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ORIGINAL RESEARCH

Diagnostic Performance of Clinical Metagenomic Next-Generation Sequencing for Suspected Central Nervous System Infections in a Municipal Hospital: A Retrospective Study in China

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Purpose: Cerebrospinal fluid (CSF) metagenomic next-generation sequencing (mNGS) has the potential to identify the majority of pathogens in a single test. Accurate pathogen identification is vital for central nervous system infection (CNSi). However, there are few related studies investigating in a municipal hospital.

Patients and Methods: A total of 52 suspected CNSi patients were retrospectively recruited in Xinxiang central hospital between July 2019 and April 2023. The diagnostic performance of CSF mNGS, conventional microbiological tests (CMT), and the combination of CSF mNGS and CMT were evaluated by comparing to the final diagnosis.

Results: Among 52 suspected CNSi patients, 35 were diagnosed as CNSi. In comparison to the final diagnosis, the area under curves (AUC) for CSF CMT, CSF mNGS, and the combination of CMT and mNGS for the diagnosis of CNSi were 0.56 (95% CI 0.4–0.72), 0.74 (95% CI 0.61–0.84), and 0.76 (95% CI 0.63–0.88), respectively. The sensitivities were 11.43% (95% CI 4.54%–25.95%), 48.57% (95% CI 32.99%–64.43%), and 51.43% (95% CI 35.57%–67.01%), respectively. The accuracy was 40.38 (95% CI 27.01%–54.90%), 65.38% (95% CI 50.91%–78.03%), and 67.31% (95% CI 52.89%–79.67%), respectively. Furthermore, based on CSF mNGS results, seven patients confirmed initial treatment, two escalated, and one de-escalated. Additionally, we identified the optimal cutoff values as 1.75 U/L for CSF adenosine deaminase (ADA), 75.44 U/L for CSF protein, and 185 mmH_2O for CSF pressure, when these values were exceeded, CSF mNGS tended to yield positive results.

Conclusion: CSF mNGS showed superior diagnostic performance in CNSi and hence could serve as a complementary tool to CMT and conjunctively guide the precision therapy. Additionally, the values for CSF ADA, protein and pressure could assist in predicting mNGS positive result. With technical improvements for mNGS sample processing to increase throughput and reduce costs, clinicians may use mNGS more widely in municipal hospital laboratories.

Keywords: CNS infections, mNGS, CSF, conventional microbiological tests, CMT

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Introduction

Central nervous system infections (CNSi) encompass encephalitis, meningitis, and abscess, which are acute/chronic inflammatory or non-inflammatory diseases caused by pathogens invading the parenchymal tissue, meninges, or blood vessels of the central nervous system.[1,2] CNSi, particularly meningitis and encephalitis, are characterized by rapid progression, high disability, and mortality.[3] A vast diversity of infectious pathogens can cause CNSi. *Streptococcus pneumoniae* remained the most prevalent bacterial agent, followed by *Neisseria meningitidis* and *Listeria monocytogenes*.[4] Enteroviruses (EV-A71, EV-D68, and Coxsackievirus B) and herpesviruses (Herpes simplex virus 1 (HSV-1), HSV-2, varicella-zoster virus (VZV), and Epstein Barr virus (EBV)) are frequently found in patients with aseptic meningitis.[5] *Candida spp., Cryptococcus neoformans, Aspergillus spp., Mucor spp*., and *Rhizopus spp*. are frequently detected in patients with fungal infections.[5] *Toxoplasma gondii* and free-living amoebae (*Acanthamoeba, Balamuthia*, and *Naegleria*) are noteworthy causative agents in patients with parasitic infections.[5] While *Mycobacterium tuberculosis* is the most common atypical pathogen in CNSi.[6] Currently, the conventional microbiological tests (CMT) for CNSi consist of CSF smears, cultures, antigen-antibody tests, and polymerase chain reaction (PCR) tests.[7] Nevertheless, numerous neuroinvasive pathogens are rare and not easily detected by CMT, leading to missed diagnosis and ultimately poor prognosis of the patients.[3,8] Within the clinical setting, approximately 40% to 60% of patients with CNS infections were etiologically undetermined.[9] Therefore, there is an urgent need for an accurate and rapid etiological test in the CNSi.

When compared to capital hospitals, municipal hospitals equip with fewer financial and technological resources, rely more on phenotypic detection and culture-dependent methods, have a longer detection cycle, and are less able to detect drug-resistant bacteria or unusual pathogens. Besides, there is less interdisciplinary collaboration in municipal hospitals, the use of antibiotics depends on empirical treatment, which readily results in antibiotics resistance.[10,11] In terms of patient population, rural people who are often afflicted with basic diseases like diabetes, account for the majority of municipal hospitals. Therefore, a high percentage of immunosuppressive states, and a higher risk of infection may be observed. Drug resistance is a severe issue, yet the surveillance capability is inadequate. Moreover, due to financial or transportation constraints, patients put off getting medical help, which causes infections to advance to the middle and late stages and makes diagnosis and treatment more challenging.[12]

Based on the second-generation high-throughput sequencing, metagenomics next-generation sequencing (mNGS) can detect almost all microorganisms' nucleic acid in a clinical sample without requiring predefined pathogen ranges.[13] When compared to CMT, mNGS offers the advantages of high sensitivity, short turnaround time, and broad coverage of pathogens.[14] Recent studies have demonstrated the significant role of mNGS in diagnosing infectious diseases, particularly bloodstream infection, lower respiratory tract (LRT) infection, and CNSi. A single-center retrospective study on the community-acquired CNSi showed that a total of 41 different pathogens were solely identified by mNGS, indicating that besides common pathogens, there were a large number of pathogens that cannot be detected by traditional methods.[15] Some studies have reported that mNGS can increase the diagnostic rate of CNSi by 25%.[16–18] A prospective cohort study exhibited that mNGS had a higher detection rate (34.5% vs 7.56%, McNemar test, $p < 0.0083$) compared to culture especially in patients with empirical therapy.[19] Another study reported that the positive rate of CNSi for mNGS was 60.6%, significantly higher than 20.2% ($p < 0.01$) using conventional methods.[20] As a results, mNGS demonstrated significant advantages in the application of suspected CNSi. Optimizing the roadmap for encephalitis/meningitis with mNGS is crucial for precise diagnosis and treatment.[21]

However, there are few studies of the mNGS diagnosis performance in the CNSi of a tertiary hospital in a municipal city. As reported, the detection rate of CMT in CNSi is relatively low.[22,23] Moreover, the empirical treatment further reduces the detection rate, thereby delaying the target treatment. CNSi, particularly viral encephalitis, can result in severe side effects. Combing mNGS and CMT can significantly increase the detection rate of pathogens. Therefore, this retrospective study included patients with suspected CNSi. Clinical characteristics of patients were extracted from the electronic medical records. The CSF samples were subjected to CMT in parallel with the mNGS assay. A comparison analysis was conducted to assess the diagnostic efficacy of two distinct detection methods in order to investigate the diagnostic advantages of mNGS in CNSi, thus contributing to the optimization of CNSi diagnostic and therapeutic efficiencies in clinical settings.

Materials and Methods

Study Design

This retrospective study comprised 52 patients with suspected CNS infections who were hospitalized to Xinxiang Central Hospital from July 2019 to April 2023 (Figure 1A). A total of 19 patients were admitted from the intensive care units (ICU), whereas 33 patients were admitted from the general wards (Supplementary Table 1). Patients enrolled in this study should meet the following inclusive criteria: (1) at least one of the following symptoms: fever (>38°C), headache, nausea/vomiting, seizures, meningeal irritation, localized neurological dysfunction, and impaired consciousness; (2) at least one of the following changes: infected cerebrospinal fluid and/or infectious changes on images; and (3) having CSF CMT and mNGS results. The exclusion criteria were as follows: (1) age <14 years, who are pediatric cases; (2) case loss (intermediate discharge or

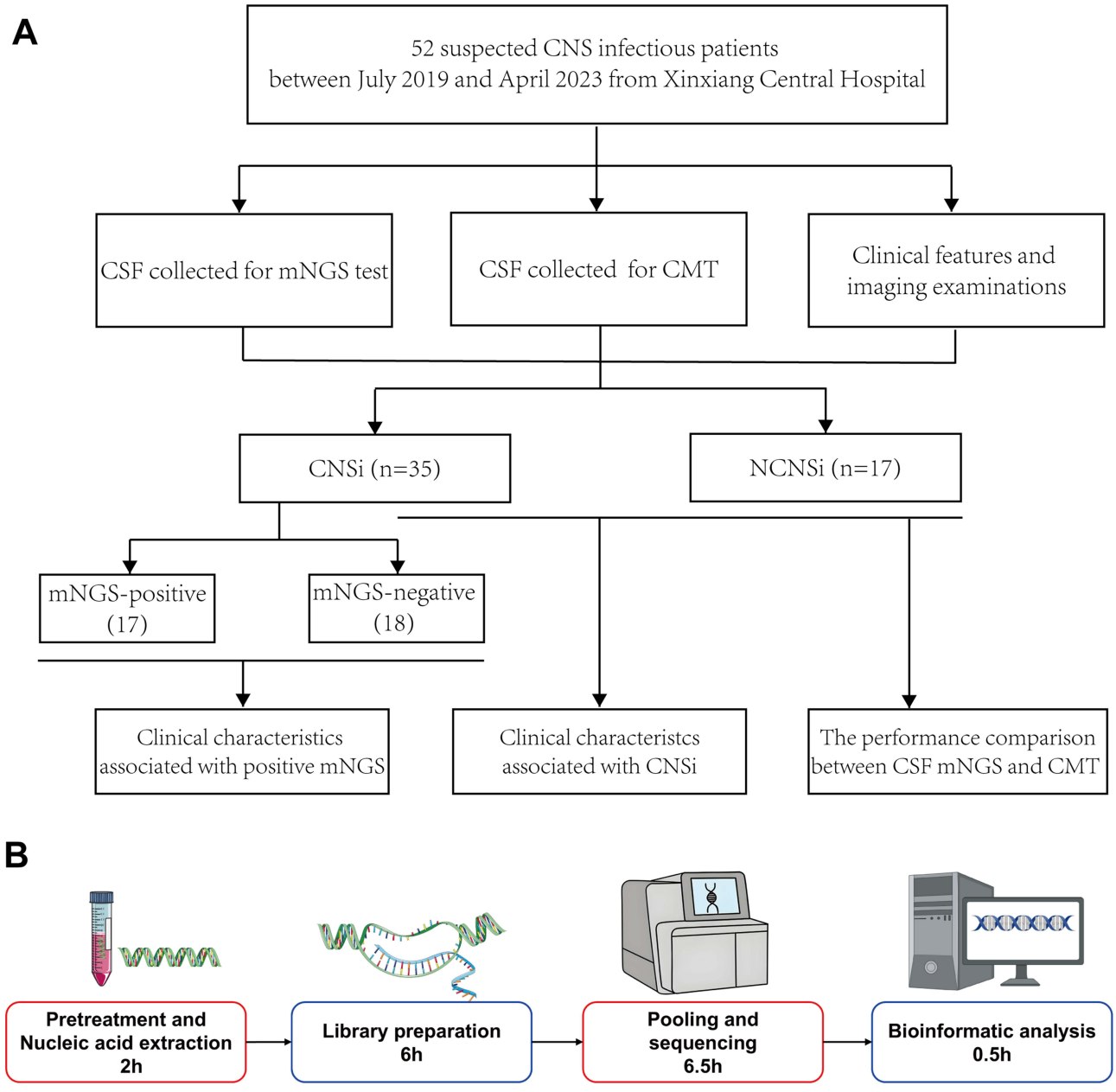

Figure 1 The flowchart of this study. (**A**) Samples screening and inclusion. (**B**) Workflow of mNGS.
Abbreviations: CSF, cerebrospinal fluid; CNSi, central nervous system infection; CMT, conventional microbiological tests; NCNSi, non-CNSi; mNGS, metagenomics next-generation sequencing.

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incomplete clinical data); and (3) initial diagnosis of non-CNS infection. Data on symptoms, laboratory findings, computed tomography images, microbiological testing, and other relevant information were extracted from electronic medical records for each case. This study followed STROBE guidelines.

Conventional Microbiological Tests (CMT)

The CSF samples were centrifuged, and the precipitate was taken for Indian ink staining and acid-fast Bacillus staining. The *M. tuberculosis* real-time fluorescence polymerase chain reaction (RT-PCR) was conducted using a SLAN-96P real-time PCR System (Hongshi, Shanghai, China). The CSF samples were subjected to the Xpert MTB/RIF assay following the instructions provided by the manufacturer's instructions (Cepheid, Sunnyvale, CA, USA). The CSF cultures were performed with the BD BACTEC FX 40 automated system (Becton Dickinson, New Jersey, USA). Microbial identification was conducted using the MALDI-TOF MS (Auto bio Diagnostics, Zhengzhou, China) and Phoenix 100 (Becton Dickinson and Company, New Jersey, USA). Bacterial antimicrobial susceptibility tests were carried out using the BD Phoenix NMIC/ID-5 (Becton Dickinson, New Jersey, USA) for gram-negative rods and the BD Phoenix PMIC/ID-4 (Becton Dickinson, New Jersey, USA) for gram-positive cocci. Fungal antimicrobial susceptibility tests were conducted using the TDR YEAST-96 with an AF-600 Automated Microorganisms analysis system (Mindray, Shenzhen, China), respectively.

Metagenomics Next-Generation Sequencing (mNGS)

The workflow of mNGS was briefly demonstrated in Figure 1B. DNA was extracted using a QIAamp® UCP Pathogen DNA Kit (Qiagen) following the manufacturer's instructions. Total RNA was extracted with a QIAamp® Viral RNA Kit (Qiagen), and ribosomal RNA was removed by a Ribo-Zero rRNA Removal Kit (Illumina). cDNA was generated using reverse transcriptase and dNTPs (Thermo Fisher). Libraries were constructed for the DNA and cDNA samples using a Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA). The library was quality assessed by the Qubit dsDNA HS Assay kit, followed by the High Sensitivity DNA kit (Agilent) on an Agilent 2100 Bioanalyzer. Library pools were then loaded onto an Illumina Nextseq CN500 sequencer for 50 cycles of single-end sequencing, resulting in approximately 20 million reads per library. To monitor the sources of potential contamination, we also prepared sterile deionized water in parallel with each batch to serve as non-template controls (NTC), using the same protocol. In addition, we used sterile cotton swabs dipped in sterile deionized water to wipe the surfaces of the centrifuge and biosafety cabinet to generate the background microorganism list in our laboratory.

Trimmomatic was used to eliminate low-quality reads, duplicate reads, adapter contamination, and those shorter than 40 bp.[24] Low-complexity reads were removed by Kcomplexity's with default settings. The human sequence data were eliminated by mapping to the hg38 reference genome using SNAP (v1.0beta.18). Microbial reads were aligned to the microbial genome database from the NCBI Assembly and Genome databases (https://benlangmead.github.io/aws-indexes/k2) using Burrows-Wheeler Aligner software.[25] The reads with 90% identity of reference were defined as mapped reads. In addition, reads with multiple locus alignments within the same genera were excluded from the secondary analysis. Only reads that mapped to the genome within the same species were considered.

Interpretation of mNGS

The clinical reportable range (CRR) for pathogens was established according to a previous study.[26] The suspected pathogens were obtained after removing the common background microorganisms and contaminants compared with negative controls. They were analyzed in terms of genus-level relative abundance, species-specific read number (SSRN), genome coverage (%), depth, and species-specific reads per million (RPM) ratio. A virus was considered positively detected if SSRN ≥ 3 covered at least three non-overlapping regions in the genome.[27] For identification of bacteria, fungi, and parasites, we developed a RPM ratio metric, or RPM-r, defined as RPM-r = RPM_{sample}/RPM_{NTC}, or RPM ratio = RPM_{sample} if $RPM_{NTC}=0$ (Miller et al, 2019). A positive detection of a bacterium, fungus, or parasite required an RPM ratio ≥ 10.[18,27] The results of mNGS can be considered positive when they are consistent with the pathogenicity of positive microorganisms, clinical characteristics, and therapeutic efficacy.

Diagnostic Performance of mNGS and CMT

Three clinicians determined the final diagnosis based on the patient's clinical manifestation, laboratory examination, clinical images (CT or MR), lumbar puncture and general characteristics examination of CSF, microbiological tests (positive culture is the gold standard for diagnosis, but it is necessary to exclude specimen contamination, and negative culture cannot be ruled out) and response to treatments. According to Expert Consensus, cases met the diagnosis criteria were classified as CNSi, otherwise were NCNSi.[28] We assessed the diagnostic performance of mNGS and CMT using final diagnosis as references,[29] in which true positive (TP) was determined as the mNGS or CMT positive in the CNSi groups, whereas false positive (FP) referred to the positive in the NCNSi groups. Consequently, the sensitivity was TP/CNSi. True negative (TN) was defined as the negative results of mNGS or CMT in the NCNSi groups, and false negative (FN) as the negative results of mNGS or CMT in the CNSi groups. The specificity was TN/NCNSi. Positive predictive value (PPV) and negative predictive value were calculated as follows: PPV = TP/(TP+FP), and NPV=TN/(TN+FN). And accuracy equaled to (TP+TN)/(TP+FP+TN+FN).

Statistics Analysis

For baseline characteristics and CSF laboratory tests, continuous variables that fitted a normal distribution were expressed as the mean ± standard deviation, and the two groups were compared using the independent *t*-test, and those that did not were expressed as the median and quartiles, and two groups were compared using the Mann–Whitney *U*-test. Categorical variables were expressed as frequency and percentages, and the two groups were compared using the Chi-square tests or Fisher's exact tests. Variables with P-values < 0.05 were considered statistically significant. A 2-sided McNemar test was used to compare differences in the diagnostic value of mNGS and CMT.[19] Receiver operating characteristic (ROC) curves were calculated to identify a cutoff value that maximized the Youden index.[30] SPSS 26.0 software and GraphPad Prism 9 software were used for statistical analysis and data processing.

Results

Comparison of Clinical Characteristics Between CNSi and NCNSi Groups

The analysis comprised a total of 52 patients with suspected CNSi (Figure 1). The baseline demographic characteristics, medical history, clinical symptoms, and laboratory investigations were summarized in Table 1. The participants had a median age of 44.35, with 29 males accounting for 55.77%. Eleven patients were smoking, and six were drinking alcohol. A total of 32 (61.54%) patients had underlying diseases. Specifically, ten patients had hypertension, ten had undergone surgical operations, five had diabetes, and three had coronary heart disease. The primary clinical symptoms observed were fever (37 cases, 71.15%), impaired consciousness (30 cases, 57.69%), and headache (23 cases, 44.23%). The median interval between the onset of symptoms and hospital admission was four days. The peak body temperature was 38.4 °C. The pulse, respiratory rate, and heart rate were 83 beats/min, 19 beats/min, and 83 beats/min, respectively. The blood biochemical testing revealed minor increases in C-reaction protein (CRP, 4.21 mg/L), procalcitonin (PCT, 0.16 μg/L), fasting blood glucose (6.35 mmol/L), and lactic dehydrogenase (LDH, 326.4 U/L). In contrast, there was a considerable increase in D-dimer. Upon analysis of the CSF examination, it was noted that the chloride (118.62 mmol/l) was slightly lowered while the protein (62.54 U/L) was somewhat increased. However, the CSF LDH (22 mg/L) was largely decreased. The CSF was predominantly colorless (26/50, 52%) and clear (24/50, 48%). The hospitalization duration had a median value of 16.5 days.

According to the CNS infection guidelines,[27] 35 cases were classified as CNS infection (CNSi) and 17 instances as non-CNS infection (NCNSi). As shown in Table 2, the clinical data was compared between CNSi and NCNSi. In terms of clinical symptoms, 30 cases and 19 cases exhibited fever ($p < 0.0001$) and headache ($p = 0.036$) in CNSi, respectively, which was a higher occurrence compared to NCNSi. The duration from symptom onset to hospitalization ($p = 0.04$) was substantially longer in the CNSi group (Figure 2A). The blood routine and biochemical testing revealed a notable reduction in D-dimer in CNSi ($p = 0.02$) (Figure 2B). In the CSF investigation, the level of adenosine deaminase (ADA, $p = 0.04$) was shown to be considerably lower in CNSi, though within the normal range (Figure 2C). The color of the

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Table 1 The Clinical Data of Patients Upon Admission

Variables	Total (N=52)	CNSi (N=35)	NCNSi (N=17)	P value
Age, years, mean (SD)	44.35 ± 21.54	42.97 ± 21.91	47.18 ± 21.12	0.514[a]
Gender, male, n (%)	29 (55.77)	19 (54.29)	10 (58.82)	0.757[b]
Smoking, n (%)	11 (21.15)	9 (25.71)	2 (11.76)	0.757[b]
Drinking, n (%)	6 (11.54)	4 (11.43)	2 (11.76)	0.972[b]
Duration from initial symptoms to hospitalization, days, median (IQR)	4 (1, 9.75)	6 (2, 15)	1 (1, 5)	0.039[c]
Underlying diseases, n (%)				
Hypertension	10 (19.23)	7 (20)	3 (17.65)	0.840[b]
Diabetes	5 (9.62)	4 (11.43)	1 (5.88)	0.525[b]
Coronary heart disease	3 (5.77)	3 (8.57)	0 (0)	0.214[b]
Post-surgical operation	10 (19.23)	9 (25.71)	1 (5.88)	0.089[b]
Clinical manifestation, n (%)				
Fever	37 (71.15)	30 (85.71)	7 (41.18)	0.001[b]
Headache	23 (44.23)	19 (54.29)	4 (23.53)	0.036[b]
Abnormal mental behavior	6 (11.54)	5 (14.29)	1 (5.88)	0.374[b]
Consciousness disorder	30 (57.69)	20 (57.14)	10 (58.82)	0.908[b]
Epileptic seizures	11 (21.15)	8 (22.86)	3 (17.65)	0.666[b]
Urine and stool disorders	5 (9.62)	4 (11.43)	1 (5.88)	0.525[b]
Arrhythmia	12 (23.08)	8 (22.86)	4 (23.53)	0.957[b]
Sleepiness	4 (7.69)	2 (5.71)	2 (11.76)	0.442[b]
Movement disorders	4 (7.69)	3 (8.57)	1 (5.88)	0.733[b]
Sensory disorder	2 (3.85)	2 (5.71)	0 (0)	0.315[b]
Clinical signs				
Peak body temperature (°C)	38.4 (36.93, 38.68)	38.4 (37.4, 38.9)	37.4 (36.45, 38.65)	0.092[c]
Pulse (beats/min)	83 (76, 95)	84 (76, 96)	78 (71, 95.5)	0.262[c]
Respiratory rate (beats/min)	19 (18, 21.75)	20 (18, 22)	18 (15.5, 20)	0.097[c]
Heart rate (beats/min)	83 (76, 95)	84 (76, 96)	78 (71, 95.5)	0.262[c]
WBC, ×10^9/L, median (IQR)	9.19 (7.45, 14.59)	9.15 (7.43, 12.82)	10.06 (7.57, 15.84)	0.359[c]
Basophils percentage, %, median (IQR)	0.1 (0.1, 0.2)	0.1 (0.1, 0.2)	0.1 (0.1, 0.25)	0.484[c]
Neutrophil count, ×10^9/L, median (IQR)	7.88 (5.12, 13.27)	7.47 (5.02, 11.85)	8.96 (5.17, 15.87)	0.215[c]
Neutrophil percentage, %, median (IQR)	80.55 (69.18, 88.4)	77.2 (68.9, 88)	84.1 (72.1, 89.6)	0.266[c]
Eosinophils Percentage, %, median (IQR)	0.13 (0, 0.68)	0.1 (0, 0.6)	0.2 (0.05, 0.75)	0.606[c]
Lymphocyte count, ×10^9/L, median (IQR)	1.23 (0.82, 1.91)	1.31 (0.87, 1.96)	1.17 (0.73, 1.6)	0.359[c]
Lymphocyte percentage, %, mean (SD)	16.52 ± 12.81	17.67 ± 13.4	14.14 ± 11.53	0.356[a]

(*Continued*)

Table 1 (Continued).

Variables	Total (N=52)	CNSi (N=35)	NCNSi (N=17)	P value
Monocyte percentage, %, median (IQR)	5.45 (3.7, 6.88)	5.6 (3.7, 7.2)	5.1 (3.5, 6.45)	0.704[c]
RBC, ×10^12/L, mean (SD)	4.29 ± 0.58	4.36 ± 0.51	4.13 ± 0.71	0.191[a]
HB, g/L, mean (SD)	129.02 ± 18.9	130.11 ± 18.17	126.76 ± 20.72	0.554[a]
PLT, ×10^9/L, mean (SD)	235.23 ± 54.05	240.91 ± 44	223.53 ± 70.59	0.281[a]
Blood biochemistry				
CRP, mg/L, median (IQR)	4.21 (1.4, 35.85)	4.52 (1, 37.92)	3.9 (1.7, 32.51)	0.969[c]
PCT, ng/mL, median (IQR)	0.16 (0.01, 0.4)	0.1 (0.01, 0.55)	0.17 (0.08, 0.31)	0.419[c]
ALT, U/L, median (IQR)	23 (12, 36)	22 (12, 41)	30 (11.5, 36)	0.800[c]
AST, U/L, median (IQR)	29 (20.75, 45)	27 (20, 47)	30 (27.5, 37.5)	0.482[c]
TP, g/L, mean (SD)	65.77 ± 6.84	66.11 ± 6.73	65.06 ± 7.23	0.609[a]
TBIL, umol/L, median (IQR)	13.75 (10.6, 18.28)	13.7 (9.7, 18.2)	13.8 (10.95, 20.7)	0.654[c]
Indirect bilirubin, umol/L, mean (SD)	10.59 ± 5.14	10.36 ± 5.44	11.05 ± 4.6	0.653[a]
Direct bilirubin, umol/L, median (IQR)	4.05 (2.23, 6.53)	4 (2.1, 6)	4.4 (2.3, 8.85)	0.725[c]
ALP, umol/L, median (IQR)	83.4 (63.88, 99.64)	77 (63, 99.64)	85 (69.8, 98.75)	0.619[c]
Glutamine transferase,U/L, median (IQR)	23.35 (16.88, 41.75)	22.9 (16.8, 34.2)	25 (16.45, 51.5)	0.807[c]
Fasting blood glucose, umol/L, median (IQR)	6.35 (5.41, 7.7)	6.21 (5.43, 7.46)	6.73 (5.14, 7.82)	0.762[c]
Urea nitrogen, Mmol/L, median (IQR)	4.3 (3.18, 5.38)	4.35 (3.41, 5.21)	3.74 (2.8, 5.87)	0.501[c]
Creatinine, umol/L, median (IQR)	55.1 (42.4, 62.35)	55.4 (43.9, 62.5)	54.8 (38.5, 63.3)	0.704[c]
Uric acid, umol/L, median (IQR)	238.75 (142.4, 340.55)	237.5 (141.8, 344.2)	243.1 (143.3, 369.65)	0.661[c]
ALT, U/L, median (IQR)	326.4 (199.65, 524)	324.8 (197, 454)	518 (205.5, 635)	0.185[c]
Serum albumin, g/L, median (IQR)	39.95 (34.53, 42.2)	40.2 (34.6, 42.2)	39.5 (33.45, 42.65)	0.891[c]
Serum Potassium, mmol/L, mean (SD)	3.67 ± 0.55	3.63 ± 0.56	3.75 ± 0.55	0.484[a]
Serum Sodium, mmol/L, mean (SD)	135.48 ± 6.32	135.67 ± 6.05	135.09 ± 7.04	0.760[a]
Serum Calcium, mmol/L, median (IQR)	2.19 (2.01, 2.31)	2.19 (2.05, 2.31)	2.12 (1.94, 2.38)	0.718[c]
PT, s, mean (SD)	12.59 ± 1.63	12.63 ± 1.75	12.52 ± 1.4	0.821[a]
APTT, s, median (IQR)	29.95 (26.7, 34.1)	30.4 (27.3, 34.2)	29.1 (25.85, 33)	0.258[c]
D-dimer, mg/L, median (IQR)	174.5 (21, 707)	95 (16, 286)	472 (98, 1182)	0.023[c]
Fibrinogen, g/L, median (IQR)	3.03 (1.89, 4.79)	3.04 (1.8, 4.5)	2.88 (2.18, 8.9)	0.418[c]
Cerebrospinal Fluid Examination				
Pressure,mmH_2O, median (IQR)	180 (140, 223.75)	180 (140, 230)	160 (130, 200)	0.470[c]
Glucose, mmol/L, mean (SD)	3.5 ± 1.31	3.34 ± 1.24	3.83 ± 1.41	0.208[a]
Chloride, mmol/L, mean (SD)	118.62 ± 8.12	117.1 ± 7.95	121.75 ± 7.77	0.052[a]
Protein, U/L, median (IQR)	62.54 (32.92, 192.91)	80.48 (40.77, 196.33)	49.12 (29.59, 166.36)	0.215[c]
LDH, U/L, median (IQR)	22 (14.95, 77.53)	22.6 (15.1, 52.9)	20.2 (14.4, 169.6)	0.876[c]

(*Continued*)

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Table 1 (Continued).

Variables	Total (N=52)	CNSi (N=35)	NCNSi (N=17)	P value
HsCRP, mg/L, median (IQR)	0.9 (0.52, 1.69)	0.92 (0.28, 1.71)	0.88 (0.57, 1.76)	0.612[c]
ADA,U/L, median (IQR)	2.4 (0.93, 4.35)	2 (0.6, 2.9)	4 (2.05, 5.45)	0.039[c]
Color[†], n (%)				0.000[b]
Colorless	26 (52)	22(62.85)	4 (26.67)	
Clear and transparent	12 (24)	5 (14.28)	7 (46.67)	
Pale yellow	5 (10)	5 (14.28)	0 (0)	
Light red	4 (8)	3(8.57)	1(13.33)	
Transparency[†], n (%)				0.001[b]
Clear	24 (48)	22 (62.85)	2 (13.33)	
Transparent	15 (30)	5 (14.28)	10 (66.67)	
Micro turbidity	7 (14)	6 (17.14)	1 (6.67)	
Outcome				
Length of hospitalization, days, median (IQR)	16.5 (11.25, 21.75)	17 (12, 21)	16 (7.5, 25.5)	0.675[c]
ICU duration, days, median (IQR)	0 (0, 7.75)	0 (0, 7)	4 (0, 9)	0.679[c]
Hospitalized survival, days, median (IQR)	1 (1, 1)	1 (1, 1)	1 (1, 1)	1.000[c]

Notes: [a]P values were calculated using the independent *t*-test between CNSi and NCNSi groups. [b]P values were calculated using Chi-square tests or Fisher's exact tests between CNSi and NCNSi groups.[c]P values were calculated using the Wilcoxon rank sum test between CNSi and NCNSi groups.[†]50, 35, 15 patients had recorded the CSF color and transparency in the groups of total, CNSi, and NCNSi, respectively. The bolded values represent significant differences between CNSi and NCNSi groups.
Abbreviations: CRP, C- reactive protein; PCT, Procalcitonin; WBC, White Blood Cell; RBC, Red blood cell count; HB, Hemoglobin; PLT, Platelet count; ALT, Alanine transferase; AST, Aspartate transferases; TP, Serum total protein; TBIL, Total bilirubin; ALP, Alkaline phosphatase; LDH, Lactate dehydrogenase; APTT, Activated partial thromboplastin time; PT, Prothrombin Time; ADA, Adenosine deaminase; HsCRP, High-sensitivity C-reactive protein.

Table 2 The Diagnostic Performance of CSF mNGS, CMT, and the Combination of CMT and mNGS in CNSi

		Composite Reference Standard		AUC (95% CI)	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV% (95% CI)	NPV% (95% CI)	Accuracy% (95% CI)
		Positive	Negative						
CMT	**Positive**	4	0	0.56 (0.40–0.72)	11.43 (4.54–25.95)	100 (81.57–100)	100 (81.57–100)	35.42 (32.74–38.18)	40. 38 (27.01–54.90)
	Negative	31	17						
mNGS	**Positive**	17	0	0.74 (0.61–0.84)	48.57 (32.99–64.43)	100 (81.57–100)	100 (51.01–100)	48.57 (40.63–56.58)	65.38 (50.91–78.03)
	Negative	18	17						
CMT+mNGS	**Positive**	18	0	0.76 (0.63–0.88)	51.43 (35.57–67.01)	100 (81.57–100)	100 (81.47–100)	50.00 (41.56–58.44)	67.31 (52.89–79.67)
	Negative	17	17						

CSF was found to be significantly different between two groups ($P < 0.0001$). In the CNSi group, the CSF was colorless in 22 cases (62.85%), while in the NCNSi groups, it appeared clear in seven cases (46.67%).

CSF mNGS Outperformed CMT in Diagnosing CNSi

The positive rate of CSF mNGS was 32.69% for all recruited patients, which was significantly higher than that of CSF CMT (Figure 3A). When focusing solely on the 35 CNSi cases, the positive rate of CSF mNGS climbed to 48.57%, and

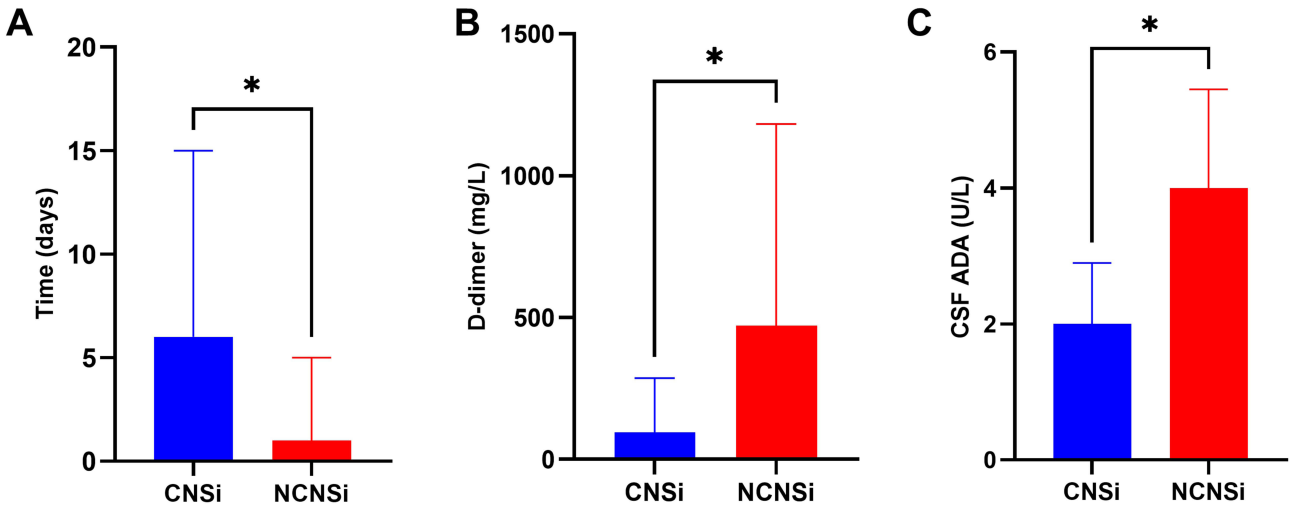

Figure 2 The significant differences in clinical data between the CNSi and NCNSi groups. (**A**) Duration from initial symptoms to hospitalization. (**B**) Serum D-dimer. (**C**) CSF adenosine deaminase (ADA). Data are presented as a box plot overlaid by a dot plot with a line at the median. P values were calculated using the Wilcoxon rank sum test. *p value < 0.05.

CSF CMT rose to 11.43% (Figure 3A). Based on the CNSi criteria and clinical diagnosis, a total of 13 pathogens were considered as causative causes of central nervous system infections in 14 CNSi patients. Among these pathogens, HHV-7, EBV, and *Escherichia coli* ranked the top 3 (Figure 3B). The CSF CMT test detected four pathogens, with *Staphylococcus hominis* and *Stenotrophomonas maltophilia* being solely detected by CMT. *S. pneumoniae* and *Pseudomonas putida* were detected by both CSF mNGS and CMT. The CSF mNGS test detected a total of 13 pathogens. Notably, the presence of *A. fumigatus* and viruses was exclusively found by mNGS. Based on McNemar test, we found that the diagnostic efficiency of CSF mNGS and CSF CMT significantly differed ($p < 0.001$). The area under the ROC curve (AUC) of CSF CMT and mNGS was 0.56 (95% CI 0.40–0.72) and 0.74 (0.61–0.84), respectively (Figure 3C and Table 2). The sensitivity of CSF CMT and mNGS was 11.43% (95% CI 4.54%–25.95%) and 48.57% (32.99%–64.43%), respectively. NPV was 35.42% (95% CI 32.74%–38.18%) for CSF CMT and 48.57% (40.63%–56.58%) for CSF mNGS. However, the specificity and PPV of CSF CMT and mNGS both achieved 100%. The accuracy of CSF CMT and mNGS was 40.38% (95% CI 27.01%–54.90%) and 65.38% (50.91%–78.03%), respectively. The integration of CSF mNGS and CMT resulted in higher values in AUC, sensitivity, NPV and accuracy, as demonstrated by the following: 0.76 (0.63–0.88) for AUC, 52.43% (35.57%–67.01%) for sensitivity, 50% (41.56%–58.44%) for NPV, and 67.31% (52.89%–79.67%) for accuracy.

Clinical Impact of mNGS and CMT on Antibiotic Adjustment

Out of the 35 patients in the CNSi group, 19 did not undergo antibiotic adjustment. Among these, seven patients confirmed the initial treatment based on the mNGS results (Figure 4A). In the CNSi groups, antibiotic treatment was escalated in ten patients. Out of them, four patients received escalation based on empirical evidence, two patients based on mNGS results, two patients based on CMT results, and two patients on both mNGS and CMT results (Table 3). Antibiotic de-escalation (ADE) was conducted on six patients, with one patient undergoing a change in treatment based on the mNGS results (Table 3). Out of the 17 patients in the NCNSi group, seven received ADE based on the negative results of CSF mNGS and CMT (Figure 4B).

Clinical Characteristics Associated with Positive mNGS

In order to find out the clinical data associated with the positive mNGS results, we divided the CNSi into a mNGS-positive group (n = 17) and a mNGS-negative group (n = 18) (Supplementary Table 2). In terms of blood biochemical testing, the fasting blood glucose levels (7 mmol/L, $p = 0.27$) showed a significant increase, while the uric acid levels (180.8 μmol/L, $p = 0.29$) showed a significant decrease in the mNGS-positive groups. In relation to the analysis of CSF,

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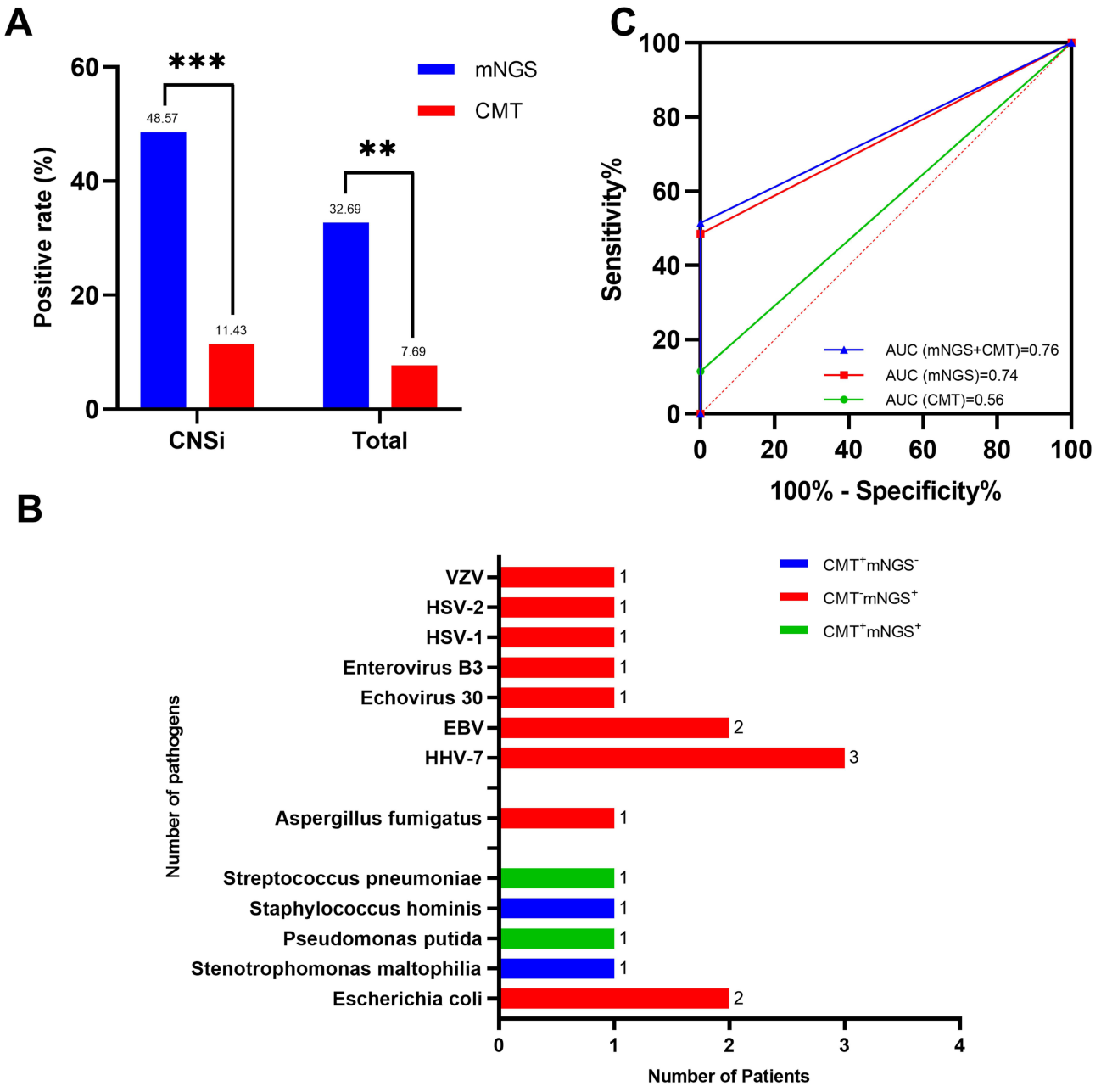

Figure 3 The diagnostic performance of mNGS and pathogen spectrum in CNS infections. (**A**) CSF mNGS and CMT in CNSi. (**B**) Pathogen distribution in CNSi. (**C**) ROC curves of CSF mNGS, CMT, and the combination of mNGS and CMT in CNSi. P values were calculated using the Chi-square tests. **p value < 0.01; ***p value < 0.001.

the levels of CSF ADA (2.6 U/L, $p = 0.007$) were found to be higher in the mNGS-positive groups even within the normal range (Figure 5A). The levels of CSF protein (100.5 U/L, $p = 0.032$) and CSF pressure (220 mmH_20, $p = 0.029$) were found to be considerably higher in the mNGS-positive groups (Figure 5B and C). The CSF ADA, CSF protein, and CSF pressure were further analyzed using the ROC curve, and the AUC values for CSF ADA, protein, and pressure were determined to be 0.76 (95% CI 0.60–0.93), 0.71 (95% CI 0.54–0.88), and 0.71 (95% CI 0.54–0.89), respectively (Supplementary Table 3). Furthermore, the optimal cutoff value, determined by maximizing the Youden index, was found to be 1.75 U/L for CSF ADA, 75.44 U/L for CSF protein, and 185 mmH_2O for CSF pressure (Supplementary Table 3). Of the 20 cases with CSF ADA ≥1.75 U/L, 14 had positive mNGS results with a positive rate of 70%. This rate was significantly higher compared to the 20% positive rate observed in the groups with CSF ADA <1.75 U/L ($p = 0.003$) (Figure 5D). Out of 18 cases with CSF protein ≥75.44 U/L, 12 had positive mNGS results with a detection rate of

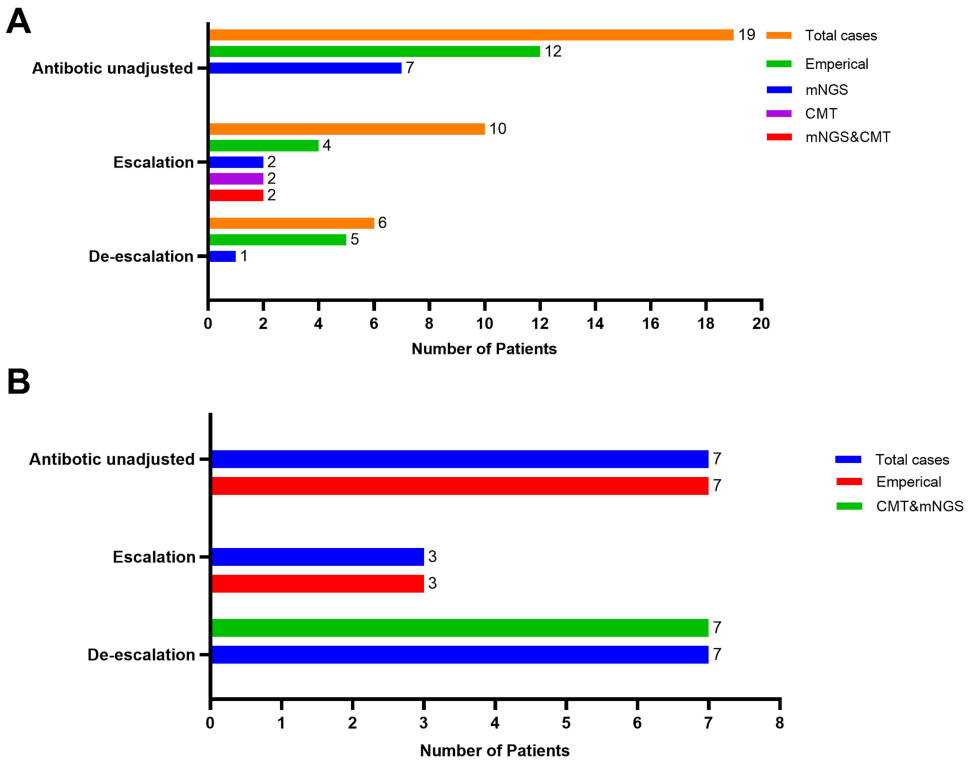

Figure 4 The adjustment of initial antimicrobial treatment. (**A**) Adjustment of initial antibiotics in CNSi. **(B)** Adjustment of initial antibiotics in NCNSi.

66.67%. This rate was significantly higher compared to the 29.41% detection rate observed in the groups with CSF protein <75.44 U/L ($p = 0.028$) (Figure 5E). Among the 15 cases with CSF pressure ≥ 185 mmH_2O, 73.33% cases had positive mNGS results, which was significantly higher than 30% observed in the groups with CSF pressure <185 mmH_2O ($p = 0.011$) (Figure 5F).

Discussion

The main aim of this study was to compare the effectiveness of CSF mNGS and CMT in diagnosing and treating patients with CNSi, and examine the clinical features associated with positive mNGS results in CNSi. mNGS has the potential to identify rare pathogens and viruses.[4] In our study, 52 patients suspected of having CNS infections were recruited, and ultimately 35 patients were diagnosed with proven CNS infections. All enrolled patients received CSF mNGS and CMT assays. Thirteen pathogens, identified using CSF mNGS or/and CMT, were conclusively determined to be the cause of the CNS infections. Among 13 causative pathogens, CSF mNGS was able to identify 11 of them. These included seven viruses (HSV-1, HSV-2, VZV, EBV, HHV-7, Enterovirus B3, and Echovirus 30), one fungus (*A. fumigatus*), and three bacteria (*E. coli, S. pneumoniae*, and *P. putida*). On the other hand, CSF CMT only detected four bacteria (*S. pneumoniae, S. hominis, P. putida*, and *S. maltophilia*).

Viruses are the main causative agents responsible for CNS infections.[31] Specifically, viral agents were responsible for 69% of encephalitis causes.[32] mNGS has significant potential for diagnosing viral CNS infections.[33,34] Our investigation found that CSF mNGS exclusively detected all seven viruses, demonstrating its superiority over CMT in virus detection. Both *S. pneumoniae*, and *P. putida* were detected using CSF mNGS and CMT. *S. hominis* and *S. maltophilia* were solely detected by CSF CMT. A prospective and multicenter study of CNS infections showed that only 22% of pathogens were solely identified by mNGS.[27] This percentage is much lower compared to our study. The discrepancy may be attributed to the relatively narrow spectrum of CMT employed in this particular study. As a result, mNGS can be used as a supplementary test for the CMT in CNS infections, particularly in the municipal city tertiary hospitals.

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Table 3 The Escalation and De-Escalation Antibiotic Therapy Based on mNGS or CMT

Patient ID	Clinical Diagnosis	CMT Results	mNGS Results	Causative Pathogen	Initial Antibiotic Treatment	Treatment After Pathogen Diagnosis	The Basis for Antibiotics Adjustment	Antibiotic Adjustment Strategy
P18	Viral meningitis	Negative	EBV, HHV-7	EBV, HHV-7	None	Ganciclovir	mNGS	Escalation
P28	Intracranial infection	CSF culture: *S. hominis*	Negative	*S. hominis*	TZP	MEM, VAN, CSL, LNZ	CMT	Escalation
P33	Intracranial infection	CSF culture: *P. putida*	*P. putida*	*P. putida*	TZP, VAN	MEM, VAN, LEV, TZP	mNGS+CMT	Escalation
P38	Bacterial meningitis	Negative	*E. coli*	*E. coli*	Acyclovir, CAZ	MEM TZP	mNGS	Escalation
P44	Viral meningitis	Negative	EBV, HHV-7	EBV, HHV-7	CRO, LEV, Acyclovir	Acyclovir, CRO	mNGS	De-scalation
P45	Bacterial meningitis	CSF culture: *S. maltophilia*	EBV, Torque teno virus	*S. maltophilia*	MEM, Acyclovir	Acyclovir, VAN, CRO	CMT	Escalation
P49	Bacterial meningitis	CSF culture: *S. pneumoniae*	*S. pneumoniae*	*S. pneumoniae*	Peramivir, Oseltamivir, CRO, VAN, Ganciclovir	VAN, TGC, CSL, MFX	mNGS+CMT	Escalation

Abbreviations: TZP, Piperacillin/Tazobactam; CSL, Cefoperazone/Sulbactam; MEM, Meropenem; CRO, Ceftriaxone; VAN, Vancomycin; LEV, levofloxacin; MFX, Moxifloxacin; TGC, Tigecycline; CAZ, Ceftazidime; LNZ, Linezolid. *E. coli, Escherichia coli; S. hominis, Staphylococcus hominis; P. putida, Pseudomonas putida; S. maltophilia, Stenotrophomonas maltophilia; S. pneumonia, Streptococcus pneumonia.*

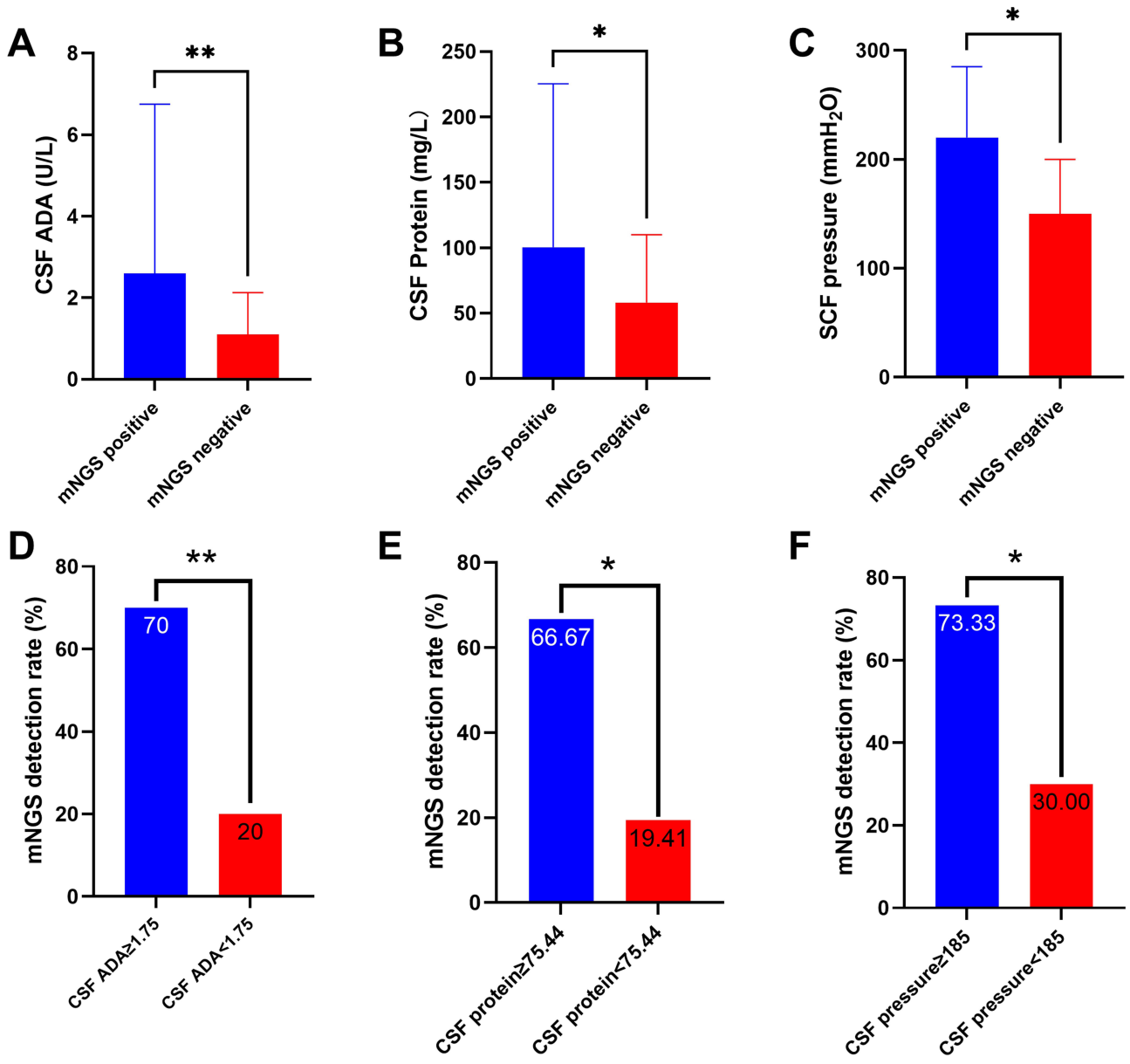

Figure 5 The CSF clinical data related to the positive mNGS results. CSF ADA **(A)**, CSF protein **(B)**, and CSF pressure **(C)** were significantly different between mNGS-positive and mNGS-negative groups. The mNGS detection rate was significantly higher when CSF ADA ≥ 1.75 U/L **(D)**, CSF protein ≥ 75.44 U/L **(E)**, and CSF pressure ≥ 185 mmH_2O **(F)**. (**A–C**) Data are presented as a box plot overlaid by a dot plot with a line at the median, and P values were calculated using the Wilcoxon rank sum test. *p value < 0.05; **p value < 0.01.

In our study, we compared the diagnostic accuracy of CSF mNGS and CMT in 35 patients with clinically proven CNS infections. The AUC for the mNGS assay in detecting CNS infections achieved 0.74, surpassing the AUC of 0.56 for CMT. This indicates that the mNGS assay outperforms CSF CMT in diagnosing CNS infections. In this study, the mNGS test demonstrated a sensitivity of 48.57% (CI 32.99%–64.43%), which is much lower than the 90% sensitivity when compared to culture reported in a retrospective cohort study conducted by Huashan Hospital of Fudan University.[22] The difference can be explained by the fact that this study included patients with confirmed CNSi and regarded the clinical diagnosis as the standard. Besides, they excluded patients with effective treatments before admission, whereas CSF samples that were collected after antibiotics treatments were also included in this study. The mNGS assay in this study demonstrated a specificity of 100% (CI 81.57%–100%), which is comparable to the 99% specificity observed in a previous study.[18]

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Timely detection of the causative pathogens in patients with CNS infections can assist in directing the precise treatment. In this study, 10 out of 35 confirmed CNS infections (28.57%) received antibiotic treatment adjusted or unadjusted according to mNGS results. This percentage was lower than the 48.1% reported in previously published research.[35] mNGS, as a cutting-edge diagnostic method, has the ability to overcome the restrictions posed by various types of pathogens and accurately identify almost 20, 000 pathogens without bias.[13] Moreover, it can also provide information about virulence and drug resistance,[36,37] hence facilitating personalized medication. mNGS is currently facing several challenges, such as nucleic acid interference of the host cell, contamination during the detection process, lack of unified experimental and bioinformatics standard operation procedure (SOP), and lack of interpretation standard (discrimination between colonization and infection).[38] Therefore, the utilization of mNGS in combination with traditional testing methods is expected to improve diagnostic rates for pathogens.[39]

To investigate the clinical factors associated with positive results of mNGS, we conducted a comparative analysis of the clinical data between mNGS-positive and mNGS-negative groups. A previous study reported that CSF protein ≥ 500 mg/L, CSF WBC ≥ 300 ×10^6 /L, and CSF/Serum glucose ratio ≤0.3 had a higher mNGS detection rate in the CNS infection.[19] We demonstrated that levels of CSF ADA, CSF protein, and CSF pressure were significantly elevated in mNGS-positive groups. And the optimal cutoff values were identified as 1.75 U/L for CSF ADA, 75.44 U/L for CSF protein, and 185 mmH_2O for CSF pressure. When these values were exceeded, CSF mNGS tended to yield positive results. Consistent with the previous study, CSF protein may serve as an indicator for the positive mNGS results. A bigger cohort of studies is needed to validate the finding in this study that CSF pressure and CSF ADA could serve as additional markers for positive mNGS results.

Our study has several limitations. First of all, as a retrospectively observational study, our study included the small sample size of CNSi. Secondly, mNGS RNA sequencing was conducted in a limited number of patients suspected of RNA viral infection, which may lead to false-negative results of CSF mNGS. Finally, the final clinical diagnosis was used as the reference in this study, of which nearly half was not confirmed by etiological evidence. The sample size was determined by practical limitations (clinical cohort availability in a municipal hospital). Despite this, we prioritized rigorous methodology, including rigorous criteria for inclusion and exclusion, robust control variables, validated and comprehensive evaluation based on clinical diagnosis. Furthermore, to transparently address uncertainty, we have reported confidence intervals alongside p-values in the results. This allows readers to evaluate both the magnitude and directionality of effects, rather than relying solely on binary significance testing. Therefore, to validate the application value of mNGS in CNSi and also clarify seasonal variations in CNSi in the future, a larger sample size, multiple centers, and a prospective study are required. The combination of mNGS and CMT enhances clinical diagnostics by combining unbiased pathogen identification with targeted, cost-effective assays. The high costs and technical complexities of mNGS necessitate strategic cost-benefit analysis-reserving it for immunocompromised patients or complex infections where traditional methods fail.[40] Standardization of bioinformatics processes is essential to maximize utility and minimize data interpretation variability. Specificity would be increased by addressing contamination through enhanced sample processing and host DNA depletion procedures. Accessibility could be further enhanced by cooperative initiatives to create shared reference libraries and cost-cutting measures like selective sequencing panels.[13] This two-pronged strategy advances precision medicine in infectious diseases by striking a balance between diagnostic accuracy and resource efficiency.

Conclusion

This retrospective study demonstrated that in cases of suspected CNSi, CSF mNGS considerably outperformed CMT for the overall detection rate of potential pathogens, and the combination of mNGS and CMT would improve the microbiological diagnosis. In addition, seven patients were administered targeted therapy based on the results of CSF mNGS. Our research also revealed a positive correlation between raised levels of CSF ADA, CSF protein, and CSF pressure and a higher incidence of mNGS positivity in CNSi. The high costs and technical complexities of mNGS necessitate strategic cost-benefit analysis-reserving it for immunocompromised patients or complex infections where traditional methods fail. Technical improvements in hardware are needed for mNGS sample processing to increase throughput and automation and to reduce costs, therefore eventually enable clinicians to use mNGS more widely in municipal hospital laboratories or point-of-care settings. Our results need to be further validated via a larger sample size, multiple centers, and prospective study.

Data Sharing Statement

Sequencing data with human sequences removed was deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database (BioProject: PRJNA1118056).

Ethics Statement

This study was reviewed and granted by the Ethics Committee of the Xinxiang Central Hospital on October 10, 2021, with the assigned approval number of 2021-294. The requirement for informed consent was waived by the Ethics Committee of the Xinxiang Central Hospital due to the retrospective study. We confirmed that the data was anonymized or maintained with confidentiality in line with the Declaration of Helsinki.

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Disclosure

The author(s) report no conflicts of interest in this work.

References

1. Varici Balci FK, Sayiner AA. A seven-year evaluation of viral central nervous system infections. *Mikrobiyol Bul*. 2019;53(4):434–441. Viral Etkenlere Bagli Santral Sinir Sistemi Enfeksiyonlarinin Yedi Yillik Degerlendirmesi. doi:10.5578/mb.68012
2. Chen YY, Yan G, Xue XH, Pang F. Application of metagenomic next-generation sequencing in the diagnosis of infectious diseases of the central nervous system after empirical treatment. *World J Clin Cases*. 2022;10(22):7760–7771. doi:10.12998/wjcc.v10.i22.7760
3. Stahl JP, Azouvi P, Bruneel F, et al. Guidelines on the management of infectious encephalitis in adults. *Med Mal Infect*. 2017;47(3):179–194. doi:10.1016/j.medmal.2017.01.005
4. van de Beek D, Cabellos C, Dzupova O, et al. ESCMID guideline: diagnosis and treatment of acute bacterial meningitis. *Clin Microbiol Infect*. 2016;22(Suppl 3):S37–62. doi:10.1016/j.cmi.2016.01.007
5. Le Govic Y, Demey B, Cassereau J, Bahn YS, Papon N. Pathogens infecting the central nervous system. *PLoS Pathog*. 2022;18(2):e1010234. doi:10.1371/journal.ppat.1010234
6. Park M, Gupta RK. Central nervous system mycobacterium infection: tuberculosis and beyond. *Neuroimaging Clin N Am*. 2023;33(1):105–124. doi:10.1016/j.nic.2022.07.006
7. Gu J, Chen L, Zeng C, et al. A retrospective analysis of metagenomic next generation sequencing (mNGS) of cerebrospinal fluid from patients with suspected encephalitis or meningitis infections. *J Healthc Eng*. 2022;2022:5641609. doi:10.1155/2022/5641609
8. He T, Kaplan S, Kamboj M, Tang YW. Laboratory diagnosis of central nervous system infection. *Curr Infect Dis Rep*. 2016;18(11):35. doi:10.1007/s11908-016-0545-6
9. Piantadosi A, Mukerji SS, Ye S, et al. Enhanced virus detection and metagenomic sequencing in patients with meningitis and encephalitis. *mBio*. 2021;12(4):e0114321. doi:10.1128/mBio.01143-21
10. Chen J, Xie X, Zhang A, He Y. Is there a genuine correlation between blood glucose control and anti-tuberculosis drug concentrations, or is it merely an illusion? *Clin Infect Dis*. 2024. doi:10.1093/cid/ciae532
11. Cui X, Xie Z, Wu Z, Xu LQ. Current trends and future directions in probiotics research for HIV/AIDS. *Front Microbiol*. 2024;15:1444552. doi:10.3389/fmicb.2024.1444552
12. Zang F, Liu J, Wen Y, et al. Adherence to guidelines and central-line-associated bloodstream infection occurrence during insertion and maintenance of intravascular catheters: evidence from 20 tertiary hospitals. *J Hosp Infect*. 2024;150:17–25. doi:10.1016/j.jhin.2024.05.011
13. Chiu CY, Miller SA. Clinical metagenomics. *Nat Rev Genet*. 2019;20(6):341–355. doi:10.1038/s41576-019-0113-7
14. Ramachandran PS, Wilson MR. Metagenomics for neurological infections-expanding our imagination. *Nat Rev Neurol*. 2020;16(10):547–556. doi:10.1038/s41582-020-0374-y
15. Zhang S, Wu G, Shi Y, et al. Understanding etiology of community-acquired central nervous system infections using metagenomic next-generation sequencing. *Front Cell Infect Microbiol*. 2022;12:979086. doi:10.3389/fcimb.2022.979086
16. Miao Q, Ma Y, Wang Q, et al. Microbiological diagnostic performance of metagenomic next-generation sequencing when applied to clinical practice. *Clin Infect Dis*. 2018;67(suppl_2):S231–S240. doi:10.1093/cid/ciy693
17. Gu W, Miller S, Chiu CY. Clinical metagenomic next-generation sequencing for pathogen detection. *Annu Rev Pathol*. 2019;14(1):319–338. doi:10.1146/annurev-pathmechdis-012418-012751
18. Miller S, Naccache SN, Samayoa E, et al. Laboratory validation of a clinical metagenomic sequencing assay for pathogen detection in cerebrospinal fluid. *Genome Res*. 2019;29(5):831–842. doi:10.1101/gr.238170.118
19. Zhang Y, Cui P, Zhang HC, et al. Clinical application and evaluation of metagenomic next-generation sequencing in suspected adult central nervous system infection. *J Transl Med*. 2020;18(1):199. doi:10.1186/s12967-020-02360-6

20. Liu Y, Zhu W, Jiao M, Guo W, Luo Y. Clinical application value of metagenomic next-generation sequencing in the diagnosis of central nervous system infections. *Front Bioeng Biotechnol*. 2023;11:885877. doi:10.3389/fbioe.2023.885877
21. Zhang Y, Zhang H, Deng B, et al. Optimal encephalitis/meningitis roadmap via precise diagnosis and treatment (IMPROVE): a study protocol for a randomized controlled trial. *BMC Infect Dis*. 2022;22(1):40. doi:10.1186/s12879-021-06943-6
22. Lin K, Zhang HC, Zhang Y, et al. Clinical application and drug-use-guidance value of metagenomic next-generation sequencing in central nervous system infection. *Am J Transl Res*. 2023;15(1):47–62.
23. Shangguan L, Xue L, Shang J, Wang H. The application value of metagenomic next-generation sequencing in community-acquired purulent meningitis after antibiotic intervention. *BMC Infect Dis*. 2023;23(1):683. doi:10.1186/s12879-023-08672-4
24. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014;30(15):2114–2120. doi:10.1093/bioinformatics/btu170
25. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*. 2009;25(14):1754–1760. doi:10.1093/bioinformatics/btp324
26. Jing C, Chen H, Liang Y, et al. Clinical evaluation of an improved metagenomic next-generation sequencing test for the diagnosis of bloodstream infections. *Clin Chem*. 2021;67(8):1133–1143. doi:10.1093/clinchem/hvab061
27. Wilson MR, Sample HA, Zorn KC, et al. Clinical metagenomic sequencing for diagnosis of meningitis and encephalitis. *N Engl J Med*. 2019;380(24):2327–2340. doi:10.1056/NEJMoa1803396
28. Shang Y, Wu J, Liu J, et al. Chinese Society of Critical Care Medicine; Chinese Medical Association. Expert consensus on diagnosis and treatment of infection in severe neurosurgical patients in China (2017). *NatI Med J China*. 2017;97(21):1607–1614. doi:10.3760/ema.j.issn.0376-2491.2017.21.005
29. Kanaujia R, Biswal M, Angrup A, Ray P. Diagnostic accuracy of the metagenomic next-generation sequencing (mNGS) for detection of bacterial meningoencephalitis: a systematic review and meta-analysis. *Eur J Clin Microbiol Infect Dis*. 2022;41(6):881–891. doi:10.1007/s10096-022-04445-0
30. Unal I. Defining an optimal cut-point value in ROC analysis: an alternative approach. *Comput Math Methods Med*. 2017;2017:1–14. doi:10.1155/2017/3762651
31. Swanson PA, McGavern DB. Viral diseases of the central nervous system. *Curr Opin Virol*. 2015;11:44–54. doi:10.1016/j.coviro.2014.12.009
32. Glaser CA, Honarmand S, Anderson LJ, et al. Beyond viruses: clinical profiles and etiologies associated with encephalitis. *Clin Infect Dis*. 2006;43(12):1565–1577. doi:10.1086/509330
33. Saha S, Ramesh A, Kalantar K, et al. Unbiased metagenomic sequencing for pediatric meningitis in Bangladesh reveals neuroinvasive chikungunya virus outbreak and other unrealized pathogens. *mBio*. 2019;10(6):e02877–19. doi:10.1128/mBio.02877-19
34. Liu Q, Wang X, Xie C, et al. A novel human acute encephalitis caused by pseudorabies virus variant strain. *Clin Infect Dis*. 2021;73(11):e3690–e3700. doi:10.1093/cid/ciaa987
35. Feng L, Chen J, Luo Q, et al. mNGS facilitates the accurate diagnosis and antibiotic treatment of suspicious critical CNS infection in real practice: a retrospective study. *Open Life Sci*. 2023;18(1):20220578. doi:10.1515/biol-2022-0578
36. Zhou M, Wu Y, Kudinha T, et al. Comprehensive pathogen identification, antibiotic resistance, and virulence genes prediction directly from simulated blood samples and positive blood cultures by nanopore metagenomic sequencing. *Front Genetics*. 2021;12. doi:10.3389/fgene.2021.620009
37. Serpa PH, Deng X, Abdelghany M, et al. Metagenomic prediction of antimicrobial resistance in critically ill patients with lower respiratory tract infections. *Genome Med*. 2022;14(1):74. doi:10.1186/s13073-022-01072-4
38. Diao Z, Han D, Zhang R, Li J. Metagenomics next-generation sequencing tests take the stage in the diagnosis of lower respiratory tract infections. *J Adv Res*. 2022;38:201–212. doi:10.1016/j.jare.2021.09.012
39. Haslam DB. Future applications of metagenomic next-generation sequencing for infectious diseases diagnostics. *J Pediatric Infect Dis Soc*. 2021;10(Supplement_4):S112–S117. doi:10.1093/jpids/piab107
40. Boers SA, Jansen R, Hays JP. Understanding and overcoming the pitfalls and biases of next-generation sequencing (NGS) methods for use in the routine clinical microbiological diagnostic laboratory. *Eur J Clin Microbiol Infect Dis*. 2019;38(6):1059–1070. doi:10.1007/s10096-019-03520-3