

Haemophilus aphrophilus and *Eikenella corrodens* Coinfection of Brain: An Unusual Case from China

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Background: The HACEK group comprises *Haemophilus* spp., *Aggregatibacter actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*, are Gram-negative bacteria that are slow-growing and fastidious. These organisms are common causes of culture-negative endocarditis. However, brain abscesses caused by *Haemophilus aphrophilus* and *E. corrodens* have been rarely reported. The case we describe, which was promptly identified and successfully treated, will be meaningful for the diagnosis and treatment of such infectious diseases.

Case Presentation: Herein, we report a case of brain abscess in a young man who was infected with *Haemophilus aphrophilus* and *E. corrodens*. The patient was admitted to the hospital with sudden onset of vomiting, coma, and fever. Magnetic resonance imaging (MRI) of the brain and cerebrospinal fluid cell counts suggested cerebral abscess, he underwent drainage of the abscess and empirical antimicrobial therapy of meropenem (2 g every 8 hours) and linezolid (0.6 g every 12 hours) for more than 10 days without significant improvement. Metagenomic next-generation sequencing (mNGS) of drainage fluid and matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) detection for isolated bacteria from samples suggested the presence of *H. aphrophilus* and *E. corrodens*. After 7 weeks of ceftriaxone (2 g every 12 hours) and meropenem (2 g every 8 hours) intravenously, the patient was discharged with a normal temperature and brain MRI showed improvement of the lesion.

Conclusion: Similar cases reported in previous studies were always associated with bacterial blood dissemination after dental surgery or myocarditis; however, the patient in our case had no any associated risk factors. As far as we know, this is the only case of central nervous system infection caused by *H. aphrophilus* and *E. corrodens* that has utilized combined mNGS and MALDI-TOF MS in the diagnosis.

Keywords: *Haemophilus aphrophilus*, *Eikenella corrodens*, HACEK, brain abscess

Introduction

The acronym HACEK refers to a group of fussy-growing Gram-negative bacteria, including *Haemophilus* spp., *Aggregatibacter actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*. HACEK group bacteria are common colonizers of the oropharyngeal, respiratory, gastrointestinal, and genitourinary tracts, and cause a majority of Gram-negative bacterial endocarditis: 1.2%–3% of all infective endocarditis.¹ However, its fussy growth environment renders identification using conventional microbiological cultures inefficient, and this usually leads to delayed diagnosis and disease aggravation. *H. aphrophilus* and *E. corrodens* are microaerobic Gram-negative bacilli, mostly colonize the oral cavity, and trigger invasive infections, including infective endocarditis, intracranial infections, liver abscess, and lung infections,² under specific circumstances, such as immunosuppression, trauma, and others. Brain abscesses of these organisms commonly result from the spread of neighboring infections, with approximately a third associated with odontogenic infections, the remainder being severe otitis media, sinus tract infections, or mastoiditis. Patients usually present with headache, brain-nerve damage, and fever.³ Intracranial infections secondary to *H. aphrophilus* and *E. corrodens* are unusual. Existing reports suggest that they are mostly related to dental surgery,

travel, drug abuse, contact with pets, or spinal surgery.^{4–7} Imaging can be a effective diagnostic tool, but final treatment mainly relies on pathogenetic diagnosis. Our study retrospectively analyzed a case of brain abscess infected with HACEK-group bacteria to investigate the diagnostic and therapeutic course, emphasizing the importance of hypothesis-independent molecular techniques such as metagenomic next-generation sequencing (mNGS) and matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) in rapid diagnosis.

Case Presentation

The patient was admitted to our hospital due to a sudden outbreak of vomiting and coma for 5 hours on October 23, 2022. Brain MRI suggested rounded nodular abnormal-density foci in the right frontoparietal lobe. The size of the largest one was about 2.9×3.6 cm, and brain abscess was considered. The physical examinations showed that his general situation was well. The patient had developed weakness of the left side of the limbs, fever, and headache 3 months prior, and recovered gradually after being treated with linezolid empiric anti-infective therapy. This admission was considered for abscess recurrence. It is worth noting that the patient had no history of pet ownership, travel, drug abuse, dental disease, or surgery. He underwent “transcranial endoscopic brain abscess incision and drainage combined right fronto-temporal parietal decompression of cranial bone flap” under general anesthesia on the day of admission, and the operation went smoothly. Intraoperative histopathology of the brain abscess revealed large numbers of inflammatory cells (Figure 1).

He was then transferred to the emergency ICU for further treatment after the operation and given meropenem (2 g every 8 hours) and linezolid (0.6 g every 12 hours) to fight infection, antiepileptic treatment, dehydration treatment to lower the cranial pressure, and nutritional support. During the hospitalization, blood tests showed white blood cells - $15.06 \times 10^9/L$ (neutrophils 87%), hemoglobin 91 g/L, platelets $278 \times 10^9/L$, CRP 18.48 mg/L, and PCT 0.28 ng/mL. Renal and liver function indices were within normal range. In order to continue CSF drainage, lumbar large pool drainage was performed, and three tubes drainage fluid were sent for conventional counting, biochemistry, bacterial culture, and mNGS examination. On routine CSF examination, total leukocyte count was $950/\mu L$, of which neutrophilic lobulated nucleated granulocytes occupied 70% and lymphocytes occupied 30%. The Pandy test was positive, and CSF biochemical testing showed glucose was 2.34 mmol/L, chloride 111.33 mmol/L, and total protein 641.61 mg/dL. The mNGS results were suggestive of *H. aphrophilus* and *E. corrodens* sequences.

Meanwhile, CSF samples were inoculated with 5% sheep blood, chocolate agar, MacConkey agar, and Sabouraud dextrose agar. These were all incubated at 37°C for 48 h, and the chocolate agar plate was inoculated in a CO₂ environment at a concentration of 5%. Figures 2 and 3 show the colony morphology on the chocolate plate and the microscopic bacterial morphology, respectively. Samples were then analyzed by MALDI-TOF MS (Vitek MS Biomedical Sciences), and the results suggested consistency with the mNGS. The taxonomic profile is shown in Figure 4. Following consultation with the pharmacy department, ceftriaxone (2 g every 12 hours) and meropenem (2 g every 8 hours) were given against *H. aphrophilus* and *E. corrodens*. The patient was discharged in good general condition after a total of 7 weeks of antibiotic

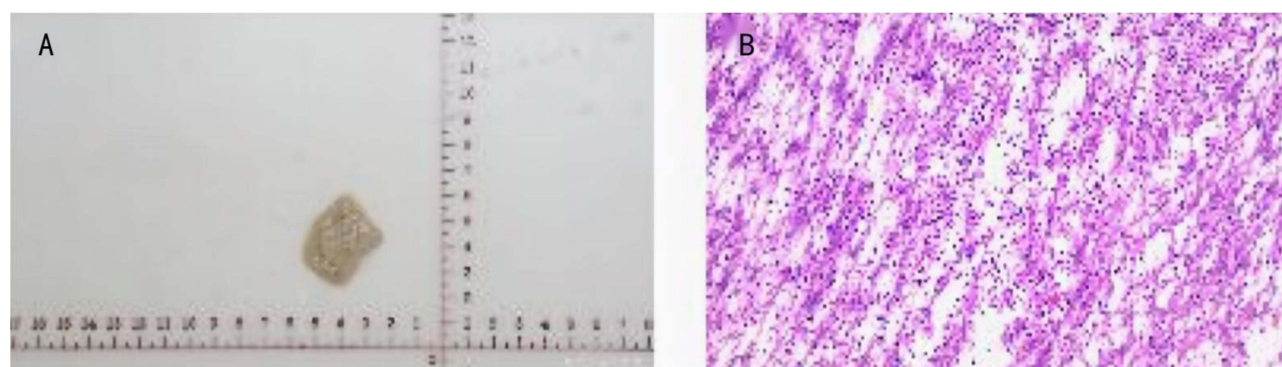


Figure 1 Histopathology of the brain abscess tissue specimen obtained from the mediastinal mass. Hematoxylin and eosin (H&E) staining showed extensive neutrophil cell infiltration. (A) Brain abscess tissue isolated during surgery. (B) H&E staining of brain abscess tissue.

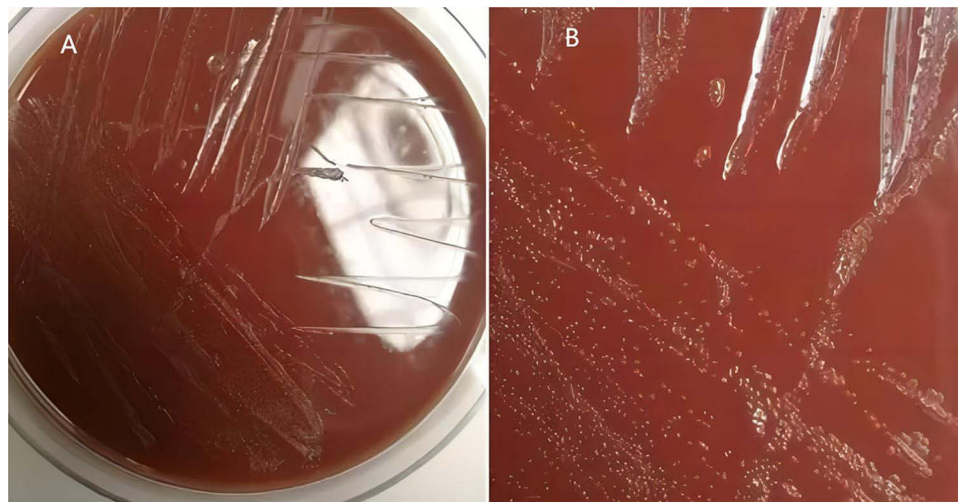


Figure 2 Colony morphology on chocolate agar plate. (A) Slow-growing colonies on chocolate agar plate in a CO₂ environment with a concentration of 5% after 48 h. (B) Image of the colonies after magnification.

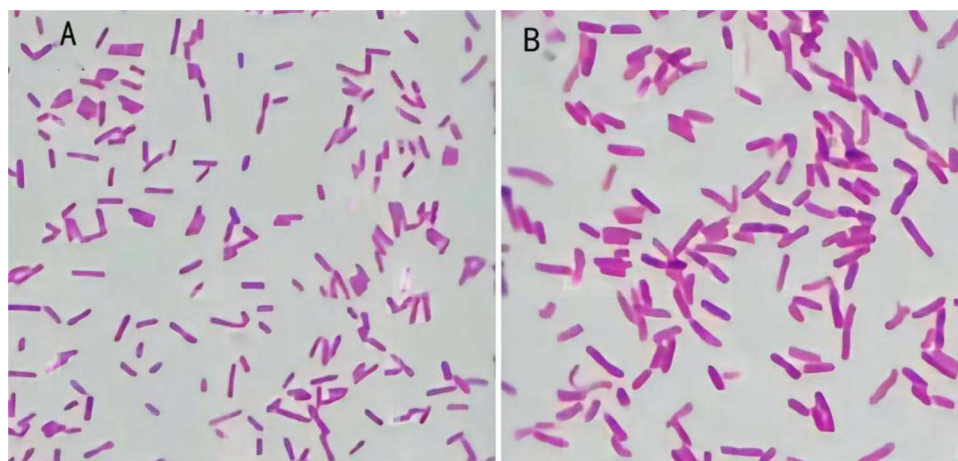


Figure 3 Gram-stained morphological observations of two pathogenic bacteria under the microscope. (A) *Haemophilus aphrophilus* is a small Gram-negative bacillus. (B) *Eikenella corrodens* is similar in morphology to *H. aphrophilus*, except that the organism is slightly larger.

treatment. Brain MRI showed that the lesion had disappeared. Images taken before and after treatment are shown in Figure 5. The diagnosis and treatment details are listed in Figure 6.

Discussion

Herein, we have presented a case of previously healthy young man with a complex brain abscess with both *H. aphrophilus* with *E. corrodens* infection. According to the literature, HACEK-group bacteria are fussy-growing Gram-negative organisms frequently seen in patients with infective endocarditis, as well as in periodontal infections, abscesses, and nonendocarditis bacteremia secondary to focal infections. Both *H. aphrophilus* and *E. corrodens* are considered commensal organisms of the oropharynx that are encountered in brain abscesses infrequently. No cases of these two bacteria infecting the brain had been reported in China until April 2023, when Lo Biundo et al⁸ published a case of brain infection resulting from *H. aphrophilus* and *E. corrodens* in a 26-year-old woman admitted to hospital with a history of left buccal margin excursion and ipsilateral monoplegia of the upper limb for 7 days. That was the only case reported with mixed infection by these two pathogens until now. MRI confirmed the presence of brain abscess and revealed a hypodense lesion in the left frontal lobe with extensive perifocal edema. The two pathogens were identified using MALDI-TOF MS. Similar to our report, there was no history of relevant risk factor exposure, as mentioned in previous

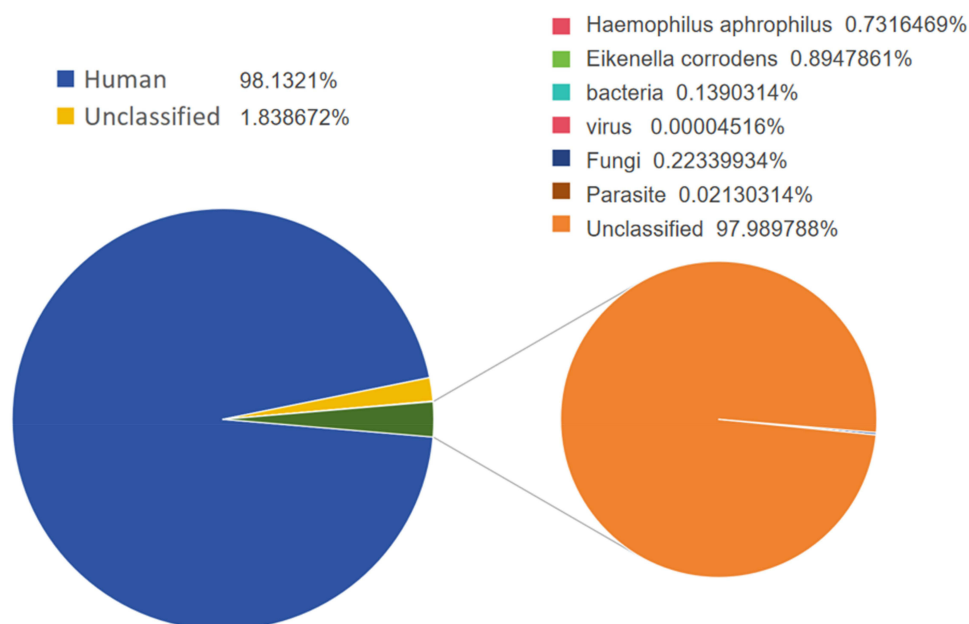


Figure 4 mNGS analysis reveals the mapping of *Haemophilus aphrophilus* with a coverage of 3.7% and *Eikenella corrodens* reads with a coverage of 0.89%. (Left) Total read distribution in the sample and (right) read distribution without the human host.

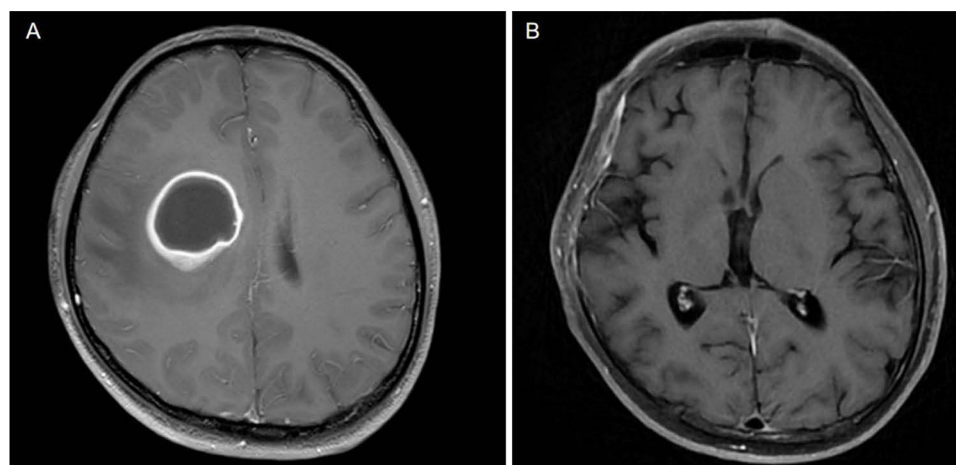


Figure 5 Enhanced cranial magnetic resonance imaging in patients with intracranial infections on T₁-weighted images. (A) On October 23, 2022, rounded nodular abnormal-density foci about 2.9×3.6 cm in size could be seen in the right frontoparietal lobe. (B) After 7 weeks of treatment, no significant foci of enhancement could be observed.

literature.⁹ However, our case had limb weakness and headache up to 3 months prior to hospitalization successfully diagnosed by mNGS and MALDI-TOF MS. This might have been due to a failure in identifying the pathogens and improper use of antibiotics that allowed the bacteria to remain in the brain tissue, which would have led to the proliferation of the bacteria and worsening infection when the patient's immune system became compromised. What is noteworthy is the site of abscess, both located in the frontal lobe in the two cases, which rationally explains their similar behavioral changes and weakness and paralysis of the lateral limbs, as neurological signs depend on the location of lesions.

The young man had the symptoms of headache, fever, and weakness of the left limbs, with a maximum temperature of 39.5°C and positive signs of meningeal irritation. CSF cell count showed a leukocyte level in the CSF of 950/μL, decreased glucose and chloride, and significantly increased intracranial pressure; therefore, the initial clinical diagnosis was purulent meningitis. The clinician excluded intracranial tumor based on MRI and histopathologic examination, and

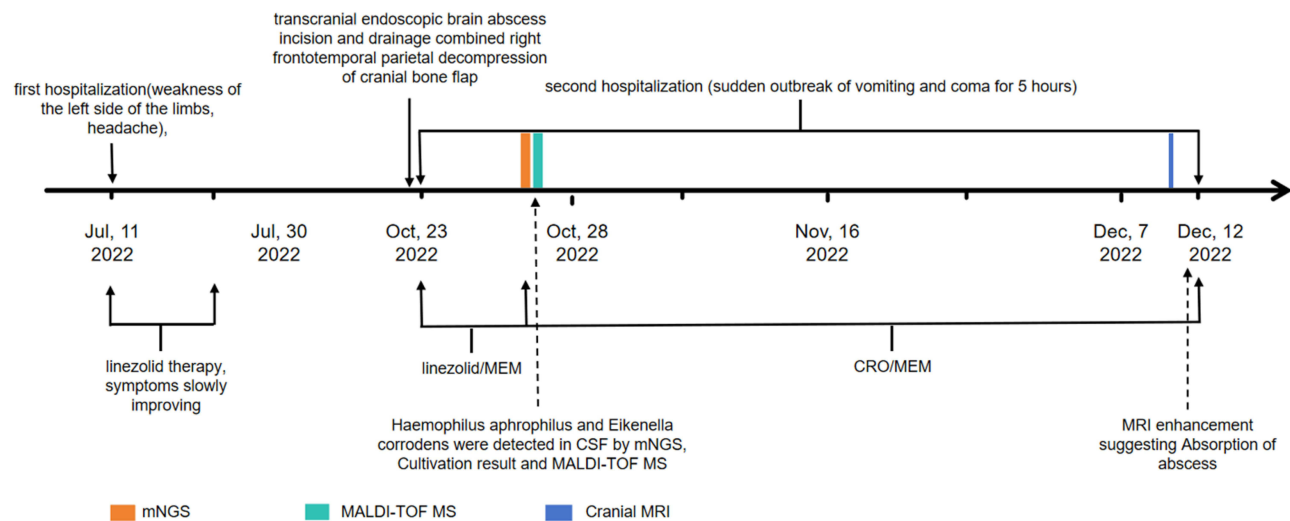


Figure 6 Timeline of patient's diagnosis and treatment.

Abbreviations: CRO, ceftriaxone; MEM, meropenem.

H. aphrophilus and *E. corrodens* were ultimately confirmed based on CSF mNGS and MALDI-TOF MS. HACEK-group bacteria are Gram-negative with high nutritional requirements. *H. aphrophilus* grows slowly on chocolate plates in the CO₂ environment with a concentration of 5% instead of ordinary plates,¹⁰ thus adding to the difficulty in identifying this organism. Our case highlights the importance of these two novel diagnostic technologies in the identification of pathogens that are difficult to culture.

MALDI-TOF MS shows good performance in identifying slow-growing caustic bacteria, providing a rapid and accurate method.^{11,12} In contrast, mNGS is a high-throughput technology¹³ that sequences DNA or RNA nucleic acids directly¹³ and does not require culture. This allows mNGS to identify multiple pathogens, including exceptional, newly discovered, and mixed-infection bacteria with high accuracy and efficiency.¹⁴ Numerous studies have shown that mNGS improves the diagnostic efficiency of culture-negative cases when patients have been treated with antimicrobial drugs,¹⁵ and demonstrated strong diagnostic ability in central nervous system infection, with an overall positive-detection rate of 15.7%-57%, sensitivity of 73%-92%, and specificity of 96-99% according to previous reports.^{16,17} Traditional tests for isolation of *H. aphrophilus* and *E. corrodens* are time-consuming and laborious. We innovatively combined MALDI-TOF with mNGS and identified the pathogens complemently and accurately in this case. This means that MALDI-TOF MS and mNGS are alternative diagnostic tools for microbiological evidence to prevent further aggravation of infections with shorter turnaround.

Therapeutic methods for brain abscesses consist mainly of neurosurgical aspiration and antibiotic therapy. The empirical antibiotic regimen is usually third-generation cephalosporins combined with metronidazole.¹⁸ However, commonly drugs in infectious cases of *H. aphrophilus* or *E. corrodens* are triple cephalosporins, meropenem, and vancomycin.^{19,20} So far, there have been few cases, and the drug selection needs further study. A recommended drug following antibiotic-susceptibility testing is usually the first choice, yet most hospitals have not developed antibiotic-susceptibility testing for these scarce bacteria,^{21,22} which makes the treatment difficult and can lead to an unsatisfactory treatment result. The patient in this case received incision drainage of the brain abscess, intravenous anti-infection drugs, high-flow oxygen, and nutritional support. In terms of antibiotic use, the initial treatment regimen was meropenem combined with linezolid in order to cover most Gram-negative and Gram-positive bacteria, though it did not work well. After the pathogens had been clarified, the symptoms significantly improved after 7 weeks of adjusted antibiotic treatment of a tertiary cephalosporin-ceftriaxone-meropenem combination. The patient continued oral linezolid treatment for 2 weeks after discharge from hospital, and MRI suggested the abscess was almost absorbed and his condition was stable with no recurrence at follow-up 2 months later.

In a previous study, a treatment course of 6 weeks or more was necessary due to the recurrent nature of these bacterial infections,²³ recommending that antimicrobials be taken for at least 4-6 weeks and the duration of antibiotic treatment should be

extended depending on the patient's condition. Despite advances in diagnosis and treatment, central nervous system infections retain high morbidity and mortality rates.²³ Early diagnosis and treatment are crucial, especially in cases of rare pathogen infections. To alleviate the pain of the patient, shorten the duration of the illness, and improve their prognosis, we should apply CSF mNGS in the early stages of onset. Antibiotic-susceptibility testing for HACEK-group bacteria is impractical for many laboratories because of fastidiousness of these organisms' growth, and hence published reports and guidelines frequently provide the only guidance for antimicrobial selection.^{8,24,25} The therapeutic procedure and dosing regimen in this report are valuable for the treatment of HACEK-group bacteria infections, especially in immunocompetent patients. Additional antibacterial drugs and aggressive interventions, such as abscess drainage and nutritional support, could improve the effectiveness of treatment.

Conclusion

This case of brain abscess caused by coinfection with *H. aphrophilus* and *E. corrodens* without apparent incentive is the first to be reported in a patient in China. The patient was discharged in good condition after prompt and appropriate anti-infective treatment. The diagnosis and treatment process of this report is thus informative for similar cases. To avoid serious consequences of aggravation, clinicians should consider the possibility of infection with rare pathogens in cases of difficult diagnosis. Furthermore, this case highlights the significant advantages of mNGS and MALDI-TOF MS in detecting unique and mixed infections.

Abbreviations

MRI, magnetic resonance imaging; CSF, cerebrospinal fluid; mNGS, metagenomic next-generation sequencing; MALDI-TOF MS, matrix-assisted laser desorption ionization–time-of-flight mass spectrometry.

Data Sharing

All the data that support the findings of the case report have been provided in the article, and are available from the corresponding author upon reasonable request.

Ethics and Consent

Written informed consent to have the case details and any accompanying images published was provided by the patient. The Ethics Committee of the First Affiliated Hospital of Nanchang University approved the waiver in this case report based on Chinese ethics guidelines for clinical research to publish the case details.

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This paper has been uploaded to ResearchGate as a preprint: https://www.researchgate.net/publication/377519464_Haemophilus_aphrophilus_and_Eikenella_corrodens_co-infection_of_brain_a_unusual_case_from_china.

Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas, took part in drafting, revising, or critically reviewing the article, gave final approval to the version to be published, have agreed on the journal to which the article has been submitted, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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