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CASE REPORT

Application of Metagenomics Next-Generation Sequencing on Diagnosis of Disseminated Infection Caused by *Rhizomucor pusillus* in an Acute Lymphoblastic Leukemia Patient

Hengxin Chen[1,2,*], Mingjin Qiu[1,2,*], Hongxu Xu[1,2], Penghao Guo[1], Yaqin Peng[1]

[1]Department of Clinical Laboratory, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, 510080, People's Republic of China; [2]Department of Clinical Laboratory, Nansha Hospital, First Affiliated Hospital of Sun Yat-sen University, Guangzhou, 511455, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yaqin Peng, Department of Clinical Laboratory, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510080, People's Republic of China, Tel +86 20 87618461, Email pyqdream@163.com

Background: *Rhizomucor pusillus* is one of the major pathogens in mucormycosis. Infection due to *R. pusillus* is rare and has a high mortality rate, especially disseminated mucormycosis infections. Rapid and accurate pathogen identification is important for the development of targeted antifungal therapies.

Case Presentation: We presented a case of disseminated *R. pusillus* infection, identified through metagenomics next-generation sequencing (mNGS), in a 4-year-old patient with acute lymphoblastic leukemia. On days 22, 23, and 28, *R. pusillus* was detected in bronchoalveolar lavage fluid, blood, and hydrothorax, respectively, through mNGS. The patient developed lung, pleural, and blood disseminated lesions caused by *R. pusillus* infection. Subsequently, the patient was treated with antifungal therapy, including posaconazole, amphotericin B, and isacconazole, with supportive treatment. However, with the continuous deterioration of symptoms, the patient's family decided to give up treatment. The patient eventually died of multiple-organ failure on day 34.

Conclusion: mNGS facilitates prompt diagnosis of disseminated *R. pusillus* infections. The successful application of mNGS provided a new perspective for the clinician, underscoring the technique's potential for rapid diagnostic etiology. mNGS has the capability to identify pathogens at the species level, which is a significant asset in guiding clinical medication decisions.

Keywords: *Rhizomucor pusillus*, metagenomics next-generation sequencing, disseminated infection, diagnosis

Background

Mucormycosis is a common invasive fungal disease that occurs after candidiasis and aspergillosis.[1] *Rhizomucor pusillus* (*R. pusillus*) is one of the major pathogens in mucormycosis. The main route of infection seems to be through inhalation, feeding, or trauma infection of sporangiospores.[2] According to the site of infection, the main clinical manifestations of mucormycosis are pulmonary mucormycosis, cutaneous and soft-tissue mucormycosis, and rhino-orbito-cerebral mucormycosis. Gastrointestinal mucormycosis and renal mucormycosis are rare.[1] *R. pusillus* infection can cause infections in individuals with diabetes, hematological malignancies, immune deficiency, or underlying diseases.[3] In the mid-20th century, diabetes evolved as a major risk factor for mucormycosis. Rhino-orbito-cerebral mucormycosis is most commonly observed in individuals with diabetes. Lack of insulin is a condition that favors the development of the fungus.[4] Pulmonary mucormycosis is ordinarily observed in immunocompromised patients.

The mortality rate of mucormycosis infections is as high as 40–80%, especially disseminated mucormycosis infections.[5] The diagnosis of *R. pusillus* is difficult due to the nonspecific clinical signs, symptoms, and imaging manifestations of *R. pusillus*. However, the sensitivity and detection time of traditional methods fall short of clinical

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requirements, which may result in treatment delays or failures. Metagenomics next-generation sequencing (mNGS) is a potential new technology for identifying pathogens that are difficult using traditional methods.[6,7] Here, we report a case of disseminated *R. pusillus* infection in a child with acute lymphocytic leukemia (ALL). The child was rapidly diagnosed with *R. pusillus* infection using mNGS. The patient was subsequently treated with antifungal therapy, including posaconazole, amphotericin B, and isacconazole, with supportive treatment. However, the effects were not satisfactory, and the patient eventually died of septic shock and multiple organ failure.

Case Presentation

A 4-year-old male was admitted to the hospital (the First Affiliated Hospital of Sun Yat sen University, Guangzhou, China) who was diagnosed with ALL for 1 week and returned to the hospital for chemotherapy. The patient underwent a series of bone marrow tests. Bone marrow chromosome examination revealed 26% primitive cells. Flow cytometry indicated that the leukemic cells expressed HLA-DR, CD34, CD10, CD19, CD20, CD22, CD79a, CD38 (+), sIgM, and cyu (-), and the proportion of naive B cells was approximately 41.4%. The patient was admitted to the hospital for prednisone trial.

On day 6, the child developed hyperthermia (38.1°C). Laboratory examinations revealed leukocytosis (0.40×10^9/L) with a high serum procalcitonin (PCT) level (1.23 ng/mL, normal range = 0–0.5 ng/L) and C-reactive protein (67.94 mg/L, normal range = 0–10 mg/L). Hemoglobin level (88 g/L, normal range = 120–160 g/L) decreased. The presence of C-reactive protein and PCT was separated, which suggested fungal infections. Galactomannan (GM test) and 1,3-β-D glucan (G test), blood cultures, and etiological examinations of bronchoalveolar lavage fluid (BALF) were obtained. However, BALF and blood cultures did not reveal any pathogens. In addition, GM and G tests were negative in the serum. At the same time, empirical antibiotics treatment was started with voriconazole (0.4 g iv.drip Q8h), levofloxacin (0.15 g iv.drip Q12h), imipenem (0.7 g iv.drip Q8h), and caspofungin (70 mg iv.drip QOD and 35 mg iv.drip QD). After 7 days of antibiotic treatment, the patient's body temperature tended to decrease.

On day 18, the child displayed recurrent fever, with a maximum temperature of 38.3°C. Digital Radiography (DR) showed right bilateral pulmonary inflammation and revealed a right pulmonary exudation with pleural effusion (Figure 1). Etiological examinations of BALF and blood cultures were taken again, but the cultures still did not reveal

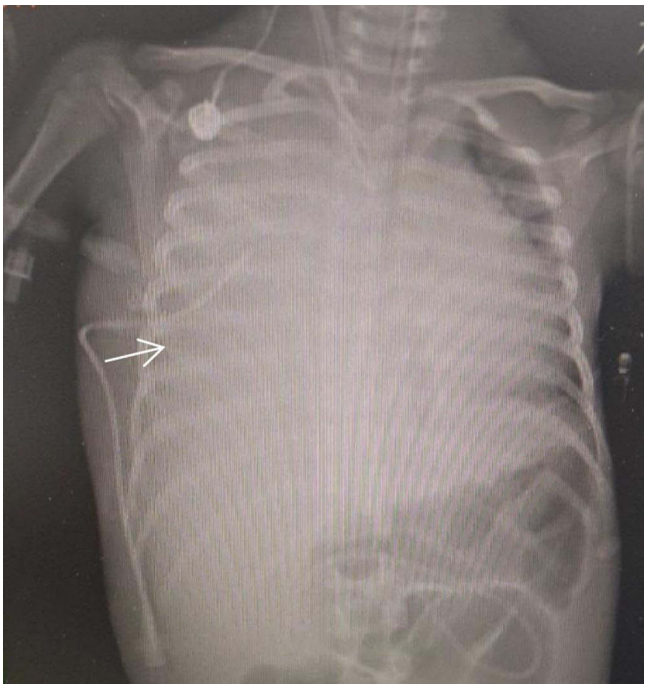

Figure 1 Digital Radiography manifestations of the patient, showing right bilateral pulmonary inflammation, a right pulmonary exudation with pleural effusion, and right chest drain indwelling. The arrow indicated the pulmonary exudation.

any pathogens. Meanwhile, mNGS for BALF and the peripheral blood specimen was performed. Voriconazole 122 mg iv. drip Q12h was added because fungal infection was suspected. On day 22, mNGS detected 1,458 total reads corresponding to *R. pusillus* in BALF, with 0.3% coverage and 95.4% relative abundance of fungi (Table 1). Meanwhile, a large number of filamentous fungal hyphae were observed in BALF (Figure 2). The hyphae were broad, non-septal, and branched near the right angle. This finding was consistent with the clinical diagnosis of pulmonary infection. The patient was started on posaconazole (90 mg iv.drip QD). The treatment was adjusted to teicoplanin (0.18 g iv.drip QD), cefoperazone sodium/sulbactam sodium (1.2 g iv.drip Q8H), levofloxacin (0.15 g iv.drip Q12H), caspofungin (70 mg iv.drip QOD and 35 mg iv.drip QD), and posaconazole (90 mg iv.drip QD). On day 23, mNGS detected 34,303 total reads corresponding to *R. pusillus* in the blood, with 5.6% coverage and 98.8% relative abundance in fungi. Five days later, the child still had a fever and 26,202 total reads corresponding to *R. pusillus* were detected in the hydrothorax, with 5.3% coverage and 99.7% relative abundance in fungi. Mucormycosis was detected by mNGS at multiple sites in the patient. Imaging results of DR were similar to those on day 18, showing pleural effusion in the right lung. Based on the clinical presentation and laboratory results, disseminated mucormycosis was considered. Amphotericin B liposomes (4–5 mg/kg/d) were used for antifungal treatment (Figure 3). On day 32, the patient appeared to have a suspected allergic reaction to amphotericin B; therefore, isaconazole (150 mg iv.drip Q12H) was used for antifungal treatment However, with the continuous deterioration of symptoms, the patient's family decided to give up treatment, and the patient eventually died of multiple-organ failure on day 34.

Discussion

R. pusillus is one of the major pathogens in mucormycosis The risk factors for mucormycosis include uncontrolled diabetes mellitus, neutropenia, hematological malignancies, corticosteroid use, and hematopoietic stem cell transplantation.[8] Rhino-orbito-cerebral mucormycosis and pulmonary mucormycosis were predominant. Pulmonary mucormycosis is most commonly observed in patients with leukemia receiving chemotherapy and in those undergoing hematopoietic stem cell transCT. Disseminated mucormycosis infections are rare in the clinical setting. Herein, we report a case of disseminated infection caused by *R. pusillus* in a child with ALL. mNGS plays an important role in the

Table 1 The Micro-Organisms in Bronchoalveolar Lavage Fluid (BALF) Were Detected by Metagenomics Next-Generation Sequencing

Category	Pathogens	Reads	Cover Age (%)	Relative Abundance (%)
BALF				
Bacteria	–	–	–	–
Fungi	*R. pusillus*	1458	0.3%	95.4%
Virus	–	–	–	–
Parasite	–	–	–	–
Human microecological microflora	*Pachyderm Malassezia*	11	0.1%	2.2%
Blood				
Bacteria	–	–	–	–
Fungi	*R. pusillus*	34303	5.6%	98.8%
Virus	*Nerpes virus hominis*	4	0.1%	67.9%
Parasite	–	–	–	–
Human microecological microflora	–	–	–	–
Pleural				
Bacteria	–	–	–	–
Fungi	*R. pusillus*	26206	5.3%	99.7%
Virus	*Nerpes virus hominis*	48	0.8%	100.0%
Parasite	–	–	–	–
Human microecological microflora	–	–	–	–

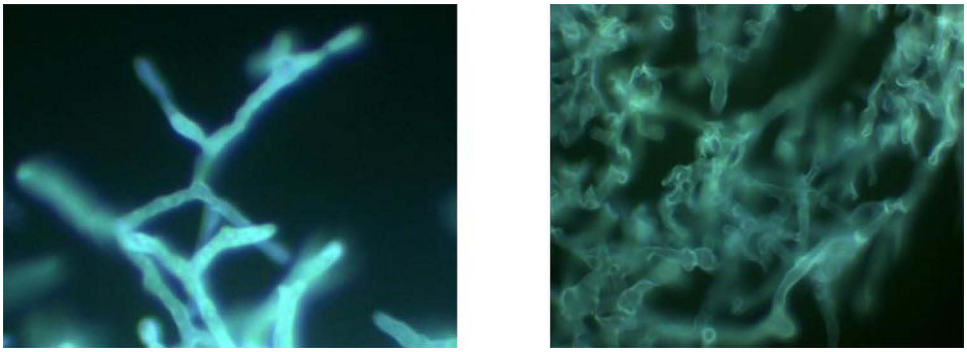

Figure 2 Filamentous fungal hyphae fluorescent staining in bronchoalveolar lavage fluid.

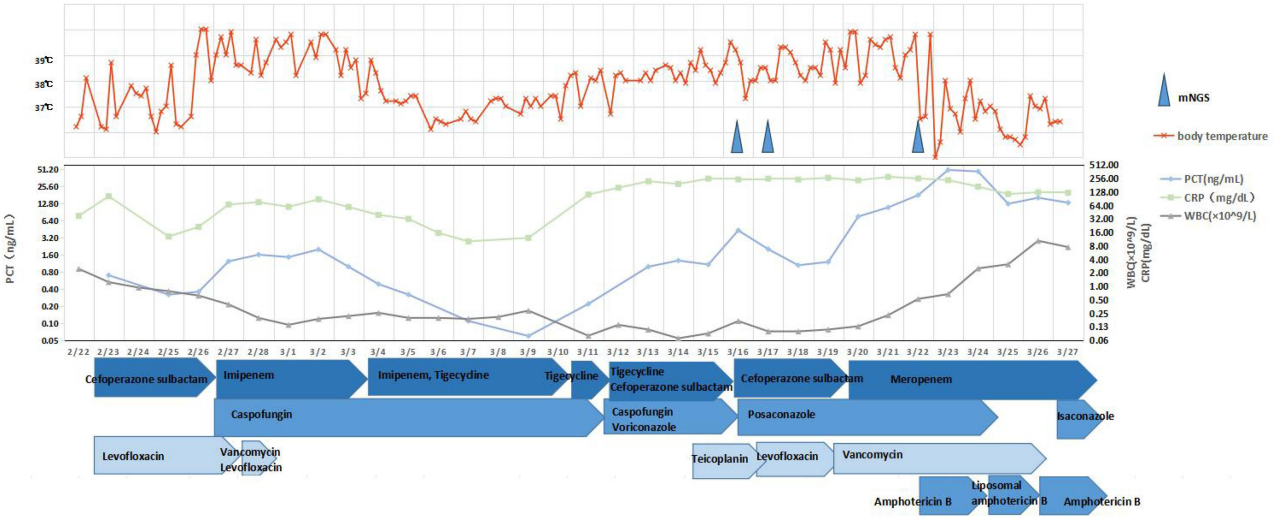

Figure 3 Treatment timeline including changes of WBC, PCT, CRP, and etiology detection timepoints.
Abbreviations: WBC, white blood cell; PCT, procalcitonin; CRP, c-reactive protein.

detection of *R. pusillus*. This case indicates that infection caused by *R. pusillus* could involve more organs than other mucormycotic infections.

Disseminated *R. pusillus* infections are progressive and invasive. However, the nonspecific clinical manifestations (fever, dyspnea, and chest pain) of *R. pusillus* make the diagnosis difficult. In this case, the patient also had atypical symptoms such as shortness of breath and fever. Consolidation, reverse halo signs, multiple nodules or masses, and pleural effusion can be observed on CT scans of patients with hematologic malignancies and pulmonary mucormycosis. Tissue biopsy was suggested when mucormycosis could not be excluded based on the clinical symptoms and imaging findings. However, histopathological diagnosis is invasive and the hyphae of the Mucorales are sometimes difficult to distinguish from Aspergillus filaments.[9] Fungal culture is time-consuming, and identifying fungi requires skilled technicians.[10] Serological antigen testing serves as a valuable adjunct to conventional mycological assays, facilitating early diagnosis of invasive fungal infections. However, serological detection methods for *R. pusillus* are limited, and GM and G tests have been reported to be negative for mucormycosis infection, as in our case.[11] Therefore, a rapid and precise diagnostic method is required.

mNGS can directly conduct high-throughput sequencing of nucleic acids in samples. Moreover, prior antibiotic exposure has less impact on the detection of mNGS.[12–14] Through comparison and analysis with the database, pathogenic microbial results were obtained, which are of great value for the diagnosis of acute and critical infections. However, mNGS results should be interpreted with caution, particularly at non-sterile sites with low sequences (<10 articles) of *R. pusillus*. Contamination must be excluded based on clinical and conventional

methods.[15] Our previous study also confirmed combining mNGS has an advantage in diagnosis.[16] mNGS has the capability to identify pathogens at the species level, which is invaluable for guiding clinical decisions regarding medication. However, in clinical practice, mNGS is a costly procedure and presents challenges for adoption in community hospitals. Moreover, mNGS demands a skilled technical workforce, a well-equipped laboratory, and adherence to standardized operating protocols. The interpretation of mNGS results must be approached with caution. In this case, the traditional culture methids were unable to detect *R. pusillus*. However, the sequences of *R. pusillus* were quickly detected by mNGS in the BALF, blood samples, and hydrothorax, indicating that mNGS is more sensitive than the traditional methods for identifying *R. pusillus*. Moreover, the patient was a hypoimmune ALL patient, whose lung imaging findings supported the presence of inflammation. Although the continued progression of this infection suggested that the treatment was ineffective, the successful application of mNGS has offered clinicians a fresh perspective, underscoring the technique's potential for rapid diagnostic etiology. The absence of pathological examination is a limitation of this case.

Mucorales are naturally resistant or insensitive to most antifungals. In general, routine in vitro drug susceptibility testing is not necessary. In this case, we failed to isolate the microroot mold and drug susceptibility experiments were not performed. Effective antimicrobial agents against the infections caused by *R. pusillus* are few. Guidelines recommend amphotericin B, posaconazole, and alaxonazole and actively combine surgical treatment.[17,18] High-dose liposomal amphotericin B is strongly recommended as a first-line treatment, while delayed release tablet posaconazole or intravenous isavuconazole and intravenous are recommended with moderate strength. Both triazoles are strongly recommended salvage treatments. Despite its substantial toxicity, amphotericin B deoxycholate is recommended against, but it may remain the only viable option in resource-limited settings. The management of mucormycosis hinges on recognizing disease patterns and achieving early diagnosis. In numerous case series, liposomal amphotericin B has been successfully used to treat mucormycosis with various organ involvement patterns.[19] The dosage should not be incrementally increased over several days; instead, the full daily dose should be administered from the outset of treatment. Isavuconazole has demonstrated efficacy comparable to an external matched control group treated with amphotericin B formulations, leading to its licensing in the USA for first-line treatment of mucormycosis. Posaconazole oral suspension has been successfully employed in first-line treatment. Recently, a delayed release tablet and an intravenous infusion formulation have been developed.[20] Double or triple antifungal combinations, such as liposomal amphotericin B with either posaconazole or voriconazole, are often utilized as first-line antifungal combination therapy in clinical practice. While some patient series have shown promising results with first-line antifungal combination therapy, there are no definitive data to guide the use of such combination therapies.[21,22] The widespread use of voriconazole prophylaxis has been linked to breakthrough *mucormycosis*.[23] In this instance, the patient received caspofungin for antifungal prophylaxis. However, it's important to note that the use of caspofungin as a preventive measure can potentially lead to severe infections due to the resistance of *R. pusillus* to this drug.[24]

There are some limitations in this manuscript. Firstly, the conventional culture in this case did not detect *R. pusillus*, and a histopathological diagnosis was lacking in this case, such as Schiff iodate or silver hexamine staining. Secondly, although fluorescent staining found the fungal hyphae, the hyphae morphology was not typical. mNGS was required to determine the fungal species. Finally, due to the costs, we only performed DR without a CT. The typical imaging features reported in the literature could not be seen.

Briefly, conventional methods to identify *R. pusillus* are time-consuming and lack sensitivity. The application of mNGS provided a prompt diagnosis of disseminated *R. pusillus* infection. This may provided a new perspective for clinicians and aid in achieving a precise and swift diagnosis of *R. pusillus* infections.[25] mNGS has the capability to identify pathogens at the species level, which is a significant asset in guiding clinical medication decisions. It allows for more targeted and effective drug therapy, ultimately a favorable outcome for the patient.

Abbreviations

Q12h, every 12 hours; Q8h, every 8 hours; iv, intravenous injection; QD, Quaque die.

Data Sharing Statement

Data and materials of this report are publicly available.

Ethics Approval and Consent to Participate

All methods were carried out in accordance with the Declaration of Helsinki. Written informed consent, including the publication of the patient's images, was obtained from a legal guardian of the patient. This study and the publication of the case details were approved by the Clinical Research and Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University.

Consent for the Publication

The authors and participant consent to the publication.

Author Contributions

All authors read and approved the final manuscript. All authors made a significant contribution to the work reported, whether that is 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 of 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 that they have no competing interests.

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