Original Article

Evaluation of the BioFire FilmArray meningitis/encephalitis panel for the detection of bacteria and yeast in Chinese children

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Background: Meningitis and encephalitis are life-threatening syndromes with high morbidity and mortality in children. Due to limitations of traditional laboratory approaches in etiological diagnosis, the rate of misdiagnoses is unacceptably high.

Methods: We retrospectively compared the potential clinical impact of the FilmArray meningitis/encephalitis (ME) panel *vs.* conventional cerebrospinal fluid (CSF) culture in children with central nervous system (CNS) infections. Sixty-eight pediatric patients (<18 years of age) with an initial diagnosis of meningitis or encephalitis were enrolled at 2 children's hospital from January to October 2017.

Results: Fifteen specimens were found to be positive after CSF culture, with a positive rate of 22.1% (15/68). For the FilmArray ME panel, 26 bacteria and fungi from 25 samples were detected, and the positive rate was 36.8% (25/68). The FilmArray ME panel identified 14 pathogens in previously pathogen-negative patients.

Conclusions: This study demonstrated the capability of the FilmArray ME panel in the diagnosis of bacterial and fungal meningitis and therefore its potential use in facilitating enhanced patient care.

Keywords: Meningitis; encephalitis; rapid diagnosis; molecular diagnostic tests; FilmArray ME panel

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Introduction

Meningitis and encephalitis are life-threatening syndromes in children that can be caused by bacteria, yeasts or viruses. The morbidity and mortality of these infections can be high, particularly with bacterial-fungal meningitis. In China, the incidence of acute bacterial meningitis ranges from 6.95 to 22.3 cases/10,000 children <5 years of age (1,2). It has been reported that *Neisseria meningitidis* (*N. meningitidis*), *Haemophilus influenzae* (*H. influenzae*) *type b* and *Streptococcus pneumoniae* (*S. pneumoniae*) are among the most prevalent pathogens in children (3,4). Prompt diagnosis and appropriate antibiotic utilization are necessary to minimize adverse outcomes.

Despite being time-consuming and having low sensitivity (particularly in patients pretreated with antibiotics), routine culture remains the gold standard for the diagnosis of bacterial and fungal meningitis (5). However, the limitations of traditional laboratory approaches lead to unnecessarily prolonged empirical antibiotic treatment and an increase in the number of hospital admissions as well as the duration of

hospital stays. In addition, the lack of routine diagnostic tests for viral causes of meningitis in China also complicates the problem (6). Rapid, sensitive and comprehensive tests, such as molecular diagnostic tests, may be helpful to overcome the limitations of conventional laboratory-based diagnosis (7).

The FilmArray meningitis/encephalitis (ME) panel (BioFire Diagnostics, Utah, USA, owned by bioMérieux) uses multiplex PCR to detect 14 common pathogens, namely, *Escherichia coli* K1 (*E. coli* K1), *H. influenzae*, *Listeria monocytogenes* (*L. monocytogenes*), *N. meningitidis*, *Streptococcus agalactiae* (*S. agalactiae*), *S. pneumoniae*, *cytomegalovirus* (CMV), *enterovirus* (EV), *herpes simplex virus 1* and *2* (HSV-1, HSV-2), *human herpesvirus 6* (HHV-6), *human parechovirus* (HPeV), *varicella zoster virus* (VZV), and *Cryptococcus neoformans/Cryptococcus gattii* (*Cr. neoformans/C. gattii*). The entire process is fully automated and takes only approximately one hour to obtain the diagnostic results. The FilmArray ME panel has been increasingly used in many western countries in recent years (8,9). However, its clinical significance in diagnosing ME etiology in the Chinese population is sparse since it is rarely utilized in China. Therefore, in this study, we retrospectively compared the potential clinical impact of the FilmArray ME panel *vs.* conventional cerebrospinal fluid (CSF) culture in children with suspected or confirmed central nervous system (CNS) infections.

Methods

Clinical specimens

This research was a retrospective study. The study was conducted at 2 children's hospitals, namely, Shanghai Children's Medical Center and Zhejiang Children's Hospital, from January 2017 to October 2017. The study was approved by the Institutional Review Board and the Ethics Committee of Shanghai Children's Medical Center (SCMCIRB-K2017059). Written informed consent was obtained from the parents of the participants when lumbar puncture (LP) was carried out.

Pediatric patients (<18 years old) with an initial diagnosis of meningitis or encephalitis were enrolled. Meningitis or encephalitis was defined according to the World Health Organization (WHO) workbook recommendations based on laboratory findings, symptoms, or signs. In addition, all patients were subjected to the following: (I) complete medical history and (II) full clinical examination. Patients were excluded from the study if they met the following criteria: (I) cases complicated with congenital diseases or chronic medical conditions and (II) cases in which other CNS disorders could not be excluded.

For FilmArray ME panel testing, specimens meeting the following inclusion criteria were selected: CSF specimens were collected by LP with adequate volume (>1 mL); specimens were stored at –80 ℃ for later testing. Duplicate specimens from the same subject were excluded.

FilmArray ME panel testing

The FilmArray ME panel testing procedure was performed at Shanghai Children's Medical Center according to the manufacturer's instructions. The operation was performed by independent researchers who were blinded to the diagnosis. The test consisted of automated nucleic acid extraction, reverse transcription and nucleic acid amplification. Comprehensive results were available within approximately 1 hour.

Bacterial and fungal conventional testing

Conventional bacterial and fungal testing programs, including CSF culture, blood culture, Gram strain, ink stain, physiology and biochemistry of CSF, latex agglutination test and serum virology were performed on every subject enrolled. Testing was performed at either hospital using the laboratories' standard procedures.

PCR and sequencing to detect CSF bacterial and fungal infection

Nucleic acid was extracted from each specimen using a QIAamp DNA minikit (Qiagen, Hilden, Germany). PCR was performed using universal primers for the bacterial 16S rDNA gene and fungal 26S rDNA gene. The sequence of the primers used for PCR amplification is provided in *Table S1*. Pathogens were identified by analyzing DNA sequences using the BLAST tool of NCBI. The results of the PCR analysis were reported as part of the data used for the discrepancy investigation.

Discrepancy analysis

Samples with discrepant results between the CSF culture and the FilmArray ME panel were reanalyzed using a targeted PCR assay. A FilmArray ME panel result was considered true positive (TP) or true negative (TN) only when it agreed with the results from CSF culture or the

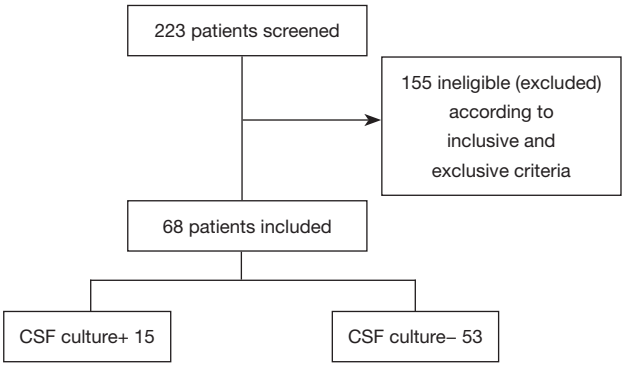

Figure 1 The enrollment process of patients with meningitis or encephalitis.

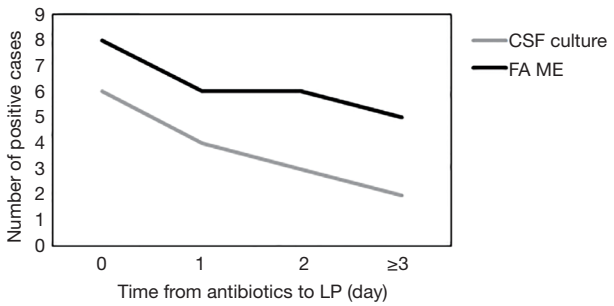

Figure 2 The correlation between the number of days that each patient was on antibiotics before lumbar puncture and the CSF culture and FA ME panel results. CSF, cerebrospinal fluid; FA ME, FilmArray meningitis/encephalitis.

comparator methods. Otherwise, the results were judged as false positive (FP) or false negative (FN).

Data analysis

Statistical analysis was performed using SPSS ver. 22. Demographic data are presented as descriptive statistics. The agreement between assays was measured using the kappa statistic. The overall percentage of agreement (OPA) was calculated as previously described (10). Briefly, OPA was calculated as [(TP+TN)/(TP+TN+FP+FN)] ×100%. The sensitivity and specificity were compared for all tests.

Results

Patient population and clinical feature

A total of 223 patients were screened, for whom 68 CSF samples met the inclusion criteria between January and October 2017 (*Figure 1*). The average age of the patients was 2.76 years (range, 3 days to 12 years), and the male/female ratio was 2.09 (46:22). There were 21 (30.9%) newborns (ages: 1–28 days), 14 (20.6%) infants (ages: 2–12 months), 23 (33.8%) preschoolers (ages: 2–6 years) and 10 (14.7%) school-aged children (ages: 7–18 years). A total of 50 patients (73.5%) in the study had received antibiotic treatment (ceftazidime, meropenem, penicillin, etc.) before LP. The correlation between the number of days that each patient was on antibiotics before LP and the CSF culture and FA ME panel results is illustrated in *Figure 2*. Thirty-five patients (51.5%) were diagnosed with bacterial meningitis, 16 patients (23.5%) with viral encephalitis and 1 patient (1.5%) with cryptococcal meningoencephalitis. Their general characteristics are presented in *Table 1*.

Table 1 General characteristics of the patients

Characteristic	Cases (%)
Total	68 (100.0)
Age	
<1 month	21 (30.9)
1–11 months	14 (20.6)
2–6 years	23 (33.8)
7–18 years	10 (14.7)
Gender	
Male	46 (67.6)
Female	22 (32.4)
Antibiotic use before LP	
Yes	50 (73.5)
No	18 (26.5)
Clinical diagnosis	
Bacterial meningitis	35 (51.5)
Viral encephalitis	16 (23.5)
Cryptococcal meningoencephalitis	1 (1.5)
Others	16 (23.5)

LP, lumbar puncture.

Pathogens detected in pediatric patients

Fifteen specimens were found to be positive after CSF

Table 2 Distribution of bacteria and yeast identified by the FilmArray ME (FA ME) panel and CSF culture

Pathogen identified	FA ME panel					CSF culture				
	No. detected	No. of positive detections by age group				No. detected	No. of positive detections by age group			
		<1 mo	1–11 mo	2–6 yr	7–18 yr		<1 m	1–11 m	2–6 yr	7–18 yr
E. coli K1	8	5	1	2	0	5	4	0	1	0
H. influenzae	0	0	0	0	0	1	0	1	0	0
L. monocytogenes	1	1	0	0	0	0	0	0	0	0
N. meningitidis	1	0	1	0	0	0	0	0	0	0
S. agalactiae	6	4	2	0	0	3	2	1	0	0
S. pneumoniae	8	0	2	6	0	5	0	0	5	0
Cr. neoformans/C. gattii	2	0	0	1	1	1	0	0	0	1
Total	26	10	6	9	1	15	6	2	6	1

CSF, cerebrospinal fluid.

Table 3 Comparison of the positive and negative results in the FilmArray ME panel and comparator assays in bacteria and yeast detection

Pathogen identified	No. of results				Discordant results		
	C–/F–	C+/F+	C+/F–	C–/F+	F+/P+	F+/P–	F–/P+
E. coli K1	58	3	2	5	4	1	1
H. influenzae	67	0	1	0	NA	NA	NA
L. monocytogenes	67	0	0	1	1	0	0
N. meningitidis	67	0	0	1	1	0	0
S. agalactiae	62	3	0	3	2	1	0
S. pneumoniae	59	4	1	4	3	1	0
Cr. neoformans/C. gattii	66	1	0	1	1	0	0

C, CSF culture; F, FilmArray ME panel; P, Target PCR assay; +, positive result; –, negative result. NA, not applicable or not able to calculate.

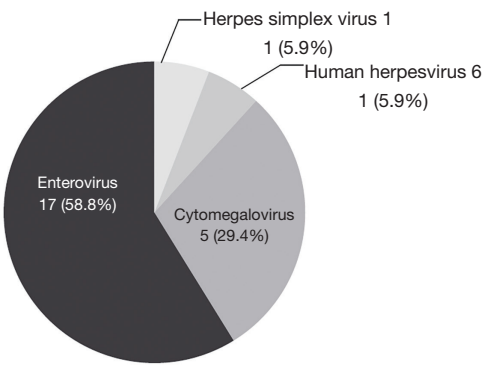

Figure 3 Virus detected by the FilmArray ME panel. ME, meningitis/encephalitis.

culture, with a positive rate of 22.1% (15/68). For the FilmArray ME panel, 26 bacteria and fungi from 25 samples were detected, and the positive rate was 36.8% (25/68) (*Table 2*). The FilmArray ME panel identified 14 pathogens in previously CSF culture-negative patients, while CSF culture identified pathogens in 4 of the 30 FilmArray ME panel-negative samples (*Table 3*). The most prevalent in the FilmArray ME panel detection were *E. coli* K1 (8/68, 11.8%), *S. pneumoniae* (8/68, 11.8%) and *S. agalactiae* (6/68 8.8%). *L. monocytogenes* and *N. meningitidis* were detected in 1 (1/68, 1.5%) sample each, and *C. neoformans/C. gattii* was detected in 2 (2/68, 2.9%) samples.

Table 4 Performance summary and characteristics of the FilmArray ME panel and CSF culture in bacteria and yeast detection

Pathogen identified	OPA (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa
E. coli K1	89.7	60	92.1	37.5	96.7	0.408
H. influenzae	98.5	0	100	0	98.5	0
L. monocytogenes	98.5	NA	98.5	0	100	0
N. meningitidis	98.5	NA	98.5	0	100	0
S. agalactiae	95.6	100	95.4	50	100	0.646
S. pneumoniae	92.7	80	93.7	50	98.3	0.577
Cr. neoformans/C. gattii	98.5	100	98.5	50	100	0.66

OPA, overall percentage of agreement; PPV, positive predictive value; NPV, negative predictive value.

Among the FilmArray ME panel results, viral pathogens were detected in 17 samples, namely, EV (10 cases), CMV (5 cases), HSV-1 (1 case) and HHV-6 (1 case) (*Figure 3*). The patients' clinical data are reported in *Table S2*.

Correlation between the FilmArray ME panel and CSF culture in detecting bacteria and yeast

In this study, a total of 476 individual FilmArray ME panel analyte tests were performed on 68 samples (for each sample, 6 bacterial and 1 fungal analyte tests were included: *E. coli* K1, *H. influenzae*, *L. monocytogenes*, *N. meningitidis*, *S. pneumoniae*, *S. agalactiae* and *Cr. neoformans/C. gattii*). The OPA between the FilmArray ME panel and CSF culture were 83.2% (396/476). For the individual target, the FilmArray ME panel had a relatively higher OPA than the CSF culture for the detection of bacteria and yeast, including *H. influenzae*, *L. monocytogenes*, *N. meningitidis*, *S. agalactiae*, and *C. neoformans/C. gattii*, while two analytes had lower sensitivities (89.7% for *E. coli* K1 and 92.7% for *S. pneumoniae*) (*Table 4*). Using CSF culture as the diagnostic gold standard, the sensitivity and specificity of the individual FilmArray ME panel's components were calculated. The FilmArray ME panel demonstrated a sensitivity of 100% for 2 analytes: *S. agalactiae* and *C. neoformans/C. gattii*. The specificity was 92.1% or greater for all analytes. Comparison of FilmArray ME panel and CSF culture for *E. coli* K1, *S. agalactiae*, *S. pneumoniae*, and *Cr. neoformans/C. gattii* showed moderate agreement (0.4< kappa <0.7).

Analysis of discrepant results

The 19 samples with discrepant results are summarized in *Table 5*. There were 12 TP cases (*E. coli* K1: 4 cases; *S. agalactiae*: 2 cases; *S. pneumoniae*: 3 cases; *L. monocytogenes*: 1 case; *N. meningitides*: 1 case; *C. neoformans/C. gattii*: 1 case) and 3 TN cases (*E. coli* K1: 1 case; *S. pneumoniae*: 1 case; *H. influenzae*: 1 case) for the FilmArray ME panel results using comparator testing results (target PCR assay). The 1 FN case (*E. coli* K1: 1 case) and 3 FP cases (*E. coli* K1: 1 case; *S. agalactiae*: 1 case; *S. pneumoniae*: 1 case) were determined with no additional evidence.

Discussion

As a major health problem in newborn infants and children worldwide, meningitis and encephalitis require early diagnosis and aggressive therapy (11,12). However, similarities exist in patients with different pathogen infections in terms of clinical manifestations, making it difficult to diagnose meningitis and encephalitis with atypical clinical symptoms and signs. Due to the lack of rapid and reliable laboratory tests in etiological diagnosis, the rate of erroneous diagnosis is unacceptably high. Until recently, the etiology was unknown for approximately 50% of cases (13,14), leading to a delay in the initiation of optimal treatment. Novel and fast molecular techniques help identify etiologies, prevent the use of unnecessary antibiotics and shorten the length of hospital stays (15). The BioFire FilmArray ME panel provides a comprehensive panel testing for 14 CNS pathogens simultaneously using a minimal amount of CSF with a rapid turn-around time (10). More recently, the use of the FilmArray ME panel for detecting pathogens has been reported to improve laboratory diagnosis (16,17). To our knowledge, this study is the first report of the performance of the FilmArray ME panel in China, where we evaluated the potential clinical benefits in testing for various pathogens.

Table 5 Discrepant investigation for samples with discordant results.

Patient	FA ME detection	CSF culture	Target PCR assay	Additional analysis or supplemental testing	Clinical diagnosis from medical records	Final resolution of FA ME result
1	Negative[a]	*E. coli*	*E. coli*	Blood culture = *E. coli*	Bacterial meningitis	False negative
2	Negative	*E. coli*	Negative	Blood culture = *E. coli*	Bacterial meningitis	True negative
3	*E. coli* K1	Negative	*E. coli*	Blood culture = *E. coli*	Bacterial meningitis	True positive
4	*E. coli* K1	Negative	*E. coli*	Blood culture = negative	Bacterial meningitis	True positive
5	*E. coli* K1	Negative	Negative	Blood culture = *S. agalactiae*	Septicemia	False positive
6	*E. coli* K1	Negative	*E. coli*	Blood culture = *E. coli*	Bacterial meningitis	True positive
7	*E. coli* K1	Negative	*E. coli*	Blood culture = *E. coli*	Bacterial meningitis	True positive
8	*S. agalactiae*	Negative	Negative	Blood culture = negative	Bacterial meningitis	False positive
9	*S. agalactiae*	Negative	*S. agalactiae*	Blood culture = negative	Bacterial meningitis	True positive
10	*S. agalactiae*	Negative	*S. agalactiae*	Blood culture = *S. haemolyticus*	Bacterial meningitis	True positive
11	*S. pneumoniae*	Negative	Negative	*S. pneumoniae* antibody = positive	Bacterial meningitis	False positive
12	*S. pneumoniae*	Negative	*S. pneumoniae*	*S. pneumoniae* antibody = positive	Bacterial meningitis	True positive
13	*S. pneumoniae*	Negative	*S. pneumoniae*	Blood culture = negative	Bacterial meningitis	True positive
14	*S. pneumoniae*	Negative	*S. pneumoniae*	Blood culture = negative	Bacterial meningitis	True positive
15	Negative	*S. pneumoniae*	Negative	*S. pneumoniae* antibody = positive	Bacterial meningitis	True negative
16	*L. monocytogenes*	Negative	*L. monocytoge*	Blood culture = *L. monocytoge*	CNS infectious	True positive
17	*Negative*	*H. influenzae*	Negative	Blood culture = negative	Bacterial meningitis	True negative
18	*N. meningitidis*	Negative	*N. meningitidis*	Blood culture = negative	Bacterial meningitis	True positive
19	*Cr. neoformans/ C. gattii*	Negative	*Cr. Neoformans/ C.gattii*	Blood culture = negative	Cryptococcal meningoencephalitis	True positive

[a], for *E. coli*, only the K1 capsular type is detected by the FilmArray ME panel.

In our study, demographic data analysis of patients revealed that there were more males than females (67.6% *vs.* 32.4%). This finding agreed with a study by Qazi *et al.*, which showed that males were more significantly affected by bacterial meningitis than females (80% *vs.* 20%) (18). This difference may signify male dominance and sex discrimination in East Asia. A total of 51.5% (n=35) of our patients were below 1 year of age, showing that meningitis is more likely to occur in younger children than in older children. A study by Seth *et al.* confirmed that meningitis is most strongly and consistently associated with a young age, in which the majority of patients (76%) were infants <12 months old (19).

The definitive diagnosis of bacterial meningitis has been historically based on culture, which has a sensitivity of ≤80% (20). As a gold standard for the diagnosis of meningitis, CSF culture was therefore used as the comparator assay in the present research. Previous studies have shown that CSF culture was positive in only 10% of antibiotic-pretreated patients in developing countries (21). Afifi *et al.* also found low rates of culture-positive CSF samples (8%) in suspected cases of bacterial meningitis (22). In our study, 50 patients (73.5%) had received antibiotic treatment before a LP was performed. Among these patients, only 10 (14.7%) samples were positive in CSF culture, while 18 (26.4%) samples were positive when detected with the FilmArray ME panel. In addition, the positive rate was influenced by the therapeutic time of antibiotics before LP. The detection number was lower with a longer use time of antibiotics in both methods. However, the FilmArray ME panel had relatively higher sensitivity than CSF culture when the use time of antibiotics was more than 1 day. Among 51 culture-negative CSF specimens, 14 were positive in the FilmArray ME panel detection. Wootton *et al.* confirmed that the

FilmArray ME panel could enhance pathogen identification in CNS-infected patients with a negative Gram stain, and the panel detected pathogens not previously identified in 11 (22.9%) of 48 patients (17). These findings show that the FilmArray ME panel can provide enhanced diagnosis in culture or Gram stain negative CSF specimens, especially after the administration of antimicrobial therapy.

Using FilmArray ME panel detection, this study identified bacteria and yeast in 25/68 patients (36.8%). *E. coli* K1 and *S. pneumoniae* were the most common organisms detected, followed by *S. agalactiae*, *C. Neoformans*, *L. monocytogenes* and *N. meningitidis*. The FilmArray ME panel had a higher sensitivity than CSF culture in detecting almost all bacteria and yeast except *H. influenzae*. Furthermore, the association between age and pathogens was analyzed. In our study, *E. coli* K1 and *S. agalactiae* were the predominant pathogens inducing neonatal bacterial meningitis. The same result was reported by Arora *et al.*, namely, that the FilmArray ME panel enhanced the identification of *group B Streptococcus* and *E. coli* in young infants with meningitis (16). Other studies in Australia, London and Canada also documented similar patterns, with *S. agalactiae* and *E. coli* being the major etiological agents for neonatal bacterial meningitis infection (23-25). *S. pneumoniae* was the predominant pathogen isolated in the 1–6 years age group, and this finding was consistent with a Korean study in which *S. pneumoniae* was the most detected etiologic pathogen beyond the neonatal period (26). Although mixed CNS infections are not uncommon in children, especially in immunocompromised individuals, infections with two or more pathogens are not easily detected by conventional methods. Hence, the biological significance of dual infections is currently not well understood. The FilmArray ME panel has the significant benefit of being able to identify coinfections. Five cases of dual infections were detected in our study, namely, a case of mixed bacterial-bacterial co-infection (*E. coli* K1 + *S. pneumoniae*), two cases of mixed bacterial-viral coinfection (*S. pneumoniae* + CMV, *S. agalactiae* + CMV), one case of mixed yeast-viral coinfection (*C. neoformans* + CMV) and one case of mixed viral-viral coinfection (HHV-6 + CMV).

Any discrepancy between CSF culture and the FilmArray ME panel was analyzed in the present study. With regard to bacteria and yeast detection, the comparator targeted PCR assay and clinical data confirmed 12 of the positive results and 3 of the negative results detected by the FilmArray ME panel. For the 3 FP results, the discrepancy investigation did not support the FilmArray ME panel results: an *S. agalactiae* FP sample from a 1-month-old girl with normal CSF parameters; a *S. pneumoniae* FP sample from a 10-month-old girl, although the detection of anti-*S. pneumoniae* antibody was positive; and an *E. coli* K1 FP sample from a 26-day-old boy whose blood culture was shown to contain *S. agalactiae*. The false-positive results (4.4%) with the FilmArray ME panel in our study were fewer than those reported in a previous study by Leber *et al.* (10), in which FP results accounted for 41% of bacterial results. Only an *E. coli* FN case was determined in a 20-day-old boy, and the reasons for the FN results were diverse. For *E. coli*, only the K1 capsular type was detected by the FilmArray ME panel, while other *E. coli* types also cause CNS infections. In addition, frozen samples or operational issues may produce negative results (27). Clinicians should be cautious when interpreting the results from the FilmArray ME panel, particularly with regard to FP results, which may lead to needless therapy and subsequent related drug toxicity (28).

There were several limitations in our study. First, we did not have viral comparative results to support the results detected by the FilmArray ME panel because a serological test is the only major available method to detect viruses in routine diagnosis in China (29). Moreover, the serological test results were not available for some samples in our study. Although many developed countries have used real-time PCR of the CSF for the daily detection of possible viruses (30), this assay was unavailable in our study. In contrast, the FilmArray ME panel fills the gap in virological testing in China. We identified 17 positive viral pathogens including EV (n=10), CMV (n=5), HSV-1 (n=1) and HHV-6 (n=1), among which only 3 samples were positive in viral serological tests. Second, the sample size was relatively small and likely had an impact on the statistical certainty of the FilmArray ME panel sensitivity and specificity calculations. Furthermore, since it was a retrospective study, selection bias is inevitable due to the criteria used for sample selection and the small volume of CSF tested.

Conclusions

This study demonstrated the capability of the FilmArray ME panel in the diagnosis of bacterial and fungal meningitis and therefore its potential use in facilitating enhanced patient care. The BioFire FilmArray ME panel may reduce diagnostic uncertainty in pediatric patients with suspected CNS infections, and its rapid diagnosis will enable optimization of antibiotic use. However, the FilmArray ME panel cannot identify some other pathogens

not included in the panel (e.g., *Mycobacterium tuberculosis*, an important cause of meningitis in China), nor could it provide information about antibiotic susceptibilities. Thus, it should be noted that the FilmArray ME panel represents an adjunctive test rather than a replacement test.

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Footnote

Conflicts of Interest: Y Xia was employed by company bioMérieux (Shanghai) Company Limited. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the Institutional Review Board and the Ethics Committee of Shanghai Children's Medical Center (SCMCIRB-K2017059). Written informed consent for specimen collection was obtained from the parents of the participants when lumbar puncture was carried out. But written informed consent for research could not be obtained as our study was a retrospective study.

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Supplementary

Table S1 Primers used in the PCR amplification

Primer	Forward	Reverse
Bacteria-16S	AGAGTTTGATCMTGGCTCAG	TACGGYTACCTTGTTACGACTT
Fungus-26S	GCATATCAATAAGCGGAGGAAAAG	GGTCCGTGTTTCAAGACGG
E.coli-mdh	TGGTTGCTAACTGGAAAGGAAT	ACGTGTATTTGAAGCATTGCTG
S. pneumoniae-ply	CCCACTCTTCTTGCGGTTGA	TGAGCCGTTATTTTTTCATACTG
S. agalactiae-cps	CAATCCTAAGTATTTTCGGTTCATT	TAGGAACATGTTCATTAACATAGC

Table S2 Summary of clinical data on patients with positive viral pathogens confirmed by the FilmArray ME panel

Patient	Clinical diagnosis from medical records	CSF cells	CSF protein	CSF glucose	CSF chlorides	Serological test	FA ME detection
1	Purulent meningitis	6929	3,529.9	2.77	114.7	–	CMV
2	Viral encephalitis	106	193	3.35	124.7	–	EV
3	Viral encephalitis	1360	244	2.61	124.6	–	EV
4	Purulent meningitis	500	418	2.85	120.4	–	EV
5	Viral encephalitis	440	193	3.33	122.8	–	EV
6	Viral encephalitis	300	1,273	1.76	121.8	–	EV
7	Viral encephalitis	61	1,546	2.3	127	HSV-1 IgM(–), IgG(–)	HSV-1
8	Purulent meningitis	90	703	2.3	118	–	EV
9	CNS infection	112	1,955	2.1	120	–	EV
10	Viral encephalitis	0	<100	3.3	127	EV71 IgM(±)	EV
11	Viral encephalitis	160	405	3.4	121	–	EV
12	Viral encephalitis	600	235	2.9	122	–	EV
13	Viral encephalitis	87	527	3.1	125	–	CMV/HHV-6