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Letter to the Editor

Microbial landscape in cerebrospinal fluid of suspected intracranial infections based on clinical metagenomics, a multicentre retrospective study

To the Editor,

In this Journal, we previously reported our experience of use of metagenomics in our network of intensive care units.[1] Intracranial infections are complex disease entities, often associated with high morbidity and mortality, and can lead to permanent neurological sequelae. Particularly in intensive care units (ICUs), many patients develop intracranial infections of unknown etiology and require advanced life support devices for treatment.[2] However, the heterogeneity of the disease and the limitations of traditional diagnostic methods, such as culture, antigen/antibody testing, and polymerase chain reaction testing, lead to slow diagnosis and low positivity rates. In fact, over 50% of encephalitis cases remain undiagnosed with traditional testing methods.[3] In recent years, clinical metagenomics, as an advanced early diagnostic technique, has been widely applied in clinical practice.[4–9] Nevertheless, large-scale studies on the pathogen distribution in cerebrospinal fluid remain scarce. This study collected clinical metagenomic data from cerebrospinal fluid samples across multiple ICU centers, aiming to depict a comprehensive distribution of intracranial infection pathogens and provide crucial reference for further clinical diagnosis.

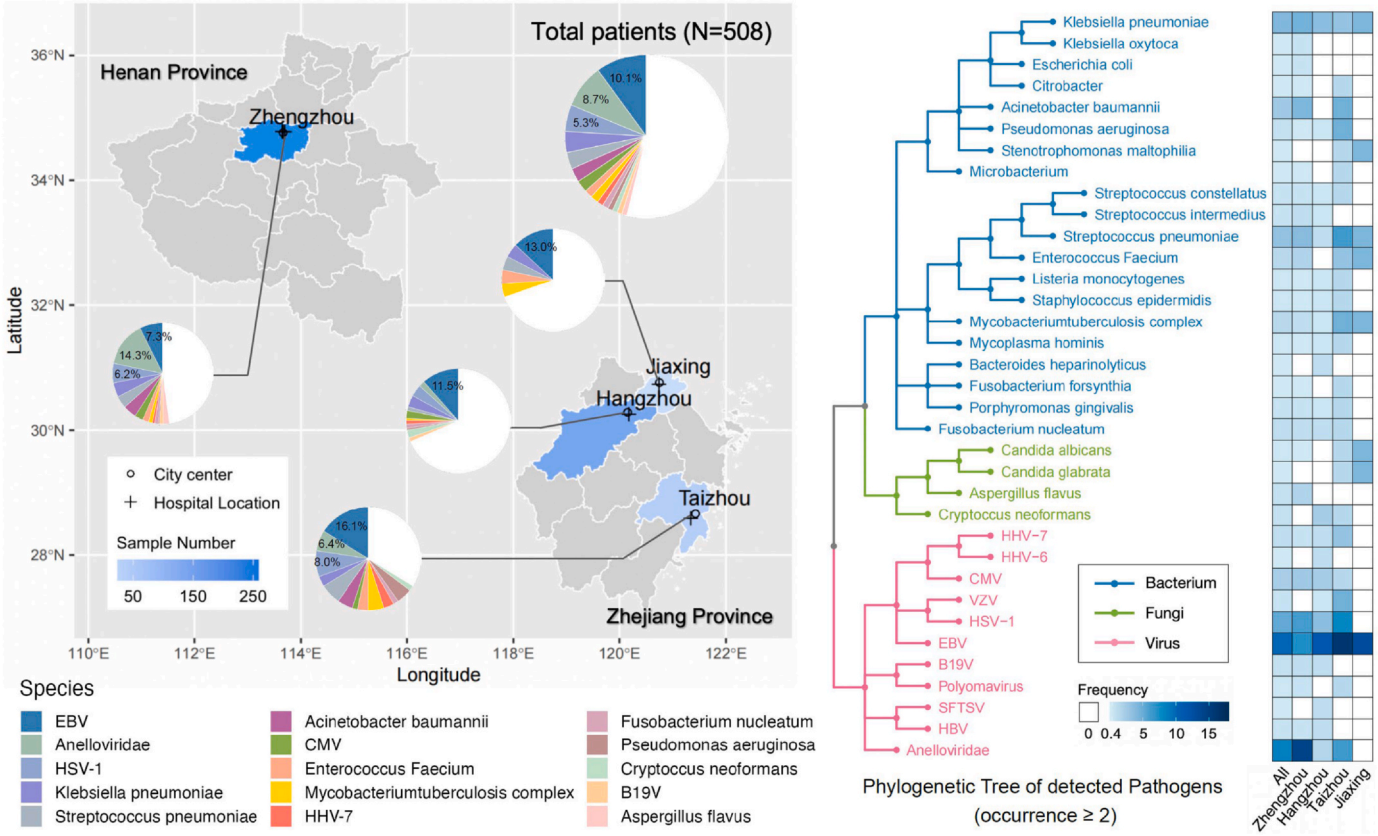

Fig. 1. Geographical distributions, frequency, and phylogeny of microbial species in four cities of mainland China from October 2019 to October 2023. Each city on the map is colored according to the sample size collected. Pie charts represent the proportional distribution of microbial species identified in each city. Each color in the pie chart corresponds to one of the 15 most frequently detected microbial species. The phylogenetic tree (on the right) shows the relationships among microbial species(occurrence ≥ 2), and the accompanying heatmap depicts their occurrence frequency across all cities.

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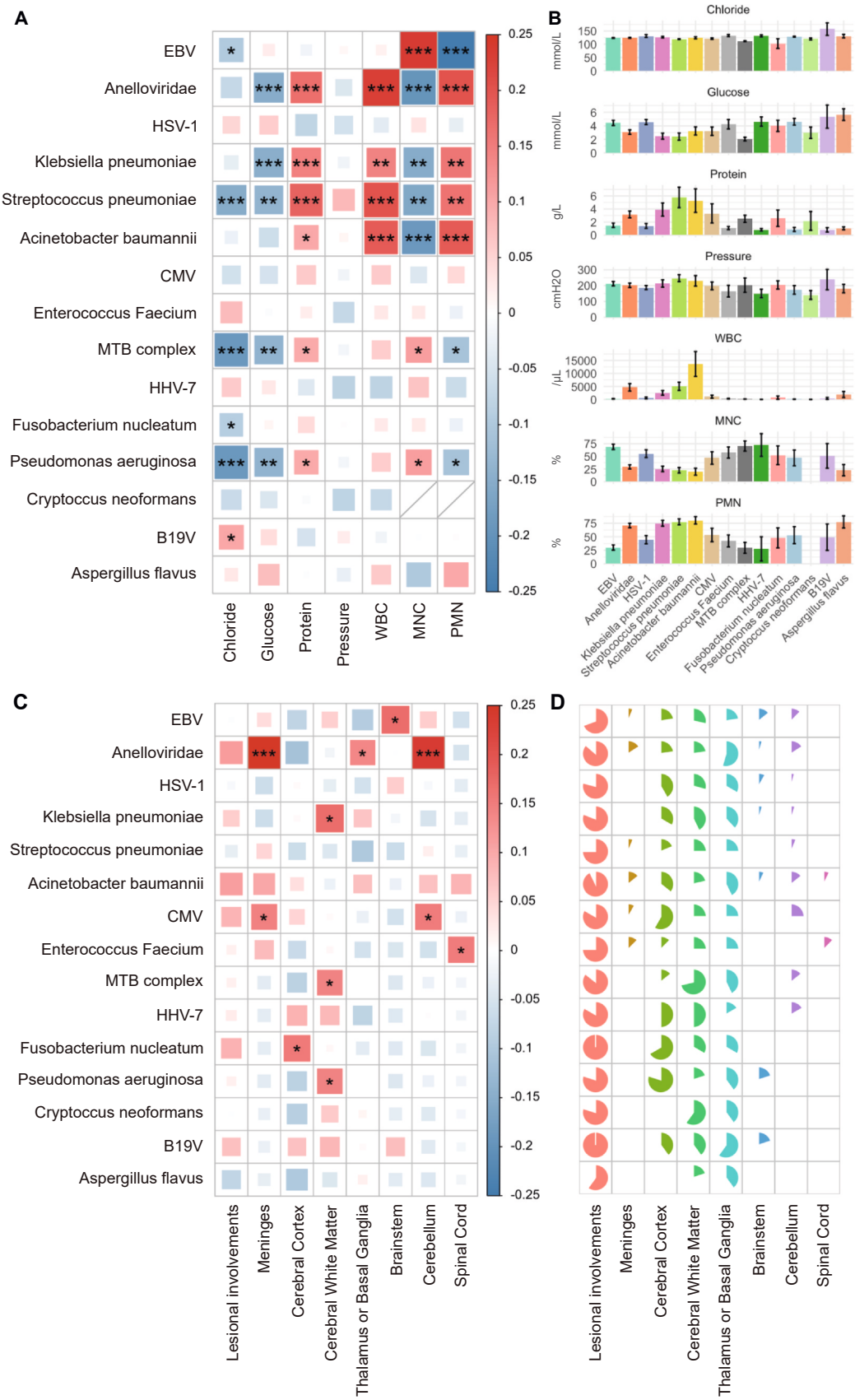

Fig. 2. Associations between pathogens and brain imaging/CSF parameters. (A) Correlation heatmap between microbial species and cerebrospinal fluid biochemical parameters. The size and color of the squares indicate the strength and direction of the correlation. (B) Cerebrospinal fluid biochemical parameters for patients positive for specific microbial species. (C) Correlation heatmap between microbial species and brain CT parameters. The size and color of the squares indicate the strength and direction of the correlation. (D) Proportion of patients with each abnormal brain CT parameter among those positive for specific microbial species. Statistically significant correlations are marked with * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$.

From December 2019 to October 2023, 507 ICU patients who received cerebrospinal fluid clinical metagenomics from 13 medical centers in 4 cities were included in the analysis. All clinical metagenomics testing requests were reviewed by senior physicians and approved if patients met one or more of the following criteria: (i) strong suspicion of central nervous system infection based on clinical presentation, laboratory testing, and/or imaging findings; (ii) recommendation for mNGS testing after consultation with neurology and/or infectious disease specialists. Demographic information and clinical data were obtained for these patients. A final composite diagnosis was determined through clinical adjudication and review of all clinical microbiologic tests by two independent clinical experts.

Data and statistical analysis

All data preprocessing, statistical analyses, and visualizations were performed in R (v4.3.1). Geographical visualizations were created using administrative boundaries of Zhejiang and Henan provinces, which were extracted from the DataV.GeoAtlas platform of Aliyun's data visualization lab. These geographical features were imported and visualized using the sf package (v1.0–14). A threshold criterion of ≥3 nonoverlapping viral reads aligning to the target viral genome was considered a positive detection for virus identification, while a threshold RPM ratio of ≥10 was considered positive for bacteria, fungi, and parasite detection.[10] Proportions of identified pathogens in each city were depicted with pie charts overlaid on a map of the study regions. Organism taxonomic hierarchies were obtained using the taxizedb package (v0.3.1), based on the NCBI taxonomy database. The phylogenetic tree, representing the relationships among identified pathogens, was constructed and visualized using the ggtree package (v3.4.4). Heatmaps of pathogen frequencies were also generated using this package. Associations between pathogen abundance (log2(RPM + 1) for bacteria/fungi and log2(reads + 1) for viruses) and brain CT/CSF biochemical parameters were assessed using Spearman's correlation, with the Hmisc package (v4.7–2), and correlation matrices were visualized using the corrplot package (v0.92). All other visualizations were created using customized plots from the ggplot2 package (v3.4.3).

A total of 507 patients were included in this study. Clinical metagenomic testing revealed that 222 patients tested positive for pathogens, with 72 patients being infected by more than one pathogen. A total of 89 microbial species were identified, including bacteria, DNA viruses, RNA viruses, and fungi, among which 35 species had a detection frequency of two or more times. The detected pathogens were distributed evenly across different cities (Fig. 1). The most common pathogens were DNA viruses (n = 161, 46.9%), followed by bacteria (n = 156, 45.5%), fungi (n = 20, 5.8%), and RNA viruses (n = 6, 1.7%).

Among the DNA viruses, the top five most frequently detected pathogens were: Epstein-Barr virus (EBV) (n = 51), Anelloviridae (n = 44), Herpes simplex virus 1 (HSV-1) (n = 27), Cytomegalovirus (CMV) (n = 12), and Human herpesvirus 7 (HHV-7) (n = 6), with Parvovirus B19 (B19V) (n = 5) also detected. The RNA viruses identified included: Severe fever with thrombocytopenia syndrome virus (SFTSV) (n = 2), Japanese encephalitis virus (JEV) (n = 1), Human metapneumovirus (HMPV) (n = 1), Hepatitis C virus (HCV) (n = 1), and Hepatitis G virus (HGV) (n = 1). A total of 141 non-fastidious bacterial pathogens, representing 56 unique bacterial species, were detected, which can be more easily identified using traditional culture methods. The three most common bacteria were: Klebsiella pneumoniae (n = 21), Streptococcus pneumoniae (n = 17), and Acinetobacter baumannii (n = 14). Additionally, 15 pathogens that are difficult to culture or grow slowly were detected, representing 6 unique bacterial species, including: Mycobacterium tuberculosis complex (MTB complex, n = 8), Mycoplasma hominis (n = 3), Mycoplasma salivarium (n = 1), Mycoplasma ureaplasma (n = 1), Leptospira interrogans (n = 1), and Afipia broomeae (n = 1). The most common fungal pathogens identified through mNGS were: Cryptococcus neoformans (n = 5), Aspergillus flavus (n = 5), and Candida albicans (n = 3).

To more intuitively demonstrate the relationship between pathogen infections and routine clinical test results, we selected pathogens with a detection frequency of five or more and conducted a correlation analysis (Fig. 2). Figs. 2A and 2B showed the correlation analysis between pathogen abundance and CSF cytology and biochemical markers. The analysis revealed characteristic patterns for Klebsiella pneumoniae, Streptococcus pneumoniae, and Acinetobacter baumannii: these pathogens were associated with reduced CSF glucose levels, elevated protein levels, and an increase in the number of nucleated cells, predominantly polymorphonuclear cells. Mycobacterium tuberculosis (MTB) infection, on the other hand, was characterized by a significant decrease in chloride levels and an increase in nucleated cells, predominantly mononuclear cells. EBV infection, although not associated with a significant increase in white blood cell count, showed a notable rise in the proportion of mononuclear cells ($p < 0.001$).

Figs. 2C and 2D showed the correlation patterns between pathogen abundance and the locations of neurological imaging abnormalities. Bacterial infections (such as Klebsiella pneumoniae, Mycobacterium tuberculosis, and Pseudomonas aeruginosa) were significantly associated with white matter lesions (all $p < 0.05$); CMV infection was significantly associated with cerebellar lesions ($p < 0.05$), which is consistent with previous reports.[11] The correlation between EBV and brainstem imaging abnormalities is consistent with our previous report.[9] Notably, Anelloviridae was significantly correlated with lesions in the meninges ($p < 0.001$), thalamus or basal ganglia ($p < 0.05$), and cerebellum ($p < 0.001$). Anelloviridae has traditionally been considered a harmless virus, usually remaining latent within the host without causing noticeable clinical symptoms.[12] Reports of intracranial infections caused by Anelloviridae are rare, and its clinical significance remains unclear. Further research is needed to elucidate its mechanistic role and clinical impact.

Conclusion

We depicted the pathogen landscape in the cerebrospinal fluid of patients with suspected intracranial infections. EBV and Anelloviridae, often overlooked viruses, were widely detected in our cohort, and these pathogens exhibited specific neurological imaging abnormalities and were correlated with certain cerebrospinal fluid biochemical and routine test results.

Ethical approval

The study has been approved by the ethics committees of Zhejiang University School of Medicine First Affiliated Hospital and other participating hospitals. As a retrospective study, informed consent was waived.

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Author contribution

QX, LTH, CJ, KW designed the study, HLZ, XDR, YJL, XHJ, XW, XHH analyzed the data and wrote the manuscript, all author participated in data collection. All investigators participated in the discussion and agreed with the final version of the manuscript.

Data Availability

The data can be obtained from the corresponding author LTH (lingtonghuang@zju.edu.cn) upon reasonable request.

Declaration of Competing Interest

None.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT in order to polishing language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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