American Journal of Biochemistry and Biotechnology

Original Research Paper

Trends in the Causes of Meningitis/Encephalitis Analyzed Using the BioFire FilmArray Panel

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Article history
Received: 05-11-2023
Revised: 16-01-2024
Accepted: 07-02-2024

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Abstract: Cerebrospinal Fluid (CSF) infections can be caused by various pathogens, including bacteria, viruses, and fungi. Therefore, accurate and timely detection of infectious agents is critical for the effective treatment of CSF infections. The FilmArray Meningitis/Encephalitis (M/E) panel is a potential complementary diagnostic tool for detecting CSF pathogens. The aim of this study was to identify trends in the causes of meningitis/encephalitis by analyzing pathogens, coinfection, age, sex, and infection timing in patients with suspected encephalitis/meningitis using the FilmArray M/E panel results. We analyzed 2,335 cerebrospinal fluid samples collected between December 2017 and February 2023. Additionally, we assessed the potential benefits and limitations of the FilmArray M/E test and identified clinically meaningful application methods. Of the total samples, 219 (9.4%) were positive; one triple infection and two double infections were also identified. Among the positive cases, 54.6% were men. Human herpes virus 6, varicella-zoster virus, and herpes simplex virus 1 were the most commonly identified pathogens with positivity rates of 49.78% (111/223), 17.94% (40/223), and 13.90% (31/223), respectively. Of the total positive samples, 56.1% (125) samples were obtained from July to December and 50.4% (113) were distributed in the 0-17-year age group. The FilmArray M/E panel provides a comprehensive and rapid way to identify CSF pathogens; it can help select appropriate treatments for target pathogens and track the epidemiology of CSF infections. Conducting research using cerebrospinal fluid is challenging owing to difficulties in its collection and manipulation. This study is novel and significant in that it comprehensively analyzed meningitis/encephalitis using FilmArray M/E panel in 2,335 large-scale CSF samples.

Keywords: Cerebrospinal Fluid, Encephalitis, Infections, Pathogens, Meningitis

Introduction

Cerebrospinal Fluid (CSF) infections vary depending on the pathogen type. Bacterial infections can lead to death within hours; despite treatment, they often cause serious adverse effects such as brain damage, hearing loss, and learning difficulties (Brouwer and van de Beek, 2017; CDCP, 2021a). Developing countries with low medical standards experience challenges in managing CSF infections, and the associated morbidity and mortality rates in these countries tend to be higher than those in developed countries (Brouwer *et al*., 2010). Viral infections are more common and often mild. However, they can still cause complications in infants with weak immunity, newborns aged <1 month, or in patients with sporadic encephalitis caused by Herpes Simplex Viruses (HSVs) (CDCP, 2021b; He *et al*., 2016). Therefore, rapid and accurate identification of CSF pathogens is crucial.

General CSF analyses are performed using microscopic cell count; CSF culture; total protein, glucose, and lactate measurements; and serological methods (such as those for Immunoglobulin [Ig] G, IgM, and IgA). Although these approaches represent the primary screening tests for CSF infection, they cannot detect non-bacterial infections, even if pathogens are identified through CSF culture (Deisenhammer *et al*., 2006; Regeniter *et al*., 2009). Furthermore, CSF culture is less sensitive and time-consuming. It requires several days to obtain results, which can lead to inappropriate use of antibiotics or delayed treatment of patients requiring immediate attention (He *et al*., 2016; Leazer *et al*., 2017).

FilmArray (BioFire Diagnostics, LLC, Salt Lake City, UT, USA) is a rapid diagnostic tool for 14 pathogens with a pre-treatment time of approximately 3 min and an inspection time of approximately 2 h (Park *et al*., 2021). Its sensitivity is higher than that of traditional tests (Hanson, 2016). The food and drug administration has approved the FilmArray for detecting 14 pathogens-6 bacteria: *Escherichia coli K1*, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*; 7 viruses: Cytomegalovirus [CMV], Enterovirus [EV], Herpes Simplex Virus 1 [HSV-1], Herpes Simplex Virus 2 [HSV-2], Human Herpes Virus 6 [HHV-6], Human Parechovirus [HPeV], and Varicella-Zoster Virus [VZV]; and 1 yeast: *Cryptococcus neoformans*/*Cryptococcus gattii* (Hanson, 2016). We aimed to investigate the etiology and patterns of meningitis/encephalitis by analyzing pathogens, coinfection, age, sex, and infection timing using the FilmArray M/E panel on 2,335 cerebrospinal fluid samples, offering insights into clinical application and epidemiology despite the challenges of working with cerebrospinal fluid. It is significant that a more comprehensive understanding of meningitis and encephalitis through these studies can provide directions for cerebrospinal fluid biological infections, which place a great burden on global health due to high morbidity and mortality by enabling accurate diagnosis, customized treatment, results-based public health strategies, and improved management of infectious diseases (Hasbun *et al*., 2017).

Materials and Methods

Materials

This study was conducted at Dankook University Hospital in Cheonan, Korea, from December 2017 to February 2022. A total of 2,335 CSF samples were obtained through lumbar puncture. These samples were collected from male and female patients of all ages with suspected CSF infection, who were referred for infection testing. For CSF samples, a sample that was not centrifuged was used following the BioFire test instructions, and tests were performed within 1 day at 20-26°C after collection and within 7 days of refrigeration. As none of the information pertaining to the samples could be used to identify individual patients and because this was a retrospective study, the requirement for obtaining patient consent was waived by the ethics board (only information such as sex, age, and time of infection were used). The study protocol was approved by the Institutional Review Board of Dankook University (IRB file No. 2023-01-013) and was conducted in compliance with the ethical principles of the Helsinki Declaration.

Methods

The FilmArray M/E panel test was performed according to the manufacturer's instructions. All tests were conducted while using protective equipment, such as goggles, gowns, and gloves. Within a Biosafety cabinet, 200 µL of the sample was mixed with a sample buffer, and the resulting mixture was then injected into a pouch containing a hydration solution. The pouch was mounted on the device, and the test was initiated. The tests were performed in the following order: Initial extraction and isolation of nucleic acids in the sample via chemical dissolution in a sample buffer, achieved by stirring with zirconium beads using the bead beating method; subsequently, reverse transcription, multiplex PCR, and finally, automated melting curve analyses were performed.

Each FilmArray pouch contains two positive controls. The first control targeted the RNA of the yeast *Schizosaccharomyces pombe*. The yeast cells were freeze-dried in a pouch and rehydrated when the sample was injected. The positive control results indicated the successful completion of every step of the test process. A second control was used to detect the yeast DNA, which was dried in the FilmArray well. Successful multiplex PCR was indicated by the positive control results. For valid sample and control tests, the FilmArray equipment provided a "detected" or "not detected" outcomes; if either of the two positive controls failed, the equipment displayed "invalid" results, ensuring the validity of the outcomes.

Results

Of the 2,335 samples collected from individuals of all ages (men patients and women patients), 2,116 tested negative and 219 tested positive. In these samples, 223 pathogens were detected. A total of 216 samples showed single infections, 2 samples showed double infections, and 1 sample showed triple infection. A pattern of double infection was observed in samples that tested positive for HSV-1 and HHV-6 as well as for HHV-6 and VZV. The sample with triple infection tested positive for HSV-1, HSV-2, and VZV (Table 1).

We discovered that of the 14 pathogens that can be potentially detected using the FilmArray equipment, only 10 were detected in this study. Specifically, the equipment detected 6 viruses (CMV, EV, HSV-1, HSV-2, HHV-6, and VZV) and 4 bacteria *(E. coli*, *S. agalactiae*, *L. monocytogenes*, and *S. pneumoniae*). It did not detect *H. influenzae*, *N. meningitidis*, HPeV, or *C. neoformans/C. gattii*. The results showed that, of the 223 detected pathogens, viruses accounted for 93.7%, whereas bacteria accounted for only 6.3% of the pathogens. HHV-6 was the most commonly detected pathogen, accounting for 49.8% (111/223) of all detected pathogens, followed by VZV, which accounted for 17.9% (40/223), and HSV-1, which

accounted for 13.9% (31/223) of the pathogens. These three pathogens accounted for 81.6% of all detected pathogens (Table 2).

Among the positive samples, 54.7% (122) were from male participants. Men patients tested positive for 10 pathogens, with positivity rates of 26.0, 10.8 and 9.9% for HHV-6, VZV, and HSV-1, respectively. Women patients showed positive results for six pathogens, with positivity rates of 23.8, 7.1 and 6.3% for HHV-6, VZV, and HSV-2, respectively. The positivity rate was the highest for HHV-6 among both male and female participants (Table 2). Between 2018 and 2022, 22, 28, 49, 52, and 60 positive samples were detected, and the figures showed a steady yearly increase. HHV-6-positive cases steadily increased from 2020-2022 (Fig. 1). In total, 98 positive samples (43.9%) were obtained between January and June, whereas <125 positive samples (56.1%) were obtained from July to December. The highest number of positive samples was recorded in August and October (26), whereas February, April, and September had the lowest numbers (14) (Fig. 2). Of the positive samples, 59.6% were distributed in the 0-17-year age group. Specifically, 48.4% of all HHV-6-positive samples were distributed in the 0-17-year age group. VZV and HSV-1-positive samples were distributed across all age groups. In the >60-year age group, 35-60-year age group, and 18-34-year age group, the positive rates were 18.4, 13.0 and 9.0%, respectively. Notably, within the 18-34-year age group and the 35-60-year age group, Varicella-Zoster Virus (VZV) exhibited the highest positive rate in the corresponding age group at 4.0 and 5.4%, respectively. On the other hand, in the >60-year age group, Herpes Simplex Virus Type 1 (HSV-1) demonstrated the highest positive rate at 8.5% (Fig. 3).

This graph classifies the 211 pathogens from positive samples detected using the FilmArray M/E panel test conducted at a university hospital in Cheonan between 2018 and 2022. The 5-year distribution of microbial infection is expressed as a trend line, with the horizontal axis expressed as the year and the vertical axis as the positivity rate. HHV-6 was detected most frequently in this study, and it is expressed as trend l. HSV-1: Herpes simplex virus 1; HSV-2: Herpes simplex virus 2; VZV: varicella-zoster virus; HHV-6: Human herpes virus 6; CMV: Cytomegalovirus; EV: Enterovirus.

This graph shows the monthly classification of the 223 positive pathogens detected using the FilmArray M/E panel test. The horizontal axis denotes the month, and the vertical axis denotes the positivite samples.

This graph classifies the 223 positive pathogens detected using the FilmArray M/E panel test conducted at a university hospital in Cheonan between December 2017 and February 2023 by age group. The horizontal axis denotes the age group, and the vertical axis represents the positivity rate.

This table shows the number of total samples, negative samples, positive samples, and coinfections. In addition, coinfection pathogens are presented.

Table 1: Positivity rate of the FilmArray Meningitis/Encephalitis panel and coinfection pathogens

	Positivity	
Parameter	Samples	% of total
All samples (n = 2,335)		
Negative samples	2,116	90.6
Positive samples	219	9.4
Single detections	216	9.3
Coinfection	3	0.1
Coinfection pathogens		
Double infection	※Case 1 HHV-6, HSV-1 ※Case 2 HHV-6, VZV	
Triple infection	HSV-1, HSV-2, VZV	

HSV-1: Herpes Simplex Virus 1; HSV-2: Herpes Simplex Virus 2; VZV: Varicella-Zoster Virus; HHV-6: Human Herpes Virus 6

Table 2: Positivity rate for the FilmArray Meningitis/Encephalitis Panel for pathogen and sex groups

Pathogen	% of male patients	% of female patients	% of total (sex)
Virus 93.7			
CMV	0.4	0.0	0.4
EV	1.3	0.5	1.8
HSV-1	9.9	4.0	13.9
HSV-2	3.6	6.3	9.9
HHV-6	26.0	23.8	49.8
VZV	10.8	7.1	17.9
Bacteria 6.3			
E. coli	0.4	0.0	0.4
L. monocytogenes	0.9	0.0	0.9
S. agalactiae	0.9	3.7	4.6
S. pneumoniae	0.4	0.0	0.4
% of the total (pathogens)	54.6	45.4	100.0

HSV-1: Herpes Simplex Virus 1; HSV-2: Herpes Simplex Virus 2; VZV: Varicella-Zoster Virus; HHV-6: Human Herpes Virus 6; CMV: Cytomegalovirus; EV: Enterovirus

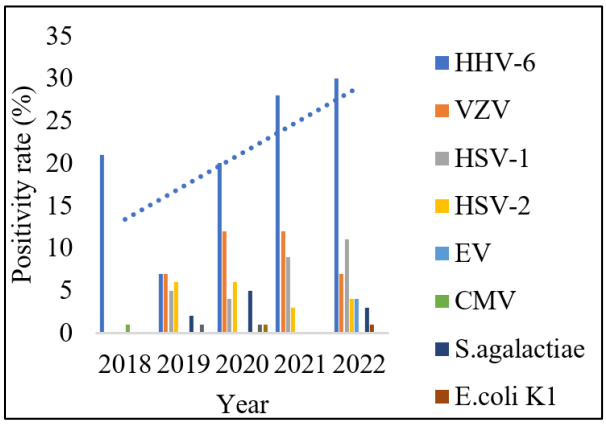

Fig. 1: Positivity rates of the FilmArray M/E panel pathogens by year

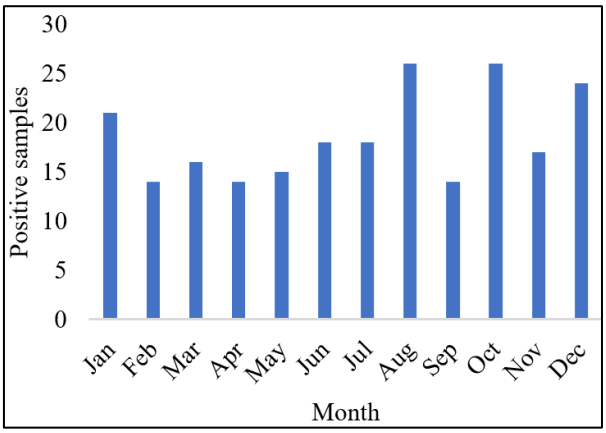

Fig. 2: Monthly FilmArray M/E panel pathogen positivite samples

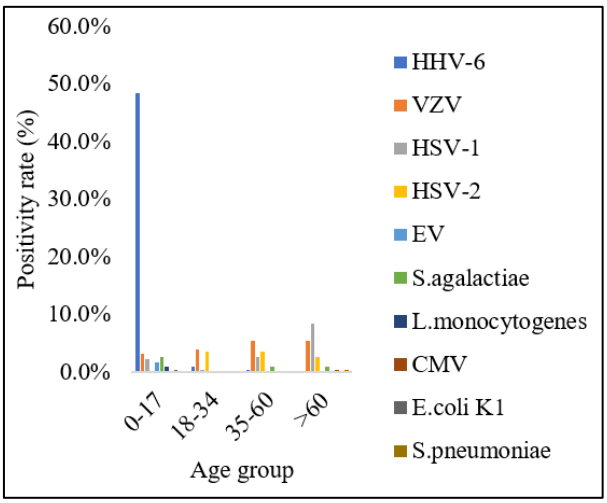

Fig. 3: Positivity rates of FilmArray M/E panel pathogens by age group

This table shows the virus and bacterial infection rates of CSF samples according to sex and also shows the positive rates of all samples from men patients and women patients.

Discussion

CSF samples are typically used to detect the presence of bacteria, leading to the classification of meningitis into aseptic and septic types, and to determine bacterial types. This is because viral culture in laboratory settings is often challenging, resulting in the differentiation of sterile and bacterial meningitis based on CSF culture results (Nigrovic, 2013; Zarrouk *et al*., 2007). In our study, most CSF-positive samples harbored viruses (93.7%) and only a few contained bacteria (6.3%). Other studies that used the FilmArray M/E panel have also reported the detection of viruses in most pathogenic cases. Notably, we observed positivity rates of 13.9, 9.9 and 49.8% for HSV-1, HSV-2, and HHV-6, respectively, with herpes viruses accounting for a positivity rate of >70% in this study. In contrast, other studies have shown that enterovirus-positive samples accounted for over 50% of the cases; HHV-6 demonstrated the highest positivity rate in our study and the second highest in other studies (Boudet *et al*., 2019; Leber *et al*., 2016; Lindström *et al*., 2022).

In this study, we discovered that 50.4% of the positive samples were from individuals aged 0-17 years. Previous studies have indicated positivity rates in adolescents below 17 years, ranging from 18-66%. However, the limited number of CSF samples from patients aged 0-17 years in these studies suggests that further research is required to confirm these findings (Broadhurst *et al*., 2020; Boudet *et al*., 2019; Leber *et al*., 2016; Lindström *et al*., 2022). HHV-6 was associated with the highest number of positive samples in the current study, and EV had the highest number of positive samples in previous studies. The distribution of both viruses was high among individuals aged 0-17 years (Broadhurst *et al*., 2020; Boudet *et al*., 2019; Leber *et al*., 2016; Lindström *et al*., 2022; Precit *et al*., 2020).

Previous studies have indicated that although bacterial meningitis shows no substantial seasonality or temporal patterns, it is associated with geographical factors. We discovered that bacterial meningitis had a positivity rate of 50.0% between July and December and 50.0% between January and June. A high positivity rate of 64.3% was observed between October and January (Paireau *et al*., 2016; Theodoridou *et al*., 2007). Previous studies have shown that viral meningitis has the highest positivity rate during summer and autumn, with a gradual decrease during winter. In contrast, in our study, viral meningitis had a positivity rate of 56.5% between July and December, 44.5% between January and June, and 51.2% between October and March. This finding contrasts with the results of previous studies, which showed a decrease in the positivity rate during the winter months (Kelly *et al*., 2013; Michos *et al*., 2007).

In this study, we identified 3 cases of coinfection (1.3%) out of 223 positive cases. Two involved dual infections with HSV-1 and HHV-6 or HHV-6 and VZV, whereas one involved a triple infection with HSV-1, HSV-2, and VZV. However, we did not observe cases of coinfection between viruses and bacteria or fungi or between bacteria and fungi. Our findings differ from those of other studies, which have shown coinfection rates of 0.4-2.7% and have identified coinfections between viruses and bacteria, viruses and fungi, and fungi and bacteria (Liesman *et al*., 2018; Rohatgi *et al*., 2019). Meningitis can lead to life-threatening complications (WHO, 2023). Furthermore, a previous study has shown that CSF coinfection cases have higher mortality rates than single infection cases (Kelly *et al*., 2012).

Nevertheless, our study had some limitations. First, our understanding of the timing and regional characteristics of M/E was limited because we used the FilmArray test results from a university hospital in Cheonan over a period of 5 years. Therefore, it is difficult to generalize the data in this study. However, the lack of such information made it impossible to study the treatment progress considering the infectious agent and the effectiveness of the drugs used. Therefore, we only assessed the overall trend in CSF infections. Finally, there were no confirmed positive cases for several pathogens in this study (*H. influenzae*, *N. meningitidis*, HPeV, *C. neoformans*, and *C. gattii*).

Despite the study limitations, the overall infection patterns from 2,335 CSF samples were identified and analyzed by sex, infection timing, and coinfection using samples that are difficult to collect and handle. The trend of CSF infectious diseases, such as meningitis and encephalitis, was examined using a relatively large number of samples, and This can be a milestone in identifying accurate diagnosis, customized treatment, outcome-based public health strategies, and improvement in infectious disease management. We gained insights into the pathogenic trends of M/E by utilizing a diverse range of patient samples across different age groups. Our study yielded data that are distinct from those of previous studies, such as the positivity rate of HHV-6 in infants and adolescents and the comparison of viral as well as bacterial meningitis. Moreover, the temporal and seasonal pathogen statistics from our study can aid in identifying the trends in CSF infectious pathogens during specific periods, thus supporting the prevention and control of local M/E outbreaks.

The FilmArray M/E panel is limited in that it can only detect the 14 types of infectious agents included in the panel. Additionally, using this panel requires specialized knowledge and expertise to correctly interpret and apply the results to clinical situations and access to a laboratory equipped with specific equipment from a particular manufacturer. However, a specific and rapid molecular biology-based method is being used to diagnose viral M/E-which is difficult to identify using CSF culture-and can diagnose several viral CSF infections, such as those caused by herpes viruses, EV, and HPeV, with high sensitivity (DeBiasi and Tyler, 2004). Furthermore, using the FilmArray for CSF testing is economical because it can simultaneously screen 14 different pathogens, including viruses, fungi, and bacteria. This enables the primary screening of pathogens and rapid treatment with appropriate antibiotics and antivirals for patients with similar symptoms, such as headache and fever (Duff *et al*., 2019; Soucek *et al*., 2019). This study conducted a comprehensive statistical analysis of 2,335 Cerebrospinal Fluid (CSF) samples over a five-year period. The analysis took into account various criteria, such as age, sex, and timing, with a specific focus on pathogens known to cause meningitis/encephalitis. By exploring these factors, the research aims to enhance our understanding of infection patterns and epidemiological characteristics associated with meningitis/encephalitis. The insights derived from this study have broader implications for public health. The identification of trends in age-specific susceptibility, sex-based variations, and temporal patterns of pathogen prevalence contributes valuable information for formulating targeted public health policies. Additionally, the findings can play a crucial role in the development of effective infection control measures, thereby helping to mitigate the effect of meningitis/encephalitis on public health.

Conclusion

The FilmArray M/E panel can be beneficial for setting up a treatment plan and managing drugs by quickly identifying various pathogens using CSF samples. Nevertheless, its applicability is limited by relatively high costs and the need for equipped laboratories and skilled personnel for data inspection and reading. The FilmArray M/E panel can be a comprehensive and expeditious method for identifying CSF pathogens in patients suspected of meningitis/encephalitis. This capability facilitates the selection of suitable treatments and medications for affected individuals. Moreover, the panel can be judiciously employed to discern epidemiological patterns and trends, thus aiding in infection prevention. Additionally, it provides a systematic means to evaluate the effect of medications or treatment outcomes. In addition, the FilmArray M/E panel should be used to prevent infectious diseases in developing countries that require rapid diagnostic testing for CSF infection or in vulnerable areas such as countries in the meningitis belts (Molesworth *et al*., 2002).

Acknowledgment

We thank the three reviewers including Professor Sung. We would like to extend our appreciation to the anonymous reviewers whose constructive feedback enhanced the quality of this study.

Funding Information

The authors have not received any financial support or funding to report.

Author's Contributions

Young Jun Hong: Conceptualization, Methodology, and Project Administration. Hong was the lead author and played a central role in developing the research design and

methodology. He was responsible for the overall project management and coordination.

Bo Kyeung Jung: Data Collection and Analysis. Emma was responsible for data collection in the field and the subsequent data analysis.

Young Jun Hong and Bo Kyung Jung contributed equally to this study.

Jae Kyung Kim: Kim critically reviewed and edited the manuscript, improving the language, structure, and overall quality of the paper. Kim takes full responsibility for this study.

All authors contributed to the writing, review, and editing of the final manuscript and approved the submission for publication.

Ethics

The study protocol was approved by the Institutional Review Board of Dankook University (IRB file No. 2023-01-013).

Data Availability

All data are available within the article or its supplementary materials.

Conflict of Interest

The authors declare that there is no conflict of interest.

Patient Consent

This study was a retrospective data-only study; therefore, the need for obtaining informed consent from the patients was waived by the Dankook University IRB.

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