Infection and Drug Resistance

Dovepress
open access to scientific and medical research

Open Access Full Text Article

CASE REPORT

Diagnosis and Treatment to a Post-Craniotomy Intracranial Infection Caused by Corynebacterium

Caixia Fan[1,2], Li Gong[2], Mo An[3], Zhenglin Li[4], Xiang Li[2], Jinzhi Fang[5]

[1]Office of Drug Clinical Trials Institution, Affiliated Longhua People's Hospital, Southern Medical University (Longhua People's Hospital), Shenzhen, Guangdong, People's Republic of China; [2]Department of Pharmacy, Affiliated Longhua People's Hospital, Southern Medical University (Longhua People's Hospital), Shenzhen, Guangdong, People's Republic of China; [3]Neurosurgery Department, Affiliated Longhua People's Hospital, Southern Medical University (Longhua People's Hospital), Shenzhen, Guangdong, People's Republic of China; [4]Department of Pharmacy, Shenzhen Boai Shuguang Hospital, Shenzhen, Guangdong, People's Republic of China; [5]Department of Medical Imaging, Affiliated Longhua People's Hospital, Southern Medical University (Longhua People's Hospital), Shenzhen, Guangdong, People's Republic of China

Correspondence: Caixia Fan, Office of Drug Clinical Trials Institution, Affiliated Longhua People's Hospital, Southern Medical University (Longhua People's Hospital), Shenzhen, Guangdong, 518109, People's Republic of China, Tel +86-18824242858, Fax +86-75529407559, Email mydream0509@qq.com

Objective: To explore the perioperative prophylactic medication, identification of Causative pathogen and the treatment strategy of post-craniotomy intracranial infection (PCII) caused by Corynebacterium.

Patients and Methods: A 47-year-old overweight male patient with hypertension, diabetes, cerebral hemorrhage and recalcitrant syphilis was clinically diagnosed with PCII based on cerebrospinal fluid (CSF) routine examination (RT), biochemical test (BT), neuroimaging CT and MRI scans, bacterial culture and identification of CSF and clinical manifestations. The risk factors of PCII and perioperative antibiotic prophylaxis were analyzed based on some reviews. The identification of the Corynebacterium Jeikeium (C. Jeikeium) and Corynebacterium simulans (C. simulans) was confirmed by CSF bacterial culture, antibiotics sensitivity in vitro and Metagenomic next-generation sequencing (mNGS) of pathogenic microorganisms, respectively. In addition, individualized therapy schemes were modified according to antimicrobial susceptibility of pathogens and mNGS of pathogenic microorganisms combined with the pathologic and physiological conditions of patients. The efficacy was evaluated depending on the changes in patients' body temperature, clinical manifestation, CSF RT, BT, and other infection-related indicators.

Results: The patient recovered after 5 weeks of individualized comprehensive treatment and was discharged home, no recurrence had been observed for three months.

Conclusion: This is likely the first reported case of chronic PCII caused by two species of Corynebacterium simultaneously in high risk patient. The PCII can not be prevented by the perioperative antibiotic prophylaxis recommended by the guidelines, prophylaxis need to be individualized based on the risk of infection and the colonization status of the patient. Causative pathogens can be identified by CSF culture and mNGS of pathogenic microorganisms. A judicious antimicrobial therapy plan should take into account not only the in vitro antimicrobial susceptibility, but also the penetration of the antimicrobial agent into the cerebrospinal fluid. It was an excellent choice to combine intrathecal vancomycin with intravenous linezolid to treat PCII resulted from Corynebacterium.

Keywords: post-craniotomy intracranial infection, Corynebacterium jeikeiu, Corynebacterium simulans, perioperative antibiotic prophylaxis, identification of pathogenic bacteria, individual treatment schemes

Introduction

PCII is a rare but serious complication of craniotomy that can lead to increased morbidity and mortality, length of hospital stay and cost.[1,2] It is critical to determine accurate prevention strategies, including improved techniques in pre-operative skin antisepsis and antibiotic prophylaxis, to reduce the postoperative infection rates. On the other hand, since the infection outcomes depend on early and specific antibiotic therapy, early detection, accurate diagnosis and prompt treatment remain crucial to reduce morbidity and mortality[1,3]. Corynebacteria are gram-positive bacteria that are normal flora of the skin and mucous membrane.[4] They have been regarded as contaminants of clinical samples rather than as

Received: 21 April 2022
Accepted: 12 September 2022
Published: 15 November 2022

potential pathogens over a period of time. Nevertheless, in recent years, several studies have demonstrated that the bacteria can be opportunistic pathogens and cause various community-acquired infections such as pharyngitis, prostatitis, conjunctivitis, genitourinary infections, etc. and multiple nosocomial infections including bloodstream infection, catheter-related infection, endocarditis, septicemia, meningitis, pneumonia, skin and soft tissue infections, prosthetic joint infection, yelonephritis, and liver abscess predominantly among immunodeficient patients, neutropenic patients, those with prosthetic and other medical devices, and post-surgery and trauma patients.[4–7] To the best of our knowledge, there are no reports of controlled clinical trials in perioperative antibiotic prophylaxis and treatment of PCII with different Corynebacterium species. Although early reports indicated that C. jeikium and C. simulans isolated were commonly susceptible to many antimicrobial drugs, including β-lactams, tetracycline, and fluoroquinolones,[4] recent studies have demonstrated they have acquired resistance to penicillin, cefotaxime, erythromycin, clindamycin, ciprofloxacin, and tetracycline, while remaining sensitive to glycopeptides, linezolid, quinupristin/dalfopristin, daptomycin, and/or tigecycline. Some studies have shown that they can rapidly develop a high level of resistance to daptomycin resistance during therapy. More importantly, different species of Corynebacterium show different drug resistance spectra.[5–7] It was reported that almost all C. simulans isolates showed multi-drug-resistance, and each hospital had its own predominant clones.[8] Therefore, it is crucial to identify Corynebacterium species and obtain their drug sensitivity in vitro to select an appropriate antibiotic. On the other hand, antimicrobial penetration into the central nervous system is an important factor in the efficacy of PCII therapy. This depends on the status of the blood-brain barrier, as well as the physicochemical properties of antibiotic such as molecular weight, degree of ionization at physiologic pH, lipid solubility, and degree of protein binding.[9] PCII is even more difficult to treat due to multidrug-resistant Corynebacterium.

Here, we presented a chronic PCII due to a simultaneous infection of C. jeikeium and C. Simulans for the first time. We described patient risk factors for PCII and possible causes of perioperative antibiotic prophylaxis failure, and demonstrated the importance of identification of pathogen and target treatment options for the pathogen.

Case Report

A 47-year-old male with hypertension, diabetes, multiple cerebral infarction and recalcitrant syphilis was brought to our hospital emergency department (ED) because of a sudden left limb weakness accompanied by vomiting for an hour on July 13, 2020. The physical examination on admission showed a height of 180 cm, a weight of 100 kg, lethargy, unable to follow orders. Head CT in our hospital indicated a hemorrhage in the right basal ganglia region. He was then admitted to neurosurgery, where he vomited again and rapidly deteriorated with progressive loss of consciousness. Shallow coma, GCS decreased to 8 points (E2V2M4). Cefuroxime prophylaxis with a dose of 750 mg was injected intravenously, and emergency craniocerebral operations consist of top right frontotemporal craniocerebral open, multiple hematoma removal, dura repair, and bone disc decompression, implantation intracranial pressure probe and catheter placement outside the epidural (also known as external ventricular drains or EVDs) were performed under general anesthesia in the operating room. The whole operation lasted 455 minutes with 1600mL of bleeding, of which 1000mL was autotransfusion, and then he was admitted to the intensive care unit (ICU) for further treatment. He was treated with mechanical ventilation, dehydration, cranial pressure reduction, neuro-nourishing, cerebral protection and cefuroxime prophylaxis with 750 mg every 8 hours for a total duration of 48 hours after surgery in ICU according to the guideline.

On 17 July, He had serious chill, high fever with the highest temperature to 39.5 °C, the infected indicator increased, the catheter was removed and then on 18 July, tracheotomy was done. CSF culture and sputum culture were taken. Then, hospital-acquired pneumonia (HAP) due to Klebsiella pneumoniae (ESBLE+) was confirmed by the results of chest CT scan, sputum culture and antibiotics sensitivity *in sequence*. While CSF culture, CSF BT, CSF RT as well as the clinical manifests did not support the diagnosis of intracranial infection. Piperacillin sodium/tazobactam was administered every 8 hours with a dose of 4.5g for 23 days, on August 11. WBC and Granulocyte ratio decreased, PCT decreased to 0.035 ng/mL, high sensitivity C-reactive protein (hs-CRP) increased to 53.0mg/L. Then, the antibiotic was modified to meropenem 1g every 8h, However, the patient had a recurrent fever and was delirious as before. The highest body temperature reached 39.6°C on August 13. Brain CT plain scan showed postoperative cerebral hemorrhage in the right basal ganglia region, and thalamus and the coronal radiator region showed a small amount of subdural/extradural effusion, in the operative area increase than before, no abscess was

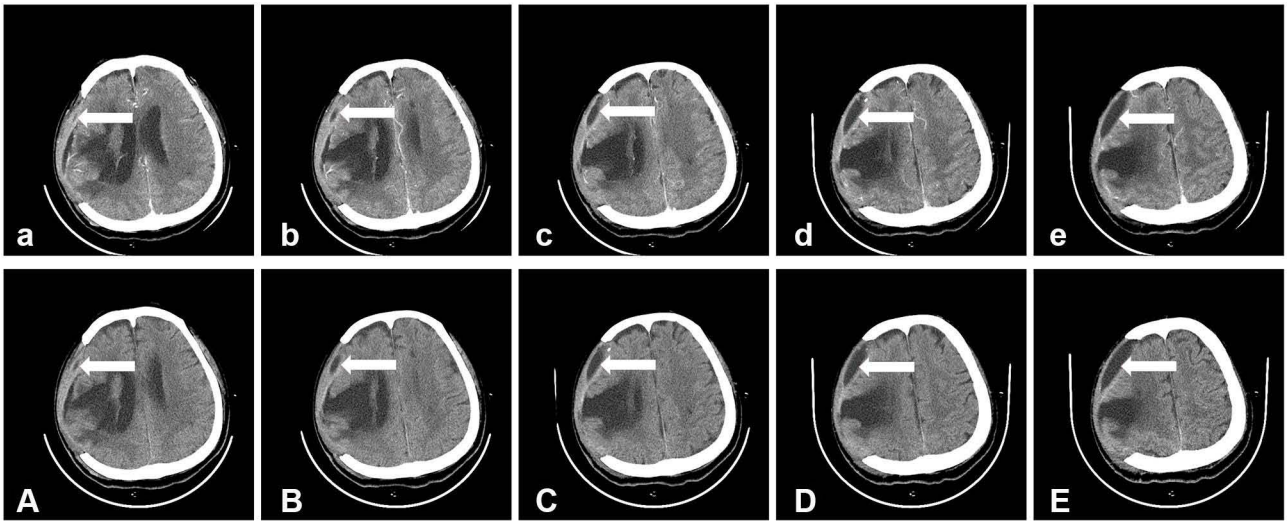

Figure 1 Brain CT plain scan results. Postoperative reexamination of intracerebral hemorrhage in the thalamus and Corona radiata areas in the right basal ganglia region revealed meningoencephalitis in the right cerebral hemisphere, and a small amount of subdural/extradural effusion in the operative area (arrow). Multiple softening foci in the brain, roughly the same as before (**a-e**). A contrast-enhanced brain CT on August 18 revealed a small amount of fluid within the subdural space adjacent to the right frontal lobe. An enhanced scan showed mild to moderate enhancement. There was a strip-like low-density shadow in the operative area of the right brain and a softening lesion in the right vertebral temporal lobe, which was partially connected with the adjacent ventricle. Small patches of low-density shadow were observed in bilateral basal ganglia, right midbrain, and left Corona radiate, and some edges were blurred.(**A**–**E**) No obvious abnormal enhanced shadow was observed on the enhanced scan.

found (Figure 1). Bacterial PCII was suspected. The anti-infection treatment regimens were adjusted to vancomycin plus meropenem intravenously 2 g every eight hours) according to the guidelines and a consensus review[10,11] (vancomycin 1.25 g intravenously every eight hours to aim for a vancomycin concentration of 15–20 μg/mL; the blood concentration was found to be 17.8μg/mL). Then, PCII resulting from two species of Corynebacterium was confirmed by CSF BT, RT, bacteremia culture, the drug sensitivity and mNGS of pathogenic microorganisms and MRI scan of brain (Figure 2): CSF BT on August 14 revealed that the cerebrospinal fluid protein (PROT) was 3000 mg/L, glucose (Glu) was 1 mmol/l, chlorine was 111.1 mmol/L. CSF RT showed that the red blood cells (RBCs), white blood cells (WBCs), and the proportion of neutrophils were 99×10^6/L, 1014.7 ×10^6/L, and 50.6%,

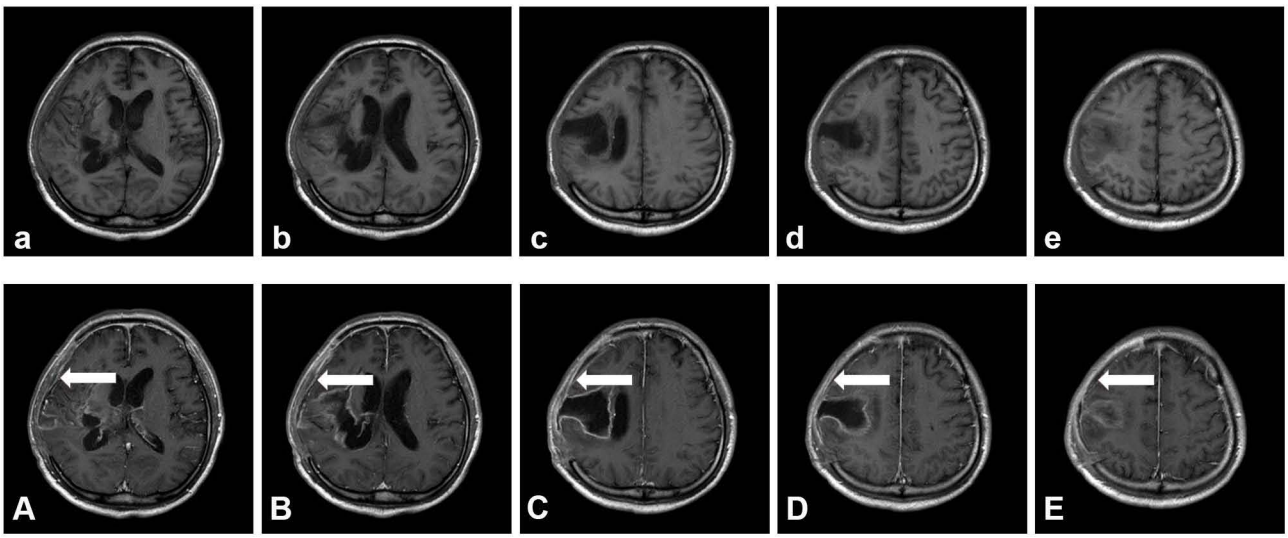

Figure 2 Brain MRI images on August 25 demonstrated post-cerebral hemorrhage in the right frontal lobe, temporal lobe, right basal ganglia, and thalamus, and the softening of the focus (**a-e**). The marginal enhancement of the operative area was considered to be a consequence of postoperative repair changes. The meninges indicated with the arrow was located in the right temporal region were enhanced and was considered to be inflammatory, Epidural marked curve demonstrated significant contrast enhancement following intravenous gadolinium administration (**A**–**E**) and there was slight subdural effusion in the right frontal area.

Table 1 Minimum Inhibitory Concentration (MIC) and Antibiotics Sensitive Test (AST) of Several Antibiotics for C. Jeikeium Isolated from the CFS Culture

Antibiotics	MIC/μg/mL	AST Results
Penicillin	1	S
Ceftriaxone	4	S
Meropenem	≤0.25	S
Gentamicin	≤1	R
Erythromycin	≥1.5	R
Clindamycin	4	R
Teicoplanin	≤1	S
Doxycycline	≥1	R
Ciprofloxacin	≤1	S
Sulfamethoxazole trimethoprim	>2/38	R
Vancomycin	≤0.5	S
Rifampicin	≤1	S
Linezolid	≤1	S

Table 2 Relative Abundance of Pathogenic Microorganisms in Cerebrospinal Fluid (CSF) Detected by Metagenomic Next-Generation Sequencing (mNGS)

Genera			Species		
Latin Name	**The Sequence Number Checked**	**Relative Abundance (%)**	**Latin Name**	**The Sequence Number Checked**	**RPM-Ratio**
Corynebacterium	19	13.57	*C. simulans*	11	60.42

Note: RPM-ratio refers to the ratio between the RPM (reads per million, number of pathogen sequences per million) of the pathogen and the RPM of the pathogen in the background database Value. The higher the RPM-ratio, the less likely the pathogen is to be the detection background.

respectively. The results of CSF culture on August 17 (accepted on August 14) indicated that C. jeikeium was sensitive to penicillin, vancomycin, and meropenem (Table-1), Administration of vancomycin and meropenem were continued, and penicillin sodium 3.2 million IU every 4 hours was prescribed on August 17. After a series of treatments, the CSF RT and BT showed the PCII was improved, and the temperature decreased to 38.0°C. Vancomycin was terminated on August 19, and penicillin sodium was adjusted every four hours to 4.8 million IU. On the 20th of August, the patient was again seized with a violent fever. The highest temperature reached 39.5° C. To confirm the possible pathogen, blood culture of aerobic and anaerobic bacteria as well as fungi was taken and CSF cultures were taken from August 20 to September 18 every other day. The return results showed that no bacteria were growing. While mNGS of pathogenic microorganisms on August 21 (samples were received on August 20.) confirmed C. simulans, the sequence number of C. simulans was 11 and the RPM-ratio was 60.42 (Table 2), then the antibiotic regimen was changed to linezolid 0.6 g every 6 hours intravenously combined with 20 mg vancomycin was administrated intrathecal daily. As a result, complete blood cell, CSF BT, CSF RT, CRP and PCT revealed that the patient recovered gradually and was discharged on September 21. There had been no recurrence for three months. The clinical course is shown in Figure 3.

Discussion

PCII is a complication related to craniotomy, which can lead to severe morbidity and higher hospital costs during the postoperative period. In the case the patient received clean craniocerebral operations under general anesthesia in the operating room, risk factors of intracranial infection after craniotomy were involved as followed: other infection (HAP), the number of operation was 4 >1, foreign body placement, duration of operation was 455min>4h, intraoperative blood loss was 600mL >400mL, three days catheter drainage, repeated samples of cerebrospinal fluid, coma, the operations were done in autumn (compared with Spring) and the complications

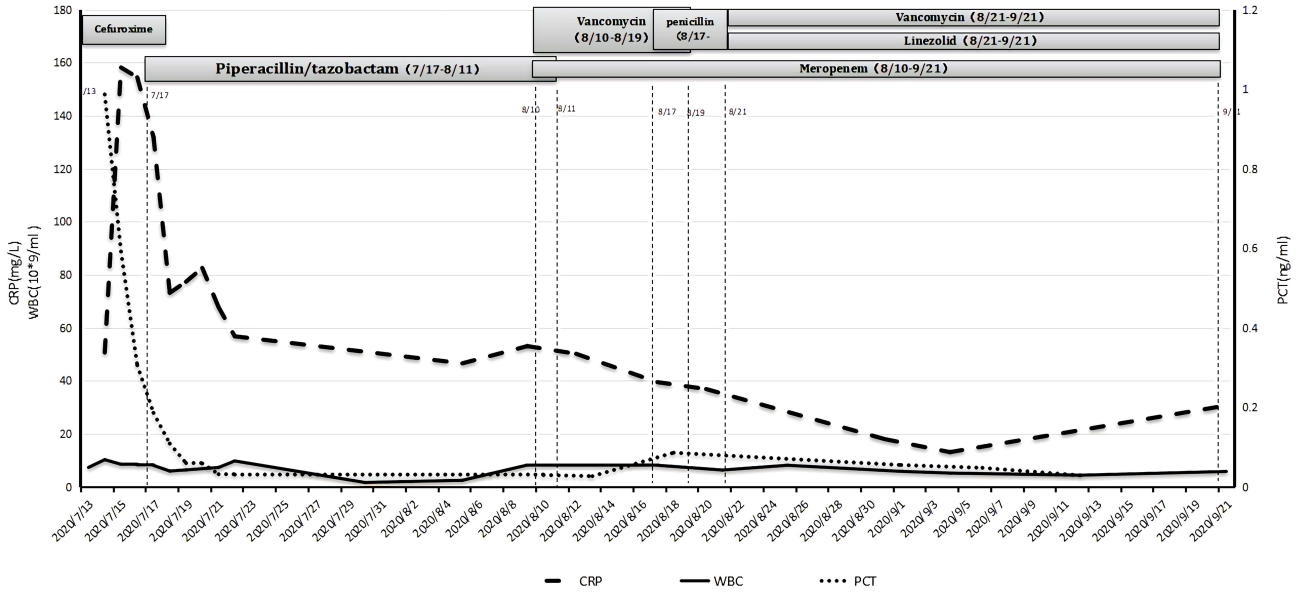

Figure 3 Clinical course of the patient with HAP and PCII in hospital.
Abbreviations: CRP, C-reactive protein; WBC, white blood cell count; PCT, procalcitonin.

including hypertension, diabetes and latent syphilis, which may be involved in the process of cellular immune dysfunction.[9,12,13] Prophylactic antibiotic, cefuroxime, was administered before beginning craniotomy and for at least 2 days after surgery based on guideline[15]. Although neuroimaging CT and MRI showed that there was no abscess cavity, the scalp incision healed well without redness and heat. The PCII was confirmed 30 days after the neurosurgical procedure. While Corynebacteria are normal flora of the skin and mucous membrane, they are opportunistic pathogen of nosocomial infection and chronic PCII caused by Corynebacterium is rare. Risk factors for Corynebacterium infection include prolonged hospitalization, granulocytopenia, multiple or prolonged courses of antibiotic therapy, disruption of mucocutaneous barriers and presence of a medical device. Prophylactic antibiotics can significantly reduce PCII, but can not completely prevent infection.[14] One of the reason is that they are resistant to multiple antibiotics, including beta-lactams, aminoglycosides, macrolides and tetracycline, except for vancomycin, daptomycin and linezolid.[4–7] And it was reported systemic antibiotics were only effective if they maintain therapeutic concentrations in the tissue, if they cover future pathogens.[14] Because of the increased detection rate of pan-drug resistant bacteria, the postoperative infection can not be prevented by empirical perioperative antibiotic prophylaxis with cefazolin and cefuroxime which are recommended based on the guidelines.[15] It is advised that prophylaxis would need to be individualized depending on the risk of infection and the colonization status of the patient. The use of vancomycin by intravenous administration or topical administration in clean craniocerebral surgery for prophylaxis may be appropriate when there is a high incidence of SSI due to MDR- Corynebacterium.[16,17]

Detection of the bacterial pathogens responsible for PCII is crucial for proper diagnosis and optimal treatment. The culture of CSF is the gold standard for confirming the diagnosis of bacterial meningitis. It is recommended to determine the Corynebacterium to causative pathogen when the bacteria are cultured/collected from generally sterile sites, such as blood (two or more positive blood) or CSF cultures (CSF). Nevertheless, it is common for CSF culture to fail to detect the causative microorganism, especially when antibiotics are administered.[4] In the case, CSF cultures were taken from August 20 to September 18 every other day. The subsequent results were negative. mNGS might be a good alternative method. It was reported that the sensitivity and specificity of mNGS for bacterial meningitis exceed 90%.[18] mNGS indicated that the pathogenic microorganisms were C. simulans. We noticed that the bacterial culture results in CSF on August 17 (accepted on August 14) were different from the mNGS of pathogenic microorganisms on August 21 (samples were received on August 20). We hypothesized that two Corynebacterium spp: C. jeikeium, and C. simulans might have

been present simultaneously. In the beginning, the predominant species was C. jeikeium, but the sensitive bacteria (C. jeikeium) were killed under the pressure of antibiotics, whereas the insensitive bacteria (C. simulans) multiplied fast and became the predominant pathogen.

It is well known that empiric antibiotic treatment should be initiated without delay when the bacterial meningitis is suspected. Once a causative pathogen is identified, antimicrobial therapy should be modified. The choice of pathogen-targeted therapy depends on in vitro antimicrobial susceptibility and the penetration of the antimicrobial agent into cerebrospinal fluid.[14,15,17] Broader spectrum antimicrobial agents are required to treat nosocomial meningitis, Vancomycin, combined with either third- or fourth-generation cephalosporins or a carbapenem, are appropriate empiric antimicrobial regimens.[1,14] In the study, as soon as the bacterial meningitis was suspected, the empiric antimicrobial regimens that Vancomycin combined with meropenem were administrated. But the clinical manifestation did not improved untill antimicrobial therapy was modified to according to in vitro antimicrobial susceptibility after the C. jeikeiu and C. simulans were detected by CSF culture and mNGS, respectively. Some studies showed that most C. simulans clinical isolates are commonly resistant to beta-lactams, aminoglycosides, macrolides, and tetracycline, and empiric antibiotic therapy with vancomycin is the optimal drug of choice.[13] Given the large molecular weight and hydrophilic properties of vancomycin, which could lead to poor blood-brain barrier permeability, the concentration of vancomycin in CSF is far lower than that in blood. On the other hand, Corynebacterium species form biofilms that are refractory to vancomycin therapy[11,16,17] To solve the above problems, many researchers have proposed that intraventricular administration could be considered to bypass the BBB. On the other hand, some studies found that several newer antimicrobials such as teicoplanin, telithromycin, and linezolid worked particularly well.[1,19] Drug combination is a good way to deal with PCII caused by multidrug resistant Corynebacterium, in our case, linezolid administered intravenously combined with vancomycin by intrathecal administration to deal with the PCII (C. jeikeium) and (C. simulans), respectively.

Conclusions

This is likely the first reported case of chronic PCII caused by two species of Corynebacterium simultaneously in high risk patient. PCII can not be prevented by the perioperative antibiotic prophylaxis recommended by the guidelines, prophylaxis need to be individualized based on the risk of infection and the colonization status of the patient. Causative pathogens can be identified by CSF culture and mNGS of pathogenic microorganisms. A judicious antimicrobial therapy plan should take into account not only the in vitro antimicrobial susceptibility, but also the penetration of the antimicrobial agent into the cerebrospinal fluid. It was an excellent choice to combine intrathecal vancomycin with intravenous linezolid to treat PCII resulted from Corynebacterium.

Ethical Approval and Consent to Participate

The patient provided informed consent for publication of the case. No ethical committee approval was required for this study as the data had been analyzed in a retrospective manner.

Consent for Publication

Written informed consent for publication of the clinical details and clinical images was obtained from the patient.

Acknowledgments

We thank Guangzhou Golden Domain Medical Laboratory Center.

Funding

This work was supported by Scientific Research Projects Medical and Health of Institutions of Longhua District, Shenzhen (2020007) and Hospital Pharmacy Research Foundation of Guangdong Province (2021A38).

Disclosure

The authors have no conflicts of interest to declare in this work.

References

1. Savin I, Ershova K, Kurdyumova N, et al. Healthcare-associated ventriculitis and meningitis in a neuro-ICU: incidence and risk factors selected by machine learning approach. *J Crit Care*. 2018;45:95–104. doi:10.1016/j.jcrc.2018.01.022
2. Ullah I, Collaborators GM, Blake N. Global, regional, and national burden of meningitis, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol*. 2018;17(12):1061–1082. doi:10.1016/S1474-4422(18)30387-9
3. honda H, Warren DK. Central nervous system infections: meningitis and brain abscess. *Infect Dis Clin North Am*. 2009;23(3):609–623. doi:10.1016/j.idc.2009.04.009
4. Bratcher DF. Other Corynebacteria - ScienceDirect. In: *Principles and Practice of Pediatric Infectious Diseases*. 5th ed. Elsevier Health Sciences; 2018:778–781.
5. Xw A, Hz A, Pd B, et al. Genomic epidemiology of Corynebacterium striatum from three regions of China: an emerging national nosocomial epidemic. *J Hosp Infect*. 2021;110:67–75. doi:10.1016/j.jhin.2020.10.005
6. Silva-Santana G, Silva CMF, Olivella JGB, et al. Worldwide survey of corynebacterium striatum increasingly associated with human invasive infections, nosocomial outbreak, and antimicrobial multidrug-resistance, 1976–2020. *Arch Microbiol*. 2021;203(5):1863–1880. doi:10.1007/s00203-021-02246-1
7. Mc Mullen AR, Anderson N, Wallace MA, et al. When good bugs go bad: epidemiology and antimicrobial resistance profiles of corynebacterium striatum, an emerging multidrug-resistant, opportunistic pathogen. *Antimicrob Agents Chemother*. 2017;61(11):e01111–17. doi:10.1128/AAC.01111-17
8. Wang X, Zhou H, Chen D, et al. Whole-genome sequencing reveals a prolonged and persistent intrahospital transmission of corynebacterium striatum, an emerging multidrug-resistant pathogen. *J Clin Micro*. 2019;57(9):327–332. doi:10.1128/JCM.00683-19
9. Stenehjem E, Armstrong WS. Central nervous system device infections. *Infect Dis Clin N Am*. 2012;26(1):89–110. doi:10.1016/j.idc.2011.09.006
10. Ng K, Mabasa VH, Chow I, Ensom MH. Systematic review of efficacy, pharmacokinetics, and administration of intraventricular vancomycin in adults. *Neurocrit Care*. 2014;20(1):158-171. doi:10.1007/s12028-012-9784-z
11. Cai Y, Zhou L, Wang H, Zhang L, Wang J, Zhang K. Comparation of vancomycin penetration into cerebrospinal fluid in postoperative intracranial infection and community-acquired meningitis patients. *J Clin Pharm Ther*. 2019;44(2):216–219. doi:10.1111/jcpt.12770
12. Wang LY, Cao XH, Shi LX, et al. Risk factors for intracranial infection after craniotomy: a case-control study. *Brain Behav*. 2020;10:e01658. doi:10.1002/brb3.165
13. Cheng Y, Ye X, Ma M, Wu K, Zhang C. Advances in prevention and treatment of intracranial infection after craniocerebral surgery. *Chin J Med*. 2019;54(12):1301–1304.
14. Alotaibi AF, Hulou MM, Vestal M, et al. The efficacy of antibacterial prophylaxis against the development of meningitis after craniotomy: a meta-analysis. *World Neuro*. 2016;90:597–603.e1. doi:10.1016/j.wneu.2016.02.048
15. Working group on revision of guiding principles for clinical application of antimicrobial agents. *Guiding Principles of Clinical Application of Antimicrobial Agents*. 2015. People's Medical Publishing House; 2015.
16. Bokhari R, You E, Zeiler FA, et al. The effect of intrawound vancomycin on surgical site infections in non-spinal neurosurgical procedures: a systematic review and meta-analysis. *World Neurosurg*. 2018;123:409–417. doi:10.1016/j.wneu.2018.10.168
17. Nailor MD, Sobel JD. Antibiotics for gram-positive bacterial infection: vancomycin, teicoplanin, quinupristin/ dalfopristin, oxazolidinones, daptomycin, telavancin, and ceftaroline. *Med Clin N AM*. 2011;95(4):723–742. doi:10.1016/j.mcna.2011.03.011
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. Deng F. *Distribution of Multidrug-Resistant G~+ Bacteria in Intracranial Infection and Evaluation of Efficacy and Safety of Vancomycin and Linezolid*. Yichun University; 2020.