

**December 30, 2021**

**Dear Editor,**

**Title:** Scedosporium apiospermum Infection of the Lumbar Vertebrae: A Case Report

**Author:** Xue-Wen Shi, Sheng-Tang Li, Jin-Peng Lou, Bo Xu, Jian Wang, Xin Wang, Hua Liu, Song-Kai Li, Ping Zhen, Tao Zhang

**Name of Journal:** World Journal of Clinical Cases

**ESPS Manuscript NO:** 72505

The manuscript has been improved according to the suggestions of reviewers:

1. Format has been updated.
2. Revision has been made according to the suggestions of the reviewer

**Manuscript NO: 72505**

#### **The first reviewer**

**1. Can you explain why this study is new or telling new things?**

Re: Scedosporium apiospermum Infection is not common in clinical work, and it is easy to be misdiagnosed and treated by doctors. After investigation, this patient is the first case of Scedosporium apiospermum Infection of the lumbar vertebrae in our province. Moreover, he has no history of drowning, working in sewage, trauma and immune deficiency.

**2. The authors mention that there are no case reports of S. apiospermum infection of lumbar vertebrae. However, I saw a case report of Cao et al in Int J Clin Exp Med 2018;11(8):8672-8676**

Re: It may be that English is not the mother tongue of our country. I missed it when searching the literature and failed to notice this literature. I'm sorry for this.

**3. Why do you use vancomycin implanted into the intervertebral space?**

Re: The main pathogenic bacteria of spinal surgical site infection are gram-positive cocci represented by Staphylococcus. With the wide application of broad-spectrum antibiotics, Methicillin resistant Staphylococcus aureus (MRSA) and coagulase negative staphylococcus (CNS) have gradually become the main pathogens of surgical site

infection after spinal surgery, and ordinary cephalosporins are ineffective. Local application of vancomycin in spinal surgery can significantly reduce the risk of surgical site infection caused by gram-positive cocci.

4. Why do you think voriconazole was administered to prevent postoperative infection in this case? Is it not for treatment?

Re: We used cefoperazone sodium sulbactam (Pfizer, USA) 3g ivgtt q8h to prevent postoperative infection. We mistakenly deleted this part of the content in the writing process, resulting in misunderstandings by reviewers when reading the manuscript. We have made modifications in the revised manuscript.

5. Do you think medical gel foam of rifamycin can be affected to voriconazole penetration into the bone ?

Re : Rifampicin is a potent CYP450 inducer and decreases voriconazole Cmax by 93% and AUC  $\tau$  Decline of 96%, so, we believe that it affects the permeation of voriconazole in bone. However, considering the possibility of tuberculosis infection of the lumbar spine in this patient, medical Gelfoam containing isoniazid and rifampicin was implanted into the intervertebral space in case.

6. In these cases, how do you deferential diagnosis between *S. apiospermum* infection and colonization. How about your criteria for diagnosis?

Re : We used blood agar medium for tissue culture twice (30 ° for 7 days). The colonies were cashmere like and the back was gray black. Under the microscope (lactic acid phenol cotton blue staining,  $\times 400$ ) display: it shows that most of the hyphae are irregularly branched, producing round or oval lateral and terminal conidia. Three microbiologists in our hospital confirmed the culture results and microscopic examination results. They all thought it was *S. apiospermum*. In most cases, the clinical symptoms and signs of the corresponding infection site will appear. Our diagnostic standard is the European diagnostic standard for endemic fungal infection in 2019.

7. Before surgery and histopathological and pathological examination, the patient was mistakenly believed to have *Mycobacterium tuberculosis* infection based on the imaging findings. Once you've proven that the patient has a fungal infection, have you

stopped your TB medication?

Re : We immediately stopped tuberculosis medication after confirming that the patient had fungal infection.

8. Did patients had history of contact contaminated water, wetlands, decaying plants within the previous 3 months or sickle cell disease?

Re : During hospitalization, we asked the patient and his family members and learned that the patient had no contact with contaminated water, wetlands, decaying plants, drowning, sickle cell disease and immunodeficiency disease. After receiving the comments from the reviewers, we contacted the patients and their families again. They all said that the patient had no contact history and medical history mentioned above.

9. Please include more *S. apiospermum* infection of lumbar vertebrae from other case report as well for comparison with this study.

Re: According to the requirements of the reviewers, we have added a comparative table on the cases of *S. apiospermum* infection to the revised manuscript.

10. Please provide more data of importance of physician around the world to recognise clinical characteristics of *S. apiospermum* infection.

Re : According to the opinions of reviewers, we have added some descriptions of the characteristics of *S. apiospermum* infection by doctors all over the world to the revised manuscript.

11 .....However, it is bacteriostatic, not bactericidal, and thus may not be effective for some patients.....Do you mean Fungistatic and Fungicidal?

Re: This part of the content is incorrectly stated, and we have deleted it.

12. Please move the dose of voriconazole from discussion to treatment section.

Re: We have moved the dose of voriconazole from the discussion to the treatment part.

## **The second reviewer**

1. History of present illness: It is still mystery what was the source/origin of the *S. apiospermum* infection and how this fungus infected the lumbar vertebrae. I do not

think that this fungus can be such infective without any trauma. Please, explain.

Re : During hospitalization, we asked the patient and his family members and learned that the patient had no contact with contaminated water, wetlands, decaying plants, drowning, sickle cell disease and immunodeficiency disease. After receiving the comments from the reviewers, we contacted the patients and their families again. They all said that the patient had no contact history and medical history mentioned above.

2. Treatment: Please, indicate the dosage of the voriconazole therapy here.

Re: We have indicated the therapeutic measurement of voriconazole in the revised manuscript.

3. Final diagnosis: This part of the manuscript is uncompleted and lack several essential information what can prove that the isolated fungus is *S. apiospermum* in fact. However, this is the most interesting and surprising part of the case report for the readers. This subchapter needs to be improved. What was the medium used for the cultivation? How was the fungus exactly identified? Based on macro- and/or micromorphology? The visual identification of a fungus can be misleading regularly. Therefore, it is essential that the authors prove that the identified fungus is *S. apiospermum* in fact based on a species-specific molecular marker. Here, I mean amplification of a species-specific partial gene sequence by PCR. Then sequencing it, and conducting a BLAST analysis, and depositing this DNA sequence in a gene data bank. In the lack of it, the manuscript cannot be accepted. It is essential, because the Figure 2 H panel is not convincing as the picture resolution and magnification is low and the conidiophores are not visible. In the Figure 2 F there is no any sign of fungal infection, however in the reported severe case the presence of fungus can be expected in the histopathology samples. Please, explain.

Re: Tissue culture on blood agar medium was performed twice (30°C, 7 days). The resultant colonies were cashmere-like and the back was gray-black. Under the microscope ( $\times 400$ ), lactic acid phenol cotton blue staining showed that most of the hyphae were irregularly branched, producing round or oval lateral and terminal conidia (Figure 2). Three microbiologists in our hospital confirmed the culture and microscopic

examination results, and all agreed on identification as *S. apiospermum*. Figure 2h in the manuscript has been replaced. We observed the pathological section of the patient again. We did not find *Cercospora apicalis*, but only observed a large number of inflammatory cell infiltration. However, by consulting the literature, we found that some reports on *Cercospora apicalis* did not find *Cercospora apicalis*, and only observed a large number of inflammatory cell infiltration, including lymphocytes, plasma cells, multinucleated giant cells, or granuloma.

4. Discussion: Please, delete the following sentences from this chapter, they are not relevant. "However, it is bacteriostatic, not bactericidal, and thus may not be effective for some patients." – As the authors talk about antifungal therapy. "The combination may have yielded better results." – As the applied voriconazole therapy was successful.

Re: We have deleted this part in the revised manuscript.

Thank you again for the useful comments to improve the paper.

**Happy New Year!**

Yours sincerely,

Tao Zhang

Orthopaedic centers, The 940<sup>th</sup> Hospital of Joint Logistics Support Force of Chinese People's Liberation Army, No. 333 Nanbinhe Road, Lanzhou 730050, Gansu Province, China.  
526199753@qq.com

January 13, 2022

1. Specific comment of re-reviewer to your manuscript: However, the authors did not reply to the comment regarding the molecular identification of the causative agent, in the attached microscopic picture it is clearly seen that they isolate *Scedosporium apiospermum* from the infection. All micromorphological features obviously indicate it. Re: The identification of *Scedosporium apiospermum* was based on tissue culture and microscopic observation. Due to the limitation of hospital testing equipment, we are unable to take a species-specific partial gene sequence by PCR, then sequence it, and conduct a BLAST analysis,

and deposit this DNA sequence in a gene data bank. In order to determine that the microorganism we isolated was *Scedosporium apiospermum*, we consulted books such as medical fungus inspection and illustration and relevant literature, repeatedly compared the identification results with them, and invited three microbiology experts from our hospital to confirm it. The final treatment and follow-up results also confirmed that our identification results were correct. 2. Please mark the specific position of "Table 1" in your manuscript. Re: I'm very sorry about this. Please put "Table 1" behind INTRODUCTION".....and invasion of the lung, bone, joints, eyes, brain, skin, and other organs.<sup>1,3,5,20</sup>"(Table 1), behind TREATMENT ".....Under the microscope ( $\times 400$ ), lactic acid phenol cotton blue staining showed that most of the hyphae were irregularly branched, producing round or oval lateral and terminal conidia (Figure 2) (Table 1),". behind DISCUSSION" .....It is most sensitive to voriconazole, followed by posaconazole, itraconazole, and amphotericin B<sup>16,23</sup>; voriconazole is the first-line therapy.<sup>6,7,17</sup>" (Table 1)。 3. I found several abbreviations of the word "month" wrong or missing in the revised manuscript of automatic typesetting. Please help me modify it, thank you. Yours sincerely, Tao Zhang Orthopaedic centers, The 940th Hospital of Joint Logistics Support Force of Chinese People's Liberation Army, No. 333 Nanbinhe Road, Lanzhou 730050, Gansu Province, China. 526199753@qq.com