Diagnostic Accuracy of the FilmArray™ Meningitis/Encephalitis Panel in Adult Patients with Suspected Bacterial Meningitis in a Tertiary Care Hospital in the Philippines

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ABSTRACT

Objective. Bacterial meningitis is associated with significant morbidity and mortality if not diagnosed and treated early. Isolation of the causative agent from cerebrospinal fluid culture is the gold standard for the diagnosis of this condition; however, it takes several days for results to be available. The FilmArray™ Meningitis/Encephalitis (ME) panel is a nucleic acid-based test that allows simultaneous detection of 14 bacterial, viral, and fungal pathogens in the cerebrospinal fluid with a rapid turnaround time. Our aim was to evaluate the diagnostic performance of the ME panel in detecting bacterial pathogens in the cerebrospinal fluid of adult patients with suspected bacterial meningitis in a tertiary hospital in the Philippines.

Methods. We performed a retrospective review of hospital records of adult patients with suspected bacterial meningitis who were admitted at our institution and underwent diagnostic testing with the FilmArray™ ME panel from January 1, 2018 to July 31, 2019. Overall percent agreement, sensitivity, and specificity for individual bacterial pathogens included in the panel were determined.

Results. A total of 88 cerebrospinal fluid samples were included in the analysis of diagnostic accuracy. The ME panel demonstrated 93.2% overall agreement, 50% sensitivity for *E. coli*, and 99–100% specificity in comparison with CSF culture in detecting bacterial pathogens that are included in the ME panel.

Conclusion. The results show that the FilmArray™ ME panel has high diagnostic accuracy and can be utilized in the rapid diagnosis and targeted treatment of patients with suspected bacterial meningitis.

Keywords: bacterial meningitis, FilmArray, diagnostics, CNS infections, ME panel

INTRODUCTION

Bacterial meningitis is an inflammatory condition involving the meninges of the brain and subarachnoid space. Worldwide, the most common bacterial pathogens causing meningitis are *Streptococcus pneumoniae*, Group B *Streptococcus*, *Neisseria meningitidis*, *Haemophilus influenzae* and *Listeria monocytogenes.*[1] Approximately 15% of cases are fatal and others result in permanent neurologic sequelae such as cognitive deficits, vision and hearing impairment, motor and sensory deficits and epilepsy.[2] Therefore, delays in the diagnosis and treatment of are associated with prolonged length of hospital stay, costs, and significant morbidity and mortality. On the other hand, unnecessary initiation of antimicrobial therapy is associated with antibiotic resistance and increased healthcare costs.[3]

The gold standard for the diagnosis of bacterial meningitis is the isolation of the causative agent via cerebro-

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spinal fluid (CSF) culture.[4] However, the turnaround time is a few days and may be falsely negative if there is prior antibiotic administration, the specimen is handled incorrectly, or the causative organism is fastidious and does not grow in conventional culture media.[5] The FilmArray™ Meningitis/Encephalitis (ME) panel (BioFire Diagnostics, Salt Lake City, Utah) is a molecular diagnostic test that uses a proprietary multiplex polymerase chain reaction (PCR) system for the simultaneous detection of 14 pathogens, including bacteria (*Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Listeria monocytogenes, Streptococcus agalactiae, Escherichia coli*), viruses (herpes simplex virus 1, herpes simplex virus 2, varicella zoster virus, cytomegalovirus, human herpes virus 6, enterovirus) and *Cryptococcus neoformans/gatti* with results available within an hour.[6] Since the ME panel was approved by the US Food and Drug Administration in 2015, various studies have evaluated its performance in different countries, with the overall agreement rate of CSF culture and the panel to be between 93%-99% with high sensitivity and specificity.[6-8] However, there is paucity of studies conducted in low-income countries and in resource-limited settings, where there is a higher incidence of bacterial meningitis. The aim of our study is to evaluate the diagnostic accuracy of the FilmArray™ ME panel in detecting bacterial pathogens in the CSF of patients with suspected bacterial meningitis admitted at a tertiary care hospital in the Philippines.

MATERIALS AND METHODS

Study design

In this retrospective chart review, we reviewed records of patients who were tested with FilmArray™ ME panel at the St. Luke's Medical Center Quezon City and Global City from January 1, 2018 to July 31, 2019. Adult patients (≥ 19 years) admitted at the wards or intensive care unit who exhibited clinical symptoms of central nervous system infection (fever, headache, stiff neck, seizures, behavioral changes or altered sensorium) and had their CSF tested with the FilmArray™ ME panel were included in this study. Patients whose CSF were not tested with FilmArray™ and not sent for CSF culture were excluded from the study.

Microbiological methods (CSF culture)

A portion of the CSF sample obtained from the patient was inoculated into blood agar plate (BAP), chocolate agar plate (CAP) and MacConkey agar plate (MAP). The BAP and CAP plates were incubated in an incubator at 35°C to 37°C and MacConkey agar at 37°C. Growth of organisms was observed until 3 days.

FilmArray™ Meningitis/Encephalitis Panel

Testing by the FilmArray™ ME panel was performed in accordance with the manufacturer's instructions for use: 200 µl of cerebrospinal fluid and hydration solution was drawn into the FilmArray™ ME reagent pouch by vacuum. The reagent pouch was then placed in the FilmArray™ instrument and the sample was tested.

Data Analysis

Patient hospital and medical records were reviewed and the following data were extracted: demographics (age, sex), clinical presentation, neuroimaging results, CSF chemistry (protein, glucose), CSF cell counts, CSF culture and FilmArray™ ME panel results. The results of the FilmArray™ ME panel were considered true positive or true negative if they agreed with the result of CSF culture. Percent agreement, sensitivity and specificity for overall and for each individual bacterial pathogen included in the panel were calculated using standard methods. Results were considered discordant when the result of the FilmArray™ ME assay did not agree with that of routine testing for the specific target represented on the panel. Data gathered were analyzed using percentage and proportion represented in tables.

RESULTS

A total 98 CSF samples collected between January 1, 2018 and July 31, 2019 from adult patients admitted in the medical wards or intensive care unit who showed clinical symptoms of bacterial meningitis were analyzed using the FilmArray™ ME panel. Among the patients, 55 (56%) were female and the median age was 51.5 years (range, 19 to 96). The most common symptoms exhibited by the patients were: (1) headache; (2) altered sensorium and; (3) seizures (Table 1).

A total of 12 (12%) CSF specimens tested positive with ME panel while 86 specimens (88%) were negative. Among the 12 specimens that tested positive in the ME panel, bacteria were detected in 2 (17%) samples, viruses in 8 (66%) samples and *Cryptococcus sp.* in 2 (17%) samples (Table 2). There were no cases where multiple infectious pathogens were detected in a single CSF sample. The clinical data and course for these patients are indicated in Table 3.

Table 1. Demographic data of subjects

Characteristics	n (%)
Total Number of Patients	98
Gender	
Male	43 (44%)
Female	55 (56%)
Median Age (years)	51.5
19 – 35	28 (29%)
36 – 50	19 (19%)
51 – 65	25 (26%)
66 – 80	17 (17%)
> 80	9 (9%)
Initial Presentation	
Headache	25 (26%)
Altered sensorium	23 (24%)
Seizures	19 (19%)
Behavioral changes	11 (11%)
Others (e.g., neck stiffness)	20 (20%)

For determination of diagnostic accuracy, samples that tested positive for viruses and *Cryptococcus sp.* using the ME panel were excluded since viral culture and cryptococcal culture (the gold standard tests for viral meningoencephalitis and cryptococcal meningitis, respectively) were not performed. A total of 88 samples were included and among these, there were 82 concordant results, 1 false positive and 5 false negative result with an overall agreement of 93.2% (95% CI 85.9 % – 96.8%) (Table 4). Analyzing the diagnostic accuracy of the ME panel across the individual bacterial pathogens, the sensitivity was 50% (95% CI 48% - 52%) for *E. coli* (sensitivity not available for the rest of the organisms) while the specificity ranged from 98.8 % - 100% (95% CI 98.7% - 100%) for the different bacterial pathogens (Table 5).

In one sample, both the ME Panel and CSF culture were able to detect the same bacterial pathogen (*E. coli*). There was one false positive result in which the ME panel detected *Streptococcus pneumoniae* but there was no growth of any organism in CSF culture. There was one false negative result

Table 2. Causative organisms detected by the FilmArray™ ME Panel

Causative Organisms	n	%
Bacteria	2	17%
Streptococcus pneumoniae	1	
Escherichia coli	1	
Viruses	8	66%
Herpes simplex (HSV) 1 & 2	4	
Varicella zoster (VZV)	2	
Enterovirus	1	
Human herpesvirus 6 (HHV6)	1	
Cryptococcus sp.	2	17%

Table 3. Clinical course of patients who tested positive for pathogens detected by FilmArray™ ME panel

Age/Sex	Chief Complaint	Pathogen Detected by FilmArray™ ME panel	Other tests	Clinical Course	Outcome
77/Male	Altered sensorium, fever	*Streptococcus pneumoniae*	CSF culture: no growth MRI: subacute infarcts on left MCA territory	Patient started on IV Meropenem	Expired
61/Male	Behavioral changes	*Escherichia coli*	CSF culture: *E. coli* Phadebact: *E. coli* MR: ventriculitis	Started on IV Ceftriaxone; referred to IDS; Prolonged course of treatment	Improved but prolonged hospital stay
44/Female	Headache, body malaise	Herpes simplex virus 1 (HSV-1)	CSF culture: no growth	IV acyclovir; supportive treatment	Improved
55/Female	Headache	Herpes simplex virus 1 (HSV-1)	CSF culture: no growth MRI: gyral enhancement	IV acyclovir	Improved
20/Male	Headache	Enterovirus	CSF culture: no growth CT scan: unremarkable	Supportive treatment; no anti-viral started	Improved
18/Female	Headache	Human herpes virus 6 (HHV-6)	CSF culture: no growth MRI: unremarkable	Supportive treatment; no anti-viral started	Improved
50/Male	Headache	Herpes simplex virus 2 (HSV-2)	CSF culture: no growth CT scan: unremarkable	IV acyclovir; supportive treatment	Improved
30/Female	Headache, fever	Varicella zoster virus	CSF culture: no growth	Supportive treatment	Improved
83/Female	Headache	Varicella zoster virus	CSF culture: no growth	IV acyclovir	Expired due to other comorbidities
55/Female	Headache	Herpes simplex virus 1 (HSV-1)	CSF culture: no growth MRI: restricted diffusion at insula, right temporal lobe	IV acyclovir	Prolonged hospital stay; tracheostomy
27/Male	Headache	*Cryptococcus neoformans*	CSF culture: no growth CALAS: positive	Amphotericin B, Fluconazole	Improved
55/Male	Headache	*Cryptococcus neoformans*	CSF culture: no growth CALAS: positive	TMP-SMX, Anti-TB meds	Improved

Table 4. Overall Diagnostic Accuracy of the FilmArray™ ME Panel in patients with suspected bacterial meningitis

Interpretation	FilmArray™ ME panel	CSF Culture	No. of samples	%
Concordant	Positive	Positive	1	93.2%
	Negative	Negative	81	
Discordant	Negative	Positive	1	5.7%
	Negative	Positive (pathogens are not included in the ME panel)	4	
	Positive	Negative	1	1.1%
Total			88	100.0%

Table 5. Diagnostic Accuracy of the FilmArray™ ME panel by each bacteria

Bacteria	FilmArray™ ME panel	True positive	False positive	True negative	False negative	Sensitivity (95% CI)	Specificity (95% CI)
E. coli	1	1	0	84	1	50% (48% - 52%)	100%
S. pneumoniae	1	0	1	85	0	n/a	98.8% (98.7 – 98.9%)
H. influenzae	0	0	0	86	0	n/a	100%
N. meningitides	0	0	0	86	0	n/a	100%
L. monocytogenes	0	0	0	86	0	n/a	100%
S. agalactiae	0	0	0	86	0	n/a	100%

Table 6. Patients with bacterial meningitis diagnosed by CSF culture due to pathogens that were not included in the FilmArray™ ME panel

No	Age/Sex	Total Cell Count	WBC	Differential Count	CSF/ Serum Glucose	CSF Gram Stain	CSF Culture
1	68/F	562	542	All lymphocytes	131/190	Gram + cocci in pairs	*Staphylococcus hominis*
2	73/F	1760	640	Neutrophils: 4 Lymphocytes: 96	1/113	No microorganism	*Streptococcus suis*
3	54/M	2500	2240	Neutrophils: 93 Lymphocytes: 7	1/172	Gram-filamentous	*Klebsiella pneumoniae*
4	60/F	1916	1836	Neutrophils: 84 Lymphocytes: 16	28/161	No microorganism	*Pseudomonas aeruginosa*

seen where the sample was negative in the ME panel but CSF culture showed growth of *E. coli*. Among the 86 samples that tested negative on ME panel, CSF culture was able to isolate the causative organism in 4 samples, which included: (1) *Staphylococcus hominis*; (2) *Streptococcus suis*; (3) *Klebsiella pneumoniae* and; (4) *Pseudomonas aeruginosa.* All the bacteria were not included in the current ME panel (Table 6).

DISCUSSION

Our study investigated the performance of the FilmArray™ ME Panel in the detection of bacterial pathogens in the CSF. The results showed that the ME panel showed high correlation with cerebrospinal fluid culture in detecting bacterial pathogens, with 93.2% (95% CI 85.9 % - 96.8%) overall agreement and 98.8 % - 100% (95% CI 98.7% - 100%) specificity, respectively. These results are consistent with previous studies showing an overall agreement of 93–99% between the ME panel and CSF culture.[7-10] A systematic review and meta-analysis of the diagnostic accuracy of the ME panel involving 3764 subjects across 13 studies also showed high diagnostic accuracy, with sensitivity and specificity of 90% and 97% respectively.[11] However, a contrasting result seen in this study is the low sensitivity (50%, 95% CI 48% – 52%) of the ME panel for *E. coli*. This can be attributed to a single false negative result (discussed later), which greatly diminished the sensitivity due to the small sample size of the study.

Although the overall agreement between the ME panel and CSF culture was high, there was one false positive result where the ME panel detected *S. pneumoniae* in the CSF sample analyzed but there was no growth in both CSF and blood cultures. This patient was a 77-year-old man admitted due to decreased sensorium and was noted to have subacute infarcts on the left middle cerebral artery territory on magnetic resonance imaging (MRI). However, the patient also presented with fever a few days prior and was noted to have nuchal rigidity on neurologic assessment. A lumbar puncture was performed and CSF analysis revealed presence of 96 white blood cells, majority of which were lymphocytes. The patient was started on intravenous meropenem and was still continued on the antibiotic despite negative growth on CSF culture. However, a repeat lumbar puncture was not performed to confirm the presence of *S. pneumoniae* or to determine response to the antibiotic.

Among all the organisms included in the ME panel, *S. pneumoniae* has the highest proportion of false positive results,[7,11] followed by *S. agalactiae*. The specific mechanism for this was not fully determined, but it was postulated that since *Streptococcus pneumoniae* can be shed from the respiratory tract of healthy individuals, contamination of the specimen from oral flora of the patient and during the specimen handling may account for the false positive results.[7]

Meanwhile, a false negative ME panel but CSF culture positive for *E. coli* was seen in one adult patient. A review of this patient's medical records showed presence of gram-positive cocci and bacilli singly and in pairs in the CSF gram stain, CSF cell count with WBC of 4000 cells/*u*L, and CSF/serum glucose ratio of 0.01 (1/88). These CSF findings were highly suggestive of an ongoing bacterial infection. A possible explanation for this result is that the ME panel is only capable of detecting the K1 capsular type

of *E. coli*. Although this is the most common capsular type which causes majority of cases of meningitis, other capsular types of *E. coli* can also cause meningitis and/or encephalitis, which may not be detected by this test.[12] In addition, the narrowed range of specificity of the ME panel for this particular organism was designed so as to prevent detection of contaminating *E. coli* nucleic acid encountered in reagents used in polymerase chain reaction such as DNA polymerase and reverse transcriptase.[13]

Due to the limited number of organisms included in the ME panel, virulent bacterial pathogens that can cause nosocomial and hospital-acquired meningitis and ventriculitis such as *Klebsiella sp., Pseudomonas sp.* and *Staphylococcus sp.* cannot be detected. This may impact on the initiation of antimicrobial treatment of patients whose CSF profile points to a likely bacterial cause. In addition, other common causative agents of community acquired bacterial meningitis in the Philippine setting, such as *Mycobacterium tuberculosis* and arboviruses (Dengue virus and Japanese B virus), are also not included in the ME panel.

CONCLUSION

The FilmArray™ ME panel has high overall agreement with CSF culture in detecting bacterial pathogens that cause meningitis. While the ME panel cannot replace all CSF diagnostic methods, the capability of this test to simultaneously detect 14 different pathogens with a rapid turnaround time can be utilized in the diagnostic algorithm and targeted antimicrobial therapy in patients with suspected bacterial meningitis.

Ethics Approval

This study was approved by St. Luke's Medical Center Institutional Review Board and was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Statement of Authorship

Both authors participated in the data collection and analysis and approved the final version submitted.

Author Disclosure

Both authors declared no conflicts of interest.

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