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CASE REPORT

Metagenomic Next-Generation Sequencing Direct Diagnosis of Mixed Bacterial Meningitis in a Child: A Case Report

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Background: Bacterial meningitis is a major cause of mortality and morbidity in children worldwide. Meanwhile, mixed bacterial meningitis is a rare entity. However, it is difficult to identify anaerobic pathogens using traditional culture methods. Disorders such as neurosurgical interventions are considered risk factors for the development of such cerebral infection. Early diagnosis and appropriate therapy may contribute to patient survival.

Case Presentation: We report the use of metagenomic next-generation sequencing (mNGS) to facilitate the diagnosis and treatment of polymicrobial meningitis in a 35-month-old male child with a history of detethering procedure for tethered cord syndrome (TCS). The mNGS tests identified six bacterial species from CSF specimens, including four ones of anaerobic bacteria. The subsequent examination of magnetic resonance imaging (MRI) revealed abnormal imaging findings of the lumbosacral area. The patient eventually recovered from severe infections due to long-term antibiotic treatment and radical surgery.

Conclusion: This case demonstrates the advantages of mNGS for the rapid and accurate diagnosis of mixed bacterial meningitis, highlighting its application of identifying uncommon pathogens in severe infections. For patients who suffered from exacerbated brain infection with history of detethering surgery, anaerobic cultivation is needed and empiric antibiotic therapy should consider mixed infection in these situations.

Keywords: meningitis, metagenomic next-generation sequencing, children, case report

Introduction

Bacterial meningitis (BM) is a devastating disease, with a mortality rate of up to 30%, an adverse neurological outcome in up to 50% of survivors, and can be fatal within 24 hours.[1,2] When BM is suspected, a lumbar puncture is essential, and the cerebrospinal fluid (CSF) should be sent for complete cell count, culture, Gram staining, and glucose and protein levels.[3] These traditional methods are cheap and well-validated but may lack sensitivity or specificity. Therefore, early and accurate identification of pathogens and directed antibiotic therapy are important. Delays in the administration of antibiotics for 3–6 hours were associated with increased mortality.[4]

Polymicrobial meningitis including anaerobes is a rare condition. Anaerobic meningitis is associated with significant neurological sequelae and a high mortality rate.[5,6] However, precise detection of these anaerobic pathogens remains difficult. First, anaerobic bacteria cultivation was difficult which required suitable equipment, specific atmosphere and adequate laboratory conditions.[7] Second, the anaerobic bacterial culture of CSF was not recommended[8] and is not performed routinely in most hospitals. A range of predisposing factors are known to increase the likelihood of intracranial anaerobic (mixed) infections, some of which are secondary to mastoiditis, sinusitis, dental caries, traumatic penetrating injuries, neurosurgical procedures[5] and chronic suppurative otitis media (CSOM).[6] Among them, tethered

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cord syndrome (TCS) is a neurological disorder which involves incomplete development of the spinal column and spinal cord. One common cause of TCS is dermal sinus tract (DST).[9] Dermal sinuses provide a pathway for infection, and meningitis or intraspinal abscess occurs in nearly half of all cases. The most common organisms are *Staphylococcus aureus* and *Escherichia coli*, followed by *Proteus species* and anaerobic organisms like *Bacteroides, Peptococcus*[10] and *Bifidobacterium* spp.[11] et al. Combined aerobic-anaerobic organisms may be cultured in 20% of cases.[12]

Metagenomic next-generation sequencing (mNGS) can overcome the limitations of the conventional diagnostic approaches. We report a case of polymicrobial mixed aerobic-anaerobic meningitis secondary to DST in which six species of bacteria were identified using mNGS, which illustrated that mNGS, as a novel culture-independent approach, demonstrated the capability of rapid, sensitive, and accurate pathogen identification.

Case Presentation

A 35-month-old boy was admitted for meningitis as he presented with a fever of 6 days duration associated with right-leg pain, unstable walking, and dysuria. He was treated outside the hospital with amoxicillin sodium clavulanate potassium (dosage unknown) for 3 days for a presumed common infection. On the 4th day, his condition worsened, with headache, occasional vomiting, lethargy, and uracratia. He then presented to the local Pediatric Intensive Care Unit (PICU). Lumbar puncture revealed turbid cerebrospinal fluid (CSF) with a white blood cell count of 183×10^6/mL and 96% polymorphonuclear leukocytes. CSF glucose was 0.04 mmol/L and CSF protein level was 8.19 g/L. Gram staining revealed the presence of both gram-positive and Gram-negative bacteria. After 3 days of empirical therapy consisting of intravenous vancomycin (60 mg/kg/day) and meropenem (40 mg/kg/day), the patient was transferred to our hospital and the same antimicrobial therapy was continued. Past history revealed the patient had had surgical repair of "tethered cord syndrome (TCS)" at 13 months of age without complications. Physical examination revealed clear meningeal syndrome with neck stiffness and Brudzinski signs. Other neurological examinations included reduced power in the right leg (grade 0) and left leg (grade 3) with an upgoing left plantar response. Magnetic resonance imaging (MRI) of the head showed a thickened bilateral cerebral sulcus and left frontotemporal dura with bilateral ventricular empyema (Figure 1). Lumbar puncture was performed on day 2 and his CSF sample was also sent to the laboratory for mNGS analysis besides traditional tests. CSF was creamy white, turbid, and purulent. CSF cell count was $457{,}530 \times 10^6$/L, with $120{,}000 \times 10^6$/L red cells, 82% polymorphs, and 18% lymphocytes. CSF glucose was 0.36 mmol/L compared to a serum glucose of 14.2 mmol/L. The CSF protein level was 14.13 g/L. A CSF smear was reported for Gram-positive bacteria. The mNGS results suggested the existence of aerobic and anaerobic bacteria (Table 1) on day 3. CSF culture revealed *Enterococcus avium* (*E. avium*) and *Proteus Vulgaris* (*P. vulgaris*) on day 5. *E. avium* was sensitive to a high-level gentamicin disk, ampicillin, linezolid, vancomycin and teicoplanin. *P. vulgaris* was sensitive to cefoperazone-sulbactam and piperacillin-tazobactam. He developed a flushed complexion and neck after an intravenous drip of vancomycin, so the antibiotics were changed to linezolid (0.15 g, q8h), meropenem (0.6 g, q8h), and rifampicin (0.15 g, q12h). Considering the anaerobic bacteria, metronidazole (0.11 g, q8h) was added. We performed further investigations (skull base-chest-abdomen computed tomography (CT) scans and whole-spine MRI (Figure 2)) to identify the source of infection. Unfortunately, these

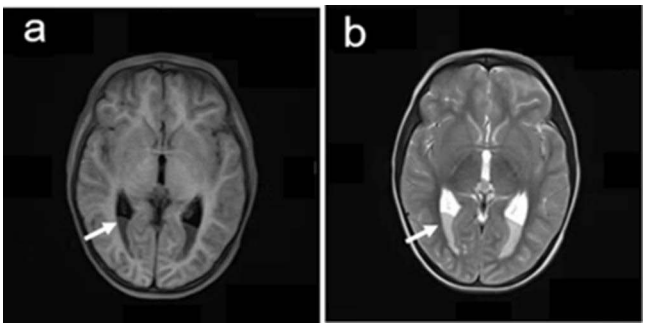

Figure 1 Brain MRI images of the head on the 2th day in our hospital. (**a**). T1-weighted imaging (T1WI) showed ventricles empyema (the white arrow). (**b**). T2-weighted imaging (T2WI) showed ventricles empyema (the white arrow).

Table 1 mNGS Results of the Patient

Species Name	Sequence Number	Coverage	Relative Abundance
P. uenonis	30757	38.19%	4.36%
E. lenta	33131	39.24%	5.91%
M. curtisii	22214	6.87%	1.66%
B. thetaiotaomicron	12773	14.09%	2.18%
E. avium	9662	14.35%	1.29%
P. penner	2253	4.1%	0.16%

investigations did not yield positive results. His fever continued over the next 14 days and then resolved for 13 days. His consciousness was clear, but there is no significant recovery in muscle strength. However, no cerebrospinal fluid outflowed on day 27 (We inferred the reason lied in the poor fluidity of CSF because of its high protein concentration), and he developed a fever of 38.8°C on day 29. The patient was discharged to another hospital on day 30 and became apyrexial after 11 days of antibiotic therapy. Repeated brain MRIs showed conspicuously enlarged ventricles.

The patient was referred to a neurosurgery hospital. The patient underwent emergency ventricular drainage and was treated with intraventricular injections (drugs, dosage, and duration unknown). An external ventricular–abdominal wall drainage system was installed; thereafter, the patient showed steady but slow improvement. On day 73, the patient gradually regained power in his limbs, except for the right leg (grade 4). He was extubated on day 111 and discharged on day 117. Enhanced lumbosacral CT revealed multiple enhancements in the spinal canal and a subcutaneous soft tissue abscess that was subsequently radically removed through resection of DST, tethered cord release, and L4–L5 laminectomy. After 12 months of follow-up, no signs of relapse were observed. The patient almost completely recovered from his neurocognitive impairment, although subtle claudication of the right foot persisted.

mNGS Analysis

CSF specimens were delivered to the Central Laboratory of Shandong Provincial Hospital for pathogen(s) detection. The mNGS results showed six species of bacteria, namely, *Porphyromonas uenonis* (*P. uenonis), Eggerthella lenta* (*E. lenta), Mobiluncus curtisii* (*M. curtisii), Bacteroides thetaiotaomicron* (*B. thetaiotaomicron), E. avium*, and *Proteus penneri* (*P. penneri*) (Table 1). *P. uenonis, E. lenta, M. curtisii*, and *B. thetaiotaomicron* which are all anaerobic bacteria, whereas *P. penner* is a facultative anaerobe. Due to mNGS only profiles nucleic acid in a sample, no antimicrobial susceptibility test was performed.

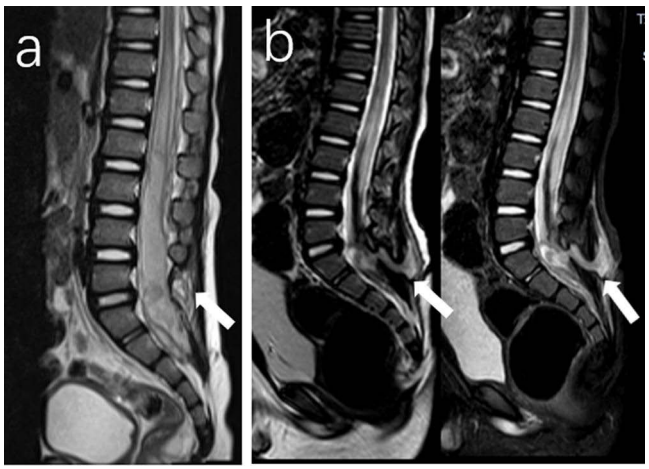

Figure 2 Lumbosacral MRI images on the 5th day and 124th day. (**a**). T2-weighted imaging (T2WI) magnetic resonance image taken on the 5th day in our hospital shows postoperative changes (the white arrow). (**b**). T2-weighted imaging (T2WI) magnetic and Enhanced Short Tau Inversion Recovery (eSTIR) resonance image taken on the 124th day in another hospital shows abscess from subcutaneous soft tissue to the dura (the white arrow).

Discussion and Conclusion

We present a unique case of a pediatric patient with mixed aerobic and anaerobic bacterial meningitis who had previously undergone TCS surgery when he was 13 months old. It was thought that an untethering procedure had been excised successfully. However, the defect was finally found to be a dermal sinus. DST is a deep epithelium-lined tract, sometimes containing hair, that ascends from its external opening over the spine to terminate at a deeper level, sometimes communicating with the dura.[13] Spinal DSTs in the lumbosacral region are usually not recognized, especially when they are not associated with other cutaneous lesions. Many DST cases (48.6%) had some kind of infection on admission including life-threatening conditions such as meningitis.[14] Delay in diagnosis may cause neurological deficit or infectious complications as was seen in our case. A definitive operation with intradural exploration and complete excision of the tract should be undertaken at the initial operation after the control of meningitis in an attempt to obviate future complications.

Most studies have focused on monomicrobial meningitis. *Streptococcus pneumoniae* and *Neisseria meningitidis* are the predominant pathogens of BM cases in children.[15] The peculiarity of our case was the polymicrobial etiology of the intracranial infection, with a rare combination of Gram-positive and Gram-negative aerobic and anaerobic pathogens. We presume that the microorganisms that are all atypical agents identified by mNGS were responsible for this case of meningitis, whereas CSF culture yielded growth of *E. avium* and *P. vulgaris* only. This result suggests that some microorganisms cannot be detected using conventional laboratory methods. *E. avium* and *E. lenta* are Gram-positive bacteria that are part of the normal intestinal flora. *E. avium* has been responsible for approximately 1% of infections in humans,[16] including bacteremia, peritonitis, splenic abscess, intracranial suppurative infection and osteomyelitis.[17] *E. lenta* is also a relatively rare pathogen that has been commonly associated with illnesses of abdominal origin.[18] *E. lenta* infections in humans have been reported, referring to bloodstream infection, liver abscess, and meningitis.[19] The patient did not have any abdominal symptoms and a previous lumbosacral CT showed a subcutaneous abscess, so the probable routes of infection were through the skin. *B. thetaiotaomicron* was reported in the case of a 15-year-old boy with a brain abscess due to recurrent otitis media.[20] *P. uenonis*, which was detected in fecal specimens of six of 30 children, seems to be of relatively low virulence since it was always found in a mixed culture.[21] *M. curtisii* is primarily related to bacterial vaginosis and rarely causes infections of other organs.[22] Our patient had a mixed infection involving four species of anaerobic agents. The prognosis of anaerobic meningitis is challenging and the mortality rate is high. Anaerobic culture testing of CSF is not always a routine practice in our hospital because meningitis cases due to anaerobic agents are rarely encountered. The introduction of mNGS technology has facilitated bacterial identification in clinical practice. It has provided a promising means for pathogen-specific diagnosis and has updated diagnostic strategies for infectious diseases in different organ systems. It also guides efficient targeted clinical medication and treatment, which informs the development of novel therapeutic strategies and, in turn, improves patient outcomes and saves lives.

The etiological empirical treatment for acute BM is primarily based on guidelines, patient age, and local epidemiology. Vancomycin plus a third-generation cephalosporin was the initial empiric antibiotic used when a patient was suspected prior to a lumbar puncture. Vancomycin is active against most Gram-positive aerobic cocci and achieves an active concentration in the CSF after parenteral administration. Meropenem is a better choice because of its activity against most aerobic and anaerobic bacteria. In our case, the meropenem–vancomycin combination was initiated as the most appropriate choice in accordance with the extremely high number of CSF cells, and metronidazole was added to the anaerobes based on the results of mNGS. Clinical evolution was marked by an improvement in the clinical status through broad-spectrum antimicrobial therapy, which was finally resolved through a subsequent surgical approach.

In conclusion, the identification of each microorganism is critically important. Although the prevalence of community-acquired polybacterial meningitis is extremely low, it should be considered and identified rapidly and safely using mNGS. Our case presenting with infectious complications serves as an example of how early diagnosis and initiation of appropriate antibiotic treatment as early as possible can be life-saving. Surgery for DST resection contributed to full recovery.

Abbreviations

mNGS, metagenomic next-generation sequencing; BM, bacterial meningitis; CNS, central nervous system; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; CT, computed tomography; DST, dermal sinus tract; PICU, pediatric intensive care unit.

Data Sharing Statement

All the data in this study are included in the published articles.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Shandong Provincial Hospital Affiliated to Shandong First Medical University. Informed consent was obtained from the patient's parents for the publication of this case. No institutional approval was required to publish the case details.

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Disclosure

The authors declare that they have no competing interests.

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