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CONTRIBUTION OF MOLECULAR BIOLOGY TO THE ETIOLOGICAL DIAGNOSIS OF INFECTIOUS MENINGITIS

MOHAMMED TAHA MEGHRAOUI[1-3], KENZA EL BAZI[1-2*], MOUHCINE MILOUDI[1-2], YOUSSEF EL KAMOUNI[1-2], SAID ZOUHAIR[1-2], LAMIAE ARSALANE[1-2]

[1]Bacteriology and Virology Department, Avicenne Military Hospital, Marrakesh, Morocco
[2]Faculty of Medicine and pharmacy of Marrakesh – Cadi Ayyad University, Marrakesh, Morocco
[3]Faculty of Medicine and pharmacy of Rabat, Mohammed V University, Rabat, Morocco
*Corresponding author: Kenza El Bazi, e-mail address: kenzaelbazi@gmail.com, postal address: Microbiology Department, Avicenne military hospital, Marrakesh, Morocco, 40000

ABSTRACT

Objectives: Our study aims to assess the contribution of the Filmarray Meningitis/Encephalitis panel in the diagnosis and management of infectious meningitis by comparing it to conventional culture, while highlighting the epidemiology of the pathogens involved.

Methods: This is a retrospective study that was carried out on 115 CSF samples from patients clinically suspected of meningitis and analyzed by conventional methods and by the Filmarray M / E panel test. This work was carried out over a period of 36 months from January 2018 toDecember2020 at the microbiology laboratory of the Avicenne Military Hospital in Marrakech.

Results: 115 samples were documented. The filmarray M/E panel detected at least one pathogen in 26 samples, representing a positivity rate of 22.6%. The infectious agents implicated in meningitis were predominated by viruses (52%, n=14), followed by bacteria (44%, n=11); yeasts are clearly in the minority making 4% (n=1) of the positive samples. The cultures were positive in 6 cases. *Streptococcus pneumoniae* was the most involved microbial agent, constituting a rate of 50% (n=3) of the bacteria detected in the cultures.

The concordance rate was 75% for the detection of *Streptococcus pneumoniae*. In this study, only one sample was positive on the filmarray Meningitis / Encephalitis panel and negative on culture.

Conclusion: The Syndromic Meningitis / Encephalitis FilmArray-type multiplex PCR is a simple, rapid and reliable diagnostic tool allowing the implementation of an early and adapted therapeutic strategy. However, given the importance of susceptibility testing, the use of molecular tests should be carried out in addition to conventional microbiology.

Keywords: Multiplex PCR, Conventional culture, Meningitis, Cerebrospinal fluid

INTRODUCTION

Infectious meningitis is the inflammation of the meninges that surround the brain and spinal cord due to the penetration and multiplication of a pathogenic agent in the subarachnoid spaces. It is most often of viral and bacterial origin, the other causes (parasitic or fungal) being less frequent.

In general, the viral cause most often remains benign, while bacterial meningitis constitutes a serious and heavy pathology because of its lethality (which can go up to 50% in the absence of treatment)[1]. As a result, infectious meningitis is a real diagnostic and therapeutic emergency.

The FilmArray® Meningitis/Encephalitis PCR Panel (BioFire® ME Panel) is a multiplex PCR assay that is able to identify 14 viral, bacterial, and fungal organisms that cause meningitis or encephalitis with high diagnostic specificity and sensitivity[2].This diagnostic test was similar in price per patient when compared to a classic CSF culture[3]. With the FilmaArray® ME Panel providing a reliable diagnosis and cost-effective method, the question arises about the effect the test has on the course of treatment. This study assessed if the utilization of the FilmArray® ME Panel was associated with a decrease in length of hospital stay and duration of antimicrobials when used in the diagnosis of meningitis or encephalitis.

MATERIALS AND METHODS
This is a retrospective descriptive study, carried out on 115 cerebrospinal fluid samples from patients clinically suspected of microbial meningitis. The samples were treated in the microbiology-virology and molecular biology laboratory at the Avicenna Military Hospital in Marrakech. This work was spread over 03 years, from January 2018 to December 2020.

1. Sampling
The study is done oncerebrospinal fluid (CSF) samples taken by lumbar punctures. Apart from any contraindication, the lumbar puncture is performed in the interspinous space of L3-L4, L4-L5, or L5-S1.

2. Methods
The filmarray® cassette is a disposable closed system that contains all the chemical and enzymatic reagents necessary for the extraction, amplification, and detection of nucleic acids of pathogens present in the sample.
The processes that occur during a film array run are:
• Nucleic acid extraction and purification
• Boost
• Detection

Complete multiplexing solutions capable of detecting bacterial, viral, and fungal pathogens in a single test have revolutionized the diagnostic approach in infectiology. Indeed, filmarray ® is a multiplex PCR solution that allows the detection of various pathogens from different clinical samples. This resulted in the marketing of various panels in our context, the filmarray® Meningitis / Encephalitis panel allows the direct identification, from a sample of cerebrospinal fluid, of infectious agents responsible for meningitis and encephalitis. (2)

The evaluation of the diagnostic performances of meningitis/encephalitis filmarray ® panel is done on viruses, bacteria, yeasandt.

Inclusion criteria
The inclusion criteria for our study are as follows all patients clinically suspected of infectious meningitis, having undergone a classic bacteriological study and a CSF filmarray M/E PCR test in the microbiology-virology department and molecular biology.

Exclusion criteria
Are excluded from the study:
• Redundant samples.
• Invalid filmarray results.
• CSF specimens are processed only by conventional methods.

RESULTS
The samples documented during the study period came mainly from hospitalized patients (91.5%) against (8.5%) from external samples. Of the 105 patients hospitalized with suspected meningitis:

• 34% (n=36) came from emergencies.
• 29% (30) from the intensive care unit
• 17% (n=18)were from internal medicine.
• 12.5% (n=13)were from neurology.
• 8% (n=8) of neurosurgery.

1-Breakdown by gender
Of the 115 patients included in the study:

- 75 were male patients (65%).

- 40 were female patients (35%).

- The M/F sex ratio was 1.87.

We noted a male predominance in our series.

2-Positive sampling
Among the 115 samples received at the microbiology laboratory during the study period, the filmarray M/E panel detected at least one pathogen in 26 samples, representing a positivity rate of 22.6%.

3-Distribution according to the pathogens involved in meningitis
The infectious agents implicated in meningitis included in our study were predominated by viruses (52%, n=14), followed by bacteria (44%, n=11); yeasts are clearly in the minority making 4% (n=1) of the positive samples. The pathogens involved are detailed in the following figure (Figure1).
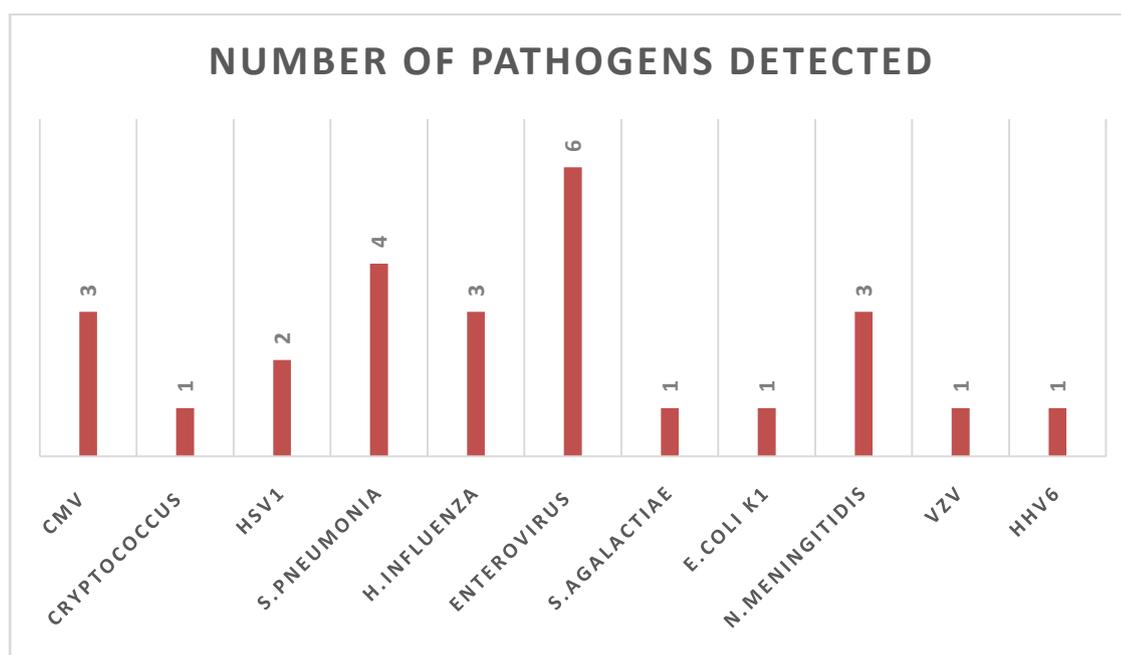
Figure 1: Pathogens detected by FilmArray.

- **Distribution of viral meningitis**
In our series, the most common virus was the enterovirus group with a positivity rate of 26%, followed by cytomegalovirus with a rate of 11%, then Herpes Simplex virus 1 with a rate of 7.5%, finally Human Herpesvirus 6 and Varicella-Zoster virus with a rate of 3.7% for each. (Figure2)
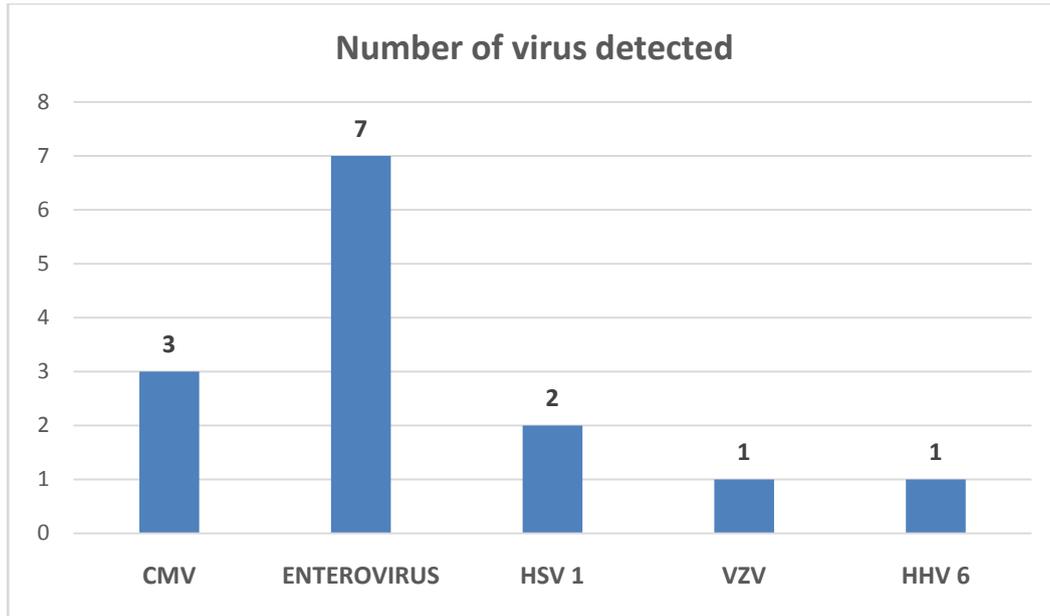
Figure 2: Viruses detected by FilmArray ®.

- **Distribution of bacterial meningitis**

Streptococcus pneumoniae had the most important positivity rate of the bacteria implicated in meningitis (15%), followed in a second position by *Haemophilus influenzae* along with *Neisseria meningitidis* with a common positivity rate of 11%; while *Streptococcus agaclatiae* and *Escherichia Coli K1* occupied the 3rd place; with a positivity rate of 3.7% for each. There was no detection of *Listeria monocytogenes* in our sample series during the study period. (Figure3)
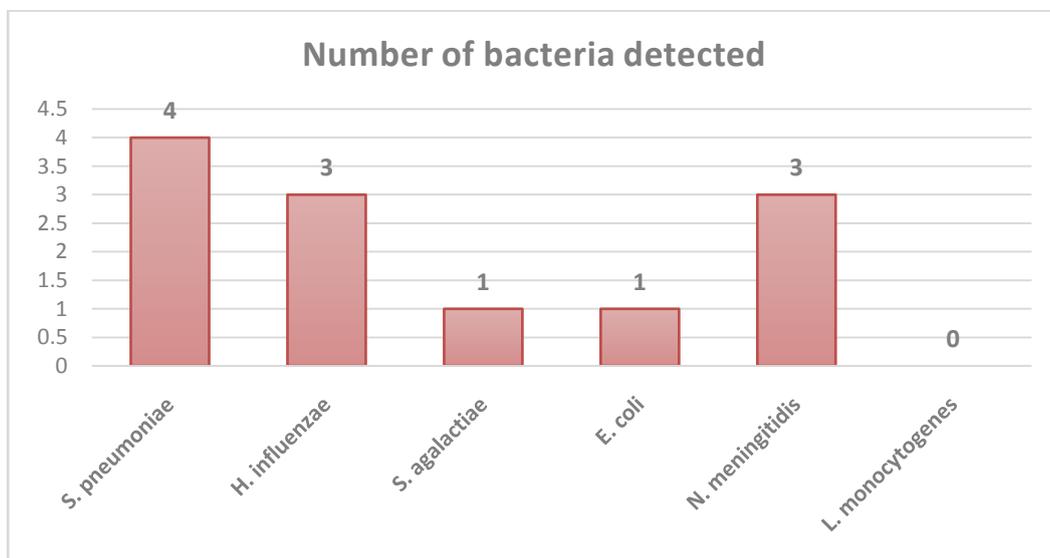

Figure 3: Bacteria detected by FilmArray®.

- **Distribution of fungal meningitis**

Of the 26 samples that tested positive by the filmarray M/E panel, Cryptococcus neoformans /gattii was responsible in 4% (n=1) of meningitis in immunocompromised patients.

4-Detection of co-infections

The analysis of the samples by the M/E filmarray® panel revealed the involvement of two pathogens (CMV and Cryptococcus neoformans/gattii) in a single sampleout of 26. The co-infection rate was 3.8%.

5- Pathogens detected on Filmarray panel and on positive cultures

The cultures were positive in 6 cases. *Streptococcus pneumoniae* was the most involved microbial agent, constituting a rate of 50% (n=3) of the bacteria detected in the cultures.

The concordance rate was 75% for the detection of *Streptococcus pneumoniae*. In this study, only one sample was positive on the filmarray Meningitis/Encephalitis panel and negative on culture.

The other germs detected were *Acinetobacter baumannii*, *Staphylococcus aureus,* and *Klebsiella pneumoniae.* These bacteria are not included in the filmarray ® Meningitis/Encephalitis panel.

6- Susceptibility testing results

The susceptibility of each of the identified bacterial strains to different was tested by referring to the antibiogram committee of the French and European Microbiology Association (CASFM/EUCAST) (5). Of the *Streptococcus pneumoniae* detected, two strains were sensitive to all antibiotics tested, while only one was resistant to penicillin G and oxacillin.

7- Concorances of the results

The following tables show concordance rates found between conventional culture and filmarray results.

Bacteria detected	Culture positive	Filmarray positive	Culture/filmarray concordance rate
Streptococcus pneumoniae	3	4	(75%)

Table 1: Concordance of filmarray with conventional culture in the detection of bacteria.

The following table reports the results of the filmarray panel for the cultures found positive:

Number of samples	Pathogen detected in culture	Result of the filmarray on the same sample
3	*S. Pneumoniae*	S. *Pneumoniae*
1	*Acinetobacter Baumannii*	Cytomegalovirus
1	*Staphylococcus aureus*	Negative
1	*Klebsiella Pneumoniae*	Negative

Table 2: Multiplex PCR results for positive culture sample

The FilmArray also allowed the diagnosis of 23 infectious meningitis whose conventional culture is negative.

Number of samples	Culture	PCR Multiplex type FilmArray
1	Negative	CMV/*Cryptococcus*
2	Negative	CMV
2	Negative	HSV 1
3	Negative	*H. Influenzae*
1	Negative	*S. Pneumoniae*
1	Negative	*S. Agalactiae*
7	Negative	*Enterovirus*
1	Negative	*E. Coli K1*
3	Negative	*N. meningitidis*
1	Negative	VZV
1	Negative	HHV-6

Table 3: Multiplex PCR filmarray results for negative culture samples.

DISCUSSION

According to a study carried out between November 2008 and March 2014 in the division of Infectious Diseases, Department of Internal Medicine, University of Texas (UT) Health Science Center, out of 149 patients with community-acquired meningitis, 48 (32.2%) had residual CSF (38 adults, 10 children < age 18) available for FA ME testing.[4]

Pathogens were identified in 14 (29.2%) out of the 48 samples by routine assessment and 15 (31.2%) by FA ME. Positivity concordance rate was of 75% percent [4].

Among the FA ME results, bacteria are the pathogens most often detected [S. pneumoniae (2), which is identical with our study.

Co-detections were present in six patients (12.5%); EBV was present in all (n=6) with VZV (n=2), HSV1 (n=1), HSV2 (n=1), Cryptococcus gattii/neoformans (n=1) and S. pneumoniae (n=1) which is somewhat higher compared to the co-infection detected in our study.

In 11 (22.9%) patients, FA ME identified previously unidentified pathogens. This is consistent with detection of (n=23) Positive sample by FA ME in our studies found to be negative by culture.[5]

In another study carried out in a pediatric intensive care unit at the Mohamed VI hospital center of Marrakesh, neuromeningeal infection was documented in 12 patients with an overall positivity rate of 23% (n=52) by FA ME. Neuromeningeal infection was documented in 3 patients with an overall positivity rate of 6% (n=52) by

culture. [6] In all the documented neuromeningeal infections with FA ME, the most common germ was *Streptococcus pneumonia*e (42%, n=5), followed by *Haemophilus influenzae* type B (25% n=3), followed by *Herpes simplex virus* type 1 (7%, n=2), *Enterovirus* (8%, n=1) and, *N. Meningitidis* (8%, n=1). On the other hand, in this work, no co-infection was detected. [6]

The results of this study made it possible to highlight many advantages related to the diagnosis of meningitis by syndromic approach using the filmarray panel platform Meningitis/Encephalitis:

- **Speed of analysis**[7]**:**
Significant improvement in lead times. From the same study the median time to obtain the results of the ME panel was 1.5 hours
  - Initiation or early therapeutic adaptation and therefore the decrease in the prevalence of bacterial resistance.
  - Improved prognosis of meningitis.

- **The syndromic approach targeting various pathogens has made possible**[7]**:**
- The detection of associated pathogens and therefore of unsuspected co-infections.
- The confirmation of one etiology and the exclusion of another.

- **High analytical performance**[7]:
- High overall sensitivity and specificity
- Diagnostic robustness not altered by the prior intake of probabilistic antibiotic therapy.
- Detection of pathogens on samples tested negative by classic techniques.

However, limitations have been identified that should be considered for the optimal use of this technology:
- High cost of the filmarray test compared to other techniques.

- Detection of pathogens absent in the panel.
- Determination of the sensitivity and resistance profile of the detected bacteria.
- Improved comprehensive management of meningitis

This study shows the improvement of the delivery time of the results and the early therapeutic orientation; a decrease in the average length of hospital stay is allowed thanks to the filmarray Panel Meningitis / Encephalitis.

CONCLUSION
Despite advances in the diagnosis and treatment of infections, meningitis remains an important cause of mortality and morbidity. The outcome of patients suspected of meningitis is based on a timely and reliable diagnosis of the pathogen involved.

Our work was carried out on 115 CSF samples taken from patients with a meningeal picture and analyzed in the microbiology-virology and molecular biology Department of the Avicenna Hospital in Marrakech between January 2018 and December 2020, and made it possible to evaluate the impact of the use of a multiplex PCR type panel filmarray® Meningitis / Encephalitis in the diagnosis of meningitis by comparing it to conventional diagnostic methods.

All the results of our study made it possible to highlight many advantages and disadvantages related to the diagnosis of meningitis by syndromic approach using the filmarray panel platform Meningitis/Encephalitis.

Abbreviation
FA ME:filmarray® Meningitis / Encephalitis
CSF: cerebro spinal fluid

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