panel is depicted in Figure-4 and 5

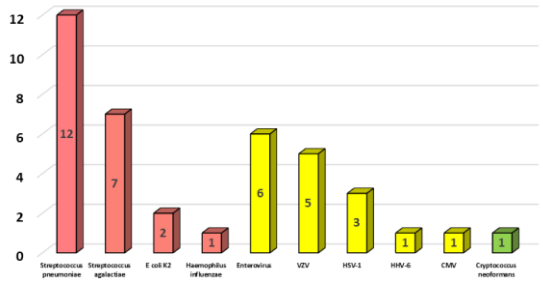

Figure-5 : Organisms distribution in Meningitis/Encephalitis Panel positive cases

CSF cell counts were elevated in all 39 positive patients. Clinical history and CSF parameters, including glucose and protein levels, correlated with the test results in all 39 patients. With appropriate antibiotics, all 39 patients recovered. In

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a subset of patients (11) with elevated CSF cell counts, no organisms were detected by the BioFire panel, and CSF culture was also negative. These patients were still treated as CNS infections despite negative FA-ME panel results.

In 201 patients suspected of meningitis who presented with altered sensorium and/or fever, CSF cell counts were within normal limits. The alternative diagnoses in these patients included autoimmune, malignant, metabolic, and vascular etiologies.

The yield of CSF bacterial culture was low, being positive in only 5 patients (1.9%) with suspected meningitis, and these results matched the FA-ME panel. The organisms isolated on culture were *Streptococcus pneumoniae* (2), *Streptococcus agalactiae* (2), and *Cryptococcus neoformans* (1). [Figure-6]

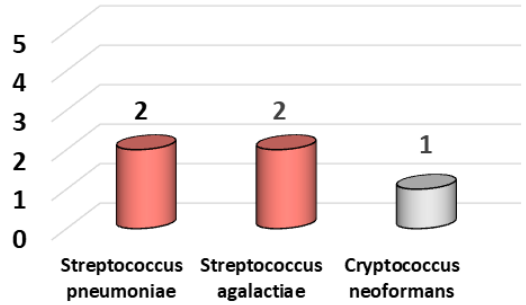

Figure-6: CSF culture growth

DISCUSSION:

Diagnostic Yield

The results of the current study demonstrate that the BioFire FilmArray Meningitis/Encephalitis (FA-ME) panel is a rapid and highly effective tool for diagnosing central nervous system (CNS) infections, significantly outperforming traditional culture methods. By detecting pathogens in 15.5% (39/251) of suspected cases with a turnaround time of approximately two hours, the panel facilitates timely clinical intervention compared to conventional cultures, which can take several days to yield results.

This yield is comparable with published studies from India including a tertiary-care study from Kerala, with 23.6% and 10.4% in New Delhi [1,2]. Globally the positivity ranged from 6.4% in USA to 21.6% in Israel (21.6%) and 26.8% in Spain (26.8%) [6,7,10]. Such variation across studies likely reflects differences in true CNS infection probability, pre-test clinical selection, timing of lumbar puncture, and local epidemiology. Importantly, the rapidity of FA-ME testing is consistent with other evaluations showing the panel produces results within about two hours supporting its value in early decision-making [11].

Pathogen Distribution: Bacteria vs Viruses

In the current study, bacterial agents (22/39, 56.4%) were more prevalent than viral pathogens (16/39, 41%), with *Streptococcus pneumoniae* being the most common bacterial isolate and enterovirus and varicella-zoster virus among the common viral causes. *Streptococcus pneumonaie* was also the most common in studies from China(11/26), Morraco (8/68), USA (41/132) and Sweden (33/315) [11-14]. In both the Indian studies enterovirus was the leading detection, followed by VZV and pneumococcus among bacterial causes [1,2] .

Similarly in Israel, the panel findings were predominantly viral, with enterovirus comprising 62% of positives [7]. Furthermore, the detection of Varicella-zoster virus (VZV) as the second most common virus (5/16) in the present study , mirrors the Israeli adult cohort [7]. Barcelona data also highlight viral predominance (HSV-1 and VZV) with a smaller contribution from pneumococcus [10]. In contrast, a U.S. inpatient series reported HHV-6 and VZV as the most frequent detections and a Swedish evaluation showed frequent VZV detections and notable HHV-6 positivity [3,6,8]. These contrasts emphasize that pathogen distribution is strongly influenced by regional epidemiology , viral circulation, and vaccination coverage. However, the current study aligns more closely with the Nigerian pediatric cohort, which also reported a high burden of bacterial pathogens, specifically *S. pneumoniae* [5] .

A significant observation in our study was the low yield of conventional CSF culture (1.9%) compared with the molecular panel. This aligns with published evidence that BioFire substantially increases diagnostic yield over standard methods: Nigeria reported conventional detection of 9.3% versus 26.8% with BioFire and in Israel, only 1/9 (11.1%) bacterial detections were confirmed by CSF culture [5,7] . Similarly, among FilmArray bacterial positives in a Brazilian cohort, CSF culture positivity was only 24%, with many detections being FilmArray-only [11].

These findings support the role of syndromic PCR in improving early etiologic diagnosis, particularly in settings where antibiotics may be initiated before lumbar puncture or where pathogen load is low, while emphasizing the need to interpret results in conjunction with CSF parameters and clinical syndrome [4,14,15,16].

CSF Parameters:

A notable observation in the current results was that all 39 positive patients had elevated CSF cell

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counts (pleocytosis) and correlated biochemical markers. This strong correlation supports findings in Spain and New York, where the positive predictive value (PPV) of the FA-ME panel was found to be significantly higher in the presence of pleocytosis. Conversely, the sources warn that the PPV can drop to as low as 36.9% in cases without pleocytosis, where positive results often represent clinically insignificant incidental findings or latent virus reactivation (e.g., HHV-6) [9,10,15].

The current study noted 11 patients with elevated CSF cell counts who were treated for CNS infections despite negative results. This highlights a limitation also discussed by Chandran et al., who noted the panel does not include nosocomial pathogens (e.g., *Klebsiella*, *Pseudomonas*) and regional infections like tuberculosis (TB), scrub typhus, and dengue. Meta-analyses further suggest that while the panel is excellent for "ruling in" pathogens, its sensitivity for *Listeria monocytogenes*, *H. influenzae*, and *E. coli* may be suboptimal [1, 4].

An important limitation of Biofire ME panel is cost, especially in a developing country. Although the direct cost of the analysis is higher than that of routine methods, several studies have confirmed that this technique can be used to detect a wide range of pathogens, allows for shorter hospital stays, allows for more frequent use of narrow-spectrum targeted antibiotic therapy, and reduces the number of prophylactic treatments initiated [1,12].

The current study also identified 201 patients with normal cell counts who were ultimately diagnosed with alternative causes, including autoimmune, malignant, metabolic, and vascular conditions. This matches findings in a Swedish study where 86.1% of samples came from patients not diagnosed with CNS infection; their common alternative diagnoses included primary headache disorders, seizures, cranial nerve palsy and neuroinflammatory disorders [6,15].

CONCLUSION:

The current results confirm that the BioFire FA-ME panel is a robust diagnostic adjunct that improves pathogen identification and supports antimicrobial stewardship by enabling rapid, targeted therapy. Though the test cannot replace clinical judgment or conventional methods, as evidenced by the cases of culture-negative pleocytosis and alternative non-infectious diagnoses, Biofire FA-ME must be integrated into a comprehensive clinical algorithm. Clinicians must continue to interpret results within the broader context of biochemical analysis, local epidemiology, and patient history.

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