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Integrated Diagnostic Approaches and Clinical Predictors in Central Nervous System Infections: Evidence from Resource-Limited Settings in Vietnam

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I. INTRODUCTION

Central nervous system (CNS) infections affect important parts of the body, including the brain, spinal cord, and the protective layers around them. These infections are more dangerous than many others because they can get worse very quickly and may cause serious problems with brain function. The way these infections appear can vary depending on the type of pathogen, the anatomical site of involvement, and host-specific aspects (Dando et al. 2014). For example, viral meningitis is usually mild and self-limiting, while bacterial meningitis is much more serious and can be life-threatening if not treated quickly. CNS infections can range from mild illnesses to extremely severe, life-threatening conditions. Therefore, early diagnosis is timely to initiate effective patient management.

Even though, we have the availability of effective antibiotics to treat bacterial meningitis, it still causes considerable mortality and morbidity, especially in older adults (Zunt et al. 2018). For example, a large study in the U.S. found that while about 9% of people aged 18 to 34 with bacterial meningitis died, the death rate soared to nearly 23% in those 65 and older (Thigpen et al. 2011). The type of bacteria causing the infection are associated with clinical outcomes. Infections caused by *Streptococcus pneumoniae* or *Listeria monocytogenes* were associated with higher mortality than those with *Neisseria meningitidis* (Durand et al. 1993). Also, people with weakened immune systems, such as those living with HIV, are at even higher risk. A study in Botswana showed that nearly half of HIV-positive patients with this pneumococcal meningitis died within 10 weeks to 1 year (mortality rates of 47% and 49% respectively) (Tenforde et al. 2019). In resource-limited settings, timely and accurate diagnosis is often hindered by inadequate infrastructure and limited resources. As a result, delays in identifying and treating bacterial meningitis can lead to poorer outcomes and a higher risk of complications (Bodilsen et al. 2016).

Tuberculous meningitis is considered one of the most severe forms of CNS infections, often associated with high mortality and a significant risk of long-term neurological complications (Navarro-Flores et al. 2022). Clinical outcomes are closely interconnected to the stage of disease at the time of diagnosis and treatment initiation. In a study

involving 48 patients admitted to intensive care units, the mortality rate reached 65%, with poorer outcomes observed in those presenting with advanced-stage disease or experiencing treatment delays of three or more days (Verdon et al. 1996). Improved prognosis has been associated with higher Glasgow Coma Scale (GCS) scores and normal plasma sodium levels at the time of hospital admission (Thao et al. 2020).

Viral meningitis generally carries a good prognosis, with most patients making a full recovery and experiencing no long-term complications (Logan & MacMahon 2008). In contrast, viral encephalitis poses a far greater risk of mortality and lasting disability, particularly in tropical regions where arboviral infections are more common (Glaser & Bloch 2020). Several factors contribute to poor outcomes, including older age, underlying immune compromise, delayed initiation of treatment, and the severity of neurological impairment at the time of hospital admission (Feng et al. 2020; Katson et al. 2025; Franck Raschilas et al. 2002). A study conducted in France involving 167 encephalitis survivors found that only 61% fully recovered without neurological sequelae. Among the survivors, 18% experienced mild impairments, 14% were left with severe disabilities, and 1% remained in a vegetative state. Persistent problems included memory loss, difficulty concentrating, speech impairments, and behavioral changes. Among those survived, 25% of previously employed individuals were unable to return to work after recovery (Mailles et al. 2012).

Fungal meningitis, though relatively uncommon, can lead to severe and often fatal outcomes, especially in individuals with compromised immune systems. Mortality rates depend on the specific fungal pathogen and the underlying health status of the patient but may reach up to 50% despite the use of antifungal treatment (Charalambous et al. 2018). Yet again, here early diagnosis and prompt initiation of appropriate antifungal treatment are essential for improving survival and minimizing the risk of serious complications (Pappas et al. 2015).

1. Epidemiology

CNS infections including meningitis, encephalitis, and meningoencephalitis, are among the foremost causes of hospitalization worldwide and are associated with substantial morbidity and mortality in both high- and low-resource settings (Zunt et al. 2018). In

2019 alone, an estimated 2.51 million new cases of meningitis were reported globally, resulting in approximately 236,000 deaths. Children under five years of age were disproportionately affected, accounting for 1.28 million cases and 112,000 deaths, underscoring the intense burden these infections place on young children and their families (Wunrow et al. 2023). CNS infections often result in severe long-term complications, including hearing loss, visual and physical disabilities, cognitive impairments, and limb loss, all of which carry significant emotional, social, and economic consequences for affected individuals and communities (Edmond et al. 2010).

A diverse range of pathogens, including bacteria, viruses, fungi, and parasites can cause CNS infections. The frequency and aetiology of these infections vary widely based on geography, age groups, underlying health conditions, and immunization strategies (Giri et al. 2013). Globally, *S. pneumoniae* and *N. meningitidis* are recognized as the leading bacterial causes of CNS infections in both children and adults (Oordt-Speets et al. 2018). Although *Haemophilus influenzae* type b was once a major cause of bacterial meningitis in children, its incidence has significantly decreased in countries that have implemented effective vaccination programs (Peltola 2000). Notably, in parts of Asia, *Streptococcus suis*, a zoonotic pathogen associated with pig exposure has emerged as a prominent cause of adult meningitis (van Samkar et al. 2015). Meanwhile, *Mycobacterium tuberculosis* continues to be a significant cause of meningitis worldwide, with Southeast Asia and Africa accounting for roughly 70% of tuberculous meningitis cases (Dodd et al. 2021). Viral infections also play a major role in CNS diseases. Enteroviruses are the most frequent cause of viral meningitis globally (Kupila et al. 2006). In Western countries, Herpes Simplex Virus type 1 (HSV-1) is the primary cause of acute encephalitis (Mailles & Stahl 2009; Whitley 1990), whereas in Southeast Asia and the Western Pacific, Japanese Encephalitis Virus (JEV) is the leading cause of acute encephalitis, particularly affecting children and young adults (WHO 2010).

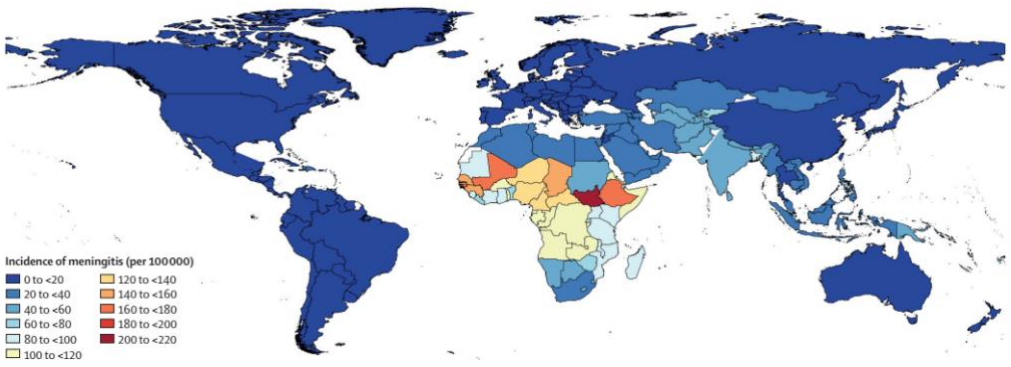

Figure 1: Meningitis incidence per 100.000 population in 2016 (Zunt et al. 2018).

Diagnosing CNS infections in low- and middle-income countries (LMICs) such as Vietnam remains particularly challenging. The causative agents of CNS infections in Vietnam show considerable variation across time and geographic regions. However, comprehensive data on the specific pathogens responsible are limited, and microbiological confirmation is frequently lacking (Gabor et al. 2022). The wide range of pathogens involved in CNS infections in Vietnam, compared to those in high-income countries, adds further complexity to diagnosis and treatment.

In Vietnam, *M. tuberculosis* remains a significant public health concern and continues to be a leading cause of CNS infections, contributing substantially to illness and death (Ngo et al. 2021; Nguyen et al. 2020). Vietnam's tropical climate and biodiversity create optimal conditions for the spread of zoonotic and vector-borne pathogens, further complicating the landscape of CNS infections. For instance, *S. suis* is the most common bacterial cause of meningitis in adults and is closely associated with the consumption of undercooked pork or raw pig blood (Wertheim et al. 2009). JEV transmitted predominantly by *Culex* mosquitoes (WHO, 2015), remains the primary cause of acute encephalitis in Vietnamese children, responsible for approximately 31% to 45% of pediatric encephalitis cases (Solomon et al. 2002; Srey et al. 2002). Despite what we know about CNS infections, a recent study found that up to 60% of these cases in Vietnam still go undiagnosed (Brindle et al. 2024). Several factors contribute to this problem. Many patients take antibiotics before reaching the hospital, which can make it harder to detect the cause of infection. Some viral infections may clear up on their own before doctors can test for them. In other cases, the available laboratory tests do not cover all possible

pathogens, or these tests are not sensitive enough to find the pathogen. Equally, delays in doing procedures like lumbar punctures and the low sensitivity of existing diagnostic tests, additionally contribute to the poor outcomes (Ho Dang Trung et al. 2012; Taylor et al. 2012).

2. Clinical Characteristics of Meningitis

Meningitis is a condition where the protective layers covering the brain and spinal cord become inflamed. It is typically characterized by an elevated white blood cell (WBC) count in the cerebrospinal fluid (CSF) in most patients (Erdem et al. 2017). While bacteria are the most common cause, meningitis can also be triggered by viruses, fungi, or even non-infectious factors like certain medications (Hoffman & Weber 2009). Pathogens can reach the fluid around the brain and spinal cord in two primary routes: (1) either by hiding inside the body's immune cells that travel to the brain, or (2) crossing directly into the brain from the bloodstream (Cain et al. 2019). The most noticeable symptoms of meningitis usually include headache, fever, a stiff neck and confusion (M. W. Bijlsma et al. 2016). However, in people with weakened immune systems, these symptoms might not appear in the usual way (Safdieh et al. 2008). Meningitis can be further classified based on the duration of symptoms, CSF findings, and underlying aetiology (see Table 1).

Table 1. Clinical Classification of Meningitis Syndromes Based on Disease Course, CSF Profile, and Aetiology. Table based on clinical and CSF parameters, as indicated in the literature (Coyle 1999).

Type	Time Course	CSF	Origin
Aseptic	Acute episode, < 4 weeks duration	Mild to moderate mononuclear pleocytosis	Infectious (viral)
		Normal glucose levels	Noninfectious
Septic	Acute episode, < 4 weeks duration	Moderate to significant polymorphonuclear pleocytosis	Infectious (bacterial)
		Low glucose levels	
Recurrent	Multiple acute episodes, < 4 weeks duration	Mild to significant mixed pleocytosis	Infectious
		Variable glucose levels	Noninfectious
Chronic	Chronic episode, ≥ 4 weeks duration	Mild to moderate mononuclear pleocytosis	Infectious (mycobacterial, fungal)
		Low glucose levels	Noninfectious

2.1. Aseptic meningitis

Aseptic meningitis is usually caused by a virus and not by bacteria. It is diagnosed in the presence of CSF pleocytosis and negative bacterial cultures, provided the patient has not received prior antimicrobial therapy (Tattevin et al. 2019). This condition is fairly common and usually goes away on its own without causing serious problems (Kumar 2005). While it can affect individuals across all age groups, it is particularly prevalent among infants and young children. Adults with aseptic meningitis often have headaches, nausea, vomiting, stiff neck, sensitivity to light, and general tiredness. In children, the illness is often accompanied by fever, respiratory symptoms, and rash (Shukla et al. 2017). Unlike bacterial meningitis, people with aseptic meningitis usually stay mentally alert and do not show confusion or changes in behaviour. This helps attending physicians to distinguish between aseptic meningitis and more serious brain infections like encephalitis, which do affect brain function (Venkatesan et al. 2013).

2.2. Septic bacterial meningitis

People with acute bacterial meningitis usually become very sick very quickly. In a large prospective study involving 1,412 confirmed cases, nearly half of the patients sought

medical care within just 24 hours of their first symptoms (M. W. Bijlsma et al. 2016). While the classic signs of bacterial meningitis, such as fever, stiff neck, and confusion, are well known, they only appear together in about 44% of episodes. However, 95% of patients will have at least two of these four symptoms: headache, fever, stiff neck, and confusion (van de Beek et al. 2004). The absence of these key clinical features strongly argues against a diagnosis of bacterial meningitis (Attia et al. 1999). Additional but less common symptoms include seizures, coma, cranial nerve deficits, aphasia, and skin rash (Merijn W. Bijlsma et al. 2016). Concurrent infections such as sinusitis, otitis media, pneumonia, or endocarditis may also be present (M. W. Bijlsma et al. 2016).

2.3. Recurrent and chronic meningitis

Chronic meningitis is defined by persistent inflammation of the protective layers around the brain and spinal cord. It is diagnosed when symptoms continue for more than four weeks, with or without abnormalities in CSF parameters (Ginsberg & Kidd 2008). People with chronic meningitis may experience anything from mild to severe headaches, often along with fever, a stiff neck, and specific nerve-related problems like weakness or trouble speaking. In certain cases, the disease follows a relapsing pattern, characterized by symptom-free intervals between episodes. During these asymptomatic periods, the inflammation may temporarily settle down (Ginsberg & Kidd 2008). The etiological range is broad, both non-infectious causes (e.g. autoimmune conditions, systemic disease, and toxic exposures) and infectious pathogens, including both conventional and opportunistic pathogens. Therefore, diagnostic evaluation should include a comprehensive history, detailed physical examination, and targeted laboratory investigations (Hildebrand & Aoun 2003).

2.4. Clinical Characteristics of Encephalitis and Meningoencephalitis

Encephalitis is a condition where the brain (brain parenchyma) itself becomes inflamed, often due to an infection, and is associated with evidence of neurological dysfunction (Tunkel et al. 2008). When inflammation extends to involve both the meninges and the brain tissue, the condition is termed meningoencephalitis (Sapra & Singhal 2019). Encephalitis frequently presents with meningitis, which overlaps in terminology and clinical features. Encephalitis patients exhibit altered mental status, which may range

from mild cognitive impairment to complete unresponsiveness/coma (Venkatesan et al. 2013).

Unlike meningitis, signs of meningeal irritation (photophobia and neck stiffness) are typically absent in encephalitis but often present in cases of meningoencephalitis. Seizures is a frequent complication, and focal neurologic abnormalities may include hemiparesis, cranial nerve palsies, and abnormal deep tendon reflexes and/or pathologic reflexes. Affected individuals may appear confused, disoriented, agitated, or increasingly lethargic (Tyler 2018). The clinical spectrum of meningoencephalitis involves both meningeal and parenchymal regions, with manifestations such as headache, fever, and neck stiffness indicating meningeal irritation, while cortical dysfunction, seizures, and focal neurological deficits reflect deeper brain involvement (Sapra & Singhal 2019).

3. CSF Diagnostics in CNS Infections

A lumbar puncture is an essential procedure used to collect CSF, which helps physicians to confirm the cause of CNS infections. The CSF is checked for cell count, glucose, and protein levels, cultures, Gram stain, and molecular diagnostics to detect pathogens responsible for CNS infections (Putz et al. 2013).

3.1. CSF Profiles in Aseptic Meningitis, Bacterial Meningitis, and Viral Encephalitis

In aseptic meningitis, CSF typically shows normal or mildly elevated opening pressure, with glucose levels that are usually normal or slightly reduced. Protein concentrations are generally modestly increased, often remaining below 2 g/L. The WBC count ranges from 10 to 1,000 cells/μL. Early in the course of illness, neutrophils may predominate, but this pattern usually shifts toward a lymphocytic predominance as the disease progresses (Putz et al. 2013).

In bacterial meningitis, CSF analysis typically reveals a high WBC count dominated by polymorphonuclear cells, reduced glucose levels (hypoglycorrhachia), and elevated protein concentrations (van de Beek et al. 2006). A widely cited diagnostic model by Spanos et al. identified several key CSF indicators predictive of bacterial meningitis, including a glucose concentration below 1.9 mmol/L, a CSF-to-blood glucose ratio under

0.23, protein levels exceeding 2.2 g/L, and a WBC count greater than 2,000 cells/mm³ (Spanos et al. 1989). While routine CSF testing includes cell counts, glucose, and protein levels, which can help differentiate between bacterial, viral, and fungal infections, these parameters alone are not sufficiently specific to establish a definitive diagnosis.

In viral encephalitis, CSF typically includes an elevated WBC count, usually not exceeding 250 cells/µL. The differential count commonly shows a predominance of lymphocytes. However, during the early phase of infection, neutrophils may be predominant. A repeat CSF analysis performed approximately eight hours later often demonstrates a shift from neutrophilic to lymphocytic dominance (Feigin & Shackelford 1973). The presence of a significant number of red blood cells in the CSF, reflecting hemorrhagic inflammation, is particularly suggestive of HSV-1 encephalitis, especially when aligned with characteristic clinical features (Whitley et al. 1989). CSF protein levels are usually mildly to moderately elevated in viral encephalitis (Tunkel et al. 2008). A reduction in CSF glucose concentration is uncommon but may occur in specific viral infections, such as those caused by mumps, varicella-zoster virus (VZV), or HSV (Davis et al. 2004).

3.2. CSF Cultures in Bacterial and Viral CNS Infections

In suspected bacterial meningitis, CSF culture remains the gold standard for diagnosing meningitis. In patients with community-acquired meningitis, standard aerobic cultures are routinely performed. However, in cases involving recent brain surgery or infections linked to CSF shunts, additional anaerobic cultures may be required (Brouwer et al. 2010). Although CSF cultures are highly valuable, they have limitations: results can take several days, and false negatives can occur. These may happen if the patient received antibiotics before the lumbar puncture, or if the infection is caused by organisms that are difficult to grow using standard laboratory methods (Leber et al. 2016).

In suspected viral encephalitis, most clinicians often request viral cultures after collecting CSF. However, the diagnostic yield is low. One review found that viruses were detected in only 6% of over 22,000 viral CSF cultures (Polage & Petti 2006). In a focused subset of 1,290 CSF samples tested for HSV using both PCR and culture methods, only nine

samples were positive for HSV, and all were identified only by PCR testing (Polage & Petti 2006).

3.3. Utility and Limitations of CSF Gram Stain in Bacterial Meningitis

The Gram staining of CSF is a quick, inexpensive, and well-established method for identifying bacteria in patients with suspected bacterial meningitis (Brouwer et al. 2010). It is especially useful when CSF cultures are negative, providing important diagnostic clues (Bryan et al. 1990). However, its effectiveness may be reduced in patients who have already received antibiotics before the lumbar puncture (Bohr et al. 1983). Furthermore, the sensitivity of the Gram stain can vary widely depending on the type of bacteria involved (Table 2).

Table 2. Diagnostic Test Sensitivities in Community-Acquired Bacterial Meningitis, adapted from literature (Brouwer et al. 2010).

Pathogen	**Sensitivity (%)**		
	Blood culture	**CSF Gram staining**	**PCR**
Haemophilus influenzae	25-90	25-65	72-92
Streptococcus pneumoniae	60-90	69-93	61-100
Neisseria meningitidis	40-60	30-89	88-94
Listeria monocytogenes	10-75	10-35	NA
Streptococcus agalactiae	80-85	80-90	NA
Streptococcus pyogenes	60-65	66-73	NA
Streptococcus suis	50	50	99
Staphylococcus aureus	75-100	20-44	NA

NA: Not Applicable.

3.4. Blood cultures

Blood cultures are frequently positive in patients with bacterial meningitis and are particularly useful when a CSF sample cannot be collected before starting antibiotics. Around 70% of bacterial meningitis cases show positive results in blood cultures (Merijn W. Bijlsma et al. 2016; van de Beek et al. 2004). However, this rate is lower for meningococcal infections (Geiseler et al. 1980). The timing of the culture is critical, especially if samples taken after antibiotic treatment and are much less likely to yield positive results, especially in meningococcal infections (Kanegaye et al. 2001; Rogers et al. 2019).

3.5. Molecular diagnostics

Nucleic Acid Testing

Molecular diagnostic tests, including nucleic acid tests (NATs) can detect the genetic material (either DNA or RNA) of infectious organisms directly from patient samples. These tests have greatly advanced the diagnosis and treatment of infectious diseases (Yang & Rothman 2004). In the context of CNS infections, the growing availability of molecular tests has transformed diagnostic practices, as they offer faster and more accurate identification of the causative pathogens, especially in cases where traditional cultures fail to provide a result. These NATs have been resourceful in the last decades, for improved clinical decision-making in patients with meningitis or encephalitis.

Multiplex Nucleic Acid Testing

Multiplex or panel-based nucleic acid tests allows for the simultaneous detection of multiple pathogens in a single tube assay. These tests combine several individual NATs into one panel, making them more valuable in diagnosing meningitis and encephalitis. In 2015, the U.S. FDA approved the BioFire® FilmArray® Meningitis/Encephalitis (FAME) panel for CNS infections. This panel detects 14 pathogens in over one hour, including *Escherichia coli K1*, *H. influenzae*, *L. monocytogenes, N. meningitidis, Streptococcus agalactiae, S. pneumoniae,* Cytomegalovirus (CMV), Enterovirus, HSV-1, HSV-2, Human herpesvirus 6, Human parechovirus, VZV, and *Cryptococcus neoformans/gattii* (US-FDA 2015).

Although, it is fast and convenient, multiplex NATs have its own limitations. They do not detect all possible CNS pathogens and do not provide information on genes carrying antimicrobial resistance. False-negative and false-positive results can occur. For example, bacterial false positives may be more frequent in low-prevalence settings where the clinical suspicion for bacterial meningitis is low (Leber et al. 2016; Trujillo-Gómez et al. 2022; Zanella et al. 2021). Moreover, the FAME test should not be used on its own to diagnose infections that develop during a hospital stay, because it does not include many of the pathogens commonly found in healthcare-associated CNS infections. To fill this gap, traditional CSF cultures should still be done in addition to the FAME test.

Importantly, all test results should be considered together with the patient's symptoms and other laboratory findings to make an accurate diagnosis. A meta-analysis of 19 studies evaluating FAME's diagnostic performance showed moderate sensitivity overall (Trujillo-Gómez et al. 2022). Sensitivity for *S. pneumoniae* was 87.5%, while it ranged from 64.9% to 74.5% for other bacterial targets such as *H. influenzae, L. monocytogenes, E. coli, S. agalactiae, N. meningitidis*. For viral infections, sensitivity was 75.5% for HSV-1, and even higher for VZV, HSV-2, and Enterovirus. However, the detection of *C. neoformans/gattii* was less reliable in several studies (Liesman et al. 2018; Van et al. 2020).

Next-generation sequencing (NGS)

Advanced sequencing technologies are transforming how infections are diagnosed, offering faster and more comprehensive pathogen detection compared to traditional culture methods (Quince et al. 2017). These technologies can be categorized into two broad categories based on the type of sequencing platform used: (1) short-read sequencing, which is mainly used for metagenomic sequencing and targeted-amplicon sequencing; (2) long-read sequencing using Oxford Nanopore Technology (ONT) reads individual DNA or RNA molecules, as they pass through tiny pores called nanopores (Gu et al. 2019).

Metagenomic NGS is a fast-evolving technology that can analyse all the genetic material from microbes in one sample, without needing to grow the organisms in culture. It provides a broad, unbiased picture of which bacteria, viruses, or fungi are present, though it is currently expensive and complex to use routinely. In a study by Wilson et al. standard diagnostic methods identified the causative organism in 45% of cases, metagenomic NGS alone in 22%, and both approaches together in 33% (Wilson et al. 2019). While the impact of prior antibiotic use was not addressed, it may explain cases where NGS succeeded, while traditional cultures were not. Although promising, routine use of metagenomic NGS in CSF infections is not yet applicable or put in routine use, as further studies are needed to better define when and how it should be used.

In contrast, ONT targeted sequencing uses a long-read sequencing approach that can overcome the limitations of PCR and metagenomic NGS (Ciuffreda et al. 2021). By focusing on specific genetic regions, such as the bacterial 16S ribosomal RNA (rRNA) gene, ONT sequencing allows for precise identification of bacteria directly from clinical samples. Studies have shown that 16S rRNA sequencing with ONT outperforms conventional culture methods, improving diagnostic accuracy and supporting better use of antibiotics (Butler et al. 2025; Lao et al. 2023). In addition, the relatively quick and user-friendly sample preparation makes ONT sequencing well-suited for timely decision-making in clinical settings especially in LMICs (M. Jain et al. 2016).

3.6. Other diagnostic methods

Several additional tests can support the diagnosis of meningitis and encephalitis, especially when standard tests are inconclusive. These include latex agglutination tests, serologic (antibody) testing, and imaging techniques such as Computed Tomography (CT), Magnetic Resonance Imaging (MRI), and Electroencephalography (EEG).

Latex agglutination is a quick test used to help diagnose bacterial meningitis. It works by detecting capsular antigens in the CSF. This test can be useful when other standard tests like the Gram stain or CSF culture do not show any results (Tunkel et al. 2004). However, the accuracy of this test has varied widely in earlier studies, depending on the type of bacteria. More research suggests that it is not very reliable, especially in patients who have already received antibiotics before the test (Brouwer et al. 2010).

Serologic tests are especially helpful when a patient with suspected viral meningitis or encephalitis is not getting better and other tests like PCR do not give a diagnosis. In most viral infections, diagnosis requires comparing two blood samples taken weeks apart, so it is important to store blood during the early phase of illness for possible later testing. For example, JEV is usually diagnosed by detecting virus-specific IgM antibodies in either CSF or blood using an ELISA test (WHO 2007). The presence of JEV-specific IgM antibodies in CSF confirms recent CNS infection. IgM antibodies in serum suggest a recent brain infection with JEV. Also, it is possible that if IgM is found only in blood, it could mean a recent infection, a mild case without symptoms, or a recent JEV vaccination.

Certain inflammatory blood markers, such as Procalcitonin (PCT) and C-reactive protein (CRP), can help differentiate between bacterial and viral meningitis in the serum of a patient (Dubos et al. 2008; Sormunen et al. 1999). Higher levels of PCT and CRP suggest a bacterial infection. Although useful, these markers are not definitive for diagnoses.

Brain imaging with CT or MRI can be helpful in evaluating encephalitis. CT scans are often used first to rule out serious complications, such as brain swelling. MRI is more sensitive and can detect damage to brain tissue, such as demyelination (loss of the protective covering of nerves), which can also occur in other clinical conditions with mental status changes. Involvement of the temporal lobes on MRI strongly suggests HSV encephalitis, though similar patterns can be seen with other herpesviruses like VZV and Epstein-Barr virus (EBV) (Glaser et al. 2006). EEG records brain electrical activity and can detect signs of brain dysfunction early in the course of encephalitis (Markand 2003). EEG findings are generally non-specific, but could offer support in making specific etiologic diagnosis of encephalitis, such as periodic lateralized epileptiform discharges in a temporal lobe in HSV encephalitis (Whitley et al. 1982).

4. Empirical and Targeted Therapy in CNS Infections

4.1. Bacterial meningitis

The bacteria that cause meningitis can differ depending on a person's age, immune status, and medical history. Therefore, antibiotic treatment should be tailored to the most likely cause in each case (see Table 3) (Brouwer et al. 2010). In addition to antibiotics, physicians sometimes give corticosteroids, anti-inflammatory drugs to reduce swelling and complications. A large review of 25 clinical trials across all age groups (Brouwer et al. 2013) found that while steroids did not significantly lower the overall risk of death, they did help in infections caused by *S. pneumoniae.* Corticosteroids were also shown to reduce the risk of hearing loss and other brain-related complications. However, these benefits were mainly observed in high-income countries. The timing of steroid treatment did not significantly affect survival, but starting it early appeared to slightly improve hearing and neurological outcomes (Brouwer et al. 2013).

Table 3. Empirical Antibiotic Regimens for Bacterial Meningitis, table adapted from literature (Brouwer et al. 2010).

Patient group	Initial therapy	Predominant bacteria
Neonates, Early onset	Ampicillin plus gentamicin or cefotaxime	*S. agalactiae, E. coli,* *L. monocytogenes*
Neonates, Late onset	Ampicillin plus an aminoglycoside or cefotaxime	*L. monocytogenes,* *S. agalactiae,* *Gram-negative bacilli*
Infants and children	Expanded-spectrum cephalosporin plus vancomycin	*S. pneumoniae,* *N. meningitidis*
Adults	Expanded-spectrum cephalosporin plus vancomycin	*S. pneumoniae,* *N. meningitidis*
Elderly	Expanded-spectrum cephalosporin plus ampicillin plus vancomycin	*S. pneumoniae,* *N. meningitidis,* *L. monocytogenes*
Immuno-compromised	Expanded-spectrum cephalosporin plus ampicillin plus vancomycin	*S. pneumoniae,* *N. meningitidis,* *L. monocytogenes*
Community-acquired recurrent meningitis	Expanded-spectrum cephalosporin plus vancomycin	*S. pneumoniae,* *N. meningitidis,* *H. influenzae*
Nosocomial meningitis	Vancomycin plus either ceftazidime, cefepime, or meropenem	*S. aureus,* *S. epidermidis,* *Gram-negative bacilli*

4.2. Viral encephalitis

Treating encephalitis should be targeted to the pathogen that causes the infection. For instance, HSV encephalitis cannot be distinguished from other viral types based on symptoms or initial test results, physicians typically begin treatment with intravenous acyclovir immediately if HSV is suspected. Starting treatment early, within the first 6 hours, has been shown to improve outcomes significantly (Tunkel et al. 2008). The dosage of acyclovir is based on ideal body weight and must be adjusted if there are any problems with kidney function. To reduce the risk of nephrotoxicity (a known side effect of acyclovir), patients should stay well-hydrated, and physicians should avoid giving other medications that could further increase the risk of nephrotoxicity (Stehman et al. 2011).

5. Clinical Outcome Measures in CNS Infections

Several validated scales are used to assess neurological recovery and long-term outcomes in patients with CNS infections, depending on the nature of the infection and the specific aspects of function being evaluated.

Glasgow Outcome Scale (GOS): The GOS is a widely accepted tool with high interobserver reliability, designed to evaluate overall functional recovery following brain injuries, including CNS infections (Jennett et al. 1976; McMillan et al. 2016). This five-point scale classifies outcomes as: 1 – death, 2 – vegetative state, 3 – severe disability (requires assistance for daily activities but can respond to commands), 4 – moderate disability (independent living possible, but unable to resume normal work or studies), 5 – mild or no disability (able to return to previous work or school activities). A score of 5 indicates a favourable outcome, while scores from 1 to 4 are considered unfavourable.

Modified Rankin Scale (mRS): Originally developed for assessing stroke recovery, the mRS is now widely applied to measure disability levels across various neurological disorders, including CNS infections (van Swieten et al. 1988). The scale ranges from 0 (no symptoms or functional limitations) to 5 (severe disability requiring constant care), providing a practical measure of a patient's degree of independence.

6. Vaccination and Chemoprophylaxis in CNS Infection Prevention

Vaccination and preventive medications help in reducing the risk of meningitis and encephalitis. For bacterial meningitis, vaccines are available for three of the most common causes in adults: *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* (Pickering & Buescher 1985). Adults with certain health conditions or risk factors are advised to get vaccinated against *S. pneumoniae* and *N. meningitidis* (Kobayashi et al. 2025; Mbaeyi et al. 2020). Routine vaccination against *H. influenzae* type b (Hib) is not needed for healthy individuals, but this is recommended for individuals with weakened immune systems (Briere et al. 2014). In some cases, such as close contact with a person infected with meningococcal or *H. influenzae* meningitis, chemoprophylaxis are recommended to stop the infection from spreading (Briere et al. 2014; Mbaeyi et al. 2020).

For viral encephalitis, prevention is especially important because many viral infections have no specific treatments and can lead to serious long-term complications. Vaccines are available to prevent diseases that can lead to encephalitis, such as measles, mumps, chickenpox (varicella-zoster), and Japanese encephalitis. These vaccines are effective in preventing both the initial infection and the risk of brain involvement. For example, after

the introduction of the varicella vaccine in Germany, cases of CNS varicella dropped by 60% (Streng et al. 2017), and in Nepal, a nationwide Japanese encephalitis vaccination campaign led to a 78% reduction in cases over five years (Upreti et al. 2017). Vector-borne viruses such as those transmitted by mosquitoes or ticks can also cause encephalitis. Protective measures include using mosquito nets, checking for ticks after outdoor activities, and applying insect or tick repellents. Travelers can also reduce their risk by avoiding high-risk areas during peak transmission seasons. e.g., in spring and early summer (Garber & Glauser 2024).

7. Scope and specific objectives

This thesis investigates the diagnostic challenges, pathogen spectrum, and clinical outcomes associated with CNS infections in resource-limited settings, with a particular focus on Vietnam. CNS infections are often life-threatening and remain underdiagnosed due to overlapping clinical features, delayed presentation, and limited diagnostic capacity. The scope of the research encompasses the evaluation of conventional and novel diagnostic tools, identification of etiological agents, and analysis of clinical predictors associated with poor outcomes. The thesis draws on data from three sequential studies: (1) a multicentre evaluation of the BioFire FilmArray Meningitis/Encephalitis (FAME) panel in comparison to conventional diagnostics, (2) the diagnostic utility of 16S Oxford Nanopore Technology (ONT) sequencing, and (3) an analysis of pathogen-specific impacts and predictors of unfavourable outcomes. Collectively, these studies aim to provide evidence for optimizing CNS infection diagnostics and improving patient management strategies in LMICs.

Specific Objectives:

1. The first study aimed to evaluate the diagnostic performance and clinical utility of the FAME panel in Vietnamese hospitals. It also aimed to compare FAME with routine microbiological diagnostics, including culture and targeted PCR, and to assess the range of pathogens detectable by FAME relative to those commonly found in the local epidemiological context. The study also aims to identify classical clinical and CSF parameters that predicted FAME positivity and thus aims to offer recommendations for tailoring molecular diagnostic panels to better align with regional pathogen prevalence.

2. The second study aimed to investigate the potential of 16S ONT sequencing as an untargeted and rapid diagnostic tool for CNS infections. It aimed to compare pathogen detection rates between 16S ONT sequencing and conventional CSF culture, evaluating the additional diagnostic yield of ONT in culture-negative cases, and exploring the clinical impact of ONT-guided results on empirical antibiotic therapy and antimicrobial stewardship. The study also aims to highlight the limitations of conventional culture and targeted molecular panels in detecting emerging or rare bacterial pathogens associated with CNS infections.

3. The third study aimed to determine the impact of causative pathogens and clinical predictors on the outcomes of CNS infections in Vietnam. It aimed to identify the spectrum and distribution of causative agents, including bacteria, viruses, fungi, and dual infections, and stratified CNS infections based on aetiology to analyse associated clinical features linked to outcomes. The study further aimed to determine key predictors of unfavourable outcomes, such as delayed presentation, altered mental status at admission, and the adequacy of empirical therapy. These findings were aimed to inform targeted clinical management strategies and guide empirical treatment recommendations in resource-constrained settings.

II. RESULTS

Chapter 1

Optimizing Diagnostic Strategies for Central Nervous System Infections in Vietnam: Evaluation of the BioFire FilmArray Meningitis/Encephalitis Panel

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Publication

Optimization of the Diagnosis of Central Nervous System Infections in Vietnamese Hospitals: Results from a Retrospective Multicentre Study

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MAJOR ARTICLE

Optimization of the Diagnosis of Central Nervous System Infections in Vietnamese Hospitals: Results From a Retrospective Multicenter Study

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Introduction. Central nervous system infections pose significant health challenges, particularly in low- and middle-income countries, because of high morbidity and mortality rates. Rapid and accurate diagnosis is essential for effective treatment to prevent adverse outcomes. Traditional culture-based diagnostics are often slow and lack specificity. This study evaluates the BioFire FilmArray Meningitis/Encephalitis (FAME) Panel against standard diagnostics in Vietnam to assess its clinical impact and suitability for local epidemiology.

Methods. We conducted a prospective study involving 330 patients with suspected central nervous system infections at 4 hospitals in northern Vietnam from July 2022 to April 2023. Cerebrospinal fluid samples were analyzed using routine culture methods and FAME. We compared pathogen detection rates and assessed the potential clinical impact of FAME results on patient management.

Results. Of the 330 cerebrospinal fluid specimens, 64 (19%) were positive by either conventional diagnostics (n = 48) and/or FAME (n = 33). The agreement between FAME and conventional diagnostics was 87%. Key pathogens *Mycobacterium tuberculosis* (n = 7), *Klebsiella pneumoniae* (n = 5), *Streptococcus suis* (n = 5), Epstein-Barr virus (n = 3), *Acinetobacter baumannii* (n = 1), and *Trichosporon asahii* (n = 1) were not detected by FAME. Classical meningitis parameter clinical symptoms, altered glucose, protein, and pleocytosis were good predictors of FAME positivity, indicating their utility in optimizing local diagnostic algorithms.

Conclusions. FAME complements traditional diagnostics by offering rapid and broad pathogen detection, crucial for timely and appropriate therapy. However, its effectiveness varies with local epidemiology, and it should not replace conventional methods entirely. Tailoring diagnostic panels to regional pathogen prevalence is recommended to enhance diagnostic accuracy and clinical outcomes in low- and middle-income countries.

Keywords. cerebrospinal fluid (CSF); CNS infections; diagnostics; FAME; Vietnam.

Central nervous system (CNS) infections represent 1 of the more severe infections associated with high morbidity and mortality, particularly in low- and middle-income countries (LMICs) [1]. Timely diagnosis and appropriate antibiotic therapy are crucial for effectively managing CNS infections because any delay in initiating such treatment is associated with unfavorable outcomes and potential sequelae [2]. Surviving individuals may experience substantial long-term consequences such as cognitive deficit, bilateral hearing loss, motor deficit, seizures, and visual impairment [3].

The diagnosis of CNS infections is challenging and typically involves the collection of cerebrospinal fluid (CSF) by lumbar puncture for culture-based microbiological diagnostics. Routine CSF diagnostics, including evaluation of cytological and biochemical parameters and clinical features, can provide insight into the etiology of the infection and attempt to distinguish between bacterial, viral, or fungal origins [4]. However, these parameters often lack the specificity to distinguish the

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infecting agent for informed decisions on appropriate empirical antimicrobial therapy. Consequently, this limitation often results in the widespread prescription of broad-spectrum antibiotics, contributing to selection pressure and the emergence of antibiotic resistance.

In addition, the traditional culture-based microbiological approach, suffers from time constraints and potential sensitivity issues, especially when patients have received prior antibiotic treatment [5]. Recognizing these limitations, molecular diagnostics have emerged as a valuable addition to the diagnostic toolkit because of their rapid turnaround time. Most commercially available molecular tests for the diagnosis of CNS infections are targeted polymerase chain reaction (PCR) assays designed to identify the most common pathogens. However, it is important to note that these tests often reflect general epidemiologic trends rather than being tailored to specific regional contexts, particularly in LMICs where epidemiologic patterns can differ significantly from those in high-income countries. In particular, there has been limited research in resource-limited settings, with only 1 study identified in Myanmar [6], whereas other studies in Asia have predominantly been conducted in non-LMIC settings, such as Taiwan [7] and Korea [8].

In this context, we evaluated the additional benefit of implementing the BioFire FilmArray Meningitis/Encephalitis Panel (FAME) compared with the standard-of-care diagnostics (culture and an in-house multiplex PCR panel) in 4 hospitals around Hanoi, northern Vietnam. The primary objective was to investigate whether the targets included in FAME were suitable for the local epidemiology in Vietnam; the secondary objective was to assess whether the implementation of such a panel would have had a meaningful clinical impact.

METHODS

Ethical Approval Statement

Written informed consent was obtained from all hospitalized patients and/or their relatives and from parents if subjects were age <18 years old. The study protocol was approved by the 108 Military Central Hospital (108 MCH) institutional review board (108MCH/RES/MENTNGITIS-V-D3-25042017). All experiments were conducted in accordance with International Council for Harmonization-Good Clinical Practice/Good Clinical Laboratory Practice (ICH-GCP/GCLP) guidelines and regulations.

Study Population and Study Design. Patients with suspected CNS infections with clinical signs of CNS infection were recruited prospectively in 4 hospitals in northern Vietnam, including 108 MCH, National Hospital for Tropical Diseases (NHTD), 103 Military Hospital (103 MH), Hanoi, and Viet Tiep Friendship Hospital (VT), Haiphong, Vietnam, between 1 July 2022 and 30 April 2023. Patients were eligible for the study if they had clinical signs of suspected CNS infections, based on the World Health Organization case definition modified by Dubot-Pérès et al [9]. Patient recruitment was at the discretion of the treating physician and followed local clinical practice. The inclusion criteria required patients to have a fever or an axillary temperature >37.5 °C and to exhibit a combination of at least 2 of the following symptoms: focal neurologic deficits, Glasgow Coma Scale abnormalities, seizures, neck stiffness, and signs of altered mental status. Exclusion criteria included: (1) patients with noninfectious, noninflammatory neurological disorders; patients not presenting to the emergency department; patients transferred from other hospitals; or those lacking an acute indication for a lumbar puncture in the emergency department; patients with contraindications to lumbar puncture, such as an intracranial space-occupying lesion with mass effect, a mass in the posterior fossa, abnormal intracranial pressure, or a local skin infection at the lumbar puncture site; (2) patients with an incomplete clinical history; and (3) patients who have not consented to the study.
A total of 330 CSF samples from 330 patients with suspected CNS infections were analyzed (199 samples collected on admission and 131 during hospitalization). Case record forms were used to collect data on demographics, medical history, clinical features on admission and subclinical findings on admission or during hospitalization, clinical course, treatment, outcome, and neurological findings on discharge. Patients were diagnosed and treated according to the clinical algorithm and management of the respective hospital. Clinical outcome was defined according to the Glasgow Outcome Scale [10]. The CSF samples were collected at various hospital sites in Vietnam and stored at −80 °C. They were then transported to Germany, where all CSF samples were analyzed using the FAME assay. The cold chain was rigorously maintained throughout the entire process to ensure the samples integrity during transportation.

Standard-of-care Laboratory Diagnostics. Routine CSF tests included cell counts and differential counts, glucose and total protein analyses and CSF bacterial cultures. If required, additional tests such as CSF fungal culture and polymerase chain reaction (PCR) tests for specific bacteria, and viruses were also performed. The microbiological culture procedures were similar on all study sites. Briefly, 1 mL of CSF was collected from a lumbar puncture for microbiological culture. Cultures were performed using the BACTEC Plus Aerobic/F System (Becton–Dickinson, Franklin Lakes, NJ, USA) at 36 °C with CO_2 for 18–72 hours. Each positive culture was grown on a selective medium such as blood agar, chocolate agar, and MacConkey agar (Merck, Kenilworth, NJ, USA). When bacterial growth was detected, colonies were selected for species identification using the matrix-assisted laser desorption/ionization time of flight VITEK MS system for automated microbial identification and antimicrobial susceptibility testing was performed using the automated VITEK®2 compact system

(BioMérieux, Lyon, France). PCR is performed at the request of physicians, in addition to routine diagnostics. An overview of the coverage of the PCR panel at the different study sites is summarized in Supplementary Table 1.

BioFire FilmArray Meningitis/Encephalitis Assays. All frozen CSF samples collected from patients by lumbar puncture were analyzed with FAME following manufacturer's instructions. In brief, frozen, noncentrifuged CSF (200 µL) was placed in the bag after the hydration solution had been injected and processed according to the manufacturer's instructions. FAME panel included 14 pathogens, namely *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Streptococcus agalactiae*, *Haemophilus influenzae*, *Listeria monocytogenes*, *Escherichia coli K1*, herpes simplex virus (HSV) 1 and HSV 2, human herpes virus 6, cytomegalovirus (CMV), Enterovirus, human parechovirus, varicella zoster virus (VZV), and *Cryptococcus neoformans/gattii*.

Data Analysis. Descriptive analysis was performed using SPSS Statistics 28 (IBM). The presence of pleocytosis, was quantitatively defined by 2 separate cutoff ranges for the corrected white blood cell count in CSF: ≥ 5 cells/mm^3 and ≥10 cells/mm^3 [11]. Abnormal CSF glucose and protein levels were defined for all patients as values of <2.8 mmol/L or >4.2 mmol/L and <0.10 g/L and >0.25 g/L, respectively, based on the 108 MCH criteria. Discordant results were defined as divergent results only when the pathogen was included in the standard of care diagnostic panel. The association of the clinical parameters with the positivity of detection by conventional or FAME method was evaluated using a Random Forest model using the positive/negative status as a prediction of the importance (Gini) of all available clinical parameters as predictors using the package Random Forest in R 4.3.3. Missing values were handled using the command na.roughfix, which replaced the quantitative missing value with the overall population medians and the qualitative values with the most frequent values in the population.

RESULTS

General Characteristics of the Study Cohort

A total of 330 patients from 456 patients with suspected CNS infections (111 fulfilling exclusion criteria and 17 nonconsent) were recruited in various hospitals around Hanoi, Vietnam (Supplementary Figure 1). Recruitment numbers varied by site: 40 patients from 103 MH, 52 from 108 MH, 136 from the NHTD, and 102 from VT Hospital. At 108 MH, 52 of 81 eligible patients were recruited, whereas the remaining 29 were excluded because of reasons such as transfer from other hospitals (n = 6 patients), incomplete clinical histories (n = 6), death (n = 2), not presenting directly to the emergency department (n = 4), lack of consent (n = 5), and family decisions to take elderly patients home (n = 6). At NHTD, 136 of 175 patients were recruited, with 6 declining consents and 33 fulfilling the exclusion criteria. At 103 MH, 40 of 60 patients were recruited, with 2 not consenting and 18 fulfilling the exclusion criteria. No further data were available from these 2 hospitals. At VT, 102 of 140 patients were recruited, with the remaining 38 patients excluded because of contraindications to lumbar puncture (n = 5), transfers from other hospitals (n = 13), lack of consent (n = 4), diagnosis was uncertain and inconclusive (n = 7), and no retrievable clinical history (n = 9). The median age of the cohort was 54 years (range, 11–97 years). Of these, 225 (68%) were male. The main clinical characteristics are summarized in Table 1. Regarding preexisting medical conditions, 27% of patients had hypertension, 19% had diabetes, and 6% had cardiac disease. Common clinical presentations included fever (83%), headache (67%), neck stiffness (63%), and altered mental status (defined by a Glasgow Coma Scale score below 14) in 43% of cases. Notably, 83% of patients had at least 2 of the following symptoms: headache, fever, neck stiffness, and altered mental status.

Detected Pathogens by FAME and Conventional Diagnostics

In total, 64 of 330 (19%) samples yielded positive results for bacterial, fungal, and/or viral pathogens. In our study, tuberculous meningitis was the leading cause of community-acquired CNS infections with community onset in 7 of 8 positive cases. The leading causes of community-acquired bacterial CNS infections were *S pneumoniae* with community-onset in 5 of 6, *Streptococcus suis* with 4 of 5, and *H influenzae* with 4 of 5 cases. Meanwhile, *Klebsiella pneumoniae* with hospital-onset in 5 of 7 positive cases, is the most common bacterial pathogen detected in hospital-acquired CNS infections. Among viral pathogens, VZV and HSV were predominant and equally represented in community-onset and hospital-onset viral CNS infections. *C neoformans* was the most common fungal pathogen but was only sporadically detected. *E coli,* HSV-2, human herpes virus 6 and human parechovirus were not detected in the study with FAME (Figure 1).

The laboratory procedures for culture-based diagnostics were consistent across all 4 study sites. However, there were variations in the range of pathogens covered by the local molecular diagnostics (PCR) panels (Supplementary Table 1). Consequently, the evaluation of the results of the FAME and the conventional diagnostics results (culture plus molecular method) differed between the centers (Figure 2). Overall, in 48 CSF samples, pathogens were detected by conventional method and in 33 samples, a positive result was obtained in the FAME.

The positivity rates at the different study sites were 10% (4/40) for 103 MH, 37% (19/52) for 108 MCH, 24% (33/136) for NHTD, and 8% (8/102) for VT, detected by either conventional methods or FAME. As expected, the highest concordance was observed at 108 MCH, which had the most extensive PCR panel, with 63% (5/8 pathogens) concordance in

Table 1. Demographic and Clinical Characteristics of Vietnamese Patients With Central Nervous System Infections

General Characteristics	CNS Infections (n = 330)	Routine Positive Specimens (n = 48)	Routine Negative Specimens (n = 282)	*P* Value	FAME-positive Specimens (n = 33)	FAME-negative Specimens (n = 297)	*P* Value
Demographics							
Age (mean ± SD)	54 ± 19	53 ± 18	55 ± 19	.47	53 ± 18	55 ± 19	.62
Male sex—no. (%)	225 (68)	37 (77)	188 (67)	.15	24 (73)	201 (68)	.56
Underlying condition—no. (%)							
Hypertension	90 (27)	8 (17)	82 (29)	.07	10 (30)	80 (27)	.68
Diabetes	62 (19)	2 (4)	60 (21)	.005	4 (12)	58 (20)	.30
Cardiac disease	19 (6)	1 (2)	18 (6)	.33[a]	0 (0)	19 (6)	.24[a]
Alcoholism	17 (5)	8 (17)	9 (3)	<.001[a]	2 (6)	15 (5)	.68[a]
Chronic liver disease	18 (6)	4 (8)	14 (5)	.31[a]	1 (3)	17 (6)	1[a]
Chronic lung disease	17 (5)	3 (6)	14 (5)	.72[a]	3 (9)	14 (5)	.24[a]
Kidney disease	15 (5)	2 (4)	13 (5)	1[a]	1 (3)	14 (5)	1[a]
Immunosuppressive drugs	13 (4)	1 (2)	12 (4)	.7[a]	1 (3)	12 (4)	1[a]
Cancer	13 (4)	0 (0)	13 (5)	.23[a]	0 (0)	13 (4)	.38[a]
HIV-positive	9 (3)	1 (2)	8 (3)	1[a]	0 (0)	9 (3)	.61[a]
Risk factor—no. (%)							
Post neurosurgery	29 (9)	5 (10)	24 (9)	.59[a]	3 (9)	26 (9)	1[a]
Head trauma	31 (9)	2 (4)	29 (10)	.28[a]	3 (9)	28 (9)	1[a]
CSF shunt	5 (2)	0 (0)	5 (2)	1[a]	0 (0)	5 (2)	1[a]
Clinical features—no. (%)							
Fever (>37.5 °C)	273 (83)	46 (96)	227 (81)	.009	32 (97)	241 (81)	.02
Headache	221 (67)	37 (77)	184 (65)	.11	27 (82)	194 (65)	.06
Neck stiffness	207 (63)	40 (83)	167 (59)	.001	27 (82)	180 (61)	.02
Nausea/vomiting	99 (30)	21 (44)	78 (8)	.025	13 (39)	86 (29)	.21
Seizure	29 (9)	3 (6)	26 (9)	.78[a]	1 (3)	28 (9)	.34[a]
Focal neurologic deficits	20 (6)	4 (8)	16 (6)	.51[a]	2 (6)	18 (6)	1[a]
Glasgow Coma Score	13 ± 2	12 ± 2	13 ± 2	<.001	13 ± 2	13 ± 2	.62
< 14 (indicating altered mental status)—no. (%)	142 (43)	36 (75)	106 (38)	<.001	19 (58)	123 (41)	.08
At least 2 of 4 symptoms (headache, fever, stiff neck, and altered mental status)	273 (83)	45 (94)	228 (81)	.03	32 (97)	241 (81)	.02
Outcome							
Death	32 (10)	4 (8)	28 (10)		3 (9)	29 (10)	
Vegetative state	29 (9)	10 (21)	19 (7)		3 (9)	26 (9)	
Severe disability	64 (19)	8 (17)	56 (20)		4 (12)	60 (20)	
Moderate disability	62 (19)	5 (10)	57 (20)		5 (15)	57 (19)	

Table 1. Continued

General Characteristics	CNS Infections (n = 330)	Routine Positive Specimens (n = 48)	Routine Negative Specimens (n = 282)	*P* Value	FAME-positive Specimens (n = 33)	FAME-negative Specimens (n = 297)	*P* Value
Mild or no disability	143 (43)	21 (44)	122 (43)		18 (55)	125 (42)	
Cerebrospinal fluid							
Cell count (cells/mm^3)[b]							
mean ± SD	1032 ± 5897	4898 ± 14437	376 ± 1726	<.001	3315 ± 7751	761 ± 5601	.007
Pleocytosis of 5 cells/mm^3	225 (69)	42 (89)	183 (66)	.001	29 (88)	196 (67)	.015
Pleocytosis of 10 cells/mm^3	175 (54)	39 (83)	136 (49)	<.001	26 (79)	149 (51)	.003
Protein (g/L)[c]							
Mean ± SD	1.56 ± 2.78	3.54 ± 4.01	1.22 ± 2.36	<.001	2.38 ± 2.36	1.47 ± 2.82	.07
Abnormal protein	307 (94)	48 (100)	259 (93)	.089[a]	33 (100)	274 (94)	.24[a]
Glucose (mmol/L)[c]							
Mean ± SD	4.16 ± 2.96	2.11 ± 1.85	4.50 ± 2.98	<.001	3.35 ± 3.85	4.25 ± 2.84	.09
Abnormal glucose	188 (58)	36 (77)	152 (54)	.005	23 (70)	165 (56)	.10

Abbreviation: CSF, cerebrospinal fluid; FAME, BioFire FilmArray Meningitis/Encephalitis.

[a]Fisher exact test.

[b]The CSF leukocyte count was determined in 324 patients; CSF specimens from 6 patients had too many leukocytes for an exact count to be performed (missing values, n = 6 for FAME negative, n = 1 for routine test positive and n = 5 for routine test negative).

[c]The protein and glucose levels in the CSF were determined in 326 patients; the remaining 4 CSF samples were not measured (missing values, n = 4 for FAME negative and n = 4 for routine test negative).

pathogen detection between conventional diagnostics and FAME. Conversely, the lowest concordance was observed at VT, the center without molecular diagnostics. At the study site VT, of the 8 pathogens covered by FAME, none was detected by conventional diagnostics.

After a thorough review of patient data and charts of discrepancies between FAME results and conventional diagnostic methods, there were 286 matched sample results with an overall match rate of 87% (286/330). In 6 CSF specimens, FAME yielded positive results, primarily for the detection of viral pathogens (3 CMV and 1 HSV-1), as well as for *H influenzae* (n = 2) (Table 2). For the samples with positive CMV and HSV-1 signals in FAME, another pathogen (*K pneumoniae*, *A baumannii*, and *M tuberculosis*) could be detected by blood culture or other molecular methods, which may better explain the clinical presentation. However, no specific PCR was ordered for the remaining two samples with *H influenzae* detection by FAME, so we cannot be sure whether these samples were false positives. In addition, there were 4 pathogens, *E coli* (1/1), *C neoformans* (2/3), HSV-1 (3/8), and VZV (1/9), that were included in the FAME but yielded negative results (Supplementary Tables 2 and 3). Although *M tuberculosis*, *K pneumoniae*, and *S suis* were among the most detected bacterial pathogens, these targets were not included in the FAME panel.

Implications for Antimicrobial and Diagnostic Stewardship

The national recommendation for antibiotic treatment regimens for patients with suspected bacterial meningitis is summarized in Supplementary Table 4. The clinical data and the laboratory results were analyzed retrospectively to determine the potential impact on antibiotic prescribing in our study cohort. Of the 33 FAME-positive cases, 20/33 (61%) patients were indicated for inappropriate empirical antibiotic therapy. If the FAME results had been available earlier, 20 patients would have benefited from FAME diagnosis, including antibiotic discontinuation in 8 patients, antibiotic de-escalation in 5, antibiotic change in 3, optimization of therapy in 2, antibiotic de-escalation and antiviral drug discontinuation in 1, and antiviral drug discontinuation in 1 (Supplementary Table 2).

After extensive analysis by machine learning algorithms of various clinical parameters and patient data collected for this study to predict pathogen positivity using either conventional methods or FAME indicated that conventional diagnostic markers emerge as the most important predictors (error rate of 10.61% for FAME and 11.52% for routine diagnostics). Specifically, these markers include CSF cell counts, abnormal (low) glucose levels, and elevated protein levels. Consequently, the integration of complementary and broadly targeted molecular diagnostics for CNS infections has the potential to improve diagnostic accuracy in

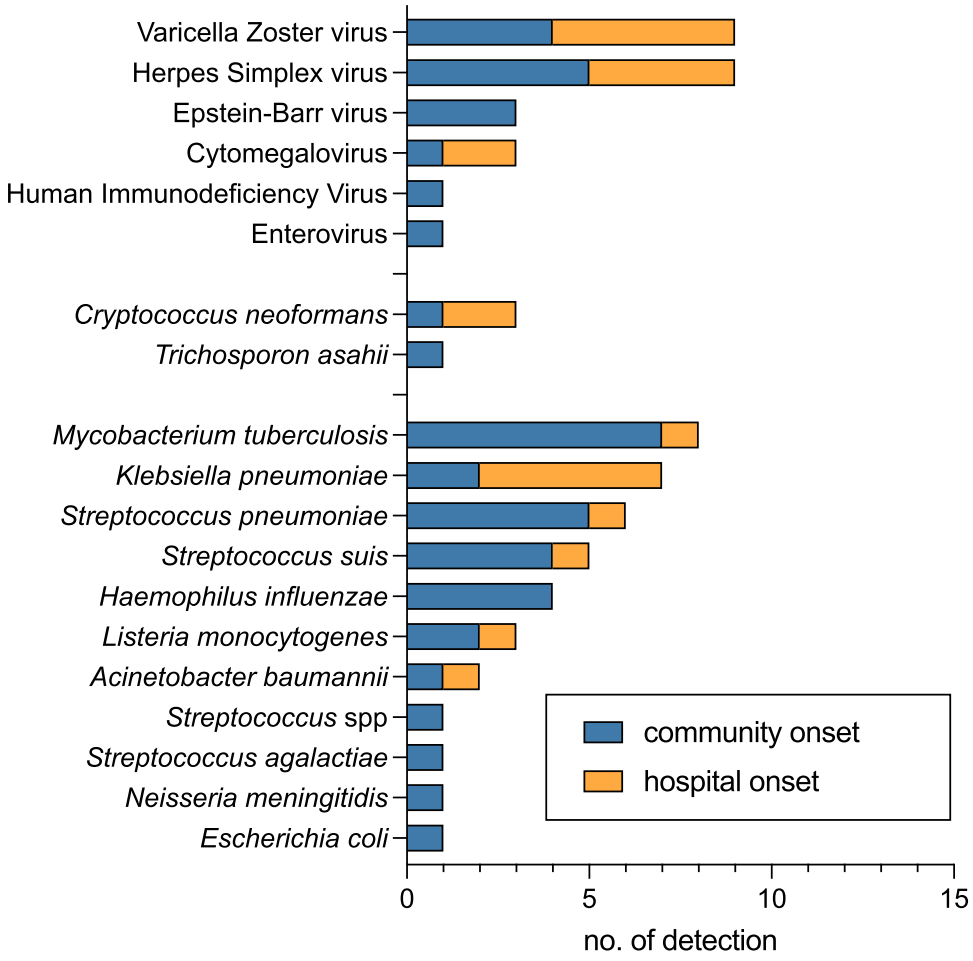

Figure 1. Distribution of bacterial, viral and fungal pathogens by onset of infection.

patients with suspected CNS infections who present with these clinical parameters (Supplementary Figure 2).

DISCUSSION

Our study showed that targeted molecular diagnostics, such as FAME or in-house molecular diagnostics, can complement conventional culture-based microbiological diagnostics in detecting CNS infections. Furthermore, clinical presentation and laboratory parameters such as headache, neck stiffness, and elevated CSF cell count, protein, and glucose levels can be used to improve the diagnostic algorithm for CNS infections in Vietnam. Implementing an evidence-based diagnostic algorithm in the sense of combined antimicrobial/diagnostic stewardship could increase cost-effectiveness by avoiding overtesting or overordering of expensive molecular tests [11, 12]. A study by Broadhurst et al [13]. showed that the positivity rate of FAME could be increased from 11.5% (53/459) to 18.6% (49/263) by implementing a testing algorithm.

Inappropriate administration and/or overuse of antibiotics to patients is one of the main causes of the emergence of resistant bacteria, leading to significantly longer hospital stays and considerable costs for the healthcare system as well as for patients and their families [14]. The implementation of rapid molecular diagnostics, such as FAME, could reduce the turnaround time of microbiological diagnostics of CSF, potentially leading to faster optimization/adjustment of antimicrobial therapy (Supplementary Table 2). Thus, complementing FAME to antimicrobial stewardship programme interventions may help to enhance clinical impact avoiding the overuse of antimicrobial substances.

Another benefit of implementing molecular diagnostics for diagnosing CSF infection is the possibility of simultaneous detection of bacterial, viral, and fungal pathogens. Moreover, molecular diagnostics have been shown to have a higher sensitivity for patients previously treated with antibiotics before sampling [15]. Diagnostic results should always be interpreted in conjunction with the clinical features, CSF analysis, and other available microbiological results. In our study, FAME contributed to the identification of more than 9 bacteria in culture-

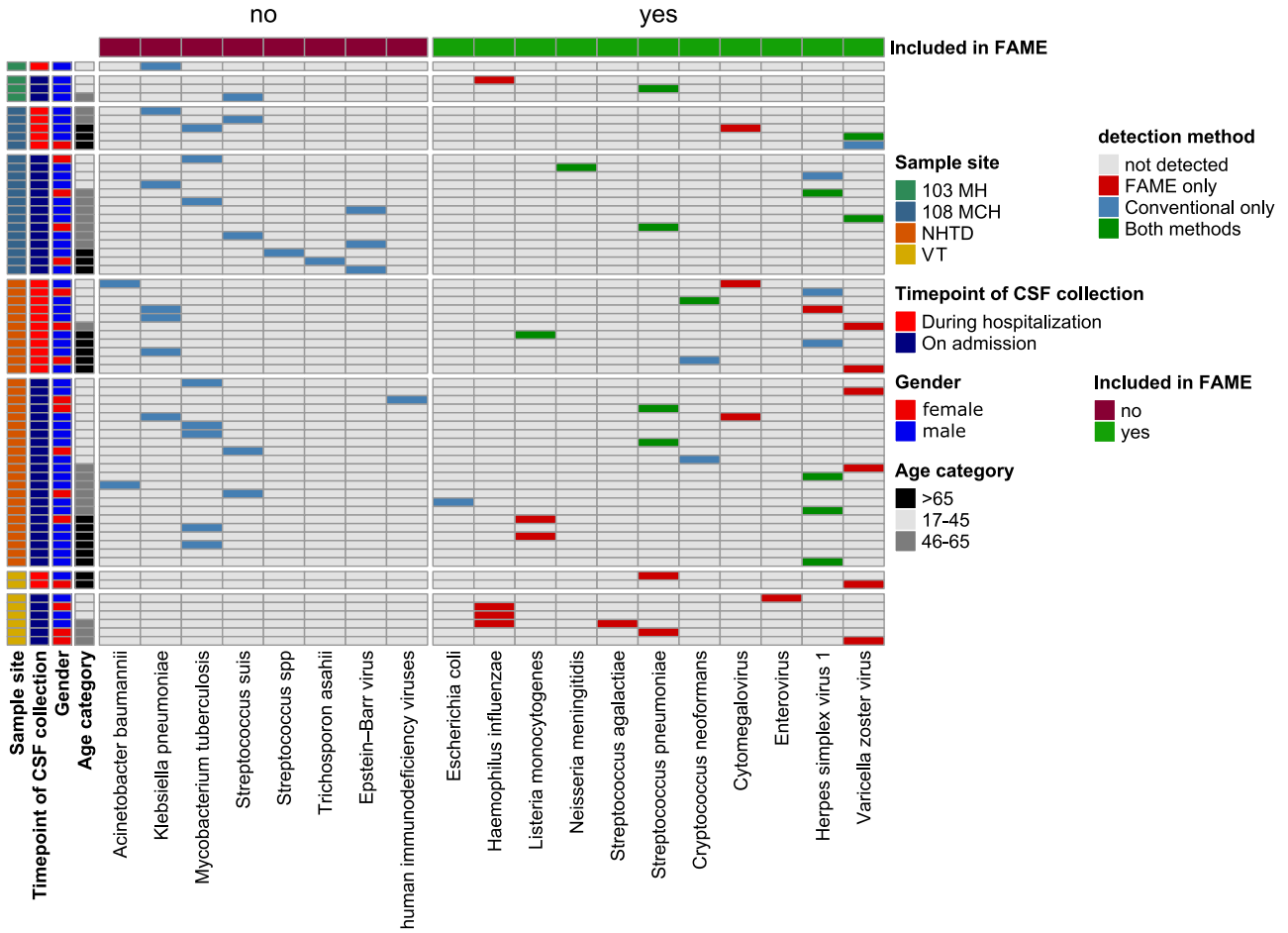

Figure 2. Overview of pathogen detection by various diagnostic algorithms. 103 MH, 103 Military Hospital; 108 MCH, 108 Military Central Hospital; NHTD, National Hospital for Tropical Diseases; VT, Viet Tiep Friendship Hospital; FAME, BioFire FilmArray Meningitis/Encephalitis Panel; CSF, cerebrospinal fluid.

negative CSF samples, including *L monocytogenes* (n = 3), *H influenzae* (n = 3), *S pneumoniae* (n = 2), and 1 case of co-detection (*H influenzae* and *S agalactiae*), of which 4 patients had taken antibiotics before lumbar puncture. Potential explanations for the discordance in the detection of *H influenzae* and *S pneumoniae* could the higher sensitivity of molecular methods than conventional culture [5] or false positive signals of the FAME assay, which has been reported previously [16].

However, we also observed several false-positive and false-negative signals by FAME in this study. After a thorough review of the patient data and charts of the inconsistencies between the results obtained by FAME and conventional diagnostic methods, there were 7 instances in which FAME produced false-negative results for pathogens included in its panel. These included HSV-1 (n = 3), *C neoformans* (n = 2), *E coli* (n = 1), and VZV (n = 1). Notably, patients in these cases had clinical symptoms consistent with a CNS infection, which were supported by the results of conventional diagnostic tests. It is of note that only *E coli* with a K1 capsule type is included in the FAME PCR panel; non-K1 *E coli* strains will therefore not be detected. Although, *C neoformans* can be detected by the FAME, implementation of *Cryptococcus* antigen testing in addition to microscopy and culture remains an important strategy in the diagnosis and management of cryptococcal disease [17]. In 6 CSF samples, FAME yielded positive results in contrast to the standard-of-care diagnostics, primarily for the detection of viral pathogens (3 CMV and 1 HSV-1), as well as for *H influenzae* (n = 2), primarily for the detection of viral pathogens (3 CMV and 1 HSV-1), as well as for *H influenzae* (n = 2). It is noteworthy that for CMV and HSV-1, microbiologic analysis of CSF and/or blood cultures also revealed positive results for other bacterial pathogens (CMV/*M tuberculosis* n = 1, HSV/*K pneumoniae* n = 1, CMV/*A baumannii* n = 1, and CMV/*K pneumoniae* n = 1), which may provide a more accurate explanation for the observed symptoms. Two cases of *H influenzae* detection were likely to be false-positive signals because CSF analysis showed no pleocytosis, normal protein and glucose levels, and no agreement with the diagnosis at discharge (Supplementary Table 1). The determination of false-positive

Table 2. Comparison of FAME and Comparator Test Results for the Diagnosis of CNS Infections in Vietnam

Results Interpretation	FAME	Comparator Methods (CSF Culture and/or PCR)	Clinical Diagnosis	No. of Patients	Identified Pathogens
Concordant results					
	Positive	Positive (concordant pathogen)	Agreement	13	*S pneumoniae* (n = 4), HSV-1 (n = 4), VZV (n = 2), *N meningitidis* (n = 1), *L monocytogenes* (n = 1), *C neoformans* (n = 1).
	Positive	Negative	Agreement	7	*L monocytogenes* (n = 3), *S pneumoniae* (n = 2), *H influenzae* (n = 1), *H influenzae* + *S agalactiae* (n = 1)
	Negative	Negative	-	266	…
Discordant results					
Expected pathogen was included in the FAME panel					
	Negative	Positive (pathogens included in FAME)	Disagreement	7	HSV-1 (n = 3), *C neoformans* (n = 2), *E coli* (n = 1), VZV (n = 1)
	Positive	Negative	Disagreement	2	*H influenzae* (n = 2)
	Positive	Positive (discordant pathogen)	Disagreement	4	CMV/*M tuberculosis* (n = 1), HSV-1/*K pneumoniae* (n = 1), CMV/*A baumannii* (n = 1), CMV/*K pneumoniae* (n = 1)
Not included in the FAME panel or standard-of-care diagnostic panel					
	Positive	Not performed/not ordered	NA	7	VZV (n = 6) and Enterovirus (n = 1)
	Not included	Positive	NA	24	*M tuberculosis* (n = 7), *K pneumoniae* (n = 5), *S suis* (n = 5), Epstein–Barr virus (n = 3), *A baumannii* (n = 1), *Trichosporon asahii* (n = 1), *Streptococcus* spp (n = 1), and HIV (n = 1)

Abbreviations: CNS, central nervous system; FAME, BioFire FilmArray Meningitis/Encephalitis; HSV, herpes simplex virus; NA, not applicable; VZV, varicella zoster virus.

rates for *H influenzae* in CSF with FAME has been described previously by Zanella et al [18], in which only 1/17 FAME-positive samples could be confirmed by culture.

Our study highlights the importance of considering local epidemiology when selecting the most appropriate test panel for targeted diagnostics. FAME covers relevant pathogens causing community-acquired CNS infections but does not detect ESKAPE pathogens (*E faecium*, *S aureus*, *K pneumoniae*, *A baumannii*, *P aeruginosa,* and *Enterobacter spp*) that are considered to cause CNS infections in healthcare settings [12, 19]. Importantly, the performance of the panel is expected to depend on the epidemiology of CNS infections in different geographic regions [7]. In Vietnam, CNS infections are complex because they can be caused by a different pathogen spectrum compared to those in high-income and industrialized countries. *M tuberculosis,* which was also not included in the panel, remains a major public health challenge in Vietnam, where the incidence ranges from 260 to 399 per 100 000 population [20]. Meanwhile, *S suis* is one of the most common pathogens causing CNS infections in Vietnam and is associated with the consumption of undercooked pork or raw pig blood [21, 22]. In line with previous studies, our data suggest a lower sensitivity of the FAME assay in detecting HSV-1 and *C neoformans* [23–25] (Supplementary Table 3). Our results suggest that the implementation of a local epidemiology-adapted molecular diagnostic panel may be a better option than a commercial molecular diagnostic platform for resource-limited settings, as demonstrated by the performance of the standard of care diagnostics of the 108 MCH in this study.

In conclusion, our study suggests that the algorithm for effective use of FAME should be applied to patients with acute CNS infections not related to neurosurgery and a CSF pleocytosis of 5 cells/mm^3. Although FAME is proving invaluable in the rapid detection of common meningitis pathogens, it should be used as a complementary rather than a replacement for conventional testing because it may not detect all pathogens associated with CNS infections. The implementation of FAME in a resource-limited laboratory setting with limited access to molecular methods could improve the diagnostic accuracy. Practitioners must exercise caution in interpreting and selecting results, considering the regional specificity of commercially available targeted molecular diagnostics.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author Contributions. T.P.V. designed, supervised the study, and contributed to the study materials and assays. D.N. and S.P. were involved in the conceptualization and contributed to the study materials. D.V.D., L.T.L.K., K.S., and V.U. performed the experimental procedures. D.V.D., D.N., S.H., J.R., and S.B. were involved in the statistical analysis and validation of the results. D.V.D., V.V.S., N.D.M., N.X.H., H.X.Q., T.T.L., V.D.T., L.H.S., and N.T.T recruited the patients and contributed to the investigation materials for sampling procedures. D.V.D. wrote the first draft. T.P.V., D.N., S.H., J.R., P.K., D.V.D., and N.H. reviewed the first draft. All authors have read and approved the manuscript.

Data availability. Not Applicable. The authors confirm that the data supporting the findings of this study are available within the article and in its supplementary material.

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Ethical approval and consent to participate. Written informed consent was obtained from all hospitalized patients and/or their relatives, and from parents if subjects were aged <18 years. The study protocol was approved by the 108 MCH institutional review board (108MCH/RES/MENTNGITIS-V-D3-25042017). All experiments were conducted in accordance with ICH GCP guidelines and regulations.

Consent for publication. All authors read and consented to publish the article.

Potential conflicts of interest. All authors: No reported conflicts.

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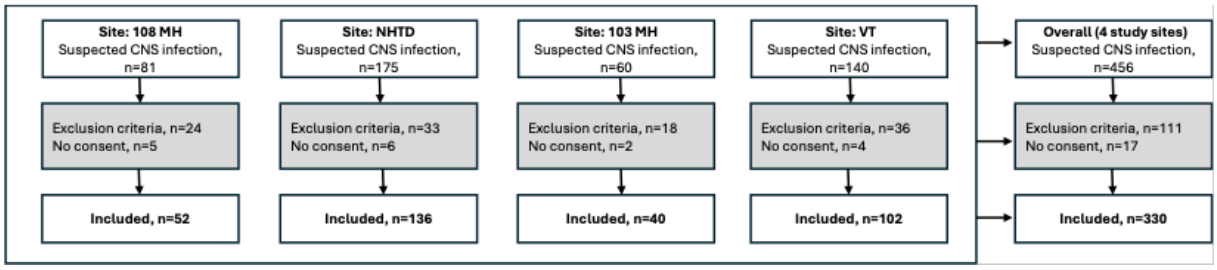
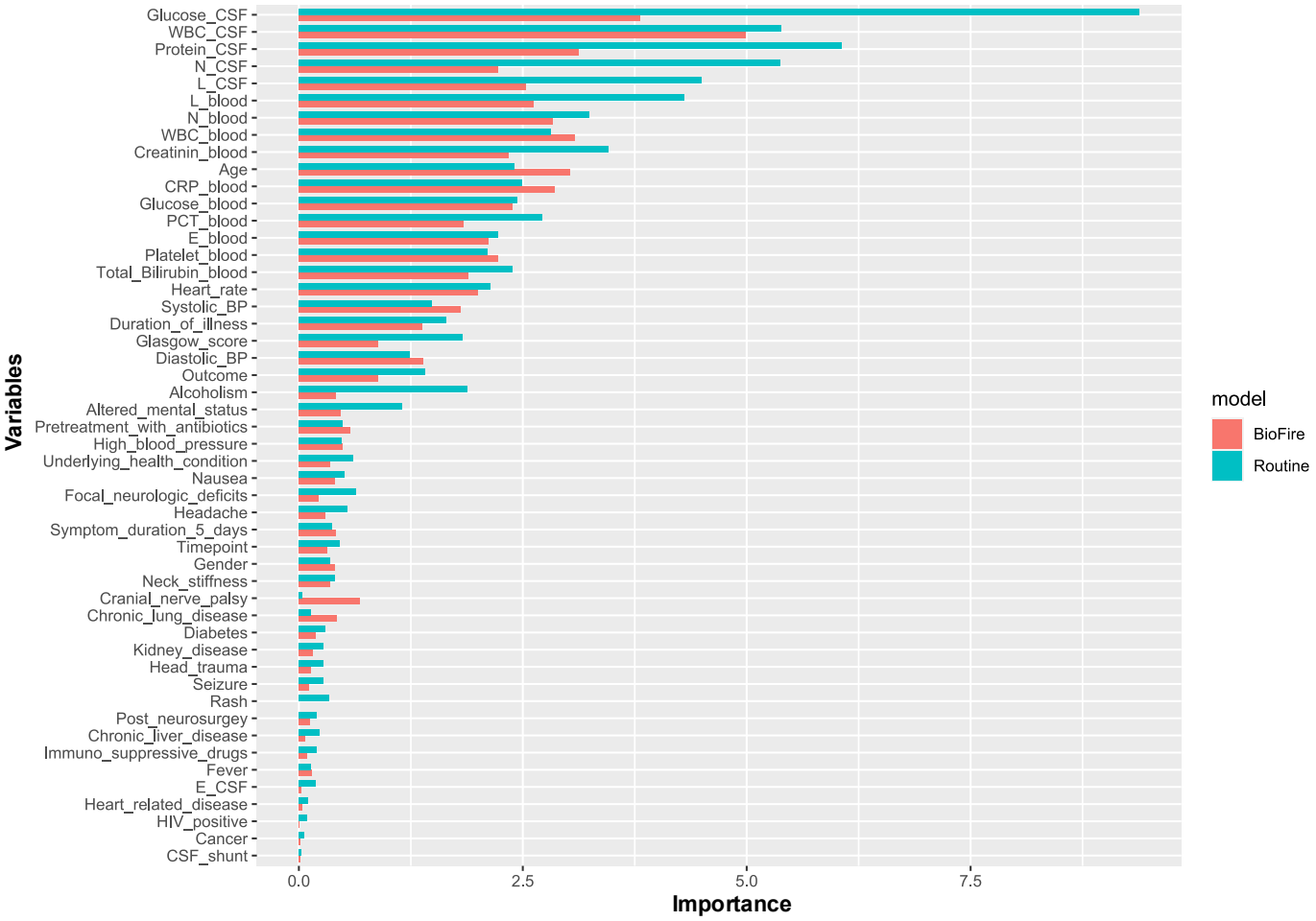
Supplementary Appendix

Supplementary Figure S1. Overview of patient inclusion at four study sites.



Supplementary Figure S2. Variable importance plot from both random forest regression model.



FAME OOB estimate of error rate: 10.61%			
Confusion matrix			
	0	**1**	**class.error**
0	294	3	0.01010101
1	32	1	0.96969697
Routine Diagnostic OBB estimate of error rate: 11.52%			
Confusion matrix			
	0	**1**	**class.error**
0	278	4	0.0141844
1	34	14	0.7083333

Variable importance plot showing the relative importance of each clinical variables as a predictor of the positivity of detection of pathogen by either routine diagnostics or FAME.

Abbreviations: BP, blood pressure; CRP, C-reactive protein; CSF, cerebrospinal fluid; E, eosinophils; FAME, BioFire® FilmArray® Meningitis/Encephalitis Panel; HIV, human immunodeficiency viruses; L, lymphocyte; N, neutrophil; PCT, Procalcitonin; WBC, white blood cells.

Supplementary Appendix

Supplementary Table S1: Standard-of-care diagnostics for diagnosing central nervous system infections in four Vietnamese hospitals.

No.	Hospital	Routine diagnostics	Note
1	108 MCH	Bacteria: CSF Gram stain, CSF culture, blood culture, CSF realtime PCR, PCR-based assays and GenXpert.	Panel realtime PCR for detection of bacteria causing meningitis: *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Streptococcus agalactiae*, *Haemophilus influenzae*, *Listeria monocytogenes*, *Escherichia coli K1*.
			PCR-based assays: *Streptococcus suis, Streptococcus spp, Enterococcus spp, Staphylococcus spp, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Mycobacterium tuberculosis.*
			GenXpert: *M.tuberculosis.*
		Viruses: CSF realtime PCR, PCR-based assays	Panel realtime PCR for detection of viruses: Herpes simplex virus 1/2, Cytomegalovirus, Epstein–Barr virus, Varicella zoster virus, Human herpes virus 6 and Human herpes virus 7.
			PCR-based assays: Japanese encephalitis virus, Dengue virus, Enterovirus.
		Fungi: CSF smear, fungal CSF culture	
2	NHTD	Bacteria: CSF Gram stain, CSF culture, blood culture, CSF PCR-based assays and GenXpert.	PCR-based assays: *M.tuberculosis*
			GenXpert: *M.tuberculosis*
		Viruses: CSF PCR-based assays	PCR-based assays: Herpes simplex virus 1/2, *S.suis*
		Fungi: CSF smear, fungal culture	
3	VT	Bacteria: CSF Gram stain, CSF culture, blood culture	
		Fungi: CSF smear, fungal CSF culture	
4	103 MH	Bacteria: CSF Gram stain, CSF culture, CSF PCR-based assays and GenXpert, blood culture	PCR-based assays: *N.meningitis, M.tuberculosis*
			GenXpert: *M.tuberculosis*
		Viruses: CSF PCR-based assays	PCR-based assays: Cytomegalovirus, Epstein–Barr virus
		Fungi: CSF smear, fungal CSF culture	

Abbreviations: CSF, Cerebrospinal fluid; PCR, Polymerase chain reaction; 108 MCH, 108 Military Central Hospital; 103 MH, 103 Military Hospital; NHTD, National Hospital for Tropical Diseases; VT, Viet Tiep Friendship Hospital.

Note: There are differences in the performance of four hospitals in routine diagnostics. 108 MCH, NHTD and 103 MH, the three national hospitals in Vietnam, are equipped with many modern devices, so the diagnostic capacity is better than VT, a provincial hospital.

Supplementary Appendix

Supplementary Table S2. Clinical and laboratory data of patients with CNS infections who were tested positive for FAME.

No.	Age/ Gender	Timepoint of CSF collection	FAME	CSF parameters				Discrepancy investigation					Appropriate empirical therapy	Possible impact of FAME result on treatment	Interpretation[a]	Outcome[b]
				WBC	Pro	Glu	CSF culture	Antibiotic use before sampling	Empirical therapy	PCR/culture[a]	Clinical diagnosis	Specific therapy				
1	59 yr/F	On admission	*S. pneumoniae*	320	4.1	0.09	*S. pneumoniae*	No	MER, AMK, DEX	CSF PCR and blood culture (+) *S. pneumoniae*	*S. pneumoniae* septic shock and meningitis	CRO, AMK, DEX	No	Antibiotic de-escalation	TP	1
2	42 yr/M	On admission	*S. pneumoniae*	18,050	6.7	0.01	*S. pneumoniae*	No	CRO, DEX	Blood culture (+) *S. pneumoniae*	*S. pneumoniae* sepsis and meningitis	CRO, VAN, DEX	Yes	Optimisation of therapy	TP	5
3	31 yr/F	On admission	*S. pneumoniae*	1960	2.13	0.2	*S. pneumoniae*	No	CRO, DEX	None	*S. pneumoniae* meningitis	MER	Yes	Optimisation of therapy	TP	5
4	41 yr/M	On admission	*S. pneumoniae*	10,460	1.65	0	*S. pneumoniae*	No	CRO, LVF, MTP	Blood culture (+) *S. pneumoniae*	*S. pneumoniae* sepsis and meningitis	CRO, MTP	No	Antibiotic de-escalation	TP	5
5	70 yr/M	During hospitalization	*S. pneumoniae*	2062	2.69	5	Negative	Yes	CRO, CIP, DEX	Blood culture (+) *S. pneumoniae*	*S. pneumoniae* sepsis and meningitis	CRO, CIP, DEX	No	Antibiotic de-escalation	TP	5
6	48 yr/F	On admission	HSV-1	3	1.13	3.4	Negative	No	CFT, ACV, DEX	CSF PCR (+) HSV-1	HSV meningoencephalitis	ACV, DEX	No	Antibiotic discontinuation	TP	5
7	79 yr/M	On admission	HSV-1	446	0.76	3.9	Negative	No	ACV	CSF PCR (+) HSV-1	HSV encephalitis	ACV	Yes	NA	TP	3
8	63 yr/M	On admission	HSV-1	245	0.58	3.81	Negative	Yes	ACV, MER, DEX	CSF PCR (+) HSV-1	HSV encephalitis	ACV, DEX	No	Antibiotic discontinuation	TP	4

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9	58 yr/M	On admission	HSV-1	58	0.63	4.91	Negative	Yes	ACV, CRO, DEX	CSF PCR (+) HSV-1	HSV encephalitis	ACV, CRO, DEX	Yes	Antibiotic discontinuation	TP	3
10	73 yr/M	During hospitalization	VZV	6	0.77	3.3	Negative	No	ACV	CSF PCR (+) VZV	VZV encephalitis	ACV, DEX	Yes	NA	TP	3
11	58 yr/M	On admission	VZV	1	0.8	5.1	Negative	No	CRO, ACV, DEX	CSF PCR (+) VZV	VZV meningoencephalitis	ACV, DEX	No	Antibiotic discontinuation	TP	5
12	20 yr/M	On admission	*N. meningitidis*	8000	7.5	0	*N. meningitidis*	Yes	MER, CIP, DEX	CSF PCR (+) *N. meningitidis*	*N. meningitidis* sepsis and meningitis	CRO, CIP	No	Antibiotic de-escalation	TP	5
13	66 yr/M	During hospitalization	*L. monocytogenes*	1624	3.53	0.68	*L. monocytogenes*	Yes	MER, VAN	None	*L. monocytogenes* meningitis	MER, VAN, AMP	No	Antibiotic change	TP	2
14	40 yr/M	During hospitalization	*C. neoformans*	445	1.71	2.4	Negative	Yes	MER, VAN	CSF fungal culture (+) *C. neoformans*	Cryptococcal meningitis	AMB, FLU	No	Antibiotic discontinuation	TP	1
15	71 yr/M	During hospitalization	CMV	54	1.31	1.7	Negative	Yes	MER, VAN, AMK	CSF GeneXpert® (+) *M. tuberculosis*	Tuberculous meningitis	MER, AMK, LNZ, LVF, DEX	No	NA	FP[c]	1
16	44 yr/M	During hospitalization	HSV-1	9217	8.83	0.1	*K. pneumoniae*	Yes	CRO, LVF, MET, DEX	Blood culture (+) *K. pneumoniae*	*K. pneumoniae* sepsis and meningitis	MER, AMK	No	NA	FP[c]	4
17	23 yr/M	During hospitalization	CMV	38,731	6.34	0.1	*A. baumannii*	Yes	MER, VAN, COL	None	*A. baumannii* meningitis, post neurosurgery	MER, COL	Yes	NA	FP[c]	2
18	37 yr/M	On admission	CMV	199	6.09	4.33	*K. pneumoniae*	Yes	MER, COL	None	*K. pneumoniae* meningitis and pneumoniae, post neurosurgery	MER, COL	Yes	NA	FP[c]	2

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19	73 yr/M	On admission	*L. monocytogenes*	828	0.94	1.9	Negative	Yes	MER, AMP ACV	None	Bacterial meningitis	MER, AMP	No	Antibiotic de-escalation, antiviral drug discontinuation	TP	3
20	69 yr/M	On admission	*L. monocytogenes*	196	3.79	3.1	Negative	Yes	MER, AMP	None	Bacterial meningitis	MER, AMP	No	Antibiotic de-escalation	TP	5
21	66 yr/F	On admission	*L. monocytogenes*	230	1.58	6.51	Negative	Yes	MER, LNZ	None	Septic shock, bacterial meningitis	MER, LNZ	No	Antibiotic change	TP	4
22	45 yr/M	On admission	*H. influenzae*	2	0.36	4.1	Negative	No	None	None	Vascular migraine	No specific treatment	NA	NA	FP[d]	5
23	40 yr/F	On admission	*H. influenzae*	3	0.35	4.3	Negative	No	CRO	None	Bacterial sepsis	CRO	Yes	NA	FP[d]	5
24	35 yr/M	On admission	*H. influenzae*	4800	1.57	21.77	Negative	No	MER	None	Bacterial meningitis	MER	No	Antibiotic change	TP	5
25	49 yr/F	On admission	*S. pneumoniae*	13,700	4.94	0.1	Negative	No	CRO, DEX	None	Bacterial meningitis	CRO, DEX	Yes	NA	TP	5
26	46 yr/M	On admission	*H. influenzae* + *S. agalactiae*	9	0.87	3.1	Negative	No	CRO, ACV, DEX	None	Bacterial meningitis	CRO, DEX	No	Antiviral drug discontinuation	TP	4
27	25 yr/M	On admission	VZV	10	0.36	3.94	Negative	No	ACV	None	Shingles on the face, aseptic meningitis	ACV	Yes	NA	NA	5
28	93 yr/M	During hospitalization	VZV	92	2.53	2.68	Negative	Yes	MER, ACV, LNZ	None	Viral meningoencephalitis and left external otitis	ACV, LNZ	No	Antibiotic discontinuation	NA	4

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29	55 yr/F	During hospitalization	VZV	158	0.27	2.92	Negative	Yes	CRO	CSF GeneXpert® (-) *M. tuberculosis*	Tuberculous meningitis	RHZ, MOX, DEX	No	NA	NA	5
30	55 yr/M	On admission	VZV	122	1.28	3.6	Negative	Yes	RHZ, S, DEX	None	Tuberculous meningitis	RHZ, S, DEX	Yes	NA	NA	5
31	50 yr/F	On admission	VZV	66	0.62	2.4	Negative	Yes	ACV	None	Viral meningoencephalitis	ACV	Yes	NA	NA	5
32	83 yr/F	During hospitalization	VZV	7	0.76	6.7	Negative	Yes	CRO	None	Viral meningoencephalitis	CRO	No	Antibiotic discontinuation	NA	5
33	36 yr/M	On admission	Enterovirus	584	1.14	4.5	Negative	No	CRO	None	Aseptic meningitis	CRO	No	Antibiotic discontinuation	NA	5

Abbreviations: ACV, acyclovir; AMB, amphotericin B; AMK, amikacin; AMP, ampicillin; CFT, cefotaxime; CIP, ciprofloxacin; CMV, Cytomegalovirus, CNS, central nervous system; COL, colistin; CSF, cerebrospinal fluid; CRO, ceftriaxone; DEX, dexamethasone; F, female; FAME, BioFire® FilmArray® Meningitis/Encephalitis Panel; FLU, fluconazole; FP, false positive; Glu, glucose (mmol/L); HSV-1, herpes virus simplex 1; LNZ, linezolid; LVF, levofloxacin; M, male; MER, meropenem; MET, metronidazole; MOX, moxifloxacin; MTP, methylprednisolone; NA, Not applicable; Pro, protein (g/L); RHZ, R = rifampin; H = isoniazid; Z = pyrazinamide; E = ethambutol; S, streptomycin; TP, true positive; VAN, vancomycin; VZV, varicella zoster virus; WBC: white blood cells (cells/mm^3).

[a]specific PCR was not ordered or performed
[b]Outcome: 1 death; 2: vegetative state; 3: severe disability; 4: moderate disability; 5: mild or no disability.
[c]false positive very likely due to the detection of other pathogens by culture or molecular methods
[d]false positive for the detection of *H. influenzae* possible (n=2). Confirmation by specific PCR was not performed.

Supplementary Appendix

Supplementary Table S3. Clinical and laboratory data from patients with CNS infections positive by routine tests but negative by FAME.

No.	Age/Gender	Timepoint of CSF collection	Clinical diagnosis	WBC	Pro	Glu	CSF culture	Empirical therapy	Molecular method/culture	Specific therapy	Interpretation	Outcome[a]
34	49 yr/M	On admission	Tuberculous meningitis	1	1.17	3.5	Negative	RHZE, DEX	CSF GeneXpert® (+) *M. tuberculosis*	RHZE, DEX	NA	5
35	17 yr/F	On admission	Tuberculous meningitis	297	4.06	2.3	Negative	CRO, DEX	CSF GeneXpert® (+) *M. tuberculosis*	RHZE, DEX	NA	5
36	68 yr/M	On admission	Tuberculous meningitis	172	1.41	2.24	Negative	MER, RHZ, MOX, DEX	CSF GeneXpert® (+) *M. tuberculosis*	MER, RHZ, S, DEX	NA	3
37	38 yr/M	On admission	Tuberculous meningitis	406	3.89	1.54	Negative	CRO, LVF, RHZ, DEX	CSF GeneXpert® (+) *M. tuberculosis*	LVF, RHZ, DEX	NA	5
38	41 yr/M	On admission	Tuberculous meningitis	20	0.5	5.3	Negative	LVF, LNZ, ZE	CSF GeneXpert® (+) *M. tuberculosis*	LVF, LNZ, ZE	NA	4
39	72 yr/M	On admission	Tuberculous meningitis	1096	3.36	0.8	Negative	VAN, MER, DEX	CSF GeneXpert® (+) *M. tuberculosis*	MER, LNZ, LVF, RHZ, DEX	NA	2
40	25 yr/M	On admission	Tuberculous meningitis	136	6.1	1.08	Negative	CRO, RHZ, S, DEX	CSF GeneXpert® (+) *M. tuberculosis*	RHZ, S, DEX	NA	2
41	45 yr/M	On admission	*K. pneumoniae* meningitis and sepsis	10,690	10.61	0	*K. pneumoniae*	MER, COL, DEX	CSF PCR and blood culture (+) *K. pneumoniae*	MER, COL, DEX	NA	5
42	44 yr/M	During hospitalization	*K. pneumoniae* meningitis	3024	2.41	0.32	*K. pneumoniae*	MER, LIN	None	MER, DEX	NA	3
43	67 yr/M	During hospitalization	*K. pneumoniae* meningitis	91,063	12.1	0.1	*K. pneumoniae*	CRO, DEX	None	MER, AMK	NA	4
44	18 yr/M	During hospitalization	*K. pneumoniae* meningitis	3910	1.02	0.72	*K. pneumoniae*	CFT	None	MER	NA	2
45	55 yr/M	During hospitalization	*K. pneumoniae* meningitis and sepsis	9	1.71	4.9	Negative	MER, AMK	CSF PCR (+) *K. pneumoniae*	MER, AMK, COL	NA	4
46	63 yr/M	During hospitalization	*S. suis* meningitis and sepsis	90	1.19	6	*S. suis*	MER, AMK, MTP	CSF PCR (+) *S. suis*	MER, AMK, MTP	NA	5

Supplementary Appendix

47	44 yr/F	On admission	*S. suis* meningitis and sepsis	9008	5.72	0.01	*S. suis*	CRO, AMP, DEX	Blood culture (+) *S. suis*	CRO, DEX	NA	5
48	61 yr/F	On admission	*S. suis* meningitis	599	6.1	1.08	*S. suis*	CRO, DEX	CSF PCR (+) *S. suis*	CRO, DEX	NA	5
49	51 yr/M	On admission	*S. suis* meningitis and sepsis	1116	1.56	ND	*S. suis*	CRO, CIP, DEX	Blood culture (+) *S. suis*	CRO, CIP, DEX	NA	5
50	59 yr/M	On admission	*S. suis* meningitis	355	4.1	0.7	Negative	CRO, TBM, DEX	CSF PCR (+) *S. suis*	CRO, TBM, DEX	NA	5
51	82 yr/M	On admission	EBV meningoencephalitis	40	1.27	3.1	Negative	MER, AMK, DEX	CSF PCR (+) EBV	MER, AMK, DEX	NA	3
52	57 yr/M	On admission	EBV meningitis	NA	1.58	3.2	Negative	MER, AMK, DEX	CSF PCR (+) EBV	MER, AMK, DEX	NA	5
53	60 yr/M	On admission	EBV meningoencephalitis	19	1.75	2.4	Negative	CRO, CIP	CSF PCR (+) EBV	CRO, CIP, DEX	NA	5
54	61 yr/M	On admission	*A. baumannii* meningitis and sepsis	6903	21.96	0.01	*A. baumannii*	MER, LNZ, DEX	None	MER, COL	NA	2
55	78 yr/F	On admission	*T. asahii* meningitis, septic shock	6	0.86	2.1	*T. asahii*	MER, DEX	Urine culture (+) *T. asahii*	VOR, DEX	NA	1
56	69 yr/M	On admission	*Streptococcus spp* meningitis	5760	7	2.5	Negative	CRO, CIP, DEX	CSF PCR (+) *Streptococcus spp*	CRO, LNZ, DEX	NA	5
57	30 yr/F	On admission	HIV related central nervous system infection, suspected Cerebral toxoplasmosis	3	0.45	1.9	Negative	SMX, CLM, FLU, DEX	CSF PCR (+) HIV	SXT, FLU, MTP	NA	3
58	35 yr/M	On admission	HSV meningoencephalitis	14	0.83	6	Negative	CRO, ACV, DEX	CSF PCR (+) HSV-1	CRO, ACV, DEX	FN	5
59	67 yr/M	During hospitalization	HSV encephalitis	101	0.87	3.19	Negative	MER, VAN ACV	CSF PCR (+) HSV-1	ACV	FN	2
60	39 yr/F	During hospitalization	HSV meningitis	122	0.41	3.48	Negative	CRO, ACV, DEX	CSF PCR (+) HSV-1	ACV, DEX	FN	5
61	92 yr/F	During hospitalization	Cryptococcal meningitis	135	0.44	3.65	Negative	CRO	CSF fungal culture (+) *C. neoformans*	AMB	FN	5

Supplementary Appendix

62	45 yr/M	On admission	Cryptococcal meningitis	117	2.37	0.34	Negative	AMB, FLU RHZ, S, MOX	Fungal culture (CSF) (+) *C. neoformans*	AMB, FLU RHZ, S, MOX	FN	2
63	62 yr/M	On admission	*E.coli* meningitis	5157	8.3	0.02	*E.coli*	MER, LNZ, LVF	None	MER, LNZ, LVF	FN	2
64	75 yr/F	During hospitalization	VZV encephalitis	2	0.49	3.1	Negative	ACV	CSF PCR (+) VZV	ACV, DEX	FN	3

Abbreviations: ACV, acyclovir; AMB, amphotericin B; AMK, amikacin; AMP, ampicillin; CFT, cefotaxime; CIP, ciprofloxacin; CNS, central nervous system; COL, colistin; CSF, cerebrospinal fluid; CRO, ceftriaxone; DEX, dexamethasone; EBV, Epstein–Barr virus; F, female; FAME, BioFire® FilmArray® Meningitis/Encephalitis Panel; FLU, fluconazole; FN; false negative; Glu, glucose (mmol/L); HSV, Herpes simplex virus; LNZ, linezolid; LVF, Levofloxacin; M, male; MER, meropenem; MOX, moxifloxacin; MTP, methylprednisolone; NA, not applicable; Pro, protein (g/L); RHZE, R = rifampin; H = isoniazid; Z = pyrazinamide; E = ethambutol; S, streptomycin; SXT, trimethoprim–sulfamethoxazole; TBM: tobramycin; VAN, vancomycin; VOR, voriconazole; VZV, Varicella zoster virus; WBC, white blood cells (cells/mm^3).

[a]Outcome: 1 death; 2: vegetative state; 3: severe disability; 4: moderate disability; 5: mild or no disability.

Supplementary Table S4. Empirical antibiotic regimen in patients with suspected bacterial meningitis according to Vietnam's Ministry of Health.

Patient group	Common bacterial pathogens	Standard therapy	Alternative therapies
0 to 4 weeks	*Enterobacteriaceae, Streptococcus agalactiae, Listeria monocytogenes.*	Cefotaxime plus Ampicillin	Ampicillin* plus Aminoglycoside**
1 to 3 months	*Haemophilus influenzae b, Neisseria meningitidis, Streptococcus agalactiae E.coli, Listeria monocytogenes.*	Ampicillin* plus Ceftriaxone (or Cefotaxime)	Vancomycin plus Ceftriaxone (or Cefotaxime)
3 months to 18 years	*Haemophilus influenzae b, Streptococcus pneumoniae, Neisseria meningitidis.*	Ceftriaxone (or Cefotaxime)	Vancomycin plus Ceftriaxone (or Cefotaxime)
Age > 18 and < 50 years	*Streptococcus pneumoniae, Streptococcus spp, Neisseria meningitidis.*	Ceftriaxone (or Cefotaxime)	Vancomycin plus Ceftriaxone (or Cefotaxime)
Age ≥ 50 years	*Streptococcus pneumoniae, Neisseria meningitidis, Listeria monocytogenes*, aerobic gram-negative bacilli.	Ceftriaxone (or Cefotaxime)	Ampicillin* plus Ceftriaxone (or Cefotaxime)
Immunodeficiency	*Streptococcus pneumoniae*, *Neisseria meningitidis*, *Listeria monocytogenes*, aerobic gram-negative bacilli.	Ampicillin plus Ceftazidime	Vancomycin plus Ampicillin* plus Ceftazidime
Head trauma, neurosurgery, CSF leak	*Streptococcus pneumoniae, Staphylococcus spp*, aerobic gram-negative bacilli.	Ceftazidime plus Vancomycin	Vancomycin plus Meropenem

* Use of ampicillin in suspected cases of *Listeria monocytogenes*

** Aminoglycoside (gentamycin or amikacin)

Chapter 2

Enhancing Diagnostic Yield Using 16S Oxford Nanopore Sequencing for CNS Infections in Resource-Limited Settings

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Publication

Evaluating the diagnostic utility of 16S Oxford Nanopore Technology Sequencing in patients with Central Nervous System infections and its usefulness in Antimicrobial Stewardship

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MAJOR ARTICLE

Evaluating the Diagnostic Utility of 16S Oxford Nanopore Technology Sequencing in Patients With Central Nervous System Infections and Its Usefulness in Antimicrobial Stewardship

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Background. Central nervous system (CNS) infections pose a significant public health challenge in resource-limited settings. Traditional culture-based and targeted molecular diagnostic methods have limitations in sensitivity and speed. This study retrospectively analyzed the data and cerebrospinal fluid (CSF) samples from our previous study to assess the diagnostic efficacy of untargeted 16S Oxford Nanopore Technology (ONT) sequencing compared to conventional CSF culture methods, with the goal of improving diagnostic accuracy, reducing time to treatment, and enhancing patient outcomes.

Methods. A total of 329 patients from 4 hospitals were enrolled in the study. CSF samples were collected and processed for both CSF culture and 16S ONT sequencing. DNA were extracted from CSF and amplified for 16S rRNA sequencing using the MinION platform. Descriptive analyses were conducted to assess pathogen detection rates and the potential impact of sequencing on antimicrobial stewardship.

Results. Of the 329 samples, 40 (12%) were positive for bacterial or fungal pathogens. 16S ONT detected pathogens in 28 samples (9%), while CSF culture identified pathogens in 23 samples (7%). 16S ONT sequencing identified 17 pathogens not detected by CSF culture, including *Streptococcus suis* and *Acinetobacter baumannii*. Based on 16S ONT findings, 61% of patients were found to have received inappropriate empirical antibiotic therapy and could have benefited from improved antimicrobial management, including de-escalation in 11, escalation in 5, and adjustments in 2 cases.

Conclusions. 16S ONT sequencing showed higher sensitivity and diagnostic yield than CSF culture, providing clinical insights for managing CNS infections through targeted antibiotic use and enhanced antimicrobial stewardship in resource-limited settings.

Keywords. central nervous system; meningitis/encephalitis; cerebrospinal fluid; Oxford Nanopore sequencing; antimicrobial stewardship.

Infections of the central nervous system (CNS), such as meningitis, encephalitis, and meningoencephalitis, can lead to severe and potentially life-threatening illnesses [1]. Timely diagnosis and prompt treatment are crucial for reducing complications, preventing irreversible damage, and improving survival rates in patients with CNS infections [2, 3]. While cerebrospinal fluid (CSF) culture remains the gold standard for diagnosing bacterial meningitis, it is time consuming with turnaround time up to 2 days, which can delay early intervention and worsen patient outcomes, especially in cases where patients have been pretreated with antibiotics [4].

CSF Gram staining is a widely used rapid diagnostic method that proves valuable in culture-negative cases. However, its sensitivity varies depending on the pathogens detected, and it is significantly reduced in patients who have received antibiotics prior to sampling, which may also contribute to underdiagnosis of antibiotic-resistant pathogens [4]. Studies comparing bacterial cultures with polymerase chain reaction (PCR)–based

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methods have demonstrated that molecular techniques, such as PCR, can detect pathogens in 30%–50% of culture-negative CSF samples, offering a valuable alternative when traditional cultures fail [5]. However, our previous study assessing the use of a targeted FilmArray Meningitis/Encephalitis panel to improve the diagnosis of meningoencephalitis in Vietnam found only a marginal improvement in diagnostic value in terms of sensitivity, and this is likely due to the specific local epidemiology [6].

The introduction of next-generation sequencing, particularly Oxford Nanopore Technology (ONT), has transformed pathogen diagnostics by offering faster turnaround times, lower costs, and the ability to simultaneously detect a wide range of pathogens from a single sample while avoiding the bias of targeted molecular diagnostic panels [7]. The 16S ribosomal RNA (rRNA) gene, a highly conserved marker found in nearly all bacteria, is a key target for sequencing. Its broad conservation across bacterial species enables the identification of a wide variety of bacterial pathogens, including fastidious organisms that may be difficult to detect with conventional culture [8]. Previous studies have shown that 16S ONT sequencing offers superiority compared to conventional microbiological cultures, with results that are valuable for both diagnostics and antimicrobial stewardship [9–12]. Moreover, the rapid and user-friendly library preparation process of ONT sequencing allows for timely pathogen identification, which can expedite clinical decision-making and improve patient management [13].

In Vietnam, a low- and middle-income country with limited diagnostic resources, CNS infections are caused by a diverse range of pathogens, which complicates diagnostic accuracy and increases the risk of undiagnosed cases. Studies have indicated that up to 75% of these cases remain undiagnosed, contributing to significant morbidity, mortality, and long-term sequelae [14]. This diagnostic gap places a significant burden on healthcare systems, leading to prolonged hospital stays, higher treatment costs, and reduced workforce productivity, all of which contribute to a considerable socioeconomic burden on the community [15, 16].

This study aims to assess the diagnostic efficacy of untargeted 16S ONT sequencing compared to traditional CSF culture methods, using CSF samples with the goal to improve diagnostic accuracy, reduce time to optimal antibiotic treatment, and ultimately enhance patient outcomes, especially in resource-limited settings.

MATERIALS AND METHODS

Study Cohort

This retrospective, multicenter, hospital-based cohort study was conducted between 1 July 2022 and 30 April 2023 across 4 hospitals in Vietnam: 108 Military Central Hospital, the National Hospital for Tropical Diseases, 103 Military Hospital, and Viet Tiep Friendship Hospital. The study cohort was from a previously published study [6]. Patients were eligible for the study if they had clinical signs of suspected CNS infections, based on the World Health Organization case definition modified by Dubot-Pérès et al [17]. Inclusion criteria were hospitalized patients with suspected meningitis or encephalitis (based on CNS infection symptoms such as fever, headache, nausea/vomiting, neck stiffness, focal neurological symptoms, seizures, and altered mental status) who underwent a lumbar puncture [18]. Exclusion criteria were (*i*) patients with contraindications to lumbar puncture, such as an intracranial space-occupying lesion with mass effect, a mass in the posterior fossa, abnormal intracranial pressure, or a local skin infection at the lumbar puncture site; (*ii*) patients with an incomplete clinical history; and (*iii*) patients who did not consent to the study.

Patient Classification and Outcome Evaluation

The level of consciousness was assessed using the Glasgow Coma Scale (GCS), with a GCS score of <14 indicating altered mental status [19]. Clinical outcomes were evaluated using the Glasgow Outcome Scale (GOS), ranging from 1 (death) to 5 (mild or no disability/recovery). A favorable outcome was defined as a GOS score of 5, while unfavorable outcomes included death, vegetative state, and varying degrees of disability (GOS score 1–4) [20]. CSF pleocytosis refers to an increased number of white blood cells (WBCs) in the CSF indicating inflammation or infection in the CNS. Two thresholds based on the corrected WBC count were used to define pleocytosis: (*i*) ≥5 cells/μL, a lower threshold commonly used in clinical practice, especially in adults, to detect even mild inflammation; and (*ii*) ≥10 cells/μL, a higher threshold that can be used to increase specificity to reduce the likelihood of false-positive results. In CSF analysis, the corrected WBC count refers to the adjustment of the measured WBC count to account for peripheral blood contamination often caused by traumatic lumbar puncture [21]. Abnormal CSF glucose and protein levels were defined for all patients as values of <2.8 mmol/L or >4.2 mmol/L and <0.10 g/L and >0.25 g/L, respectively.

Specimen Collection and Laboratory Tests

CSF sampling procedures were standardized across all centers to ensure consistency and reliability. CSF cultures were performed using the BACTEC Plus Aerobic/F System (Becton-Dickinson, Franklin Lakes, New Jersey, USA). In the event of bacterial growth, colonies were identified using the VITEK matrix-assisted laser desorption/ionization–time of flight mass spectrometry system, an automated microbial identification platform. Antimicrobial susceptibility testing was conducted with the VITEK 2 Compact System (bioMérieux, Lyon, France) to determine resistance profiles. Five hundred microliters of CSF was stored at −80°C and transported to

Germany for 16S ONT sequencing, with strict cold chain protocols maintained throughout the process.

CSF Sample Preparation and Nucleic Acid Extraction

DNA was extracted from 200 µL of CSF from each patient sample using the Quick-DNA HMW MagBead Kit (Zymo Research, Irvine, California, USA) for high-molecular-weight DNA extraction. DNA quality and quantity were assessed using the Qubit 4 fluorometer and the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, Massachusetts).

16S rRNA Sequencing With Oxford Nanopore's MinION Device

For multiplex sequencing, the 16S rRNA gene was PCR-amplified from CSF DNA using the 16S Barcoding Kit 24 V14 (SQK-16S114.24) (Oxford Nanopore Technologies, Oxford, United Kingdom), following the manufacturer's instructions. The PCR was carried out for 35 cycles at an annealing temperature of 55°C, using 10 ng of genomic DNA per reaction, with a nuclease-free water as negative control. Amplicons were purified using AMPure XP beads and quantified using the Qubit 4 fluorometer with the Qubit dsDNA HS Assay Kit. Bar-coded amplicons were pooled in equimolar ratios and sequenced on the MinION Mk1B (R10.4.1 flowcell) sequencer for 6 hours, using super-accurate base calling by MinKNOW software (v.24.11.10). After sequencing, the flow cell was cleaned using the Flow Cell Wash Kit (EXP-WSH004; Oxford Nanopore Technologies). To evaluate the validation parameters of our 16S rRNA sequencing method, we used the ZymoBIOMICS Microbial Community DNA Standard (Zymo Research Europe, Freiburg, Germany) as a positive control, which served as both an extraction control and a reference for sequencing performance. The resulting taxonomic profiles were compared to the manufacturer's theoretical composition to evaluate the accuracy and taxonomic resolution of our analysis pipeline.

Data Analysis

Descriptive analyses were performed using R version 4.4.0 software (http://www.r-project.org). Sequencing data generated by MinKNOW were analyzed on the Nanopore EPI2ME cloud platform. Low-quality reads (Q score <10) and reads outside the 1400–1700 bp length range were discarded. Each read was classified at the species level based on a 90% coverage and a 97% identity threshold. The species with the highest number of aligned reads was considered the causative pathogen, unless identified in the negative control, in which case it was excluded from further analysis.

RESULTS

Overview of the Study Cohort and Clinical Characteristics

A total of 329 patients aged ≥18 years with suspected CNS infections were recruited from 4 hospitals around Hanoi, Vietnam, based on presenting symptoms such as fever, headache, and altered mental status. The patients were distributed among the hospitals as follows: 136 from the National Hospital for Tropical Diseases, 52 from 108 Military Central Hospital, 101 from Viet Tiep Friendship Hospital, and 40 from 103 Military Hospital. The main clinical characteristics are summarized in Table 1. In this study, only samples from 329 patients were available for sequencing. The mean age of the cohort was 54 years, and 68% were male. Underlying conditions included hypertension (27%), diabetes (19%), and cardiac disease (6%). Common clinical features included fever, headache, neck stiffness, and altered mental status. WBC count, protein, and glucose levels in CSF were significantly associated with positive CSF culture and 16S ONT sequencing results (Table 1).

Pathogens Detected by 16S ONT and CSF Culture

A total of 40 of 329 (12%) samples tested positive for bacterial or fungal pathogens, using either or both methods. Of these, 16S ONT sequencing identified pathogens in 28 (9%) samples, while CSF culture identified pathogens in 23 (7%) samples (Supplementary Figure 1). 16S ONT sequencing yielded a higher positivity rate than CSF culture in detecting *Streptococcus suis* (8 cases vs 4), *Acinetobacter baumannii* (5 vs 2), *Streptococcus pneumoniae* (4 vs 4), *Neisseria meningitidis* (3 vs 1), and *Klebsiella pneumoniae* (2 vs 6). 16S ONT sequencing detected 17 pathogens in 17 samples that were negative by CSF culture. This included *S suis* (n = 4), *A baumannii* (n = 4), *S pneumoniae* (n = 1), and *N meningitidis* (n = 2) as well as 5 pathogens detected exclusively by 16S ONT: *Escherichia marmotae* (n = 2), *Staphylococcus epidermidis* (n = 1), *Streptococcus oralis* (n = 1), *Klebsiella aerogenes* (n = 1), and *Citrobacter freundii* (n = 1) (Figure 1). The case descriptions for patients with positive 16S sequencing but negative CSF cultures, including relevant clinical information such as CSF parameters, documented diagnoses, and risk factors (eg, head trauma, recent neurosurgery, presence of shunts) supporting the likelihood of true infection, are listed in Supplementary Table 1.

In contrast, CSF culture detected 12 pathogens in 12 samples not identified by 16S ONT, including *A baumannii* (n = 1), *K pneumoniae* (n = 4), *S pneumoniae* (n = 1), *Cryptococcus neoformans* (n = 3), *Trichosporon asahii* (n = 1), *Listeria monocytogenes* (n = 1), and *Escherichia coli* (n = 1) (Figure 1). Nanopore-negative results were anticipated in 4 patients (IDs: 7, 9, 10, and 11), as *C neoformans* and *T asahii* are fungi not detectable by 16S rRNA sequencing (Supplementary Table 2). Moreover, the number of species-specific reads obtained from 16S rRNA ONT sequencing was higher in both CSF culture-positive and culture-negative specimens, further supporting our findings (Figure 2; Supplementary Table 3).

Table 1. Demographic and Clinical Characteristics of Patients With Central Nervous System Infections

Characteristic		CNS Infections (n = 329)	16S ONT Positive (n = 28)	16S ONT Negative (n = 301)	*P* Value	CSF Culture Positive (n = 23)	CSF Culture Negative (n = 306)	*P* Value
Demographics								
Age, y, mean ± SD		54 ± 19	52 ± 18	55 ± 19	.386	49 ± 18	55 ± 19	.174
Male sex		224 (68)	21 (75)	203 (67)	.543	17 (74)	207 (68)	.697
Underlying conditions								
Hypertension		89 (27)	5 (18)	84 (28)	.356	3 (13)	86 (28)	.185
Diabetes		62 (19)	0 (0)	62 (21)	**.016**	1 (4)	61 (20)	.093
Cardiac disease		19 (6)	2 (7)	17 (6)	.670	1 (4)	18 (6)	1
Alcoholism		17 (5)	1 (4)	16 (5)	1	2 (9)	15 (5)	.337
Chronic liver disease		18 (6)	0 (0)	18 (6)	.382	2 (9)	16 (5)	.364
Chronic lung disease		17 (5)	2 (7)	15 (5)	.646	1 (4)	16 (5)	1
Kidney disease		15 (5)	0 (0)	15 (5)	.626	0 (0)	15 (5)	.611
Immunosuppressive drugs		13 (4)	0 (0)	13 (4)	.613	0 (0)	13 (4)	.611
Cancer		13 (4)	0 (0)	13 (4)	.613	0 (0)	13 (4)	.611
HIV		11 (3)	0 (0)	11 (4)	.608	0 (0)	11 (4)	1
Risk factors								
Post-neurosurgery		29 (9)	5 (18)	24 (8)	.086	5 (22)	24 (8)	**.041**
Head trauma		31 (9)	3 (11)	28 (9)	.737	2 (9)	29 (9)	1
CSF shunt		5 (2)	0 (0)	5 (2)	1	0 (0)	5 (2)	1
Clinical features								
Fever (>37.5°C)		272 (83)	23 (82)	249 (83)	1	22 (96)	250 (82)	.147
Headache		220 (67)	18 (64)	202 (67)	.925	19 (83)	201 (66)	.152
Neck stiffness		207 (63)	19 (68)	188 (63)	.718	20 (87)	187 (61)	**.024**
Nausea/vomiting		98 (30)	12 (43)	86 (29)	.172	12 (52)	86 (28)	**.028**
Seizure		29 (9)	2 (7)	27 (9)	1	0 (0)	29 (9)	.243
Focal neurologic deficits		20 (6)	0 (0)	20 (7)	.396	0 (0)	20 (7)	.38
GCS score, mean ± SD		13 ± 2	12 ± 3	13 ± 2	**.004**	11 ± 3	13 ± 2	**.001**
Altered mental status		142 (43)	20 (71)	122 (41)	**.003**	19 (83)	123 (40)	**<.001**
At least 2 of 4 symptoms recorded (headache, fever, stiff neck, altered mental status)		272 (83)	24 (86)	248 (82)	.798	22 (96)	250 (82)	.147
GOS score								
1: Death		32 (10)	2 (7)	30 (10)	.076	3 (13)	29 (9)	**.004**
2: Vegetative state		29 (9)	5 (18)	24 (8)		7 (30)	22 (7)	
3: Severe disability		64 (19)	5 (18)	59 (20)		1 (4)	63 (21)	
4: Moderate disability		62 (19)	1 (4)	61 (20)		2 (9)	60 (20)	
5: Mild or no disability		143 (43)	15 (53)	127 (42)		10 (44)	132 (43)	
CSF parameters								
WBC count, cells/μL[a]	Mean ± SD	1030 ± 5910	3392 ± 5072	804 ± 5937	**.016**	9601 ± 19 724	371 ± 1687	**.035**
	Pleocytosis of 5 cells/μL	224 (69)	24 (86)	200 (68)	.080	23 (100)	201 (67)	**.002**
	Pleocytosis of 10 cells/μL	174 (54)	22 (79)	152 (52)	**.011**	22 (96)	152 (51)	**<.001**
Protein, g/L[b]	Mean ± SD	1.56 ± 2.79	3.85 ± 4.82	1.35 ± 2.43	**.013**	5.36 ± 4.92	1.27 ± 2.32	**.001**
	Abnormal protein	311 (96)	24 (89)	287 (96)	.100	21 (91)	290 (96)	.26
Glucose, mmol/L[b]	Mean ± SD	4.16 ± 2.97	2.61 ± 2.22	4.30 ± 2.99	**.001**	1.01 ± 1.66	4.38 ± 2.91	**<.001**
	Abnormal glucose	187 (57)	19 (73)	168 (57)	.159	21 (96)	166 (55)	**.001**

Data are presented as No. (%) unless otherwise indicated. Significant *P* values are highlighted in bold.

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; GCS, Glasgow Coma Scale; GOS, Glasgow Outcome Scale; HIV, human immunodeficiency virus; ONT, Oxford Nanopore Technology; SD, standard deviation; WBC, white blood cell.

[a]The CSF leukocyte count was determined in 323 patients; CSF specimens from 6 patients had too many leukocytes for an exact count to be performed (missing values, n = 6 for both 16S ONT negative and CSF culture negative).

[b]The protein and glucose levels in the CSF were measured in 325 patients. For 16S ONT sequencing, 4 CSF samples were not analyzed (missing values: n = 3 for 16S ONT negative, n = 1 for 16S ONT positive). For CSF culture, the missing values were as follows: protein (n = 4 for CSF culture negative) and glucose (n = 3 for CSF culture negative, n = 1 for CSF culture positive).

Implications for Antimicrobial Stewardship

A retrospective analysis of clinical and laboratory data revealed that 61% (17/28) of patients with 16S ONT-positive results were initially prescribed unsuitable empirical antibiotics. If 16S ONT sequencing results had been available to guide therapy decisions alongside CSF culture, 18 patients would have

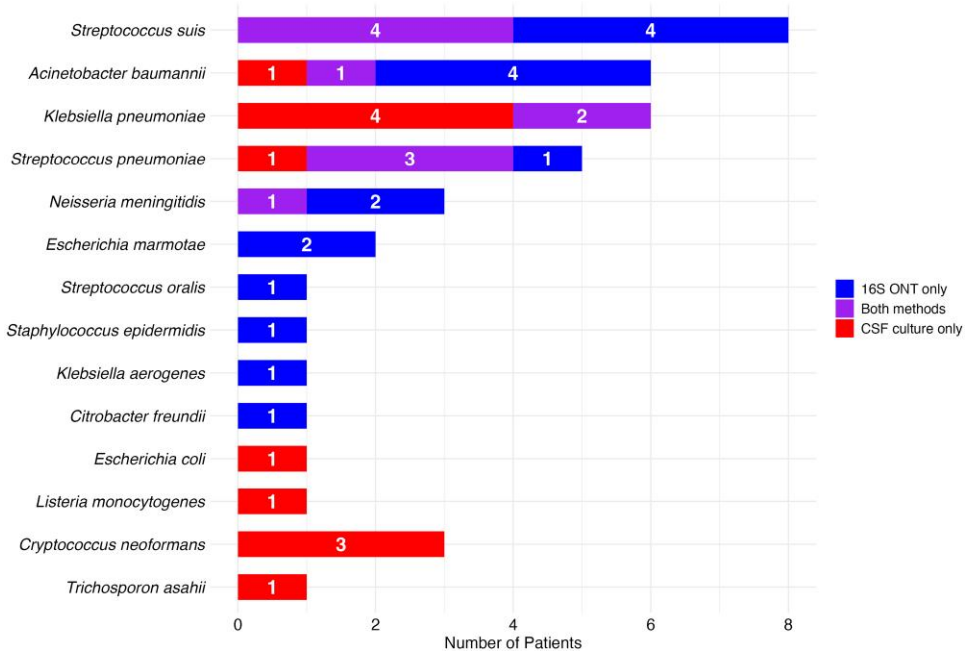

Figure 1. Bacterial pathogens detected using 16S ribosomal RNA Oxford Nanopore Technology (ONT) sequencing compared to cerebrospinal fluid (CSF) culture.

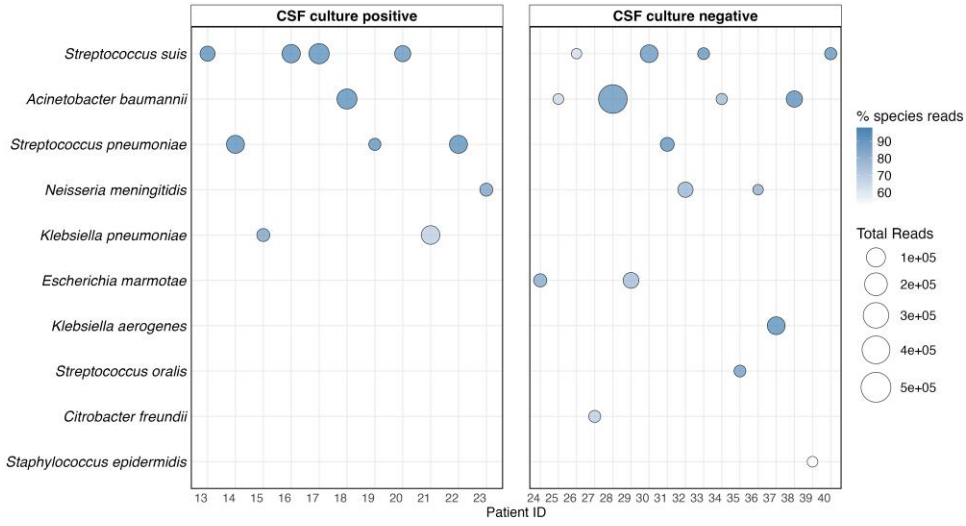

Figure 2. Causative pathogens detected with 16S ribosomal RNA sequencing using Oxford Nanopore Technology (n = 28). Size of the circles indicates the total number of sequencing reads. Abbreviation: CSF, cerebrospinal fluid.

benefited from this additional diagnostic method. This would have included de-escalation of antibiotic therapy for 11 patients, escalation for 5, and a change in antibiotics for 2 patients (Table 2).

DISCUSSION

This multicenter study aimed to evaluate the diagnostic utility of 16S ONT sequencing for bacterial CNS infections across 4 hospitals in Hanoi, Vietnam, with a focus on improving diagnostic accuracy and antimicrobial stewardship in a region with a unique pathogen landscape. Our findings highlight the significant potential of 16S ONT sequencing to enhance pathogen detection, improve antibiotic therapy, and combat

Table 2. Characteristics of Patients Positive by 16S Ribosomal RNA Oxford Nanopore Technology Sequencing (n = 28)

Patient ID	Age/ Sex	White Blood Cell Count	Glu	Pro	CSF Culture[a]	16S ONT[a]	Therapy Prior to Lumbar Puncture	Empirical Therapy	Treatment Adequacy	Appropriate Empirical Therapy	Possible Impact of 16S ONT on Treatment	Outcome[b]
13	51/M	1116	NA	1.56	*S suis*	*S suis*	No	CTX, CIP, MTP	CTX, CIP, MTP	No	Antibiotic de-escalation	5
14	41/M	10 460	0	1.65	*S pneumoniae*	*S pneumoniae*	Yes	CTX, LEV, MTP	CTX, LEV, MTP	No	Antibiotic de-escalation	5
15	37/M	199	4.33	6.09	*K pneumoniae*	*K pneumoniae*	Yes	MER, COL	MER, COL	Yes	No	2
16	61/F	599	1.08	6.1	*S suis*	*S suis*	Yes	CTX, DEX	CTX, DEX	Yes	No	5
17	44/F	9008	0.01	5.72	*S suis*	*S suis*	No	CTX, AMP, DEX	CTX, DEX	No	Antibiotic de-escalation	5
18	61/M	6903	0.01	22	*A baumannii*	*A baumannii*	Yes	MER, LIN, DEX	MER, COL	No	Antibiotic de-escalation	2
19	42/M	18 050	0.01	6.7	*S pneumoniae*	*S pneumoniae*	No	CTX, DEX	CTX, VAN, DEX	Yes	No	5
20	63/M	90	6	1.19	*S suis*	*S suis*	No	MER, AMK, MTP	MER, AMK, MTP	No	Antibiotic de-escalation	5
21	45/M	10 690	0	10.6	*K pneumoniae*	*K pneumoniae*	Yes	CTX, DEX	MER, COL, DEX	No	Antibiotic escalation	5
22	59/F	320	0.09	4.1	*S pneumoniae*	*S pneumoniae*	No	CTX, AMK, DEX	MER, AMK, DEX	No	Antibiotic de-escalation	1
23	20/M	8000	0	7.5	*N meningitidis*	*N meningitidis*	Yes	MER, CIP, DEX	CTX, CIP	No	Antibiotic de-escalation	5
24	50/M	240	3.34	0.92	Negative	*E marmotae*	Yes	CTX	MER	Yes	Antibiotic escalation	3
25	36/F	60	3.89	1.26	Negative	*A baumannii*	No	CTX, LEV	CTX, LEV	No	Antibiotic escalation	3
26	73/M	80	3.97	0.42	Negative	*S suis*	No	CFT	CFP, VAN	No	Antibiotic change	3
27	65/M	7	5.6	0.73	Negative	*C freundii*	Yes	MER, DEX	MER, DEX	Yes	No	1
28	74/M	3	4.4	0.37	Negative	*A baumannii*	No	TCC, LEV	TCC, LEV	No	Antibiotic escalation	2
29	35/F	2	3.6	0.37	Negative	*E marmotae*	No	No specific treatment	No specific treatment	NA	NA	5
30	86/F	2	4.2	0.31	Negative	*S suis*	No	CTX	CTX	Yes	No	5
31	74/M	5	7	0.67	Negative	*S pneumoniae*	No	MER, LEV, DEX	MER, LEV, DEX	No	Antibiotic de-escalation	3
32	84/F	1	4.4	0.92	Negative	*N meningitidis*	No	CPZ, LEV	CPZ, LEV	No	Antibiotic change	2
33	46/M	7641	4.42	2.01	Negative	*S suis*	Yes	MER, DEX	CTX, DEX	No	Antibiotic de-escalation	5
34	24/M	3779	0.06	2.97	Negative	*A baumannii*	Yes	MER, COL	MER, COL	Yes	No	4
35	58/M	13 993	3.78	11.7	Negative	*S oralis*	Yes	CTX, DEX	MER, VAN, DEX	Yes	No	5
36	31/M	641	3.3	1.95	Negative	*N meningitidis*	Yes	CTX, DEX	CTX, LIN, DEX	Yes	No	5
37	18/M	1308	0.23	NA	Negative	*K aerogenes*	Yes	MER, COL, DEX	MER, COL	Yes	No	2
38	68/M	38	3.55	0.6	Negative	*A baumannii*	Yes	MER, VAN, FLU	MER, VAN, FLU	No	Antibiotic de-escalation	3
39	37/M	1388	2.37	1.43	Negative	*S epidermidis*	No	CTX, ABD, DEX	ABD, DEX	No	Antibiotic escalation	5
40	59/M	355	0.7	4.1	Negative	*S suis*	Yes	CTX, TBM, DEX	CTX, TBM, DEX	No	Antibiotic de-escalation	5

Highlighted cells indicate patients who received antibiotics before sampling; 16S ONT sequencing could have potentially influenced treatment decisions in these cases if performed concurrently with CSF cultures.

Abbreviations: ABD, albendazole; AMK, amikacin; AMP, ampicillin; CFP, cefepime, CFT, cefoxitin; CIP, ciprofloxacin; COL, colistin; CPZ, cefoperazol; CSF, cerebrospinal fluid; CTX, ceftriaxone; DEX, dexamethasone, F, female; FLU, fluconazole; Glu, glucose; LEV, levofloxacin, LIN, linezolid; M, male; MER, meropenem; MTP, methylprednisolone; NA, not applicable; ND, not done; ONT, Oxford Nanopore Technology; Pro, protein; TBM, tobramycin; TCC, ticarcillin; VAN, vancomycin.

[a]*A baumannii, Acinetobacter baumannii; C freundii, Citrobacter freundii; E marmotae, Escherichia marmotae; K aerogenes, Klebsiella aerogenes; K pneumoniae, Klebsiella pneumoniae; N meningitidis, Neisseria meningitidis; S epidermidis, Staphylococcus epidermidis; S oralis, Streptococcus oralis; S pneumoniae, Streptococcus pneumoniae; S suis, Streptococcus suis.*

[b]Outcome: 1 death; 2: vegetative state; 3: severe disability; 4: moderate disability; 5: mild or no disability.

antimicrobial resistance (AMR), particularly in resource-limited settings.

Diagnostic Performance of 16S ONT Sequencing

In this study, 16S ONT sequencing demonstrated a higher sensitivity when compared to traditional culture-based methods, as shown earlier [12, 22–24]. These findings indicate that 16S ONT sequencing can serve as a reliable complementary tool to CSF culture for the detection of bacterial pathogens in CNS infections, as documented from other studies [9, 11]. The sequencing method identified 28 pathogens in 28 CSF samples, with 23 pathogens also being detected by CSF culture. Notably, 16S ONT sequencing was able to detect 9 distinct pathogens in 17 culture-negative CSF samples, including key bacteria such as *S suis*, *A baumannii*, *N meningitidis, E marmotae*, *K pneumoniae*, *K aerogenes*, *S oralis*, *C freundii*, and *S epidermidis*. It is noteworthy that 9 of the 17 patients had received antibiotics prior to sampling, which may affect the viability of the causative pathogens and, thus, the overall sensitivity of culture-based methods. This highlights the superiority of sequencing-based methods, particularly in detecting fastidious or difficult-to-culture organisms. Another challenge for Vietnam is the atypical epidemiology of causative pathogens for community-acquired CNS. While *N meningitis* and *S pneumoniae* are common causes of meningitis worldwide, *S suis* is the leading bacterial pathogen of CNS infections in Vietnam and is associated with occupational exposure to pigs or consumption of raw pig products [25–27].

Impact on Clinical Management

Vietnam is facing a major public health challenge as AMR continues to rise [28, 29]. This growing threat results from the inappropriate and excessive use of antibiotics, imposing a substantial financial burden on healthcare systems and society at large [30]. In clinical practice, when bacterial meningitis is suspected, empirical antibiotic therapy should be initiated as soon as possible. Given that microbiological results are typically unavailable at the time of initial treatment, clinicians must rely on local epidemiological data to guide their decisions. Once microbiological results are available, antimicrobial therapy should be reassessed and adjusted as necessary [31]. However, if microbiological results are negative, empirical broad-spectrum antibiotic therapy should be continued to address the possibility of antibiotic resistance [32].

The use of 16S ONT sequencing in our study sites would have a significant impact on the therapy decision and clinical management of the patients with CNS infections. In 18 of 28 cases, the sequencing results would have led to adjustments of the antibiotic therapy. Specifically, 11 patients would have benefited from antibiotic de-escalation, 5 would have required an escalation to broader-spectrum antibiotics, and 2 could have their treatment regimen tailored to match the identified pathogen. These findings underscore the value of rapid molecular diagnostics in guiding more precise and timely antimicrobial therapy in the sense of antimicrobial stewardship, which can reduce unnecessary antibiotic use and improve clinical outcomes. Moreover, by minimizing antibiotic overuse, the overall selection pressure on pathogens may be reduced, contributing to efforts to mitigate AMR in Vietnam, where the burden of infections with drug-resistant pathogens is high.

False-Negative Results

Despite the overall good performance of 16S ONT sequencing, we observed some limitations in pathogen detection. For instance, *K pneumoniae* and *S pneumoniae* were identified more frequently by CSF culture than by ONT sequencing. Potential explanations for these discrepancies could be low bacterial load in CSF samples or challenges with the DNA extraction process. Improving the DNA yield by optimizing extraction protocols to better capture bacterial DNA from low-bacterial-load samples could help minimize false-negative results.

This study has a few limitations. First, the lack of a universally accepted gold standard for diagnosing bacterial meningitis complicates the assessment of diagnostic tools such as 16S ONT sequencing. Second, due to the retrospective nature of the study, not all patients underwent uniform testing. Notably, simultaneous serum glucose measurements were often unavailable, preventing calculation of the CSF/serum-glucose ratio, a key diagnostic marker, particularly in patients with diabetes or suspected hyperglycemia. Nonetheless, our study could show that 16S ONT sequencing could detect pathogens missed by conventional culture. Although 16S ONT sequencing demonstrated greater sensitivity than targeted molecular diagnostic panels, it should be viewed as a complementary tool rather than a replacement for culture-based diagnostics, serving to enhance overall pathogen detection.

In conclusion, 16S ONT sequencing is a promising tool for the diagnosis of bacterial CNS infections, offering significant advantages over traditional culture methods in terms of diagnostic yield, speed, and impact on clinical decision-making. By improving pathogen identification, guiding more accurate antibiotic therapy, and contributing to antimicrobial stewardship, 16S ONT sequencing has the potential to improve patient outcomes and combat AMR, particularly in resource-limited settings.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole

responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Author contributions. T. P. V. and D. N. designed the study. T. P. V. conceptualized the study, supervised the study, and contributed to the study materials and assays. D. V. D. performed the experimental procedures. D. V. D., N. T. T. N., and L. T. K. L. were involved in the analysis and validation of the results. D. V. D., N. X. H., T. T. T. H., N. T. K. L., H. X. Q., T. T. L., V. D. T., V. V. S., and L. H. S. recruited the patients and were involved in sampling procedures. D. V. D. wrote the first draft. T. P. V. reviewed and revised the first draft. P. G. K. and D. N. reviewed the manuscript. All authors have read and approved the manuscript.

Ethical approval. This study adhered to ethical guidelines set by the Declaration of Helsinki to ensure participant safety and integrity. Written informed consent was obtained from all hospitalized patients and/or their relatives, and from parents for subjects <18 years of age. The study protocol was reviewed and approved by the institutional review board of the 108 Military Central Hospital, Hanoi, Vietnam (protocol number 108MCH/RES/MENTNGITIS-V-D3-25042017).

Patient consent. Informed written consent was obtained from all hospitalized patients and/or their relatives after a detailed explanation of the study procedures.

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Data availability. The 16S sequencing data have been deposited in the Sequence Read Archive under accession number PRJNA1261013. The authors confirm that the data supporting the findings of this study are available within the article and in its Supplementary Material.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Supplementary Table 1: Patients with positive 16S ONT but negative CSF culture results (n=17).

Patient ID	Timepoint of sample collection	Risk factor	16S sequencing	Blood culture	Additional analysis	Summary of Clinical Diagnosis from Medical Records
24	During hospitalization	Head trauma, post-neurosurgery	*Escherichia marmotae*	Negative	NA	Bacterial meningitis
25	During hospitalization	Non-specific	*Acinetobacter baumannii*	NA	PCR (-ve) for *A.baumannii*	Meningitis of unknown cause
26	During hospitalization	Non-specific	*Streptococcus suis*	Negative	PCR (-ve) for *S. suis*	Meningitis of unknown cause
27	On admission	Non-specific	*Citrobacter freundii*	NA	NA	Septic shock, bacterial meningitis
28	During hospitalization	Non-specific	*Acinetobacter baumannii*	NA	PCR (-ve) for *A.baumannii*	Septic shock, bacterial pneumoniae
29	On admission	Non-specific	*Escherichia marmotae*	NA	NA	Migraine
30	On admission	Non-specific	*Streptococcus suis*	NA	PCR (-ve) for *S. suis*	Neurological Complications of Acute Rhinosinusitis
31	During hospitalization	Non-specific	*Streptococcus pneumoniae*	Negative	NA	Meningoencephalitis of unknown cause
32	During hospitalization	Non-specific	*Neisseria meningitidis*	Negative	NA	Septic shock, bacterial pneumoniae
33	On admission	Non-specific	*Streptococcus suis*	Negative	PCR (+ve) for *S. suis*	Bacterial meningitis
34	On admission	Head trauma, post-neurosurgery	*Acinetobacter baumannii*	Negative	PCR (+ve) for *A. baumannii*	Bacterial sepsis and meningitis
35	On admission	Non-specific	*Streptococcus oralis*	*S. oralis*	NA	Bacterial sepsis and meningitis due to *S. oralis*
36	On admission	Non-specific	*Neisseria meningitidis*	Negative	NA	Bacterial meningitis
37	On admission	Head trauma	*Klebsiella aerogenes*	Negative	NA	Bacterial meningitis
38	On admission	Post-neurosurgery	*Acinetobacter baumannii*	Negative	PCR (+ve) for *A. baumannii*	Bacterial sepsis and meningitis
39	On admission	Non-specific	*Staphylococcus epidermidis*	Negative	NA	Meningitis of unknown cause
40	On admission	Non-specific	*Streptococcus suis*	Negative	PCR (+ve) for *S. suis*	Bacterial meningitis

Abbreviation: CSF, Cerebrospinal fluid; Patient ID; NA, Not Available; PCR, Polymerase Chain Reaction.

Supplementary Table 2: Patients characteristics positive by CSF culture but negative 16S rRNA ONT (n=12).

Patient ID	Age/Gender	Cell count	Glucose	Protein	CSF culture	16S ONT	Empirical therapy	Treatment Adequacy	Outcome
1	18/M	3910	0.72	1.02	*K. pneumoniae*	Negative	CFX	MER	2
2	66/M	1624	0.68	3.53	*L. monocytogenes*	Negative	MER, VAN	MER, VAN, AMP	2
3	31/F	1960	0.2	2.13	*S. pneumoniae*	Negative	CTX, DEX	MER	5
4	23/M	38731	0.1	6.34	*A. baumannii*	Negative	MER, VAN, COL	MER, VAN, COL	2
5	67/M	91063	0.1	12.1	*K. pneumoniae*	Negative	CTX, DEX	MER, AMK	4
6	44/M	9217	0.1	8.83	*K. pneumoniae*	Negative	CTX, LEV, MET, DEX	MER, AMK	4
7	45/M	117	0.34	2.37	*C. neoformans*	Negative	AMB, FLU, RHZ, S, MOX	AMB, FLU, RHZ, S, MOX	2
8	44/M	3024	0.32	2.41	*K. pneumoniae*	Negative	MER, LIN	MER, DEX	3
9	92/F	135	3.65	0.44	*C. neoformans*	Negative	CTX	AMB	5
10	40/M	445	2.4	1.71	*C. neoformans*	Negative	MER, VAN	AMP, FLU	1
11	78/F	6	2.1	0.86	*T. asahii*	Negative	MER, DEX	VCN, DEX	1
12	62/M	5157	0.02	8.3	*E. coli*	Negative	MER, LIN, LEV	MER, LIN, LEV	2

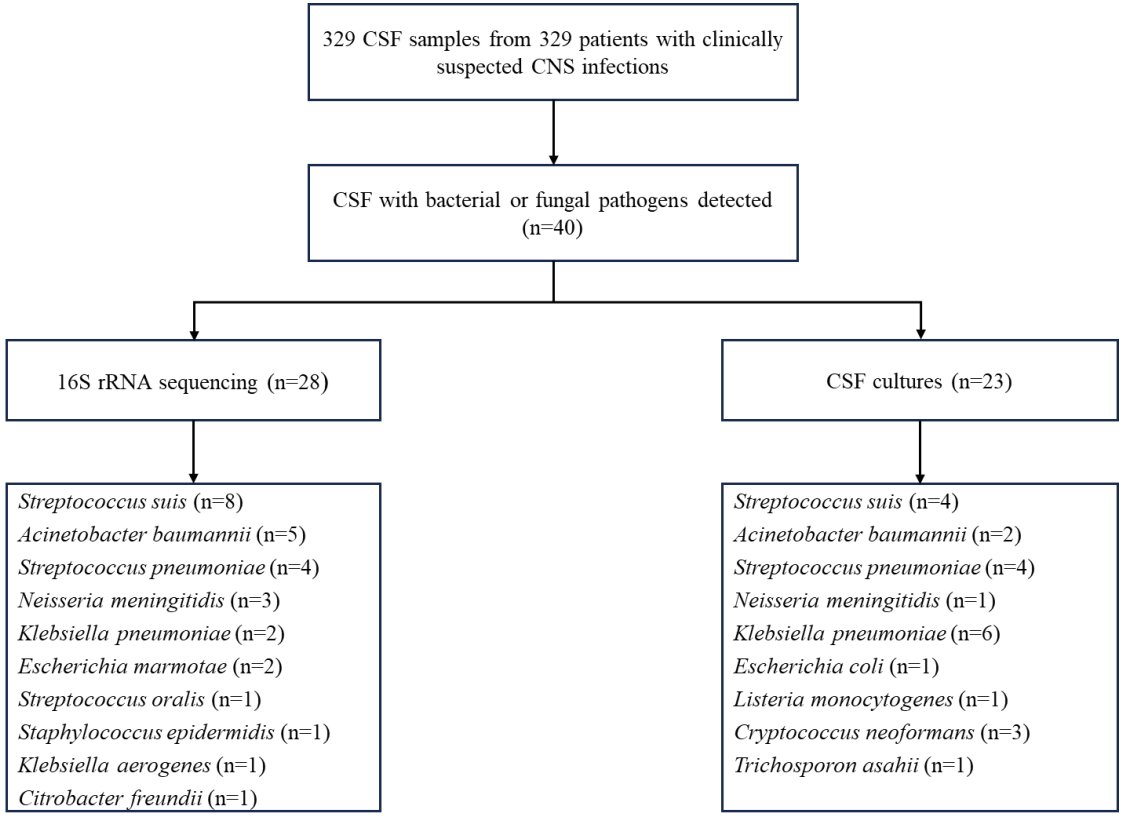
Abbreviation: Patient ID, Patient Identification; 16S ONT, 16S Oxford Nanopore Technology. F, Female; M, Male; ND, not done. AMB, Amphotericin B; AMK, Amikacin; AMP, Ampicillin; CFX, Cefotaxime; COL, Colistin; CTX, Ceftriaxone; DEX, Dexamethasone; FLU, Fluconazole; LEV, Levofloxacin; LIN, Linezolid; MER, Meropenem; MET, Metronidazole; MOX, Moxifloxacin; RHZ, Rifampicin-Isoniazid-Pyrazinamide; S, Streptomycin; VAN, Vancomycin; VCN, Voriconazole; Outcome: 1 death; 2: vegetative state; 3: severe disability; 4: moderate disability; 5: mild or no disability.

Supplementary Table 3: 16S rRNA sequencing reads at genus and species level (n=28)

Patient ID	Total reads	Results (Genus)	Genus reads	% genus reads	Results (Species)	Species reads	%
13	29166	*Streptococcus*	28586	98%	*Streptococcus suis*	28566	98%
14	76660	*Streptococcus*	74863	98%	*Streptococcus pneumoniae*	74781	98%
15	9131	*Klebsiella*	8391	92%	*Klebsiella pneumoniae*	8237	90%
16	84364	*Streptococcus*	82970	98%	*Streptococcus suis*	82899	98%
17	129709	*Streptococcus*	127728	98%	*Streptococcus suis*	127629	98%
18	124467	*Acinetobacter*	122356	98%	*Acinetobacter baumannii*	122348	98%
19	4300	*Streptococcus*	4181	97%	*Streptococcus pneumoniae*	4180	97%
20	44000	*Streptococcus*	42448	96%	*Streptococcus suis*	42418	96%
21	90235	*Klebsiella*	80579	89%	*Klebsiella pneumoniae*	64529	72%
22	77398	*Streptococcus*	75848	98%	*Streptococcus pneumoniae*	75813	98%
23	9225	*Neisseria*	8529	92%	*Neisseria meningitidis*	8277	90%
24	10556	*Escherichia*	9434	89%	*Escherichia marmotae*	9183	87%
25	194	*Acinetobacter*	134	69%	*Acinetobacter baumannii*	130	67%
26	34	*Streptococcus*	22	65%	*Streptococcus suis*	22	65%
27	3891	*Citrobacter*	3528	91%	*Citrobacter freundii*	2802	72%
28	441095	*Acinetobacter*	423520	96%	*Acinetobacter baumannii*	423511	96%
29	39166	*Escherichia*	32280	82%	*Escherichia marmotae*	31004	79%
30	72325	*Streptococcus*	68587	95%	*Streptococcus suis*	68465	95%
31	16515	*Streptococcus*	16037	97%	*Streptococcus pneumoniae*	16026	97%
32	31835	*Neisseria*	27105	85%	*Neisseria meningitidis*	26201	82%
33	3042	*Streptococcus*	2937	97%	*Streptococcus suis*	2931	96%
34	1138	*Acinetobacter*	922	81%	*Acinetobacter baumannii*	915	80%
35	3035	*Streptococcus*	2806	92%	*Streptococcus oralis*	2799	92%
36	97	*Neisseria*	83	86%	*Neisseria meningitidis*	81	84%
37	73999	*Klebsiella*	72522	98%	*Klebsiella aerogenes*	72521	98%
38	46062	*Acinetobacter*	44969	98%	*Acinetobacter baumannii*	44966	98%
39	122	*Staphylococcus*	67	55%	*Staphylococcus epidermidis*	63	52%
40	4321	*Streptococcus*	4164	96%	*Streptococcus suis*	4157	96%

Abbreviation: Patient ID, Patient Identification.

Supplementary Figure 1: Pathogens detected by CSF culture and 16S rRNA sequencing.



Note: 16S ONT sequencing yielded higher positivity rate than CSF culture in detecting *Streptococcus suis* (8 cases vs. 4), *Acinetobacter baumannii* (5 vs. 2), *Streptococcus pneumoniae* (4 vs. 4), and *Neisseria meningitidis* (3 vs. 1) and *Klebsiella pneumoniae* (2 vs. 6). 16S ONT sequencing detected 17 pathogens in 17 samples which were negative by CSF culture. This included *S. suis (n=4)*, *A. baumannii (n=4)*, *S. pneumoniae (n=1)*, *N. meningitidis (n=2)* as well as five pathogens detected exclusively by 16S ONT: *Escherichia marmotae* (n=2), *Staphylococcus epidermidis* (n=1), *Streptococcus oralis* (n=1), *Klebsiella* aerogenes (n=1) and and *Citrobacter freundii* (n=1). In contrast, CSF culture detected 12 pathogens in 12 samples not identified by 16S ONT, including *A. baumannii* (n=1), *K. pneumoniae* (n=4), *S. pneumoniae* (n=1), *Cryptococcus neoformans* (n=3), *Trichosporon asahii* (n=1), *Listeria monocytogenes* (n=1), and *Escherichia coli* (n=1). Nanopore-negative results were anticipated in four patients as *C. neoformans* and *T. asahii* are fungi not detectable by 16S rRNA sequencing.

Chapter 3

Etiological Spectrum and Clinical Predictors of Unfavourable Outcomes in Central Nervous System Infections in Vietnam

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Publication

Causative Pathogens and Predictors of Unfavourable Outcomes in Central Nervous System Infections in Resource-Limited Settings

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Causative pathogens and predictors of unfavourable outcomes in central nervous system infections in resource-limited settings

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ABSTRACT

Objectives: This study investigated the impact of causative pathogens on the outcomes of central nervous system (CNS) infections and assessed clinical parameters to identify patients at risk for unfavourable outcomes.

Methods: Patients with suspected CNS infections underwent blood and cerebrospinal fluid (CSF) culture and advanced molecular testing, including real-time PCR assays for bacterial and viral pathogens. Patients were classified into clinical categories and their outcomes assessed using the Glasgow Outcome Scale.

Results: Pathogens were identified in 24% (80/330) of patients, with *Mycobacterium tuberculosis, Klebsiella pneumoniae*, and *Acinetobacter baumannii* being the most common. Mortality was 10%, with fungal meningitis and dual infections having the highest rates. Unfavourable outcomes were observed in 57% of patients. The most common pathogens associated with unfavourable outcomes were *M. tuberculosis* followed by *K. pneumoniae, A. baumannii*, and HSV-1. Multivariate analysis identified community-onset infection as a protective factor, while a longer duration of illness before admission (≥5 days) and altered mental status on admission were significant predictors for unfavourable outcomes. Furthermore, the timely administration of appropriate empirical therapy was significantly associated with a reduced risk of mortality.

Conclusions: CNS infections in northern Vietnam have diverse causes and overlapping clinical features, complicating diagnosis and management.

Introduction

Infections of the central nervous system (CNS), including meningitis, encephalitis, and meningoencephalitis, are a significant global public health challenge, causing an estimated one million cases annually with high mortality rates. These life-threatening conditions disproportionately affect low- and middle-income coun-

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tries (LMICs), where demographic disparities, geographic factors, limited vaccination coverage, and pre-existing health conditions contribute to higher mortality and morbidity [1,2]. Despite advancements in diagnostic testing, up to 70% of cases remain etiologically undiagnosed, even after extensive investigations [3,4], even with wide-ranging investigations. Survivors often face long-term complications such as vision and hearing impairments, seizures, and cognitive deficits, underscoring the critical need for rapid and accurate diagnosis to improve outcomes [5].

Vietnam exemplifies the difficulties LMICs face in diagnosing and managing CNS infections. In large urban areas, the causative pathogens vary over time and space, and diagnostic confirmation is hindered by prior antibiotic use, the limited scope of molecular assays, and the low sensitivity of available tools. Mortality and residual disability rates remain alarmingly high, with approximately 30% of survivors experiencing lasting impairments [6,7]. These challenges highlight the urgent need for better diagnostic approaches tailored to resource-limited settings. Our previous investigations evaluated the diagnostic performance of the BioFire® FilmArray® Meningitis/Encephalitis (FAME) panel and the added value of incorporating 16S rRNA sequencing using Oxford Nanopore Technology (ONT) into the standard diagnostic algorithm, revealing that the limited pathogen coverage of the FAME panel restricts its ability to reliably detect the causative agents of CNS infections in Vietnam [8,9]. Therefore, the pathogens causing CNS infections in Vietnam may not be diagnosed correctly and in a timely manner and hence, therapy initiation may be delayed, which may influence the infection outcome.

In this study, we investigated the impact of the causative pathogen on the outcomes of CNS infections in Vietnam and evaluated additional clinical parameters that could help identify patients at risk for unfavourable outcomes. Identifying these factors may aid in improving the diagnostic and clinical management of patients with CNS infections and to ultimately reduce the incidence of unfavourable outcomes of CNS infections in Vietnam.

Methods

Ethics approval

This study adhered to strict ethical guidelines to ensure participant safety and integrity. Informed written consent was obtained from all hospitalized patients and/or their relatives after a detailed explanation of the study procedures. For subjects under 18 years of age, consent was obtained from their parents or legal guardians. The study protocol was reviewed and approved by the institutional review board of the 108 Military Central Hospital, Hanoi, Vietnam (Protocol No. 108MCH/RES/MENTNGITIS-V-D3-25042017). All procedures were conducted in compliance with ICH-GCP/GCLP guidelines and applicable regulations.

Study population and design

This retrospective, multicenter, hospital-based cohort study used a convenience sampling approach and included all patients with suspected CNS infections who had complete clinical and laboratory data available during the study period. The study was conducted across four hospitals in northern Vietnam including 108 Military Central Hospital (108 MCH, Hanoi), National Hospital for Tropical Diseases (NHTD, Hanoi), 103 Military Hospital (103 MH, Hanoi), and Viet Tiep Friendship Hospital (VT, Haiphong) from July 1, 2022, to April 30, 2023. Inclusion criteria required patients to be hospitalized with suspected meningitis or encephalitis, presenting with symptoms such as fever, headache, nausea/vomiting, neck stiffness, focal neurological signs, seizures, or altered mental status, and to have undergone a lumbar puncture for diagnostic purposes [10]. Patients were excluded if they had contraindications to lumbar puncture, including intracranial space-occupying lesions with mass effect, a mass in the posterior fossa, abnormal intracranial pressure, or localized skin infections at the lumbar puncture site, or if they had incomplete clinical histories or declined to provide consent. Demographic information, medical history, clinical presentation, laboratory findings, treatment details, microbiological diagnostic results, and discharge outcomes were extracted from medical records. Cerebrospinal fluid (CSF) samples were collected in hospitals in Vietnam and stored at -80°C for further analysis.

Patient classification and outcome assessment

Patients were classified into two groups: community-onset and hospital-onset infections. Community-onset infections were defined as those occurring within 48 hours of hospital admission, whereas hospital-onset infections were defined as those developing more than 48 hours after admission. Immunocompromised patients were identified based on the use of immunosuppressive drugs or a medical history of conditions such as diabetes mellitus, HIV infection, or splenectomy. The level of consciousness was assessed using the Glasgow Coma Scale (GCS), with an altered mental status defined by a GCS score of <14 [11]. CSF pleocytosis was defined using two thresholds for corrected white blood cell (WBC) counts: ≥ 5 cells/mm^3 and ≥ 10 cells/mm^3 [12]. Abnormal CSF glucose and protein levels were determined based on local clinical guidelines, with glucose defined as abnormal at <2.8 mmol/L or >4.2 mmol/L, and protein as abnormal at <0.10 g/L or >0.25 g/L. Clinical outcomes were evaluated using the Glasgow Outcome Scale (GOS), where scores range from 1 (death) to 5 (mild or no disability/recovery). A favourable outcome was defined as a GOS score of 5, while unfavourable outcomes included scores of 1 to 4, encompassing death, vegetative state, severe disability, and moderate disability [13]. Clinical syndromes were further categorized as bacterial meningitis (BM), viral encephalitis/meningitis (VEM) [14], tuberculous meningitis (TM) [15], and fungal meningitis (FM) [16,17], based on established diagnostic criteria and detailed in Supplementary Table S1. This classification framework facilitated a structured analysis of clinical features, laboratory findings, and outcomes.

Haematological and biochemical analysis

Peripheral blood samples were analysed using the ADVIA 2120i Haematology System (Siemens Healthcare Diagnostics, Zürich, Switzerland) to measure haematological parameters. CSF white blood cell counts were determined using the body fluid mode in the Haematology System. Biochemical and serological markers were assessed with the Beckman Coulter AU5800 automated analyser (Beckman Coulter, Singapore), following the manufacturer's instructions.

Standard laboratory diagnostics

Microbiological culture procedures were standardized across all participating study sites to ensure consistency and reliability. Blood and cerebrospinal fluid (CSF) cultures were performed using the BACTEC™ Plus Aerobic/F system (Becton–Dickinson, Franklin Lakes, NJ, USA) and incubated at 36 °C for up to 5 days, following the manufacturer's instructions and standard local laboratory protocols. When bacterial growth was detected, colonies were subjected to species identification using the MALDI-TOF VITEK® MS system, an automated microbial identification platform. Antimicrobial susceptibility testing (AST) was performed with the VITEK® 2 Compact System (BioMérieux, Lyon, France) and results were interpreted according to the Clinical and Laboratory Standards Insti-

tute (CLSI) to determine the pathogens resistance profile. In addition to these standard procedures, supplemental tests, including CSF fungal cultures and polymerase chain reaction (PCR) assays for specific bacteria and viruses, were conducted when clinically indicated. These additional diagnostics were initiated based on physician requests and completed alongside routine testing, enhancing the breadth of pathogen detection and clinical management. An overview of the standard CSF diagnostic approaches and the specific molecular testing capabilities available at each study site is provided in Supplementary Table S2.

CSF sample analysis

CSF samples that tested negative by both the BioFire® FAME panel and routine diagnostics were further screened for additional viral pathogens, including Zika, Dengue, and Chikungunya viruses, using a multiplex Real-time PCR assay with the Fast Track Diagnostics Kit (FTD) (Siemens, Erlangen, Germany). Additionally, specific real-time PCR assays were employed to screen for *Streptococcus suis, Acinetobacter baumannii, Mycobacterium tuberculosis*, and Epstein-Barr virus, while routine PCR methods were used to detect *Staphylococcus aureus* and *Klebsiella pneumoniae*. Nucleic acids were extracted from 200 µL of CSF using the QIAsymphony DSP Virus/Pathogen Kit (Qiagen, Hilden, Germany), with a final elution volume of 60 µL. PCR was conducted on a thermal cycler (Eppendorf, Hamburg, Germany), while Real-time PCR assays were carried out using the LightCycler 480 Instrument II (Roche, Basel, Switzerland). Detailed primer and probe sequences for both PCR and Real-time PCR, along with thermal cycling conditions, are provided in Supplementary Table S3. This multi-target approach allowed us for a comprehensive detection of pathogens in CSF samples, enhancing diagnostic accuracy.

Data analysis

Statistical analyses were conducted using R version 4.4.0 software (http://www.r-project.org). To identify potential predictors (unfavourable outcomes), multivariate logistic regression models were used to assess the association between various factors and clinical outcomes. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to determine the strength and significance of these associations. This multivariate analysis, corrected for age, sex, infection onset, comorbidities, days of illness before admission, and altered mental status, allowed the identification of key prognostic factors that can support early clinical decision-making and improve patient management.

Results

Study cohort overview and clinical characteristics

A total of 330 patients with suspected CNS infections were recruited from four hospitals around Hanoi, Vietnam. Recruitment numbers varied across sites: 136 patients from the National Hospital for Tropical Diseases (NHTD), 52 patients from 108 Military Central Hospital (108 MCH), 102 patients from Viet Tiep Friendship Hospital (VT), and 40 patients from 103 Military Hospital (103 MH). Among these, causative pathogens were confirmed in 24% (80/330) of patients. The main clinical characteristics are summarized in Table 1. The average age of the cohort was 54 years, with 225 patients (68%) being male. Fever, neck stiffness, and altered mental status were observed more frequently in patients with confirmed infectious etiologies compared to those with unknown causes. Among CSF parameters, cell count, protein, and glucose levels were associated with a confirmed infectious etiology.

Demographic and clinical characteristics of CNS infection types

Demographic and clinical characteristics of patients with bacterial meningitis (BM), tuberculous meningitis (TM), viral encephalitis/meningitis (VEM), fungal meningitis (FM), and dual infections (DI) are compared in Table 2. Patients with TM generally presented later for hospitalization compared to those with other types of CNS infections. Clinical features were not pathogen-specific, making it challenging to distinguish between different etiologies based solely on clinical presentation. Laboratory findings revealed that BM and DI were associated with a higher proportion of polymorphonuclear cells in CSF, while VEM was characterized by a higher proportion of lymphocytes. Protein levels and the proportion of abnormal glucose levels in CSF were significantly higher in BM and DI compared to other infections. In blood parameters, BM and DI exhibited higher WBC counts, a greater proportion of polymorphonuclear cells, and elevated C-reactive protein levels compared to other forms of meningitis. These findings highlight distinct laboratory patterns that can assist in differentiating between CNS infection types, though clinical features remained broadly similar across the groups.

Pathogen identification and distribution in CNS infections

Among the 80 cases with confirmed pathogens, 64 were diagnosed by routine diagnostics (n=48) and/or the FAME assay (n=33) [8], while the remaining 16 pathogens were identified using customized PCR. The leading pathogen causing CNS infections was *Mycobacterium tuberculosis* (n=12), followed by *Varicella Zoster Virus* (VZV) (n=9), *Klebsiella pneumoniae* (n=8), *Herpes Simplex Virus 1* (HSV-1) (n=7), *Streptococcus suis* (n=6), *Streptococcus pneumoniae* (n=6), and *Acinetobacter baumannii* (n=6). Dual infections were identified in 5 cases, including combinations such as *A. baumannii* + CMV (n=1), *Haemophilus influenzae* + *Streptococcus agalactiae* (n=1), *K. pneumoniae* + CMV (n=1), *K. pneumoniae* + HSV-1 (n=1), and *M. tuberculosis* + CMV (n=1). CSF samples were also tested for additional pathogens like *S. aureus*, HSV-2, Human Herpesvirus 6 (HHV-6), Human Parechovirus (HPeV), chikungunya virus, dengue virus, and Zika virus, but these were not detected in any of the available samples. Confirmed infectious etiologies by age group: Among the confirmed pathogens, *M. tuberculosis* was the most frequent etiology in the ≤60 years age group, while in the >60 years age group, *Listeria monocytogenes, A. baumannii*, and VZV were more commonly identified (Figure 1). In immunocompromised patients, the most common pathogens were *M. tuberculosis* and VZV, followed by *Cryptococcus neoformans, K. pneumoniae*, and HSV-1. In contrast, the distribution of causative pathogens in immunocompetent patients was more varied (Figure 1).

Risk factors for mortality and unfavourable outcomes in CNS infections

The overall mortality rate in the study cohort was 10% (32/330), with a mortality rate of 9% (7/80) for patients with confirmed causative pathogens and 10% (25/250) for those with unknown pathogens (Table 1). Mortality rates varied by etiology: bacterial meningitis (BM) (6%, 2/36), viral encephalitis/meningitis (VEM) (0%), tuberculous meningitis (TM) (17%, 2/12), fungal meningitis (FM) (40%, 2/5), and dual infections (DI) (20%, 1/5) (Table 2). The clinical and patient characteristics of 80 patients with confirmed causative pathogens and their corresponding clinical outcomes are summarized in Table 3. Deaths were primarily attributed to *M. tuberculosis* (n=2 cases), *S. pneumoniae* (n=1 case), *C. neoformans* (n=1 case), *Trichosporon asahii* (n=1 case), *M. tuberculosis* + CMV (n=1 case), and *A. baumannii* (n=1 case) (Figure 1). In the multivariate analysis, timely administration of appropriate and adequate

Table 1
Demographic and clinical characteristics of patients with central nervous system infections.

Patient characteristics		CNS infections (n=330)	Confirmed Causative Pathogen (n=80)	No Defined Diagnosis (Unknown) (n=250)	*P*-value
Demographics					
Age (Mean ± SD)		54 ± 19	52 ± 18	55 ± 19	0.202
Male sex-no. (%)		225 (68)	61 (76)	164 (66)	0.101
Underlying conditions – N (%)					
Hypertension		90 (27)	15 (19)	75 (30)	0.068
Diabetes		62 (19)	5 (6)	57 (23)	**0.002**
Cardiac disease		19 (6)	1 (1)	18 (7)	0.053
Alcoholism		17 (5)	9 (11)	8 (3)	**0.008**
Chronic liver disease		18 (6)	4 (5)	14 (6)	1
Chronic lung disease		17 (5)	6 (8)	11 (4)	0.26
Kidney disease		15 (5)	2 (3)	13 (5)	0.537
Immuno-suppressive drugs		13 (4)	3 (4)	10 (4)	1
Cancer		13 (4)	0 (0)	13 (5)	**0.043**
HIV-positive		9 (3)	2 (2.5)	7 (3)	1
Risk factors – N (%)					
Post neurosurgery		29 (9)	11 (14)	18 (7)	0.115
Head trauma		31 (9)	8 (10)	23 (9)	1
CSF shunt		5 (2)	0 (0)	5 (2)	0.341
Clinical features-N (%)					
Fever (>37.5°C)		273 (83)	77 (96)	196 (78)	**<0.001**
Headache		221 (67)	60 (75)	161 (64)	0.106
Neck stiffness		207 (63)	67 (83)	140 (56)	**<0.001**
Nausea/Vomiting		99 (30)	33 (41)	66 (26)	**0.017**
Seizure		29 (9)	3 (4)	26 (10)	0.109
Focal neurologic deficits		20 (6)	6 (8)	14 (6)	0.591
Glasgow Coma Score (GCS)		13 ± 2	13 ± 2	13 ± 2	**0.018**
Altered mental status		142 (43)	50 (62)	92 (37)	**<0.001**
at least 2/4 symptoms recorded (headache, fever, stiff neck, altered mental status)		273 (83)	76 (95)	196 (79)	**0.002**
Glasgow Outcome Score (GOS) N (%)					
1: Death		32 (10)	7 (9)	25 (10)	0.121
2: Vegetative state		29 (9)	11 (14)	18 (7)	
3: Severe disability		64 (19)	11 (14)	53 (21)	
4: Moderate disability		62 (19)	11 (14)	51 (20)	
5: Mild or no disability		143 (43)	40 (49)	103 (42)	
Cerebrospinal fluid parameters					
Cell count (cells/mm^3)[¥]	Mean ± SD	1032 ± 5897	3440 ± 11400	254 ± 1500	**0.015**
	Pleocytosis of 5 cells/mm^3	225 (69)	71 (89)	154 (62)	**<0.001**
	Pleocytosis of 10 cells/mm^3	175 (54)	66 (83)	109 (44)	**<0.001**
Protein (g/L)[€]	Mean ± SD	1.56 ± 2.78	2.76 ± 3.36	1.18 ± 2.46	**<0.001**
	Abnormal protein	307 (94)	79 (99)	228 (91)	**0.006**
Glucose (mmol/L)[€]	Mean ± SD	4.16 ± 2.96	2.88 ± 2.93	4.57 ± 2.86	**<0.001**
	Abnormal glucose	188 (58)	56 (70)	132 (53)	**0.009**

[¥] The CSF leukocyte count was determined in 324 patients; CSF specimens from 6 patients had too many leukocytes for an exact count to be performed (missing values, n=5 for no defined diagnosis, n=1 for confirmed causative pathogen).
[€] The protein and glucose levels in the CSF were determined in 326 patients; the remaining 4 CSF samples were not measured (missing values, n=3 for no defined diagnosis and n=1 for confirmed causative pathogen); Significant p-values shown in bold.

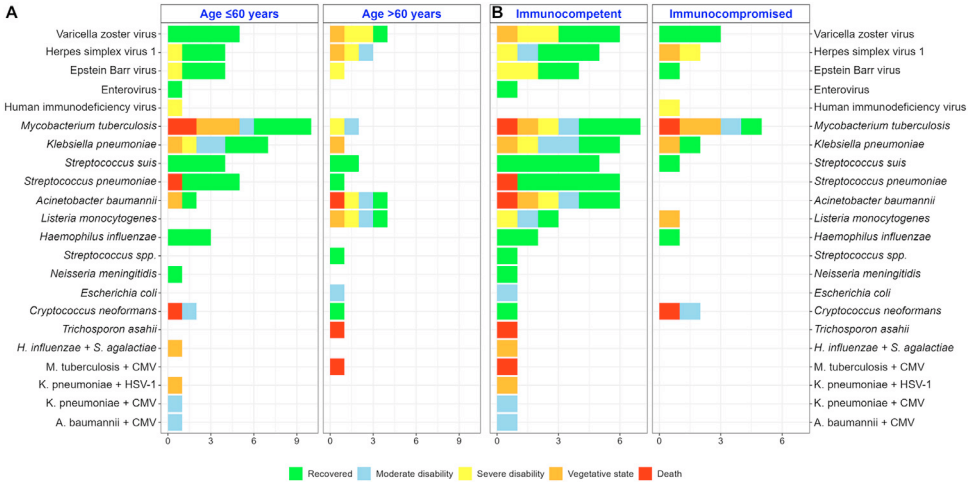

Figure 1. Clinical Outcomes of Patients with Central Nervous System Infections: Impact of Age Group (A) and Immune Status (B).
Abbreviations: CMV: Cytomegalovirus; HSV-1: Herpes simplex virus 1.

Table 2
Clinical characteristics of patients with bacterial meningitis (BM), tuberculous meningitis (TM), viral encephalitis/meningitis (VEM), fungal meningitis (FM), and dual infections (DI).

Patient characteristics		Bacterial meningitis (BM) (n=36)	Tuberculous meningitis (TM) (n=12)	Viral encephalitis/meningitis (VEM) (n=23)	Fungal meningitis (FM) (n=4)	Dual infection (DI) (n=5)	*p*-value
Demographics							
Age (Mean ± SD)		51 ± 18	44 ± 16	58 ± 18	64 ± 25	44 ± 18	0.124
Male sex-no. (%)		27 (75)	11 (92)	16 (70)	2 (50)	5 (100)	0.276
Onset of infection							
Community		28 (78)	9 (75)	15 (65)	2 (50)	2 (40)	0.315
Hospital		8 (22)	3 (25)	8 (35)	2 (50)	3 (60)	
Day of illness before admission [Median (min, max)]		3 (1-14)	10 (1-30)	4 (1-60)	8.5 (2-60)	5 (2-17)	**0.022**
Pretreatment with antibiotics		24 (67)	8 (67)	12 (52)	4 (100)	4 (80)	0.433
Clinical features							
Fever (>37.5°C)		36 (100)	12 (100)	21 (91)	3 (75)	5 (100)	0.054
Headache		27 (75)	9 (75)	16 (70)	3 (75)	5 (100)	0.825
Neck stiffness		29 (91)	11 (92)	19 (83)	4 (100)	4 (80)	0.922
Nausea/Vomiting		19 (53)	7 (58)	4 (17)	1 (25)	2 (40)	0.037
Seizure		0 (0)	0 (0)	3 (13)	0 (0)	0 (0)	0.161
Focal neurologic deficits		0 (0)	2 (17)	4 (17)	0 (0)	0 (0)	0.054
Glasgow coma score (Mean ± SD)		12 ± 3	13 ± 2	13 ± 2	13 ± 2	10 ± 3	0.138
Altered mental status		23 (64)	7 (58)	12 (52)	3 (75)	5 (100)	0.374
at least 2/4 symptoms recorded (headache, fever, stiff neck, altered mental status)		36 (100)	11 (92)	21 (91)	3 (75)	5 (100)	0.097
Glasgow Outcome Score (GOS)							
1. Death		2 (6)	2 (17)	0 (0)	2 (50)	1 (20)	
2. Vegetative state		5 (14)	2 (17)	1 (4)	1 (25)	2 (40)	
3. Severe disability		3 (8)	1 (8)	7 (30)	0 (0)	0 (0)	
4. Moderate disability		4 (11)	3 (25)	2 (9)	0 (0)	2 (40)	
5. Mild or no disability		22 (61)	4 (33)	13 (57)	1 (25)	0 (0)	
Cerebrospinal fluid parameters							
Cell count (cells/mm^3)	Mean ± SD[¥]	5930 ± 15300	483 ± 460	170 ± 313	176 ± 188	9640 ± 16700	0.185
	Pleocytosis of 5 cells/mm^3	33 (92)	11 (92)	18 (78)	4 (100)	5 (100)	0.774
	Polymorphonuclear (%)[€] [Median (min, max)]	86 (2-99)	50 (3-94)	15 (2-80)	70 (10-99)	77 (60-95)	**<0.001**
	Lymphocytes (%)[€] [Median (min, max)]	12 (1-98)	27.5 (6-97)	80 (17-98)	20 (2-80)	23 (2-39)	**<0.001**
Protein (g/L)[£]	Mean ± SD	3.94 ± 4.32	2.22 ± 1.72	1.07 ± 0.89	1.35 ± 0.86	4.69 ± 3.46	**0.009**
	Abnormal protein	35 (97)	12 (100)	23 (100)	4 (100)	5 (100)	
Glucose (mmol/L)[£]	Mean ± SD	2.77 ± 4.13	2.49 ± 1.21	3.60 ± 1.19	2.12 ± 1.36	1.87 ± 1.86	0.652
	Abnormal glucose	30 (83)	9 (75)	10 (44)	3 (75)	4 (80)	**0.012**
Blood cells and Inflammatory markers							
White blood cell (G/L)[α]	Mean ± SD	17.5 ± 9.0	12.9 ± 5.0	9.3 ± 3.7	9.5 ± 2.8	13.2 ± 4.4	**0.001**
	Polymorphonuclear (%)[Median (min, max)]	88.8 (50.6-94.3)	86.8 (58.0-92.7)	75.5 (32.7-86.8)	77.3 (67.1-92.0)	89.8 (66.6-97.0)	**<0.001**
	Lymphocytes (%) [Median (min, max)]	5.6 (1.0-36.2)	5.0 (2.9-12.2)	15.0 (5.9-45.8)	10.9 (4.8-24.8)	2.7 (1.2-25.5)	**0.001**
Procalcitonin (ng/mL)[β]		11.40 ± 23.00	0.19 ± 0.15	0.14 ± 0.13	0.67 ± 0.65	3.38 ± 4.07	0.255
C-reactive protein (mg/L)[π]		139 ± 132	28.3 ± 42.0	22.4 ± 45.2	478 (NA)	91.5 ± 42.0	**<0.001**

[¥] The CSF leukocyte count was determined in 79 patients (missing values, n=1 for viral encephalitis/meningitis).
[€] The proportion of polymorphonuclear and lymphocytes in CSF were not measured in 17 patients, including 5 for bacterial meningitis, 8 for viral encephalitis/meningitis, 2 for tuberculous meningitis, 1 for fungal meningitis, 1 for dual infection.
[£] The protein and glucose levels in CSF were determined in 79 patients; the remaining sample was not measured (missing values, n=1 for bacterial meningitis).
[α] The white blood cell in blood was measured in 79 patients (missing values, n=1 for bacterial meningitis).
[β] The level of Procalcitonin was not analyzed in 35 patients, including 17 for bacterial meningitis, 11 for viral encephalitis/meningitis, 3 for tuberculous meningitis, 1 for fungal meningitis, 3 for dual infection.
[π] The level of C-reactive protein was not measured in 28 patients, including 13 for bacterial meningitis, 7 for viral encephalitis/meningitis, 4 for tuberculous meningitis, 3 for fungal meningitis, 1 for dual infection.; Significant p-values shown in bold.

empirical therapy was associated with a significantly reduced risk of death (OR=0.1; 95% CI 0.0–0.6; p=0.029). The patient characteristics for which a defined pathogen was identified, and empirical therapy was administered, as well as appropriate treatment adequacy needed, are listed in the Supplementary Table S4. Regarding unfavourable outcomes, 57% (187/330) of patients experienced adverse outcomes, with 51% (40/80) of patients with confirmed pathogens and 58% (147/250) with unknown pathogens having unfavourable outcomes (Table 1). The proportions of unfavourable outcomes varied by etiology: BM (39%, 14/36), VEM (43%, 10/23), TM (67%, 8/12), FM (75%, 3/4), and DI (100%, 5/5) (Table 2). The most common pathogens associated with unfavourable outcomes were *M. tuberculosis* (n=8 cases), *K. pneumoniae* (n=5 cases), *A. baumannii* (n=4 cases), and HSV-1 (n=4 cases) (Figure 1). Multivariate logistic regression analysis identified community-onset infection (OR=0.2; 95% CI 0.1-0.8; p=0.03) as a protective factor, while a longer duration of illness before admission (≥ 5 days) (OR=5.1; 95% CI 1.6-20.0; p=0.01) and altered mental status on admission (OR=7.4; 95% CI 2.0-33.2; *P*=0.004) were significant predictors for unfavourable outcomes. The results indicate distinct patterns between community-onset and hospital-onset CNS infections, with notable differences in infection etiologies and clinical outcomes (Figure 2).

Discussion

This study highlights on the complex landscape of CNS infections in northern Vietnam, a region representative of the chal-

Table 3
Patients characteristics with favourable and unfavourable clinical outcomes defined by Glasgow Outcome Score (GOS).

Variables	Died (n=7)	Survived (n=73)	*p*-value	Unfavourable (n=40) (GOS 1-4)	Favourable (n=40) (GOS-5)	*p*-value
Demographic						
Age (Mean ± SD)	61 ± 16	51 ± 18	0.193	55 ± 19	49 ± 17	0.139
Age ≤ 60 years	4 (57)	49 (67)	0.683	21 (53)	32 (80)	**0.018**
Age > 60 years	3 (43)	24 (33)		19 (47)	8 (20)	
Female	2 (29)	17 (23)	0.668	5 (12)	14 (35)	**0.036**
Male	5 (71)	56 (77)		35 (88)	26 (65)	
Onset of infection						
Community	3 (43)	53 (73)	0.189	23 (58)	33 (83)	**0.028**
Hospital	4 (57)	20 (27)		17 (42)	7 (17)	
Medical history						
At least one internal comorbidity	3 (43)	42 (58)	0.693	29 (73)	16 (40)	**0.007**
Hypertension	1 (14)	14 (19)	1	7 (18)	8 (20)	1
Cardiac disease	0 (0)	1 (1)	1	0 (0)	1 (3)	1
Diabetes	1 (14)	4 (5)	0.375	1 (3)	4 (10)	0.359
Chronic lung disease	1 (14)	5 (7)	0.434	5 (13)	1 (3)	0.201
Chronic liver disease	0 (0)	4 (5)	1	4 (10)	0 (0)	0.116
Kidney disease	0 (0)	2 (3)	1	2 (5)	0 (0)	0.494
Immunosuppressive drugs	1 (14)	2 (3)	1	2 (5)	1 (3)	1
HIV infection	0 (0)	2 (3)	1	2 (5)	0 (0)	0.494
Alcoholism	1 (14)	8 (11)	0.581	7 (18)	2 (5)	0.154
Immunocompromised status	2 (29)	17 (23)	0.668	11 (28)	8 (20)	0.599
Day of illness before admission						
< 5 days	4 (57)	38 (52)	1	16 (40)	26 (65)	**0.044**
≥ 5 days	3 (43)	35 (48)		24 (60)	14 (35)	
Clinical features						
Fever >37.5°C	7 (100)	70 (96)	1	39 (98)	38 (95)	1
Headache	6 (86)	54 (74)	0.673	28 (70)	32 (80)	0.439
Nausea/Vomiting	5 (71)	28 (38)	0.118	18 (45)	15 (38)	0.65
Seizure	0 (0)	3 (4)	1	3 (8)	0 (0)	0.241
Neck stiffness	6 (86)	61 (84)	1	36 (90)	31 (78)	0.225
Focal neurologic deficits	0 (0)	6 (8)	1	5 (13)	1 (3)	0.201
Glasgow coma scale	12 ± 1	13 ± 3	0.855	12 ± 2	13 ± 2	**< 0.001**
Altered mental status	7 (100)	43 (59)	0.041	33 (83)	17 (43)	**0.001**
Heart rate	99 ± 7	95 ± 16	0.252	94 ± 16	89 ± 15	**0.003**
Tachycardia (>100 beats/min)	2 (29)	19 (26)	1	8 (20)	13 (33)	0.309
Central nervous system infections						
Bacterial meningitis	2 (29)	34 (47)	0.449	14 (35)	22 (55)	0.116
Other infections	5 (71)	39 (53)		26 (65)	18 (45)	
Blood analysis						
White blood cell (G/L)[α]	14.5 ± 7.3	13.7 ± 7.6	0.768	12.8 ± 5.2	14.6 ± 9.4	0.3
Leukocytosis >10 G/L	5 (71)	45 (63)	1	26 (65)	24 (62)	0.932
Platelet [α]	303 ± 126	260 ± 109	0.414	269 ± 119	259 ± 102	0.681
Platelet < 150 G/L	0 (0)	8 (11)	0.481	5 (13)	3 (8)	0.329
Platelet 150 to 450 G/L	6 (86)	60 (83)		31 (78)	35 (90)	
Platelet > 450 G/L	1 (16)	4 (6)		4 (9)	1 (2)	
C-reactive protein (mg/L)[β]	269 ± 296	81.5 ± 109	0.535	108.0 ± 136.0	62.9 ± 91.1	0.163
C-reactive protein >100 mg/L	1/2 (50)	14/50 (28)	0.498	11 (37)	4 (18)	0.253
Procalcitonin (ng/mL)[£]	2.4 ± 4.2	5.4 ± 16.6	0.365	6.9 ± 20.4	3.0 ± 7.6	0.401
Procalcitonin >2 ng/mL	1/5 (20)	11/40 (28)	0.227	7 (29)	5 (24)	0.946
Cerebrospinal fluid analysis						
Cell count (cells/mm^3) [¥]	370 ± 254	3741 ± 11869	**0.019**	4313 ± 15405	2549 ± 4466	0.491
Cell count < 5 cells/mm^3	0 (0)	8 (11)	0.088	2 (5)	6 (15)	0.335
Cell count 5-100 cells/mm^3	2 (29)	17 (24)		10 (25)	9 (23)	
Cell count > 100-1000 cells/mm^3	5 (71)	21 (29)		16 (40)	10 (26)	
Cell count > 1000 cells/mm^3	0 (0)	26 (36)		12 (30)	14 (36)	
Protein (g/L)[€]	1.73 ± 1.09	2.86 ± 3.49	0.066	2.99 ± 4.10	2.54 ± 2.47	0.555
Protein < 0.25 g/L	0 (0)	0 (0)	0.098	0 (0)	0 (0)	0.092
Protein ≥ 0.25 – 0.5 g/L	0 (0)	10 (14)		2 (5)	8 (20)	
Protein ≥ 0.5 – 1 g/L	1 (14)	16 (22)		12 (31)	5 (13)	
Protein ≥ 1 – 2 g/L	5 (71)	17 (24)		11 (28)	11 (28)	
Protein ≥ 2 g/L	1 (15)	29 (40)		14 (36)	16 (39)	
Glucose (mmol/L)[€]	1.90 ± 0.95	2.97 ± 3.04	**0.045**	2.21 ± 1.71	3.56 ± 3.70	**0.043**
Glucose < 2.2 mmol/L	5 (71)	26 (36)	0.105	20 (50)	11 (28)	0.08
Glucose ≥ 2.2 mmol/L	2 (29)	46 (64)		20 (50)	28 (72)	
Adequate empirical therapy[µ]						
Yes	1 (14)	47 (65)	**0.013**	24 (60)	24 (61)	1
No	6 (86)	25 (35)		16 (40)	15 (39)	

For dead and survived groups: [α]The white blood cell in blood was measured in 79 patients (missing values, n=1 for survived group).
[β]The level of C-reactive protein was not analysed in 28 patients, including 23 for survived group, and 5 for died group.
[£]The level of Procalcitonin was not measured in 35 patients, including 33 for survived group, and 2 for died group.
[¥]The CSF leukocyte count was determined in 79 patients (missing values, n=1 for survived group).
[€]The protein and glucose levels in the CSF were determined in 79 patients (missing values, n=1 for survived group).
[µ]Empirical therapy was found in 79 patients (missing values, n=1 for survived group).
For unfavourable and favourable outcome groups: [α]The white blood cell in blood was measured in 79 patients (missing values, n=1 for favourable outcome).
[β]The level of C-reactive protein was not analysed in 28 patients, including 18 for favourable outcome, and 10 for unfavourable outcome.
[£]The level of Procalcitonin was not measured in 35 patients, including 19 for favourable outcome, and 16 for unfavourable outcome.
[¥]The CSF leukocyte count was determined in 79 patients (missing values, n=1 for favourable outcome).
[€]The protein and glucose levels in the CSF were determined in 79 patients (protein: missing values, n=1 for unfavourable outcome; glucose: missing values, n=1 for favourable outcome).
[µ]Empirical therapy was found in 79 patients (missing values, n=1 for favourable outcome).
Significant p-values highlighted in bold.

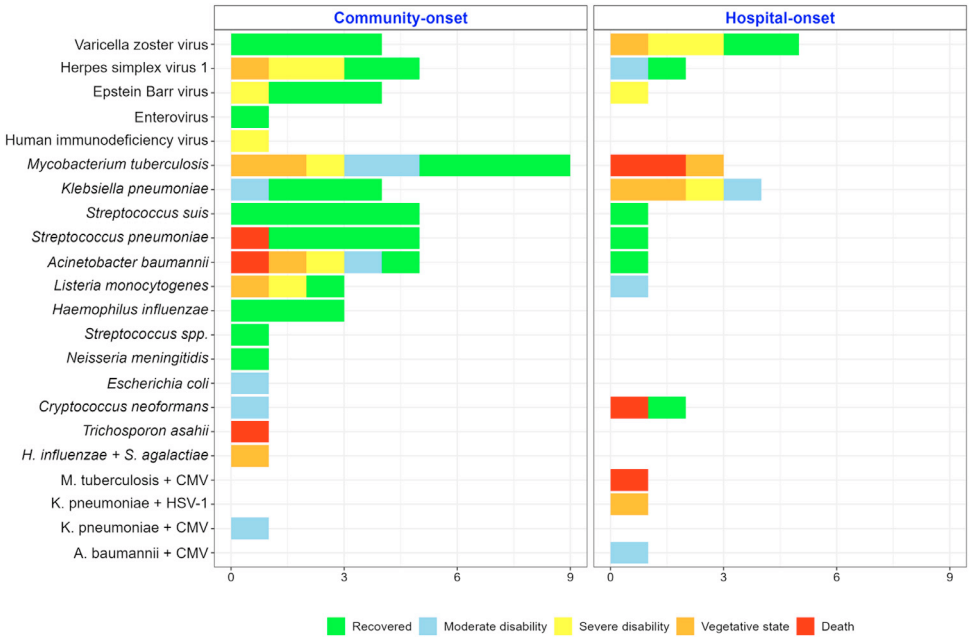

Figure 2. Clinical Outcomes of Patients with Central Nervous System Infections based on their onset: community- versus hospital-onset infections.
Abbreviations: CMV: Cytomegalovirus; HSV-1: Herpes simplex virus 1.

lenges faced in LMICs, where limited diagnostic resources, prior antibiotic use, and a constrained pathogen detection spectrum hinder effective clinical management. Our findings underscore the urgent need to enhance diagnostic frameworks and tailor them to local epidemiological patterns for improved outcomes.

CNS infections often present with classic symptoms such as fever, headache, neck stiffness, and altered mental status [18]. In our study, these symptoms were observed in 83%, 67%, 63%, and 43% of patients, respectively. However, their non-specific nature complicates differentiation between conditions like meningitis, encephalitis, and meningoencephalitis based solely on clinical presentation. Despite employing advanced diagnostic tools, including the BioFire® FAME panel [8], a significant diagnostic gap persists, as pathogens were unidentified in >70% of suspected CNS infections. This gap highlights the limitations of existing diagnostic technologies in addressing the broader spectrum of infectious agents prevalent in Vietnam. The findings also emphasize the need for more inclusive, adaptable diagnostic strategies to account for pathogens such as *Mycobacterium tuberculosis, Streptococcus suis*, and viral agents like CMV, HSV-1, and VZV, which are often underidentified in LMICs.

Clinical presentation and etiology

The overlapping clinical features of bacterial meningitis (BM), viral encephalitis/meningitis (VEM), tuberculous meningitis (TM), and fungal meningitis (FM) complicate etiological diagnosis. While certain laboratory markers, such as polymorphonuclear predominance in bacterial infections or elevated lymphocytes in viral cases, provide diagnostic clues, they lack pathogen specificity. Advanced diagnostic modalities are essential for distinguishing these conditions and facilitating timely, targeted treatment. In line with other studies from this region, BM was one of the most common causes of CNS infections, followed by VEM and TM [6,7,19]. Our findings reaffirm the regional significance of pathogens like *S. pneumoniae, S. suis*, and *A. baumannii. S. pneumoniae* underscores the potential of pneumococcal vaccination in reducing invasive disease and fostering herd immunity [20]. Meanwhile, *S. suis*, prevalent due to cultural practices involving raw pork consumption, highlights the need for public health campaigns addressing food safety [21]. The unexpected prominence of *A. baumannii* in community-onset CNS infections, typically linked to risk factors such as neurosurgical interventions and CSF shunts, underscores the importance of thorough clinical evaluations for accurate diagnosis and management. Furthermore, the association between different pathogens and clinical outcomes reveals critical insights into the management of CNS infections. For example, fungal meningitis, particularly due to *Cryptococcus neoformans*, was associated with high mortality rates, as were dual infections, which further complicate treatment and worsen prognosis. These findings suggest that dual infections might be an underappreciated cause of unfavourable outcomes and should be considered in clinical management, especially in immunocompromised patients.

Risk factors for mortality and unfavourable outcomes

CNS infections carry a high risk of unfavourable outcomes, with 57% of patients in this study experiencing complications and 10% mortality. Our multivariate analysis identified delayed hospital admission (≥5 days), altered mental status, and the lack of adequate empirical therapy as significant predictors of poor outcomes. These findings are consistent with previous studies, emphasizing the importance of early recognition and intervention [13,22,23]. Hospital-onset CNS infections were associated with higher morbidity and mortality compared to community-onset infections in our studies and is likely due to delayed detection and the involvement of resistant pathogens [24]. These findings highlight the need for robust infection control practices and proactive management strategies in hospital settings.

Pathogen distribution and age-related differences

Patient age, immune status, and infection onset are key factors that can aid physicians in predicting causative pathogens and selecting appropriate empirical treatments [25]. The distribution of pathogens varied significantly with age and immune status in this study. While *M. tuberculosis* was more prevalent in younger adults, elderly patients exhibited higher incidences of *L. monocytogenes, A.*

baumannii, and VZV infections, reflecting age-related immune decline and epidemiological trends. Immunocompromised patients, particularly those with HIV, were disproportionately affected by pathogens like *C. neoformans* and *M. tuberculosis*, reinforcing the need for targeted preventive and therapeutic measures in these vulnerable populations [26].

The role of dual infections, which were associated with worse outcomes, is particularly notable. These findings suggest that dual infections may be an underrecognized contributor to CNS infection severity and should be routinely considered in clinical evaluations, especially in immunocompromised individuals. The higher prevalence of *M. tuberculosis* and VZV in immunocompromised patients further underscores the vulnerability of these individuals to severe CNS infections, particularly in the context of limited healthcare resources. Our findings emphasize the need for targeted strategies to prevent and treat CNS infections in this high-risk group, including vaccination campaigns and early diagnostic testing for high-risk pathogens. In addition, our study found that the absence of adequate empirical therapy in patients with CNS infections is an important predictor of mortality. The determination that inadequate empirical treatment is a strong independent risk factor for death in patients with CNS infections has already been described [23].

Although our findings provide valuable insights, several limitations must be acknowledged. The first limitation of our study is the standard 5-day incubation period used for blood culture diagnostics, which may be insufficient for detecting fastidious or slow-growing organisms, such as *Burkholderia pseudomallei* [27,28]. Second, the relatively modest sample size and reliance on physician documentation for infection classification may limit generalizability and introduce misclassification bias, particularly in cases where community-onset infections, such as tuberculosis, were diagnosed later during hospitalization. Third, although we employed a combination of conventional and molecular diagnostic methods, the availability and implementation of these assays varied across participating hospitals due to infrastructure constraints and disparities in access to advanced diagnostics.

Conclusion

This study underscores the critical influence of specific causative pathogens on clinical outcomes in patients with CNS infections. Notably, *Mycobacterium tuberculosis, Klebsiella pneumoniae, Acinetobacter baumannii*, and HSV-1 were disproportionately associated with unfavourable outcomes. Moreover, clinical indicators such as delayed hospital admission and altered mental status at presentation were identified as strong predictors of poor prognosis, reinforcing their relevance in early risk stratification and clinical decision-making.

CNS infections continue to be a burden in LMICs, where timely diagnosis and effective treatment are often constrained by limited infrastructural capacities. Our findings advocate for the strengthening of diagnostic infrastructure in these settings specifically, the broader implementation of multiplex molecular panels and non-targeted approaches such as 16S rRNA ONT sequencing to improve pathogen detection beyond conventional methods. Future efforts must also address the development of tailored clinical management strategies in such settings. Equitable access to advanced diagnostics and targeted interventions is essential to mitigating the burden of CNS infections and improving patient outcomes.

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Ethical approval statement

This study adhered to strict ethical guidelines to ensure participant safety and integrity. Informed written consent was obtained from all hospitalized patients and/or their relatives after a detailed explanation of the study procedures. For subjects under 18 years of age, consent was obtained from their parents or legal guardians. The study protocol was reviewed and approved by the institutional review board of the 108 Military Central Hospital, Hanoi, Vietnam (Protocol No. 108MCH/RES/MENTNGITIS-V-D3-25042017). All procedures were conducted in compliance with ICH-GCP/GCLP guidelines and applicable regulations.

Author contributions

TPV and LHS designed the study. TPV conceptualized, supervised the study and contributed to the study materials and assays. DVD performed the experimental procedures. DVD, SP and DN were involved in the analysis and validation of the results. DVD, VVS, NXH, NVH, NTKL, BTS, NTT, LHS, HXQ, TTL and VDT collected the patient samples, clinical data and contributed to the investigation materials for sampling procedures. DVD wrote the first draft. TPV contributed to the first draft. DN, PGK, and SP reviewed the first draft. All authors have read and approved the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Thirumalaisamy P Velavan reports article publishing charges was provided by German Academic Exchange Service. Dennis Nurjadi reports financial support was provided by Shionogi and Co Ltd. Dennis Nurjadi reports financial support was provided by Cephied. The co-author Dennis Nurjadi declares that he received speakers honoraria from Shionogi and Cepheid, which outside the scope of this article. All other authors reported no conflict of interest. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2025.108002.

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Supplementary Table S1: Case definition for bacterial meningitis (BM), tuberculous meningitis (TM), viral encephalitis/meningitis (VEM), and fungal meningitis (FM)

<table>
<tr><th colspan="2">Bacterial meningitis (BM) (modified case definition of BM of WHO) (1)</th></tr>
<tr><td>Laboratory-confirmed</td><td>Probable</td></tr>
<tr><td>A suspected or probable case with microbiological confirmation via: Positive culture, Gram stain or PCR of CSF sample.</td><td>• Sudden onset of fever
• AND at least one of the following features:
✓ Neck stiffness
✓ Altered mental status
• AND CSF routine testing showing at least one of the following:
✓ Pleocytosis (≥5 cells/mm^3) AND at least one of the following criteria:
o Protein > 1g/L
o Glucose < 2.2 mmol/L or less than 50% of blood glucose
✓ Turbid appearance (when CSF leukocyte count is missing or cell count <5/mm^3)</td></tr>
<tr><th colspan="2">Viral encephalitis/meningitis (VEM) (modified case definition of acute encephalitis syndrome of WHO) (1)</th></tr>
<tr><td>Laboratory-confirmed</td><td>Probable</td></tr>
<tr><td>A suspected or probable case with microbiological confirmation via: Positive PCR in CSF sample.</td><td>• Acute onset of fever
• AND at least one of the following features:
✓ Neck stiffness
✓ Altered mental status
✓ New onset of seizures
• AND CSF routine testing showing at least one of the following:
✓ Pleocytosis (≥5 cells/mm^3) AND at least one of the following criteria:
o Protein ≤ 1g/L
o Glucose ≥ 2.2 mmol/L or more than 50% of blood glucose
✓ Clear appearance (when CSF leukocyte count is missing or cell count <5/mm^3)</td></tr>
<tr><th colspan="2">Tuberculous meningitis (TM) (2)</th></tr>
<tr><td>Laboratory-confirmed</td><td>Probable</td></tr>
<tr><td rowspan="2">A possible or probable case with microbiological confirmation via: Positive smear or PCR of CSF sample.</td><td>• Total diagnostics score ≥ 10 (when cerebral imaging is not available) or ≥ 12 (when cerebral imaging is available)
• At least 2 points should either come from CSF or cerebral imaging criteria</td></tr>
<tr><td>Possible</td></tr>
</table>

	• Total diagnostics score of 6-9 points (when cerebral imaging is not available) or 6-11 (when cerebral imaging is available) • Possible tuberculosis cannot be diagnosed or excluded without doing lumbar puncture or cerebral imaging
Fungal meningitis (FM) (3, 4)	
Laboratory-confirmed	
A suspected case with an India ink stain of CSF positive showing encapsulated yeasts, or positive culture or PCR of CSF sample	

Bacterial meningitis (BM); tuberculous meningitis (TM); viral encephalitis/meningitis (VEM); fungal meningitis (FM); WHO, World Health Organization; PCR, Polymerase chain reaction; CSF, Cerebrospinal fluid.

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Supplementary Table S2: Standard CSF diagnostics amd specific molecular testing for diagnosing central nervous system infections in four Vietnamese hospitals.

No.	Hospital	Routine diagnostics	Note
1	108 MCH	Bacteria: CSF Gram stain, CSF culture, blood culture, CSF real-time PCR, PCR-based assays and GeneXpert.	Panel real-time PCR for detection of bacteria causing meningitis: *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Streptococcus agalactiae*, *Haemophilus influenzae*, *Listeria monocytogenes*, *Escherichia coli K1*.
			PCR-based assays: *Streptococcus suis, Streptococcus spp, Enterococcus spp, Staphylococcus spp, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Mycobacterium tuberculosis.*
			GeneXpert: *M.tuberculosis.*
		Viruses: CSF real-time PCR, PCR-based assays	Panel real-time PCR for detection of viruses: Herpes simplex virus 1/2, Cytomegalovirus, Epstein–Barr virus, Varicella zoster virus, Human herpes virus 6 and Human herpes virus 7.
			PCR-based assays: Japanese encephalitis virus, Dengue virus, Enterovirus.
		Fungi: CSF smear, fungal CSF culture	
2	NHTD	Bacteria: CSF Gram stain, CSF culture, blood culture, CSF PCR-based assays and GeneXpert.	PCR-based assays: *M.tuberculosis*
			GeneXpert: *M.tuberculosis*
		Viruses: CSF PCR-based assays	PCR-based assays: Herpes simplex virus 1/2, *S.suis*
		Fungi: CSF smear, fungal culture	
3	VT	Bacteria: CSF Gram stain, CSF culture, blood culture	
		Fungi: CSF smear, fungal CSF culture	
4	103 MH	Bacteria: CSF Gram stain, CSF culture, CSF PCR-based assays and GeneXpert, blood culture	PCR-based assays: *N. meningitidis, M.tuberculosis*
			GeneXpert: *M.tuberculosis*
		Viruses: CSF PCR-based assays	PCR-based assays: Cytomegalovirus, Epstein–Barr virus
		Fungi: CSF smear, fungal CSF culture	

Abbreviations: CSF, Cerebrospinal fluid; PCR, Polymerase chain reaction; 108 MCH, 108 Military Central Hospital; 103 MH, 103 Military Hospital; NHTD, National Hospital for Tropical Diseases; VT, Viet Tiep Friendship Hospital.

Supplementary Table S3: Primers and probes utilized in this study

No	Primers and Probes	Cycling conditions	Reference
1	***S. suis*** **(Real time-PCR)**	95°C, 15 min., 40 x (94°C, 10 sec., 60°C, 60 sec.)	(1)
	S. suis F: GGTTACTTGCTACTTTTGATGGAAATT		
	S. suis R: CGCACCTCTTTTATCTCTTCCAA		
	S. suis probe: FAM-TCAAGAATCTGAGCTGCAAAAGTGTCAAATTGA-TAMRA		
2	***M. tuberculosis*** **(Real time-PCR)**	95°C, 15 min., 40 x (94°C, 10 sec., 60°C, 60 sec.)	(2)
	MTB-F: GCCGGATCAGCGATCGT		
	MTB-R: GCAAAGTGTGGCTAACCCTGAA		
	MTB-P: FAM-TTCGACGGTGCATCT-MGB		
3	***S. aureus*** **(PCR)**	1 x 94°C, 3min., 35 x (94°C,30sec.; 50°C,30sec.; 72°C,60 sec.), 72°C, 10 min.; 4°C	(3)
	SA442 F1: GTCGGGTACACGATATTCTTCACG		
	SA442 R1: CTCTCGTATGACCAGCTTCGGTAC		
4	***K. pneumoniae*** **(PCR)**	1 x 94°C, 3min., 40 x (94°C,30sec.; 57°C,30sec.; 72°C,60 sec.), 72°C, 10 min.; 4°C	(4)
	K. pneu. F: ATTTGAAGAGGTTGCAAACGAT		
	K. pneu. R: TTCACTCTGAAGTTTTCTTGTGTTC		
5	***A. baumannii*** **(Real time - PCR)**	95°C, 15 min., 40 x (94°C, 10 sec., 60°C, 60 sec.)	(5)
	OmpA F: TCTTGGTGGTCACTTGAAGC		
	OmpA R: ACTCTTGTGGTTGTGGAGCA		
	probe: FAM-AAGTTGCTCCAGTTGAACCAACTCCA-TAMRA		

6	**Epstein-Barr virus (Realtime - PCR)**	95°C, 15 min., 40 x (94°C, 10 sec., 60°C, 60 sec.)	(6)
	TS-EBV-Fwd1: CCCAACACTCCACCACACC		
	TS-EBV-Rev1: TCTTAGGAGCTGTCCGAGGG		
	TS-EBV-Pro1: FAM-CACACACTACACACACCCACCCGTCTC-Iowa Black FQ		

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Supplementary Table S4: Pathogen Identification and Empirical Therapy Adequacy Based on Routine and Retrospective Diagnostic Modalities

No.	Pathogen identified	Routine diagnostics (Hospital)	FAME (Retrospective)	Targeted PCR (Retrospective)	Empirical Therapy at Admission	Therapy Continued Based on Routine Diagnostics	Correct Empirical Therapy Administered
1	Varicella zoster virus	Positive	Negative	NA	Acyclovir	Acyclovir, Dexamethasone	Yes
2	*Streptococcus sp.*	Positive	Negative	NA	Ceftriaxone, Ciprofloxacin, Dexamethasone	Ceftriaxone, Linezolid, Dexamethasone	Yes
3	Epstein Barr virus	Positive	Negative	NA	Meropenem, Amikacin, Dexamethasone	Meropenem, Amikacin, Dexamethasone	**No**
4	*Neisseria meningitidis*	Positive	Positive	NA	Meropenem, Ciprofloxacin, Dexamethasone	Ceftriaxone, Ciprofloxacin	Yes
5	*Mycobacterium tuberculosis*	Positive	Negative	NA	Ticarcillin/Acid clavulanic	RHZE, Dexamethasone	**No**
6	Varicella zoster virus	Positive	Positive	NA	Acyclovir	Acyclovir, Dexamethasone	Yes
7	*Mycobacterium tuberculosis*/Cytomegalovirus	Positive	Positive	NA	Meropenem, Vancomycin	RHZE, Methylprednisolone	**No**
8	*Mycobacterium tuberculosis*	Negative	Negative	Positive	Meropenem, Vancomycin	Meropenem, Vancomycin	**No**
9	Herpes simplex virus - 1	Positive	Positive	NA	Cefotaxime, Acyclovir, Dexamethasone	Acyclovir, Dexamethasone	Yes
10	*Streptococcus pneumoniae*	Positive	Positive	NA	Ceftriaxone, Amikacin, Dexamethasone	Meropenem, Amikacin, Dexamethasone	Yes
11	*Mycobacterium tuberculosis*	Positive	Negative	NA	Ceftriaxone, Dexamethasone	RHZE, Dexamethasone	**No**
12	*Streptococcus suis*	Positive	Negative	NA	Ceftriaxone, Tobramycin, Dexamethasone	Ceftriaxone, Tobramycin, Dexamethasone	Yes
13	*Acinetobacter baumannii*	Negative	Negative	Positive	Cefotaxime, Dexamethasone	Cefotaxime, Dexamethasone	**No**
14	Varicella zoster virus	Positive	Positive	NA	Acyclovir, Ceftriaxone, Dexamethasone	Acyclovir, Dexamethasone	Yes
15	Epstein Barr virus	Positive	Negative	NA	Meropenem, Amikacin, Dexamethasone	Meropenem, Amikacin, Dexamethasone	**No**
16	*Klebsiella pneumoniae*	Positive	Negative	NA	Ceftriaxone, Dexamethasone	Meropenem, Colistin, Dexamethasone	**No**

17	Herpes simplex virus - 1	Positive	Negative	NA	Ceftriaxone, Acyclovir, Dexamethasone	Ceftriaxone, Acyclovir, Dexamethasone	Yes
18	Epstein Barr virus	Positive	Negative	NA	Ceftriaxone, Ciprofloxacin	Ceftriaxone, Ciprofloxacin, Dexamethasone	**No**
19	*Streptococcus suis*	Positive	Negative	NA	Meropenem, Amikacin, Methylprednisolone	Meropenem, Amikacin, Methylprednisolone	Yes
20	*Klebsiella pneumoniae*	Positive	Negative	NA	Meropenem, Amikacin	Meropenem, Amikacin	Yes
21	*Trichosporon asahii*	Positive	Negative	NA	Meropenem, Dexamethasone	Voriconazole, Dexamethasone	**No**
22	*Cryptococcus neoformans*	Positive	Positive	NA	Meropenem, Vancomycin	Amphotericin B, Fluconazole	**No**
23	*Cryptococcus neoformans*	Positive	Negative	NA	Ceftriaxone	Amphotericin B	**No**
24	*Mycobacterium tuberculosis*	Negative	Negative	Positive	Ceftriaxone, Dexamethasone, Albendazole	Albendazole, Dexamethasone	**No**
25	Herpes simplex virus - 1	Positive	Negative	NA	Meropenem, Vancomycin, Acyclovir	Acyclovir	Yes
26	Herpes simplex virus - 1	Positive	Negative	NA	Ceftriaxone, Acyclovir, Dexamethasone	Acyclovir, Dexamethasone	Yes
27	*Acinetobacter baumannii*	Negative	Negative	Positive	Meropenem, Vancomycin, Fluconazole	Meropenem, Vancomycin, Fluconazole	Yes
28	*Klebsiella pneumoniae*	Negative	Negative	Positive	Meropenem, Colistin, Dexamethasone	Meropenem, Colistin	Yes
29	*Escherichia coli*	Positive	Negative	NA	Meropenem, Linezolid, Levofloxacin	Meropenem, Linezolid, Levofloxacin	Yes
30	*Klebsiella pneumoniae*	Positive	Negative	NA	Meropenem, Linezolid	Meropenem, Dexamethasone	Yes
31	*Mycobacterium tuberculosis*	Positive	Negative	NA	Meropenem, Moxifloxacin	Meropenem, Moxifloxacin, RHZ, Dexamethasone	**No**
32	*Mycobacterium tuberculosis*	Positive	Negative	NA	Ceftriaxone, Levofloxacin, RHZ, Dexamethasone	RHZ, Levofloxacin, Dexamethasone	Yes
33	Varicella zoster virus	Negative	Positive	NA	Acyclovir	Acyclovir, Vancomycin	Yes
34	*Streptococcus pneumoniae*	Positive	Positive	NA	Ceftriaxone, Dexamethasone	Ceftriaxone, Dexamethasone, Vancomycin	Yes
35	*Cryptococcus neoformans*	Positive	Negative	NA	Amphotericin B, Fluconazole, Streptomycin, RHZ, Moxifloxacin	Amphotericin B, Streptomycin, RHZ, Moxifloxacin, Fluconazole	Yes

36	*Acinetobacter baumannii*	Positive	Negative	NA	Meropenem, Linezolid, Dexamethasone	Meropenem, Colistin	Yes
37	Varicella zoster virus	Negative	Positive	NA	Meropenem, Acyclovir, Linezolid	Acyclovir, Linezolid	Yes
38	Epstein Barr virus	Negative	Negative	Positive	Meropenem	Meropenem	**No**
39	*Klebsiella pneumoniae*/Herpes simplex virus 1	Positive	Positive	NA	Ceftriaxone, Levofloxacin, Metronidazole, Dexamethasone	Meropenem, Amikacin	**No**
40	*Mycobacterium tuberculosis*	Positive	Negative	NA	Levofloxacin, Linezolid, ZE	Levofloxacin, Linezolid, ZE	Yes
41	*Klebsiella pneumoniae*	Positive	Negative	NA	Ceftriaxone, Dexamethasone	Meropenem, Amikacin	**No**
42	*Acinetobacter baumannii*/Cytomegalovirus	Positive	Positive	NA	Meropenem, Vancomycin, Colistin	Meropenem, Vancomycin, Colistin	Yes
43	*Streptococcus pneumoniae*	Positive	Positive	NA	Ceftriaxone, Dexamethasone	Meropenem	Yes
44	Human immunodeficiency virus	Positive	Negative	NA	SMX-TMP, Clindamycin, Fluconazole, Dexamethasone	SMX-TMP, Fluconazole, Methylprednisolone	**No**
45	Varicella zoster virus	Negative	Positive	NA	Ceftriaxone	RHZ, Moxifloxacin	**No**
46	*Mycobacterium tuberculosis*	NA	Negative	Positive	Meropenem, Vancomycin	Meropenem, Fosfomycin, Colistin	**No**
47	*Listeria monocytogenes*	Negative	Positive	NA	Meropenem, Acyclovir, Ampicillin	Meropenem, Ampicillin	Yes
48	*Listeria monocytogenes*	Negative	Positive	NA	Meropenem, Ampicillin	Meropenem, Ampicillin	Yes
49	Herpes simplex virus - 1	Positive	Positive	NA	Acyclovir	Acyclovir	Yes
50	Varicella zoster virus	Negative	Positive	NA	RHZ, Streptomycin, Dexamethasone	RHZ, Streptomycin, Dexamethasone	**No**
51	*Mycobacterium tuberculosis*	Positive	Negative	NA	Vancomycin, Meropenem, Dexamethasone	Meropenem, Linezolid, Levofloxacin, RHZ, Dexamethasone	**No**
52	*Streptococcus suis*	Positive	Negative	NA	Ceftriaxone, Ampicillin, Dexamethasone	Ceftriaxone, Dexamethasone	Yes
53	*Listeria monocytogenes*	Positive	Positive	NA	Meropenem, Vancomycin	Meropenem, Vancomycin, Ampicillin	Yes
54	Herpes simplex virus - 1	Positive	Positive	NA	Acyclovir, Meropenem, Dexamethasone	Acyclovir, Dexamethasone	Yes

55	*Listeria monocytogenes*	Negative	Positive	NA	Meropenem, Linezolid	Meropenem, Linezolid	Yes
56	Herpes simplex virus - 1	Positive	Positive	NA	Ceftriaxone, Acyclovir, Dexamethasone	Ceftriaxone, Acyclovir, Dexamethasone	Yes
57	*Mycobacterium tuberculosis*	Positive	Negative	NA	Ceftriaxone, RHZ, Streptomycin, Dexamethasone	RHZ, Streptomycin, Dexamethasone	Yes
58	*Acinetobacter baumannii*	Negative	Negative	Positive	Meropenem, Colistin	Meropenem, Colistin	Yes
59	*Klebsiella pneumoniae*	Negative	Negative	Positive	Ceftriaxone	Ceftriaxone	**No**
60	*Streptococcus suis*	Positive	Negative	NA	Ceftriaxone, Dexamethasone	Ceftriaxone, Dexamethasone	Yes
61	*Klebsiella pneumoniae*/Cytomegalovirus	Positive	Positive	NA	Meropenem, Colistin	Meropenem, Colistin	Yes
62	*Streptococcus suis*	Negative	Negative	Positive	Meropenem, Dexamethasone	Ceftriaxone, Dexamethasone	Yes
63	*Streptococcus pneumoniae*	Negative	Positive	NA	Ceftriaxone, Dexamethasone	Ceftriaxone, Dexamethasone	Yes
64	*Haemophilus influenzae*	Negative	Positive	NA	No specific treatment	No specific treatment	NA
65	*Haemophilus influenzae* + *Streptococcus agalactiae*	Negative	Positive	NA	Ceftriaxone, Acyclovir, Dexamethasone	Ceftriaxone, Acyclovir, Dexamethasone	Yes
66	*Streptococcus pneumoniae*	Negative	Positive	NA	Ceftriaxone, Ciprofloxacin, Dexamethasone	Ceftriaxone, Ciprofloxacin, Dexamethasone	Yes
67	Varicella zoster virus	NA	Positive	NA	Acyclovir	Acyclovir	Yes
68	*Acinetobacter baumannii*	Negative	Negative	Positive	Levofloxacin	Levofloxacin	**No**
69	*Haemophilus influenzae*	Negative	Positive	NA	Ceftriaxone	Ceftriaxone	Yes
70	*Mycobacterium tuberculosis*	NA	Negative	Positive	Ceftriaxone	Ceftriaxone	**No**
71	*Klebsiella pneumoniae*	Negative	Negative	Positive	Meropenem, Ampicillin	Meropenem, Ampicillin	Yes
72	Varicella zoster virus	NA	Positive	NA	Ceftriaxone	Ceftriaxone	**No**
73	Enterovirus	NA	Positive	NA	Ceftriaxone	Ceftriaxone	**No**
74	*Streptococcus pneumoniae*	Positive	Positive	NA	Ceftriaxone, Levofloxacin, Methylprednisolone	Ceftriaxone, Levofloxacin, Methylprednisolone	Yes

75	*Haemophilus influenzae*	Negative	Positive	NA	Meropenem, Levofloxacin	Meropenem, Levofloxacin	Yes
76	*Klebsiella pneumoniae*	Positive	Negative	NA	Cefotaxime	Meropenem	**No**
77	*Streptococcus suis*	Positive	Negative	NA	Ceftriaxone, Ciprofloxacin, Methylprednisolone	Ceftriaxone, Ciprofloxacin, Methylprednisolone	Yes
78	Epstein Barr virus	NA	Negative	Positive	Ceftriaxone	Meropenem	**No**
79	*Mycobacterium tuberculosis*	NA	Negative	Positive	Ceftriaxone, Ciprofloxacin, Methylprednisolone	Meropenem, Ciprofloxacin, Methylprednisolone	**No**
80	*Acinetobacter baumannii*	Negative	Negative	Positive	Ceftriaxone, Fosfomycin, Ciprofloxacin, Acyclovir, Methylprednisolone	Ceftriaxone, Fosfomycin, Ciprofloxacin, Acyclovir, Methylprednisolone	**No**

RHZ: Rifampicin- Isoniazid- Pyrazinamide; RHZE: Rifampicin- Isoniazid- Pyrazinamide -Ethambutol; NA: Diagnostics not available due to limited capacities.

III. DISCUSSION

CNS infections represent a significant public health burden worldwide and are associated with high morbidity and mortality. Conventional tests, such as CSF culture, remain the gold standard in the diagnosis of bacterial CNS infections. However, their sensitivity may be reduced due to the prior administration of antibiotics before admission, and it takes several days for results to be available. CNS infections are medical emergencies, so prompt diagnosis and appropriate treatment play a crucial role in improving clinical outcomes and avoiding complications.

Chapter 1: Optimizing Diagnostic Strategies for Central Nervous System Infections in Vietnam: Evaluation of the BioFire FilmArray Meningitis/Encephalitis Panel

CNS infections, such as meningitis and encephalitis, can progress rapidly and require prompt diagnosis for effective treatment. The BioFire FAME panel is a molecular test designed to detect a broad range of common bacterial, viral, and fungal pathogens using just a small amount of CSF. It delivers results in about an hour, which makes it much faster than traditional culture-based methods (Dien Bard & Alby 2018; Leber et al. 2016). Despite its increasing global use, data on FAME's performance in LMICs in Asia is scarce. To date, only one study from Myanmar has explored its diagnostic value in such settings (Galardi et al. 2023), while most published evaluations have been conducted in high-resource countries such as Taiwan (Lee et al. 2019) and South Korea (Roh et al. 2020). This study, forming the first part of my thesis, aimed to evaluate the clinical utility and performance of FAME in the Vietnamese hospital context, thereby addressing a critical knowledge gap.

In our study, FAME showed an overall agreement rate of 87% with conventional diagnostic methods (culture and targeted PCR) and a positivity rate of 10%, aligning well with findings from previous research (Leber et al. 2016; Naccache et al. 2018; Tarai & Das 2019). Importantly, FAME demonstrated utility in detecting pathogens in patients who had already received antibiotics before lumbar puncture, a scenario in which culture often fails (Du et al. 2019). In our dataset, nine bacterial infections, including *L.*

monocytogenes, *H. influenzae*, and *S. pneumoniae* were identified in culture-negative CSF samples, with several cases occurring in pre-treated patients.

While these results are promising, FAME also showed both false-negative and false-positive findings. False-negative results were observed for pathogens such as *E. coli* (n=1), HSV-1 (n=3), VZV (n=1), and *C. neoformans* (n=2). These discrepancies could be attributed to technical limitations or pathogen-specific detection challenges. For instance, the FAME panel detects only the K1 strain of *E. coli*, which is commonly associated with neonatal meningitis and represents about 80% of such cases (Kaper et al. 2004). This selective detection was designed to minimize interference from non-pathogenic *E. coli* DNA, a known issue in PCR reagents (Hughes et al. 1994). Six false-positive results were identified, involving CMV (n=3), HSV-1 (n=1), and *H. influenzae* (n=2). In cases where CMV and HSV-1 were detected, co-infection with bacterial pathogens was also observed. For example., CMV with *M. tuberculosis* or *Klebsiella pneumoniae*, which may have better explained the clinical presentation. Given that herpesviruses can persist latently and reactivate without symptoms, interpreting their detection in CSF requires clinical correlation (Sunnerhagen et al. 2024), (Zanella et al. 2021). The *H. influenzae* false positives, which occurred in CSF samples with no concordant discharge diagnosis, are consistent with reports from other settings. A Swiss study found that 78% of FAME-positive *H. influenzae* results were likely false positives (Zanella et al. 2021).

FAME's rapid turnaround time offers a key advantage, as it facilitates earlier adjustment of empirical antimicrobial therapy, which supports antimicrobial stewardship efforts and may help reduce antibiotic resistance, hospital stay, and treatment costs utilization by 42.7% and increase diagnostic yield by 61.8% by applying specific criteria (Broadhurst et al. 2020; Ventola 2015). By targeting pathogens early, clinicians can avoid unnecessary use of broad-spectrum antibiotics, a major contributor to antimicrobial resistance.

Another important insight from our findings is the limited applicability of FAME in regions where key local pathogens are not included in the panel. In Vietnam, *M. tuberculosis* and *S. suis* are leading causes of CNS infections but are not covered by FAME (Nguyen et al. 2020; Wertheim et al. 2009) . Tuberculous meningitis is particularly

prevalent due to Vietnam's high tuberculosis burden, while *S. suis* is frequently observed in adults with occupational exposure to pigs. Furthermore, the panel does not detect multidrug-resistant ESKAPE pathogens, which are often responsible for healthcare-associated CNS infections (Tunkel et al. 2017).

Overall, our findings underscore the importance of tailoring molecular diagnostic tests such as FAME to the types of infections that are common in each region. Although FAME is a rapid and sensitive tool for detecting many causes of CNS infections, it does not cover all important pathogens, especially those that are more common in LMICs such as Vietnam. It is therefore important to continue using traditional laboratory methods such as culture and microscopy alongside molecular tests. To achieve the best results in patient care, these advanced tools should be adapted to local disease patterns and used carefully, considering both the test results and the clinical situation.

Chapter 2: Enhancing Diagnostic Yield Using 16S Oxford Nanopore Sequencing for CNS Infections in Resource-Limited Settings

Although CSF culture remains the traditional gold standard for diagnosing bacterial meningitis, one major limitation is the time it takes to get results, which is often between 48 to 72 hours after the sample is collected. This delay can affect patient care. While awaiting culture results, clinicians may administer broad-spectrum antibiotics, which can lead to overtreatment. On the other hand, if a specific pathogen is missed, needed treatment may be delayed. These delays can also impact individuals who are in close contact with the patient, as timely diagnosis is essential for deciding whether they need preventive treatment (Leber et al. 2016).

Recent advances, such as ONT, have provided new ways to detect a wide range of bacteria faster and at a lower cost. One of the most promising tools is 16S rRNA gene sequencing, which targets a genetic marker found in nearly all bacteria. Because this gene is highly conserved, it enables identification of many bacterial species, including those that are difficult to grow in culture (Pallerla et al. 2022). The ONT platform makes this process even faster, with simple and rapid sample preparation and the potential to deliver results

within hours (Miten Jain et al. 2016). In our current study, we explored how well this sequencing method performs compared to standard CSF cultures, with the goal of improving diagnostic accuracy, speeding up treatment decisions, and ultimately improving patient outcomes.

Unlike broad genome sequencing approaches, 16S rRNA amplicon sequencing is more targeted, showing higher sensitivity and fewer errors in detecting bacteria (Tessler et al. 2017). Previous research has already demonstrated that 16S ONT can match the performance of culture in diagnosing bacterial meningitis, with the added benefit of significantly faster results, even when using very small sample volumes (Pallerla et al. 2022). Building on our earlier work, we conducted this study to further evaluate its clinical benefit in the Vietnamese hospital setting.

Our findings showed that 16S ONT sequencing identified more bacterial infections than traditional CSF culture. Notably, 17 pathogens were detected in samples that were negative by culture, including common causes of meningitis such as *S. suis*, *Acinetobacter baumannii*, and *N. meningitidis*. Additionally, the sequencing approach identified five pathogens that were missed entirely by culture *Escherichia marmotae*, *Staphylococcus epidermidis*, *Streptococcus oralis*, *Klebsiella aerogenes*, and *Citrobacter freundii*. Many of these cases had a history of prior antibiotic use, which likely suppressed bacterial growth in culture. A similar pattern was reported that demonstrated that 16S ONT detected pathogens in all seven CSF samples from patients with suspected CNS infections in Vietnam, while routine tests like Gram stain and culture failed to detect bacteria in nearly half the cases (Hong et al. 2020). In Vietnam, *S. suis* is a particularly important pathogen, often associated with occupational exposure to pigs or consumption of undercooked pork (Nghia et al. 2011; Wertheim et al. 2009). Alongside *S. pneumoniae* and *N. meningitidis*, it is among the leading causes of bacterial meningitis in the region. Thus, sequencing-based detection is valuable not only for individual diagnosis but also for public health surveillance and guiding vaccine strategies.

In our study, 16S ONT failed to detect some pathogens, including *K. pneumoniae, A. baumannii, E. coli, L. monocytogenes,* and *S. pneumoniae*. These false negatives may be

due to low bacterial counts in the CSF or inefficiencies in DNA extraction. Improving extraction protocols may help recover more bacterial DNA from such low-yield samples.

Perhaps most importantly, 61% of patients with confirmed 16S ONT-positive results were initially given inappropriate antibiotics. If 16S ONT sequencing results had been available earlier, antibiotic treatment could have been optimized in 18 patients: 11 could have received narrower, more targeted therapy; 5 would have required escalation; and 2 would have needed a switch in treatment altogether. This finding is especially relevant in the context of rising antimicrobial resistance, which is worsened by the unnecessary or inappropriate use of antibiotics (Ahmed et al. 2024).

Current clinical guidelines recommend starting empirical antibiotics and corticosteroids immediately when bacterial meningitis is suspected (Tunkel et al. 2004). However, if culture results remain negative, clinicians may struggle with the decision to continue, escalate, or de-escalate treatment, especially in complex cases (Durand et al. 1993). This uncertainty contributes to overtreatment, and this can enhance the development of antimicrobial resistance. By identifying the causative bacteria quickly, even in culture-negative cases, 16S ONT provides crucial information to help resolve this clinical dilemma.

In conclusion, our study supports the use of 16S ONT sequencing as a complementary tool to traditional culture methods in the diagnosis of CNS infections. It improves pathogen detection, especially in patients who have already received antibiotics, and provides valuable data to guide antibiotic treatment decisions.

Chapter 3: Etiological Spectrum and Clinical Predictors of Unfavourable Outcomes in Central Nervous System Infections in Vietnam

According to a recent review of 12 epidemiological studies, the causative pathogen in CNS infections remains unidentified in up to two-thirds of cases (Brindle et al. 2024). This diagnostic uncertainty contributes to poor outcomes: mortality remains high, and

about 30% of survivors experience long-term neurological complications (Ho Dang Trung et al. 2012; Taylor et al. 2012).

Typical symptoms such as fever, headache, neck stiffness, and confusion are common, but not specific enough to distinguish between different CNS syndromes like meningitis, encephalitis, or meningoencephalitis. In our cohort, these symptoms occurred in 83%, 67%, 63%, and 43% of patients, respectively. As shown in Chapter 1, while rapid tests such as the BioFire FAME panel can be helpful, their pathogen coverage is limited. This means key pathogens, especially those common in Vietnam, can go undetected, leading to delayed or inappropriate treatment, which directly affects survival and recovery. This chapter therefore focuses on the clinical impact of different causative pathogens and host-related factors on patient outcomes. Our goal was to identify predictors of poor prognosis to improve clinical decision-making and patient care.

In this study, 57% of patients had an unfavourable outcome and the mortality rate was 10%. The most common pathogens identified were *M. tuberculosis*, followed by VZV, *K. pneumoniae*, HSV-1, *S. suis*, *S. pneumoniae*, and *A. baumannii*. Additionally, we identified five dual infections, including combinations such as *A. baumannii* + CMV and *M. tuberculosis* + CMV. Fungal meningitis had the highest mortality rate at 40%, followed by dual infections (20%), tuberculous meningitis (17%), and bacterial meningitis (6%). These findings align with previous research, which shows that fungal and tuberculous infections tend to have poorer outcomes due to their nonspecific presentation, diagnostic complexity, and fewer treatment options (Akaishi et al. 2024). Dual infections are often underdiagnosed and also appear to significantly contribute to disease severity, particularly in immunocompromised patients (Fang et al. 2024). Deaths in our cohort were primarily associated with high-risk pathogens including *M. tuberculosis, S. pneumoniae, C. neoformans, Trichosporon asahii, A. baumannii,* and one dual infection with *M. tuberculosis* and CMV. Supporting these findings another study equally showed that tuberculous meningitis was linked to a nearly sixfold increase in mortality risk (Asare-Baah et al. 2023).

Through multivariate analysis, we identified two significant predictors of poor outcomes: symptom duration of five days or more before hospital admission and altered mental status at presentation. These findings mirror earlier studies, which emphasized that delayed admission leads to delayed treatment, resulting in higher mortality and neurological complications (Bodilsen et al. 2018; Franck Raschilas et al. 2002). Altered consciousness is frequently linked to more severe neurological injury and has repeatedly been associated with unfavourable outcomes (Ter Horst et al. 2023; van de Beek et al. 2004). Contrary to some previous studies, our analysis did not find advanced age or comorbidities to be significant predictors of poor outcomes (Mailles & Stahl 2009; van de Beek et al. 2004). This may be due to the heterogeneity of diagnoses in our cohort. Notably, some variables shifted direction in odds ratios between univariate and multivariate analysis, possibly due to interaction effects or collinearity though this remains speculative.

Conclusion

This thesis addressed critical diagnostic and clinical challenges associated with CNS infections in resource-limited Vietnamese settings. Drawing on three interlinked studies, it demonstrated the limitations of conventional diagnostics, the added value of rapid molecular assays such as the BioFire FAME panel, and the enhanced pathogen detection capacity of 16S ONT sequencing. These diagnostic innovations improved the identification of causative agents, particularly in culture-negative cases, and provided actionable data to support antimicrobial stewardship. Furthermore, by evaluating pathogen-specific outcomes and identifying key clinical predictors such as delayed hospital presentation and altered mental status, this work offers important guidance for early risk stratification and therapeutic decision-making. Collectively, the findings advocate for the integration of tailored molecular diagnostics with conventional methods, region-specific pathogen panels, and strengthened clinical surveillance to improve patient outcomes. These insights contribute to a broader framework for optimizing the management of CNS infections in LMICs.

IV. SUMMARY

This doctoral thesis focuses on improving the diagnosis and management of central nervous system (CNS) infections in resource-limited settings, using Vietnam as a case study. CNS infections are often life-threatening, and their diagnosis is challenging due to overlapping symptoms, prior antibiotic use, and limited access to advanced diagnostics. The thesis comprises three chapters, each addressing a key aspect of this clinical problem.

Chapter 1 evaluated the BioFire FilmArray Meningitis/Encephalitis (FAME) panel, a rapid molecular test that detects 14 common pathogens in cerebrospinal fluid (CSF). The study demonstrated that FAME significantly improved diagnostic speed and yield compared to conventional methods, especially in patients who had already received antibiotics. However, it also revealed limitations such as false positives/negatives and the panel's inability to detect regionally important pathogens like *Streptococcus suis* and *Mycobacterium tuberculosis*. The findings highlight the need to adapt molecular panels to local epidemiology.

Chapter 2 explored the use of 16S Oxford Nanopore Technology (ONT) sequencing as a culture-independent diagnostic method for bacterial CNS infections. This approach successfully identified pathogens in a large number of culture-negative cases, including several rare and antibiotic-suppressed bacteria. It also demonstrated clinical impact by showing that many patients received inappropriate antibiotics initially, which could have been corrected earlier using ONT results. The chapter emphasizes ONT's potential in guiding targeted therapy and improving antimicrobial stewardship in low-resource settings.

Chapter 3 analysed the impact of specific pathogens and clinical factors on patient outcomes. The most commonly identified organisms were *Mycobacterium tuberculosis*, *Streptococcus suis*, HSV-1, and *Cryptococcus neoformans*. Fungal and tuberculous meningitis were associated with the highest mortality. Two strong predictors of poor outcome were identified: delayed hospital presentation (≥5 days of symptoms) and altered mental status at admission. These findings can help clinicians recognize high-risk patients earlier and prioritize interventions accordingly.

Together, these chapters provide actionable evidence to improve diagnostic strategies, tailor treatment, and strengthen patient care for CNS infections in settings with limited resources.

V. ZUSAMMENFASSUNG

Diese Doktorarbeit befasst sich mit der Verbesserung der Diagnostik und Behandlung von Infektionen des zentralen Nervensystems (ZNS) in ressourcenbegrenzten Umgebungen, am Beispiel Vietnams. ZNS-Infektionen verlaufen häufig lebensbedrohlich und sind aufgrund unspezifischer Symptome, vorheriger Antibiotikagabe und eingeschränkter diagnostischer Möglichkeiten schwer zu diagnostizieren. Die Arbeit gliedert sich in drei Kapitel, die jeweils einen zentralen Aspekt dieses klinischen Problems behandeln.

Kapitel 1 untersucht das BioFire FilmArray Meningitis/Enzephalitis (FAME)-Panel, einen molekularen Schnelltest zur Detektion von 14 häufigen Erregern im Liquor. Die Studie zeigte, dass FAME die Diagnostikgeschwindigkeit und -ausbeute im Vergleich zu konventionellen Methoden deutlich verbesserte, insbesondere bei vorbehandelten Patienten. Gleichzeitig wurden jedoch Limitationen aufgezeigt, etwa falsch-positive/-negative Ergebnisse und das Fehlen regional bedeutsamer Erreger wie *Streptococcus suis* und *Mycobacterium tuberculosis*. Die Ergebnisse unterstreichen die Notwendigkeit, molekulare Testpanels an lokale epidemiologische Gegebenheiten anzupassen.

Kapitel 2 befasst sich mit der Anwendung der 16S Oxford Nanopore Technology (ONT)-Sequenzierung als kulturunabhängige Methode zur Erregerdiagnostik bakterieller ZNS-Infektionen. Diese Technik konnte zahlreiche Pathogene nachweisen, insbesondere in Fällen mit negativer Kultur, und zeigte sich hilfreich bei der Identifikation seltener oder antibiotikagehemmter Bakterien. Zudem wurde ein klinischer Nutzen nachgewiesen: Viele Patienten hatten initial eine unpassende Antibiotikatherapie erhalten, die durch ONT-basierte Befunde hätte optimiert werden können. Die Studie hebt das Potenzial von ONT zur gezielten Therapie und zur Förderung eines verantwortungsvollen Antibiotikaeinsatzes hervor.

Kapitel 3 analysierte den Einfluss spezifischer Erreger und klinischer Faktoren auf den Krankheitsverlauf. Zu den häufigsten nachgewiesenen Erregern zählten *Mycobacterium tuberculosis*, *Streptococcus suis*, HSV-1 und *Cryptococcus neoformans*. Pilz- und tuberkulöse Meningitis wiesen die höchsten Sterblichkeitsraten auf. Zwei starke Prädiktoren für ungünstige Verläufe wurden identifiziert: ein verzögerter

Krankenhausaufenthalt (≥5 Tage Symptome) und eine Bewusstseinsstörung bei Aufnahme. Diese Erkenntnisse können helfen, Hochrisikopatienten frühzeitig zu erkennen und gezielt zu behandeln.

Insgesamt liefert die Arbeit praxisrelevante Erkenntnisse zur Optimierung der Diagnostik, Therapieanpassung und Patientenversorgung bei ZNS-Infektionen in Ländern mit begrenzten Ressourcen.

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VII. DECLARATION OF CONTRIBUTIONS

We hereby declare that the doctoral dissertation entitled "Integrated Diagnostic Approaches and Clinical Predictors in Central Nervous System Infections: Evidence from Resource-Limited Vietnamese Settings", submitted to the members of the PhD Board at the Faculty of Medicine, University of Tübingen, represents original work conducted by Do Van Dong and co-authors at the Institute of Tropical Medicine, University of Tübingen, under the supervision of Prof. Dr. Thirumalaisamy P. Velavan. Three publications form the backbone of this doctoral dissertation, all authored primarily by Do Van Dong.

Publication 1: Open Forum Infect Dis. 2024 Sep 13;11(9):ofae531; PMID: 39346707

Publication 2: J Infect Dis. 2025 May 26:jiaf280; PMID: 40418729

Publication 3: Int J Infect Dis. 2025 Jul 31:108002; PMID: 40752766

Do Van Dong has made substantial contributions to all three manuscripts, including study design, sampling procedures, patient recruitment, experimental implementation, data analysis, and manuscript preparation. A detailed statement of the individual contributions of all co-authors for each publication, are as follows:

Publication 1:

Dong DV, Boutin S, Sang VV, Manh ND, Hoan NX, Quang HX, Lien TT, Trang VD, The NT, Linh LTK, Schmauder K, Ueltzhöffer V, Hafza N, Hauswaldt S, Rupp J, Kremsner PG, Song LH, Nurjadi D, Peter S, Velavan TP. Optimization of the diagnosis of Central Nervous System infections in Vietnamese hospitals: Results from a retrospective multicenter study. Open Forum Infect Dis. 2024 Sep 13;11(9):ofae531. PMID: 39346707. **IF:3.8**

Do Van Dong: Methodology, Investigation, Formal analysis, Validation, Writing – Original Draft, Writing – Review & Editing. **Sébastien Boutin:** Formal analysis, Validation. **Vu Viet Sang:** Resources, Investigation. **Nguyen Dang Manh:** Resources, Investigation. **Nghiem Xuan Hoan:** Resources, Investigation. **Hoang Xuan Quang:**

Resources, Investigation. **Tran Thi Lien:** Resources, Investigation. **Van Dinh Trang:** Resources, Investigation. **Nguyen Trong The:** Resources, Investigation. **Le Thi Kieu Linh:** Resources, Investigation. **Kristina Schmauder:** Methodology, Supervision. **Viola Ueltzhöffer:** Methodology, Supervision. **Nourhane Hafza:** Writing – Review & Editing. **Susanne Hauswaldt:** Formal analysis, Validation, Writing – Review & Editing. **Jan Rupp:** Formal analysis, Validation, Writing – Review & Editing. **Peter G. Kremsner:** Writing – Review & Editing. **Le Huu Song:** Resources. **Dennis Nurjadi:** Conceptualization, Validation, Writing – Original Draft, Writing - Review & Editing. **Silke Peter:** Conceptualization, Supervision, Resources. **Thirumalaisamy P. Velavan:** Conceptualization, Supervision, Resources, Writing – Original Draft, Writing – Review & Editing, Funding acquisition.

Publication 2:

Van Dong D, Linh LTK, Nga NTT, Hoan NX, Linh NTK, Huyen TTT, Quang HX, Lien TT, Trang VD, Sang VV, Kremsner PG, Song LH, Nurjadi D, Velavan TP. Evaluating the diagnostic utility of 16S Oxford Nanopore Technology Sequencing in patients with Central Nervous System infections and its usefulness in Antimicrobial Stewardship. J Infect Dis. 2025 May 26:jiaf280. doi: 10.1093/infdis/jiaf280. PMID: 40418729. **IF: 4.5**

Do Van Dong: Methodology, Investigation, Formal analysis, Validation, Writing – Original Draft, Writing – Review & Editing. **Le Thi Kieu Linh**: Formal analysis, Validation. **Nguyen Thi Tuyet Nga**: Formal analysis, Validation. **Nghiem Xuan Hoan**: Resources, Investigation. **Nguyen Thi Khanh Linh**: Resources, Investigation. **Tran Thi Thanh Huyen**: Resources, Investigation. **Hoang Xuan Quang**: Resources, Investigation. **Tran Thi Lien**: Resources, Investigation. **Van Dinh Trang**: Resources, Investigation. **Vu Viet Sang**: Resources, Investigation. **Peter G. Kremsner**: Writing – Review & Editing. **Le Huu Song**: Resources, Investigation. **Dennis Nurjadi**: Conceptualization, Supervision, Writing – Review & Editing. **Thirumalaisamy P. Velavan**: Conceptualization, Supervision, Resources, Writing – Original Draft, Writing – Review & Editing, Funding acquisition.

Publication 3:

Van Dong D, Sang VV, Hoan NX, Ha NV, Linh NTK, Sy BT, The NT, Quang HX, Lien TT, Trang VD, Kremsner PG, Song LH, Peter S, Nurjadi D, Velavan TP. Causative Pathogens and Predictors of Unfavourable Outcomes in Central Nervous System Infections in Resource-Limited Settings. Int J Infect Dis. 2025 Jul 31:108002; PMID: 40752766. **IF 4.3**

Do Van Dong: Methodology, Investigation, Formal analysis, Validation, Writing – Original Draft, Writing – Review & Editing. **Vu Viet Sang**: Resources, Investigation. **Nghiem Xuan Hoan**: Resources, Investigation. **Nguyen Viet Ha**: Resources, Investigation. **Nguyen Thi Khanh Linh**: Resources, Investigation. **Bui Tien Sy**: Resources, Investigation. **Nguyen Trong The**: Resources, Investigation. **Hoang Xuan Quang**: Resources, Investigation. **Tran Thi Lien**: Resources, Investigation. **Van Dinh Trang**: Resources, Investigation. **Peter G. Kremsner**: Writing – Review & Editing. **Le Huu Song**: Conceptualization, Supervision, Resources, Investigation. **Silke Peter**: Validation, Writing – Original Draft. **Dennis Nurjadi**: Supervision, Validation, Writing – Original Draft, Writing – Review & Editing. **Thirumalaisamy P. Velavan**: Conceptualization, Supervision, Resources, Writing – Original Draft, Writing – Review & Editing, Funding acquisition.

I also declare that I wrote the general introduction and discussion of this dissertation, and I have only used the sources cited.

Tübingen, 4th August 2025

Doctoral Candidate

Do Van Dong, MD

VIII. ACKNOWLEDGEMENTS

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