Original Article

Application of metagenomic next-generation sequencing for the diagnosis of intracranial infection of *Listeria monocytogenes*

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Background: Intracranial infection of *Listeria monocytogenes* (LM) can lead to various manifestations, including meningitis, meningoencephalitis, brainstem encephalitis, and brain abscess, which often have a poor prognosis. Metagenomic next-generation sequencing (mNGS) is a promising new tool for the diagnosis of intracranial infection of LM. We describe the typical clinical manifestations of LM intracranial infection and highlight its rarity and severity to help physicians better understand the disease characteristics.

Methods: Six cases of severe LM intracranial infection were diagnosed by mNGS. We conducted a retrospective analysis of the data on disease progression, diagnostic tools, treatments, and outcomes, and summarized the findings. We compared the differences in diagnostic accuracy and timeliness between mNGS and etiological cultures.

Results: Among the 6 patients, 5 were males and 1 was female (age range 32–83). Three patients had a history of immunosuppressive therapy. Common symptoms included fever (100%) and a stiff neck (100%). Coma occurred early in severe patients (66%). Two healthy young patients had previously developed with meningitis, while coma occurred in 3 immunosuppressed patients and 1 elderly patient. Three immunosuppressed patients presented with brain abscess, brainstem encephalitis, and meningitis. 1 elderly patient presented with meningitis. Two patients developed septic shock complications early. Laboratory data showed normal or slightly increased leukocytes, neutrophils, and procalcitonin, and cerebrospinal fluid (CSF) tests were consistent with bacterial CSF infection. All 6 patients were examined for blood culture and CSF culture. The positive rate of blood culture and CSF culture was 50% and 16%. The average time from admission to positive culture findings was 91 h. All 6 patients were examined for CSF mNGS. Two were also examined for whole-blood mNGS. The positive rate for CSF mNGS and whole-blood mNGS results was 100%. The mean time from admission to positive mNGS report was 47 h. After diagnosis and treatment with sensitive antibiotics, 1 patient with brain abscess developed neurological sequelae, while the other 5 patients completely recovered.

Conclusions: mNGS can improve accuracy in the diagnosis of LM intracranial infection and reduce the delay in diagnosis. Intracranial infection of Listeria monocytogenes responds well to the timely use of appropriate antibiotics.

Keywords: *Listeria monocytogenes* (LM); intracranial infection; metagenomic next-generation sequencing (mNGS); early diagnosis; brain abscess

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Introduction

Listeria monocytogenes (LM) is a Gram-positive bacterium that is most common in low-temperature environments, and can survive for prolonged periods of time. Intracranial infection caused by LM is rare. The symptoms of non-specific intracranial infection and eating contaminated food, particularly unheated food that has been refrigerated, are the main criteria for its clinical diagnosis (1,2). Diagnosis of LM intracranial infection still depends on culture of LM or detection of LM in cerebrospinal fluid (CSF). As antibiotic treatment is different for different pathogens, it is important to identify the pathogens of intracranial infection after diagnosis. Conventional culture and smear can take time, and the positive rate is low. Metagenomic next-generation sequencing (mNGS) is a new tool that can quickly and accurately identify potential pathogens, which is of great clinical significance for patients with severe infection. In the present study, we describe the clinical characteristics of LM intercranial infection, as well as the accuracy and efficiency of mNGS for its diagnosis. To our knowledge, there has been no similar clinical study focusing on LM intercranial infection. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-2186/rc).

Methods

Study design

We conducted a retrospective case review of 6 patients admitted to The First Affiliated Hospital of Soochow University from April 2021 to September 2021. For each case, data on general information, clinical manifestations, laboratory tests [including routine hematology, cerebrospinal fluid (CSF) routine and biochemical, CSF culture and blood culture, and craniocerebral imaging], treatments, and outcomes were obtained from electronic medical records.

The present study was reviewed and approved by the ethics committee of The First Affiliated Hospital of Soochow University (No. 2022-074), and all data were anonymized prior to analysis. The study was conducted in accordance with the Helsinki Declaration (as revised in 2013). Individual consent for this retrospective analysis was waived.

mNGS was conducted using the following operational steps. First, CSF samples were collected from patients according to standard procedures. DNA was extracted using the TIANamp Magnetic DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China), according to the manufacturer's protocols. DNA quantity and quality of were assessed using Qubit (Thermo Fisher Scientific Co., Ltd., Shanghai, China) and NanoDrop (Thermo Fisher Scientific Co., Ltd., Shanghai, China), respectively. Second, libraries were prepared using the KAPA Hyper Prep kit (KAPA Biosystems, Roche, Switzerland), according to the manufacturer's protocols. Agilent 2100 (Agilent Technologies, Inc., Santa Clara, CA, USA) was used for quality control, and DNA libraries were 75-bp single-end sequenced on Illumina NextSeq 550Dx (Illumina Inc., San Diego, CA, USA). Third, we used an inhouse-developed bioinformatics pipeline for pathogen identification (Dinfectome Inc., Nanjing 213164, Jiangsu Province, China). Briefly, high-quality sequencing data were generated by removing low-quality reads, adapter contamination, and duplicated and shot (length: <36 bp) reads. Human host sequence was identified by mapping to human reference genome (hs37d5) using "Bowtie 2" software (2.2.6). Reads that could not be mapped to the human genome were retained and aligned with microorganism genome database for pathogen identification. Our microorganism genome database contained genomic sequences for bacteria, fungi, viruses, and parasites (download from GenBank release 238, https://ftp.ncbi.nlm.nih.gov/genomes/genbank/). Finally, we used the following criteria for positive mNGS results: for *Mycobacterium*, *Nocardia*, and *Legionella pneumophila*, the result was considered positive if a species detected by mNGS had a species-specific read number ≥1. For bacteria (excluding *Mycobacterium*, *Nocardia*, and *Legionella pneumophila*), fungi, viruses, and parasites, the result was considered positive if a species detected by mNGS had at least 3 non-overlapping reads. Pathogens detected in the negative 'no-template' control (NTC) were excluded, but only if the detected reads were ≥10-fold than that in the NTC.

Diagnostic criteria for LM intracranial infection

Patients diagnosed with LM intracranial infection met the following 3 criteria: (I) the criteria for intracranial infection (3); (II) specific fragment DNA of LM was identified using mNGS in the CSF of all patients; and (III) no other pathogens were detected in the CSF of all patients.

Statistical analysis

The categorical variables are expressed as absolute values and percentages. The continuous variables are expressed as mean and as ranges.

Results

Patient characteristics

Of the 6 patients, 5 were male and 1 was female; 3 patients had a history of immunosuppressive therapy (*Table 1*). The mean age of all patients was 56 years, with a range of 32–83 years. In the early stage of onset, all patients suffered high fever with body temperature ranging from 38.5 ℃ to 40.2 ℃; 3 patients also presented with headache. Two patients developed gastrointestinal symptoms (e.g., nausea, diarrhea). Two patients had fatigue and hemiplegia. Four of the 6 patients had severe symptoms, including disturbance of consciousness, septic shock, and acute kidney injury. For the patients with severe symptoms, the mean Acute Physiology and Chronic Health Evaluation (APACHE II) score and Sequential Organ Failure Assessment (SOFA) scores were 23.5 and 7.25, respectively.

Laboratory examination

Of the 6 patients, 3 had elevated white blood cell counts (mean: 9.25×10^9/L). Neutrophils were elevated in all patients, and lymphocytes were decreased. Procalcitonin was elevated in 2 patients, 1 patient had renal insufficiency, and 3 patients had early hyposodium and potassium. All patients completed lumbar puncture within 2 days of hospital admission. Their CSF was yellow and slightly cloudy, and in 5 cases, the pressure was significantly elevated. In the CSF of all patients, white blood cell count was significantly increased (mostly monocytes), CSF protein was severely elevated, glucose was significantly decreased, and chloride was slightly decreased.

All 6 patients were tested for blood culture and CSF culture. Among them, 3 patients had LM in blood culture and 1 patient had positive CSF culture. The average time from admission to positive culture report was 91 h. CSF mNGS was performed in all 6 patients, and whole-blood mNGS was also performed in 2 patients. LM was detected in all samples. The average number of LM sequences detected by mNGS in CSF samples was 404, and the number in the two whole blood samples was 12 and 42, respectively. The mean time from admission to positive mNGS test was 47 h, as shown in *Table 1*.

Imaging examination

All 6 patients underwent brain computed tomography (CT) scan or magnetic resonance imaging (MRI). Of these patients, CT or MRI showed that 4 patients had meningitis but no abnormalities, 1 patient had brain abscess formation (*Figure 1*), and 1 patient had low density in the brain stem, indicating brainstem encephalitis (*Figure 2*).

Treatment and outcome

After admission, all 6 patients were treated with mannitol to dehydrate and reduce cranial pressure, while piperacillin-tazobactam or third-generation cephalosporins were used for antiinfection. After confirming diagnosis of LM intracranial infection by mNGS and CSF or blood culture results, 5 patients were treated with meropenem combined with penicillin G and compound sulfamethoxazole tablets (SMZ), and 1 patient was given linezolid combined with etimicin and SMZ because of penicillin allergy. Five of the 6 patients improved following treatment. The other patient, who was misdiagnosed with cerebral infarction, developed a brain abscess with neurological sequelae.

Discussion

Six cases of intracranial infection caused by LM were retrospectively analyzed, including 4 cases of *Listeria* meningitis, 1 case of brain abscess, and 1 case of brainstem encephalitis. Intracranial infection caused by LM is more common in immunocompromised or elderly patients. Its clinical manifestations are meningitis and meningoencephalitis. Brainstem encephalitis and brain abscess are rare, and it has been documented that LM brainstem encephalitis is most common in healthy adults (4). LM-induced brain abscess only accounts for 10% of Listeria-induced intracranial infection, and mostly occurs

Table 1 Characteristics of all the patients

Characteristics	Patients, n (%)	Median value (range)
Demographics		
Male/female	5/1	–
Age, mean (range), years	–	56 (32–83)
Immunosuppressive status	3/6 (50.0)	–
Eating contaminated food/catch cold	4/6 (66.66)	–
Underlying disease	1/6 (16.6)	–
Clinical manifestations		
Fever >38.5 ℃	6/6 (100.0)	39.5 (39.0–40.2)
Headaches	3/6 (50.0)	–
Diarrhea, nausea, vomiting	2/6 (33.33)	–
Fatigue and hemiplegia	2/6 (33.33)	–
Neck rigidity	6/6 (100.0)	–
Coma	4/6 (66.66)	–
Hyperspasmia	2/6 (33.33)	–
Shock	2/6 (33.33)	–
Pathological signs were positive	2/6 (33.33)	–
APACHE II score	–	23.5 (15–27)
SOFA score	–	7.25 (1–14)
Laboratory examination		
Elevated WBC [normal: (4–10) $\times10^9$/L]	3/6 (50.0)	9.2 (5.24–15.0)
Elevated percentage of neutrophils (normal: 45–75%)	6/6 (100.0)	87.25 (84.4–92.6)
Reduced lymphocytes [normal: (1.2–3.8) $\times10^9$/L]	6/6 (100.0)	0.45 (0.24–0.50)
Increased PCT (normal: 0–0.5 ng/mL)	4/6 (66.66)	2.1 (0.11–8.7)
Abnormal liver and kidney function	1/6 (16.6)	–
Elevated CSF pressure (normal: 12–20 cmH_2O)	4/6 (66.66)	23 (15–40)
CSF white blood cells [normal: (0–8) $\times10^6$/L]	6/6 (100.0)	420 (166–765)
CSF monocyte ratio (%)	–	76.8 (35–96)
CSF glucose (normal: 2.5–4.5 mmol/L)	6/6 (100.0)	1.62 (0.5–2.3)
Elevated CSF protein (normal: 0.15–0.45 g/L)	6/6 (100.0)	3.09 (1.42–6.59)
Reduced CSF chloride (normal: 120–130 mmol/L)	6/6 (100.0)	114 (113–119)
Positive blood culture	3/6 (50.0)	–
Positive CSF culture	1/6 (16.6)	–
Time from admission to culture report (h)	–	91 (68–125)
Positive blood mNGS	2/2 (100.0)	–
Positive CSF mNGS	6/6 (100.0)	–
Time from admission to mNGS (h)	–	47 (33–56)

APACHE, Acute Physiology and Chronic Health Evaluation; CSF, cerebrospinal fluid; mNGS, metagenomic next-generation sequencing; PCT, procalcitonin; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell.

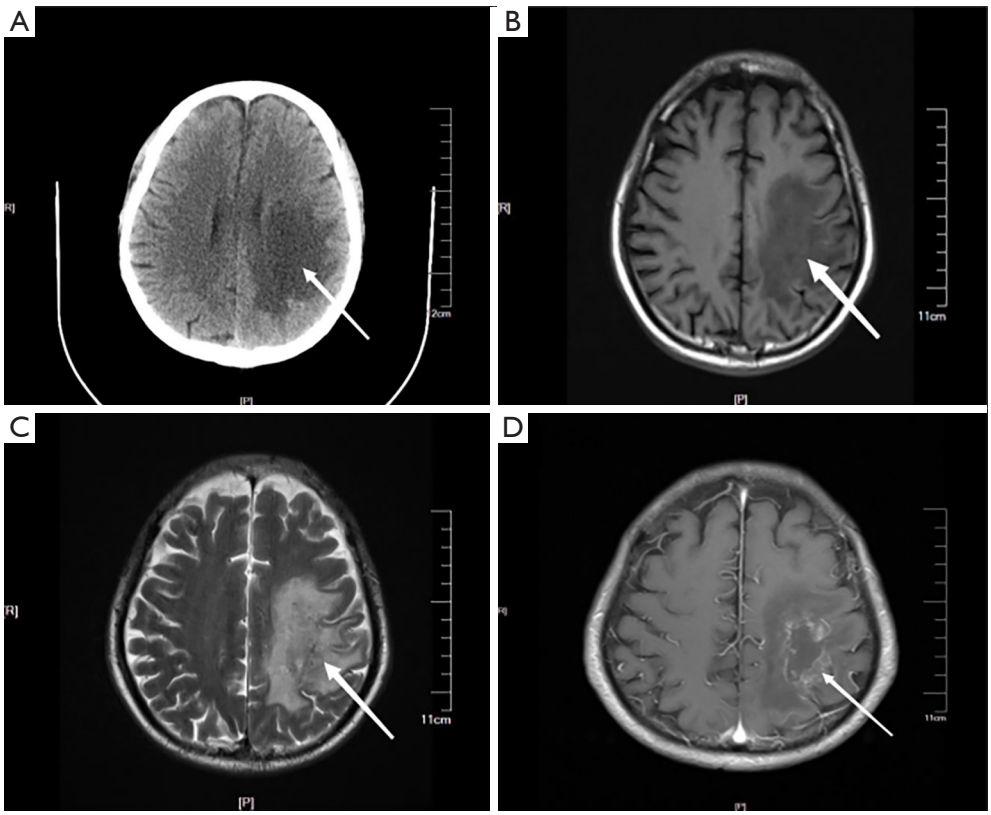

Figure 1 Brain CT scan and MRI of a patient with LM intracranial listeria infection complicated with brain abscess. Brain CT showed low density in the left frontoparietal temporal lobe, basal ganglia, and left cerebellar foot (A, arrow). MRI showed enhanced long T1 and long T2 signals (B,C, arrows), and shadow of ring enhancement in the same area (left frontoparietal temporal lobe, basal ganglia, and left cerebellar foot) (D, arrow). R represents the right side; P represents posterior side. CT, computed tomography; MRI, magnetic resonance imaging; LM, *Listeria monocytogenes*.

in elderly patients with an underlying medical history and immunosuppressed patients (5). However, its mortality has been reported to be as high as 50%, and survivors usually have severe sequelae (5). In the present study, healthy young patients developed meningitis; all had a history of eating unheated food that has been refrigerated or catch cold. Therefore, doctors should be vigilant against such infections when attending young, healthy patients. Among the other 4 patients, 3 were immunosuppressed and presented with brain abscess, brainstem encephalitis, and meningitis, respectively. The remaining 83-year-old patient also presented with meningitis.

Most of the clinical manifestations of patients with LM intracranial infection reported in the past have been non-specific, showing non-specific prodromal symptoms, such as fever, headache, nausea, and vomiting; coma and convulsion can occur in the later stage (6). Four cases of LM meningitis were reported in our study, and symptoms were consistent with the above symptoms (fever, headache, nausea, and vomiting). The other 2 patients were immunosuppressed patients, who developed fatigue and hemiplegia at disease onset; fever did not manifest until the fifth day of the disease. The patients were eventually diagnosed brain abscess and brainstem encephalitis, respectively. These findings indicate that the early manifestations of LM intracranial infection are diverse, especially in the elderly and immunosuppressed patients, whose symptoms are not typical and easily misdiagnosed as cerebrovascular disease, which requires greater vigilance. The elderly patient and the immunosuppressed patient with brain abscess developed septic shock at disease onset, while the APACHE II (Acute Physiology and Chronic Health Evaluation II) score was 27 and 23 respectively. The elderly patient also had acute renal insufficiency and received continuous renal replacement

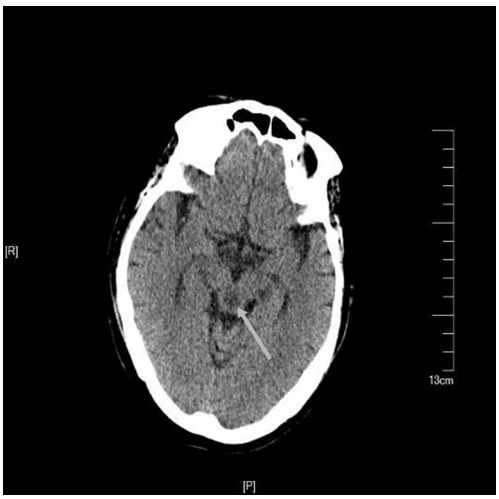

Figure 2 Brain computed tomography scan of a patient with brainstem encephalitis. Scan shows patchy low-density shadow in the brain stem (arrow). R represents the right side; P represents posterior side.

therapy (CRRT) immediately after admission. The patient with brain abscess was treated with cyclophosphamide and glucocorticoid for membranous nephropathy 2 months prior to disease onset and was eventually diagnosed with LM intercranial infection by CSF mNGS. After >2 months of treatment, the patient had stable vital signs, but had residual neurological sequelae. These findings indicated that advanced age and immunosuppression are risk factors for rapid progression of the disease and poor prognosis, and that the APACHE II score is an important marker for disease severity.

CSF examination findings during the early stage of the disease is similar to those of bacterial infection, such as increased CSF pressure, increased white blood cell count, mild-to-moderate protein increase, decreased sugar levels, and insignificant chloride changes. The diagnosis of LM intracranial infection depends on pathogen examination (7). However, conventional culture has a low positive rate and takes time, which is not conducive to diagnosis and treatment. The advantage of mNGS is its wide range of detection. It does not require prior designation of suspected pathogenic microorganisms, and can be used to diagnose meningitis, encephalitis, and lower respiratory tract infections (8). In our hospital, mNGS results can be obtained in 36–96 h. However, conventional culture results can take 3–7 days and have a lower positive rate, especially in CSF culture and for rare pathogens. CSF mNGS was performed in all 6 patients in the present study, and the results were all positive. It has a significant advantage over conventional culture in both positive rate (16% *vs.* 100%) and timeliness (91 *vs.* 47 h). Treatment of severe intracranial infections often requires early identification of the causative agent so as to provide targeted treatment. However, due to its atypical clinical features and the delay of routine culture, the diagnosis of LM intracranial infection is challenging and often misdiagnosed. Accurate and timely diagnosis of infected microorganisms is a key advantage of mNGS, which can minimize the diagnostic time of LM intracranial infection, leading to early intervention, therefore improving patient prognosis.

Early diagnosis and appropriate antibacterial treatment are the best choices to reduce mortality and sequelae of the disease (9,10). Intravenous ampicillin or penicillin combined with intravenous gentamicin has been known to be effective against LM intracranial infection, and is generally considered the first choice for treatment. Cephalosporins have no effect on LM (11). Vancomycin, meropenem, or linezolid can also be used in patients allergic to ampicillin/penicillin. When clinical manifestations are difficult to distinguish from other infections, anti-infection regimens should be used to treat LM. Meropenem or linezolid is recommended as the initial regimen, and cephalosporins alone are not recommended. Once LM intracranial infection is confirmed, combined treatment with penicillin or SMZ should be considered. The treatment course for patients with meningitis should not be less than 3 weeks, and for patients with brain abscess, the total treatment course should not be less than 6–8 weeks (9,12-15). Among the 6 patients with LM intracranial infection in our study, 4 had infections in other areas, including pulmonary and intestinal infections. In terms of anti-infection regimen, 5 patients were treated with meropenem combined with penicillin, and 1 patient was treated with linezolid combined with etimicin because of penicillin allergy. After treatment, 5 patients recovered, 1 patient with brain abscess developed left neurological sequelae. It has been reported that surgical drainage as early as possible is more effective in improving the survival rate and prognosis of brain abscess induced by LM than antibiotic therapy alone (16).

In conclusion, LM intracranial infection is mostly seen in immunocompromised or elderly patients, but it should not be ignored in healthy patients with normal immune function. Its clinical manifestations are complex and varied, and can include meningitis, brainstem encephalitis, and brain abscess. Early diagnosis and treatment with antibiotics

are key to improving the prognosis of patients. mNGS can shorten the diagnostic time of LM intracranial infection, provide a basis for effective treatment, and ultimately improve the prognosis of patients. LM infection with brain abscess usually has a poor prognosis. If conditions permit, surgical drainage should be performed as early as possible to improve patient survival, avoid neurological sequelae, and improve short- and long-term outcomes.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-2186/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-2186/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-2186/coif). JW reports a grant from Gusu Health Talents Programme (No. GSWS2020006). The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The present study was reviewed and approved by the ethics committee of The First Affiliated Hospital of Soochow University (No. 2022-074). Individual consent for this retrospective analysis was waived.

References

1. Vázquez-Boland JA, Kuhn M, Berche P, et al. Listeria pathogenesis and molecular virulence determinants. Clin Microbiol Rev 2001;14:584-640.
2. Pagliano P, Ascione T, Boccia G, et al. Listeria monocytogenes meningitis in the elderly: epidemiological, clinical and therapeutic findings. Infez Med 2016;24:105-11.
3. Committee of Neurosurgeons Branch of Chinese Medical Doctor Association: Chinese expert consensus on diagnosis and treatment of Central Nervous System Infection in Neurosurgery (2021). Chinese Journal of Neurosurgery 2021;37:2-15.
4. Desai AN, Anyoha A, Madoff LC, et al. Changing epidemiology of Listeria monocytogenes outbreaks, sporadic cases, and recalls globally: A review of ProMED reports from 1996 to 2018. Int J Infect Dis 2019;84:48-53.
5. Zhao B, Gai H, Wang Q, et al. Clinical analysis of three cases of Listerial rhombencephalitis. Chinese Journal of Neurology 2019;(8):640-5.
6. Arslan F, Ertan G, Emecen AN, et al. Clinical Presentation and Cranial MRI Findings of Listeria monocytogenes Encephalitis: A Literature Review of Case Series. Neurologist 2018;23:198-203.
7. Clauss HE, Lorber B. Central nervous system infection with Listeria monocytogenes. Curr Infect Dis Rep 2008;10:300-6.
8. Langelier C, Kalantar KL, Moazed F, et al. Integrating host response and unbiased microbe detection for lower respiratory tract infection diagnosis in critically ill adults. Proc Natl Acad Sci U S A 2018;115:E12353-62.
9. Yılmaz PÖ, Mutlu NM, Sertçelik A, et al. Linezolid and dexamethasone experience in a serious case of listeria rhombencephalitis. J Infect Public Health 2016;9:670-4.
10. Mansbridge CT, Grecu I, Li Voon Chong JS, et al. Two cases of listeria rhombencephalitis. IDCases 2018;11:22-5.
11. .Dutta NK, Mazumdar K, Park JH. In vitro synergistic effect of gentamicin with the anti-inflammatory agent diclofenac against Listeria monocytogenes. Lett Appl Microbiol 2009;48:783-785.
12. Bazooyar B. Rhombencephalitis by Listeria Monocytogens in Two Diabetic Patients. Arch Iran Med 2015;18:613-5.
13. Karlsson WK, Harboe ZB, Roed C, et al. Early trigeminal nerve involvement in Listeria monocytogenes rhombencephalitis: case series and systematic review. J Neurol 2017;264:1875-84.

14. Popescu GA, Saquepée M, Poisson D, et al. Treatment difficulties of a listerial rhombencephalitis in an adult patient allergic to penicillins. J Clin Pathol 2004;57:665-6.
15. Morosi S, Francisci D, Baldelli F. A case of rhombencephalitis caused by Listeria monocytogenes successfully treated with linezolid. J Infect 2006;52:e73-5.
16. Sonneville R, Ruimy R, Benzonana N, et al. An update on bacterial brain abscess in immunocompetent patients. Clin Microbiol Infect 2017;23:614-20.

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