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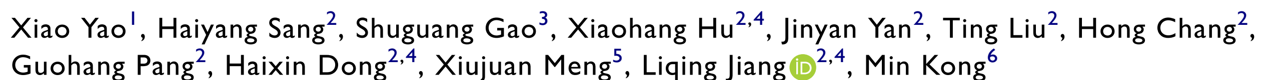
ORIGINAL RESEARCH

# Diagnostic Utility of Bronchoalveolar Lavage Metagenomic Next-Generation Sequencing for Pulmonary Mucormycosis: A Single-Center Retrospective Cohort Study



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**Background:** Although pulmonary mucormycosis is rare, it is highly invasive and carries a significant mortality rate. Due to its nonspecific clinical manifestations, it is often misdiagnosed as other invasive fungal diseases. Bronchoalveolar lavage fluid metagenomic next-generation sequencing is a rapid, precise, and comprehensive method for pathogen detection, showing great potential in the early diagnosis of pulmonary mucormycosis in a single-center retrospective series. It provides clinicians with faster and more accurate etiological information, thereby improving patient outcomes and reducing mortality rates.

**Methods:** This study conducted a retrospective analysis of the clinical data from 14 patients diagnosed with pulmonary mucormycosis between 1/6/2021 and 30/6/2024. Peripheral blood samples were collected to perform a complete blood count, measure C-reactive protein levels, and conduct 1,3-β-D-glucan and Galactomannan tests. Lung tissue samples were sent to the pathology laboratory for histological examination. Bronchoalveolar lavage fluid was subjected to fungal culture and metagenomic next-generation sequencing. Additionally, a three-month follow-up on the patients' survival status was carried out via telephone.

**Results:** Males accounted for 57.14% of the cases. Diabetes mellitus was present in 12 patients (85.71%, 12/14), and fever was observed in 12 patients (85.71%, 12/14). The 14 patients were categorized as proven cases (4 cases), probable cases (4 cases), and possible cases (6 cases). Two patients (14.29%, 2/14) were diagnosed with disseminated mucormycosis. Chest Computed Tomography scans revealed cavities in half of the patients (50.00%, 7/14). Fungal hyphae were identified in all the histopathological examinations (100%, 4/4). Metagenomic next-generation sequencing detected *Mucorales* pathogens in all the (100%, 14/14) cases, which is higher positivity than the positive rates of the 1,3-β-D-glucan test (35.71%, 5/14), Galactomannan test (42.86%, 6/14) and fungal culture (7.14%, 1/14). The turnaround time for metagenomic next-generation sequencing reports is 1–3 days, which is much shorter than the time required to obtain results from fungal culture (2–5 days). Additionally, metagenomic next-generation sequencing identified bacterial and viral co-infections, with 11 patients diagnosed as having mixed infections. All patients were treated with antifungal agents targeting *Aspergillus species*, such as voriconazole, posaconazole, isavuconazole, or amphotericin B, resulting in 9 patients improving, 2 patients being transferred to higher-level hospitals, and 3 patients discontinuing treatment. The 90-day follow-up revealed a mortality rate of 28.57%.

**Conclusion:** Metagenomic next-generation sequencing can serve as an important complement to traditional diagnostic methods, enabling rapid and accurate differentiation of *Mucorales* from other fungi. This allows patients to receive timely and targeted antifungal therapy, playing a critical role in early intervention and improving prognosis.

**Keywords:** pulmonary mucormycosis, clinical features, metagenomic next-generation sequencing, bronchoalveolar lavage fluid, treatment strategy

Received: 13 June 2025
Accepted: 1 December 2025
Published: 9 December 2025



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## Introduction

Mucormycosis is an invasive fungal infection caused by *Mucorales* fungi, with the most common being *Rhizopus, Mucorales* and *Lichtheimia*. Although relatively rare, it progresses rapidly, shows no age predilection, is highly destructive, and carries a high mortality rate (approximately 40%-80%).[1] Its incidence ranges from 1 to 12.3 cases per million people in Asia.[2] *Mucorales* can invade the lungs and other tissues, often leading to multi-organ involvement and disseminated infections.[1,3,4] In recent years, solid organ transplantation, malignancies, hypoxia, and inappropriate corticosteroid use have become significant risk factors for mucormycosis with advances in medicine.[5] However, diabetes remains the primary risk factor in developing countries.[6,7] Additionally, studies indicate that males are more susceptible to mucormycosis than females.[8,9] Pulmonary mucormycosis (PM), as the second most common type, has garnered significant attention due to its invasiveness and mortality rate exceeding 50%.[10,11] Due to its nonspecific clinical manifestations, PM is often misdiagnosed as other infectious diseases, particularly in cases of mixed infections.[12,13] Therefore, early and rapid diagnosis poses a significant challenge for clinicians.

According to the EORTC/NIAID-MSG criteria, pulmonary histopathology is the gold standard for the diagnosis of PM, but its positivity rate is only about 50%, and obtaining representative tissue samples is difficult, especially in the early stages of the disease.[11] Microbiological culture, imaging studies, and serological tests such as the 1,3-β-D-glucan test (G test) and Galactomannan test (GM-test) test can all assist in diagnosis. However, these methods have certain limitations. In microbiological diagnostics, direct microscopy has a low positivity rate and cannot differentiate fungal species, while fungal cultures are time-consuming and also exhibit low positivity rates,[12] making them inadequate for rapid diagnosis. Imaging studies, although helpful in diagnosing lung diseases, face challenges in distinguishing PM from other fungal infections. It is important to note that, although the G test and GM-test have certain value in screening for some types of invasive fungal infections (especially Aspergillosis), they are not applicable for the diagnosis of PM. In recent years, advancements in molecular diagnostic technologies have provided new possibilities for the diagnosis of PM. Techniques such as qPCR, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and restriction fragment length polymorphism have shown diagnostic value but are limited by issues such as high false-positive rates or operational complexity.[13] In contrast, metagenomic next-generation sequencing (mNGS) has demonstrated significant advantages. It can detect the genomic information of all microorganisms in a sample, enabling simultaneous detection of bacteria, viruses, fungi, and parasites. This is particularly useful for rare or difficult-to-culture pathogens, such as *Mucorale*s.[14–16] Compared to traditional methods, mNGS offers higher sensitivity and significantly reduces detection time,[14] facilitating early diagnosis and timely treatment, providing clinicians with faster and more accurate pathogen information, thereby improving patient outcomes and reducing mortality rates. Furthermore, BALF mNGS has significant advantages in the diagnosis of PM. It can directly obtain pathogens from the alveoli, reduce contamination, and improve the positivity rate and accuracy of detection. Although other types of specimens, such as sputum and serum, also have certain application values, BALF remains the preferred specimen type for the diagnosis of PM. Zhang et al[17] summarized and analyzed 63 cases of PM on PubMed from 2022 to 2024, and found that 39.7% of cases were diagnosed based on BALF, while only 17.5% of cases were diagnosed based on sputum, blood, or pleural fluid. Moreover, 36% of patients were tested with mNGS, indicating that BALF is superior to other specimens in the diagnosis of PM. However, it is important to note that fungi are widely present and it is impossible to distinguish between colonization and infection. Therefore, a comprehensive analysis in combination with the clinical manifestations of the patient is required.

Currently, most studies focus merely on the diagnosis and treatment of PM, with few comparing the rapidity and sensitivity of BALF mNGS to traditional diagnostic methods in diagnosing PM, as well as the impact of mNGS on the diagnosis, treatment, and prognosis of PM. This paper analyzes the aforementioned aspects to explore the application value of mNGS in PM.

## Materials and Methods

### Study Design and Patients

This retrospective study strictly adhered to inclusion and exclusion criteria, consecutively enrolling 14 hospitalized patients with a diagnosis of PM at the Affiliated Hospital of Jining Medical University between 1/6/2021 and 30/6/2024.

The inclusion criteria were as follows: age ≥ 18 years; clinical manifestations and radiographic findings consistent with mucormycosis, and clinical diagnosis of PM; simultaneous performance of serological tests, fungal cultures, and mNGS. Exclusion criteria: BALF volume <3 mL; single-sample mNGS sequencing depth <20 million reads; incomplete clinical data; and duplicate samples from the same admission (only the first sample retained).

All patients had received treatment at local hospitals prior to admission. The date of admission was defined as D0. To promptly identify the infectious pathogens, all enrolled patients underwent BALF fungal culture and mNGS within D4. Based on the definitions of invasive fungal diseases by the European Organization for Research and Treatment of Cancer/ National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/NIAIDMSG),[15] invasive fungal infections were classified into three categories:I) Proven cases: defined by the presence of fungi in lung tissue histopathology or lung tissue culture; II) Probable cases: meeting clinical criteria (ie, imaging, bronchoscopy, or sinus analysis) and mycological criteria (specific fungal antigens and cell wall components); III) Possible cases: meeting clinical criteria but lacking mycological evidence.

Of 14 patients in this study, patients 1 to 13 signed the informed consent by themselves, while patient 14 was in poor condition due to a high fever of 40°C, unable sign to the informed consent for BALF extraction normally, therefore her consent was signed by the family member on her behalf. After obtaining written informed consent, clinicians collected BALF through standard bronchoalveolar lavage procedures and promptly sent the samples for fungal culture and mNGS. Clinical data collected for all enrolled patients included gender, age, comorbidities, imaging and laboratory test results, clinical manifestations, treatment modalities, hospital stay duration, and discharge outcomes. A three-month follow-up on survival status was conducted via telephone.

## Traditional Detection Methods

After trypsin digestion of BALF, 10 μL was inoculated onto Sabouraud dextrose agar (Baibo Bio-Tech Co., Ltd., Jinan, China) for fungal culture. The cultures were incubated at 28°C in a Thermo Scientific 37°C $CO_2$ incubator (Thermo Fisher Scientific, USA). Following pure culture isolation, filamentous fungi were stained with phenol cotton blue by wet mount technique to observe their morphology under the microscope, and identified by matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) if necessary, but no drug susceptibility test was performed. Simultaneously, samples were smeared onto glass slides for Gram staining and morphological observation.

Lung tissue samples were sent to the pathology laboratory for histological sectioning. Tissue sections were stained with hematoxylin-eosin, periodic acid-Schiff, and Grocott's methenamine silver and examined under an optical microscope (Olympus BX53, Japan), The hyphae of *Mucor* are typically irregular, broad, and aseptate, with right-angle branching. Peripheral blood samples were collected for complete blood count, C-reactive protein (CRP), G-test, and GM-test. All patients underwent Chest Computed Tomography scans using either a GE Optima CT660 scanner or a SIEMENS Definition Flash scanner after admission, and the reports were issued by two senior radiologists.

## mNGS Detection

A 3 mL aliquot of BALF was inactivated at 65°C for 30 minutes, and DNA was extracted strictly following the instructions of the DNA purification kit (BGI Biotechnology Co.,Ltd, Wuhan, China). DNA libraries were constructed at a concentration of ≥ 1ng/μL, including DNA fragmentation, end repair, adapter ligation, and PCR amplification. PCR reaction conditions were as follows: 98 °C for 2 min, followed by 20 cycles of 98 °C for 15s, 56 °C for 15s, and 72 °C for 30s, with a final extension at 72 °C for 5min. The DNA library and sequencing library preparations were performed using the PMseq™ high-throughput DNA detection kit (BGI Biotechnology Co.,Ltd, Wuhan, China). Quality-qualified libraries were used to prepare DNA nanoballs. Finally, sequencing was performed on the MGISEQ-200 platform (MGI Tech Co., Ltd).[18] Negative and positive controls were included throughout the entire process to ensure quality control and minimize contamination. Each sample required a minimum of 20 million high-quality sequencing reads.

The data were generated by removing adapter sequences, sequences with high N base content, and low-complexity sequences using in-house software. The data were then processed by computationally extracting human host reads aligned to the human reference genome hg38 (GRCh38, Dec. 2017) using Burrows-Wheeler Alignment (BWA, version: 0.7.17-r1188)[19] and Kraken2 (Kraken version 2.1.2).[20] Subsequently, the remaining data were identified through BWA

alignment to the pathogen sequence database (PMDB), a self-built database comprising 10,989 bacterial species, 1,179 fungal species, 282 parasitic species, and 5,050 viral species. This database was curated by referencing public genome databases such as NCBI (https://www.ncbi.nlm.nih.gov/) and EupathDB (https://veupathdb.org/veupathdb/app), through processes including sequence cleaning, cluster analysis, and selection of representative reference sequences. Pathogens were identified based on the number of stringently PMDB-mapped reads (SMRN), following these criteria: I) Microorganisms identified as background were first removed. Background microorganisms were determined through a series of experiments involving laboratory environments, reagents, and experimental materials; II) The SMRN thresholds for considering a candidate species positive were as follows: for bacteria, DNA viruses, and fungi, the SMRN must be at least 3; for Mycobacterium tuberculosis (MTB) and RNA viruses, the SMRN must be at least 1; and for parasites, the SMRN must be at least 100; III) Species with no significant differences were filtered out based on the following conditions: for species with SMRN > 50, the SMRN must be at least 5-fold higher than that in the negative control; for species with SMRN ≤ 50, the SMRN must be at least 3-fold higher than that in the negative control.

However, whether the reported pathogen was truly pathogenic required further clinical judgment by physicians based on the patient's clinical manifestations.

## Statistical Analysis

Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were described as counts and percentages. Continuous variables were presented as median [first quartile (Q1), third quartile (Q3)] due to non-normal distribution (assessed by Shapiro–Wilk test). Mortality was defined as all-cause death occurring within 30 days of hospital admission, and mortality rates were calculated as the proportion of deaths among the total cohort with 95% confidence intervals estimated using the binomial exact method.

# Results

## Baseline Characteristics of Patients

This study included 14 patients with PM, with a mean age of 63.4 years (range: 38–77). Males accounted for 57.14% (8/14), and the average hospital stay was 14 days (range: 4–34). Among the 14 patients, only 1 (7.14%, 1/14) had no underlying diseases, while the remaining 13(92.86%, 13/14) had a history of comorbidities. Specifically, 12 patients (85.71%, 12/14) had diabetes, 2 (14.29%, 2/14) had a history of fungal infections, and others had conditions such as coronary heart disease and hypertension. Fever was observed in 12 patients (85.71%, 12/14), with the highest recorded temperature reaching 40°C. Chest pain was reported in 7 patients (50.00%, 7/14), and all patients exhibited symptoms of cough, sputum production, and dyspnea. However, none experienced abdominal pain or coma. Blood cell analysis on the day of admission revealed that 5 patients (35.71%, 5/14) had elevated white blood cell counts, with the highest reaching $31.31\times10^9$/L, and 8 patients (57.14%, 8/14) had elevated neutrophil percentages. All patients showed elevated C-reactive protein (CRP) levels, indicating significant inflammatory responses.

After active treatment, 9 patients improved and were discharged, 2 were transferred to higher-level hospitals due to rapid disease progression, and 3 discontinued treatment for financial reasons. The 14 patients in this study were categorized as follows: 4 proven cases (numbered 1–4), 4 probable cases (numbered 5–8), and 6 possible cases (numbered 9–14). Among them, 12 patients (85.71%, 12/14) had infections confined to the lungs, while 2 (14.29%, 2/14) had disseminated mucormycosis involving the chest and brain, respectively. Chest CT findings primarily included cavities in 7 patients (50.00%, 7/14), consolidation in 3 (21.43%, 3/14), high-density shadows in 2 (14.29%, 2/14), nodules in 1 (7.14%, 1/14), and pleural effusion in 1 (7.14%, 1/14). The baseline clinical characteristics of the enrolled patients are summarized in Table 1.

## Comparison of Serological Tests, Histopathology, and mNGS

Among the 14 enrolled patients, 5 (35.71%, 5/14) tested positive for the G-test, and 6 (42.86%, 6/14) tested positive for the GM-test. Among the 4 proven cases, only 1 showed positivity for both tests. In the 4 probable cases, all tested positive for the

**Table 1** Clinical Characteristics of 14 Mucormycosis Patients

| Characteristics | Frequency (%) |
|---|---|
| **Gender, Male** | 8(57.14) |
| **Age, years, mean (Q1, Q3)** | 63.36(56,71) |
| **Comorbidities** | |
| Diabetes | 12(85.71) |
| Cardiovascular disease | 6(42.86) |
| Pulmonary disease | 4(28.57) |
| Hypertension | 3(21.43) |
| Hematological disease | 2(14.29) |
| Cerebrovascular disease | 1(7.14) |
| **Clinical manifestations** | |
| Fever | 12(85.71) |
| Cough | 14(100.00) |
| Dyspnea | 14(100.00) |
| Sputum | 14(100.00) |
| Chest pain | 7(50.00) |
| Abdominal pain | 0 |
| Coma | 0 |
| **Clinical forms** | |
| Pulmonary | 12(85.71) |
| Dissemination | 2(14.29) |
| **Classification** | |
| Proven | 4(28.57) |
| Probable | 4(28.57) |
| Possible | 6(42.86) |
| **Surgery** | 0 |
| **Length of stay, d, means (Q1, Q3)** | 14(8,18) |
| **Outcomes** | |
| Improved | 9(64.29) |
| Transferred | 2(14.29) |
| Discontinued | 3(21.43) |
| **Survival at 90 days** | 10(71.43) |
| **Mortality at 90 days** | 4(28.57) |
| **Laboratory Examination** | |
| WBC (>10×$10^9$/L) | 5[a](35.71) |
| NEU% (>70%) | 8[b](57.14) |
| CRP (>8 mg/L) | 14[c](100.00) |
| **Imaging features** | |
| Cavities | 7(50.00) |
| Consolidation | 3(21.43) |
| High density images | 2(14.29) |
| Multiple solid nodules | 1(7.14) |
| Pleural effusion | 1(7.14) |

**Notes**: Surgery: 0 (0%) Patients underwent surgery in this cohort; [a]Patients with elevated WBC count (>10×10^9/L); [b]Patients with elevated Neutrophil percentage (>70%); [c]Patients with elevated CRP (>8 mg/L).
**Abbreviations**: WBC, White blood cell; NEU%, Neutrophil percentage; CRP, C-reactive protein.

GM-test, but only 2 (14.29%, 2/14) were positive for the G-test. Among the 14 patients, 4 underwent pulmonary histopathological examination, all of which showed the presence of acute and chronic inflammatory cell infiltration and fungal hyphae. mNGS results detected PM pathogens in all patients, identifying a total of 5 species and 16 strains. These included *Rhizopus microsporus* (43.75%, 7/16), *Rhizopus delemar* (18.75%, 3/16), *Rhizomucor pusillus* (18.75%, 3/16), *Rhizopus oryzae* (12.50%, 2/16), and *Lichtheimia ramosa* (6.25%, 1/16). In proven cases 1–4, both mNGS and histopathology were positive, but only case 1 was positive for both serological tests, while case 2 was positive only for the G-test. Among probable cases 5–8, only cases 5 and 6 were positive for both the G-test and GM-test, while cases 7 and 8 were positive only for the GM-test. In possible cases 9–14, mNGS was positive in all cases, with only case 10 showing positivity for the G-test and the remaining cases negative for serological tests. Notably, among the 4 proven cases, although the positive rates of pulmonary histopathology and mNGS were both 100% (4/4), but mNGS results were obtained prior to histopathological findings. These results demonstrate that the positivity rate of mNGS (100%, 14/14), however positivity rate of the G-test is only 35.71% (5/14), and that of the GM-test is 42.86% (6/14). The detection results for all enrolled patients are summarized in Table 2.

## Comparison of BALF mNGS and Fungal Culture Results

All patients underwent timely collection of BALF for both mNGS and fungal culture after admission. Samples were collected on D1 for 6 patients (42.86%, 6/14), D2 for 4 patients (28.57%, 4/14), D3 for 2 patients (14.29%, 2/14), and D4 for 2 patients (14.29%, 2/14). mNGS results were reported within 18–48 hours after sample submission, however fungal culture required 4.4 days[4,5] (Figure 1).

Among the 14 enrolled patients, mNGS detected a total of 42 pathogenic strains, including 10 bacteria (23.81%, 10/42), 27 fungi (62.29%, 27/42), and 5 viruses (11.90%, 5/42). Fungi accounted for the highest proportion, with *Aspergillus fumigatus* being the most common (14.29%, 6/42). The pathogens causing PM detected in this study included *Rhizopus microsporus* (11.90%, 5/42), *Rhizomucor pusillus* (7.14%, 3/42), *Rhizopus delemar* (7.14%, 3/42), *Rhizopus oryzae* (7.14%, 3/42), *Pneumocystis jirovecii* (4.76%, 2/42) and *Lichtheimia ramosa* (2.38%, 1/42). Additionally, *Candida albicans* and *Candida glabrata* were detected. Among the bacteria, besides common pathogens such as *Staphylococcus*

**Table 2** The Detection Results and Treatment Strategy for 14 PM Patients

| Case | G Test | GM Test | Histopathologic | Fungal Culture | Pathogens of PM Detected by mNGS | Days from Admission | Days from Admission to Treatment Change | Treatment |
|---|---|---|---|---|---|---|---|---|
| 1 | P | P | P | N | *Rhizopus microsporus* | 2 | 4 | VOR+AMB |
| 2 | P | N | P | N | *Rhizopus delemar* | 4 | 5 | ISA |
| 3 | N | N | P | N | *Rhizopus oryzae* | 2 | 4 | AMB |
| 4 | N | P | P | *Rhizopus arrhizus* | *Rhizopus delemar; Rhizopus microsporus* | 1 | 3 | AMB+POS |
| 5 | P | P | NT | N | *Rhizomucor pusillus* | 3 | 5 | POS |
| 6 | P | P | NT | N | *Rhizopus microsporus* | 1 | 3 | VOR+AMB |
| 7 | N | P | NT | N | *Lichtheimia ramosa; Rhizopus microsporus* | 1 | 3 | ISA |
| 8 | N | P | NT | N | *Rhizomucor pusillus* | 1 | 4 | AMB |
| 9 | N | N | NT | N | *Rhizopus delemar* | 2 | 4 | AMB |
| 10 | P | N | NT | N | *Rhizopus oryzae* | 4 | 6 | VOR+POS |
| 11 | N | N | NT | N | *Rhizopus microsporus* | 2 | 4 | AMB |
| 12 | N | N | NT | N | *Rhizopus microsporus* | 1 | 3 | POS |
| 13 | N | N | NT | N | *Rhizopus delemar* | 3 | 5 | AMB+ISA |
| 14 | N | N | NT | N | *Rhizopus microsporus* | 1 | 3 | POS |
| Positive rate (%) | 35.71 | 42.86 | 100 | 7.14 | 100 | | | |

**Abbreviations**: PM, Pulmonary mucormycosis; P, positive; N, negative; NT, no tested; AMB, Amphotericin B; POS, Posaconazole; ISA, Isavuconazole; VOR, Voriconazole; G-test, 1,3-β-D-glucan test; GM-test, Galactomannan test.

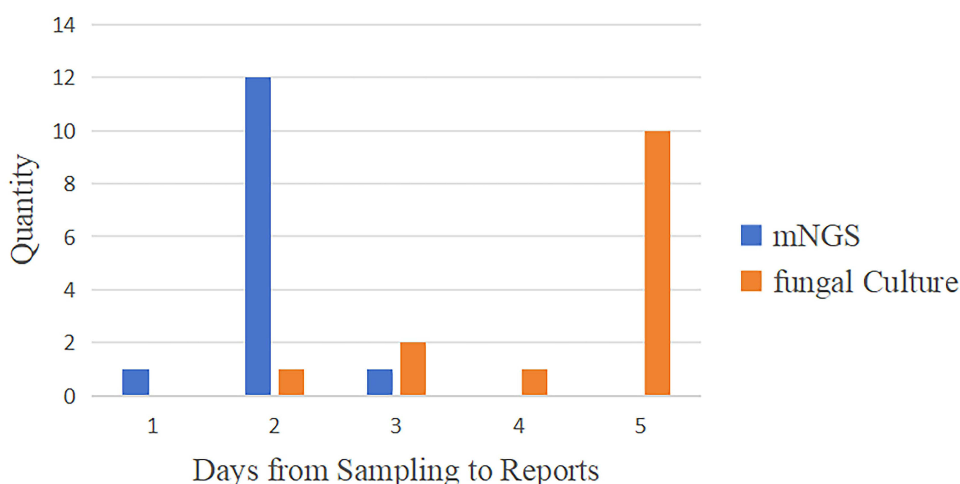


**Figure 1** Days from sampling to reports for mNGS and fungal culture. The blue line represents mNGS, the yellow line represents fungal culture.

*aureus* (7.14%, 3/42) and *Haemophilus influenzae* (4.76%, 2/42), mNGS also identified *Ureaplasma urealyticum* (2.38%, 1/42), which are difficult to detect using traditional methods. Detected viruses included *Human gammaherpesvirus type 4* (4.76%, 2/42), *Human betaherpesvirus type 5* (4.76%, 2/42), and *Human alphaherpesvirus type 1* (2.38%, 1/42).

Pathogens were detected in all patients. While 3 patients had only one fungal species detected, the remaining 11 had two or more pathogens, including cases with two fungi (4), bacteria and fungi (2, 3, 5, 6, 8 and 11), fungi and viruses (1 and 7), and bacteria, fungi, and viruses (10 and 14). Case 10 exhibited a complex mixed infection with *S. aureus, A. fumigatus, C. albicans, R. oryzae*, and *H. gammaherpesvirus type 4*. Case 14 showed *Acinetobacter pittii, P. jirovecii, Aspergillus* sp., *R. microsporus*, and *H. betaherpesvirus type 5*, representing the most complex mixed infection.

Fungal culture identified fungi in 4 cases, with only case 4 detecting *Rhizopus arrhizus*, while the other 3 cases (1, 2 and 6) identified *A. fumigatus* (Figure 2B). In cases 1, 2, 4, and 6, mNGS not only detected the corresponding *Rhizopus* and *A. fumigatus* but also identified additional bacteria, fungi, and viruses (Figure 2A). The overall mismatch rate between the two methods was 71.43% (10/14), with a partial match rate of 28.57% (4/14). These results demonstrate that mNGS significantly outperforms in positivity rate, detection speed, and breadth of pathogen identification, particularly for rare pathogens and mixed infections. The specific pathogens detected are illustrated in Figure 2A.

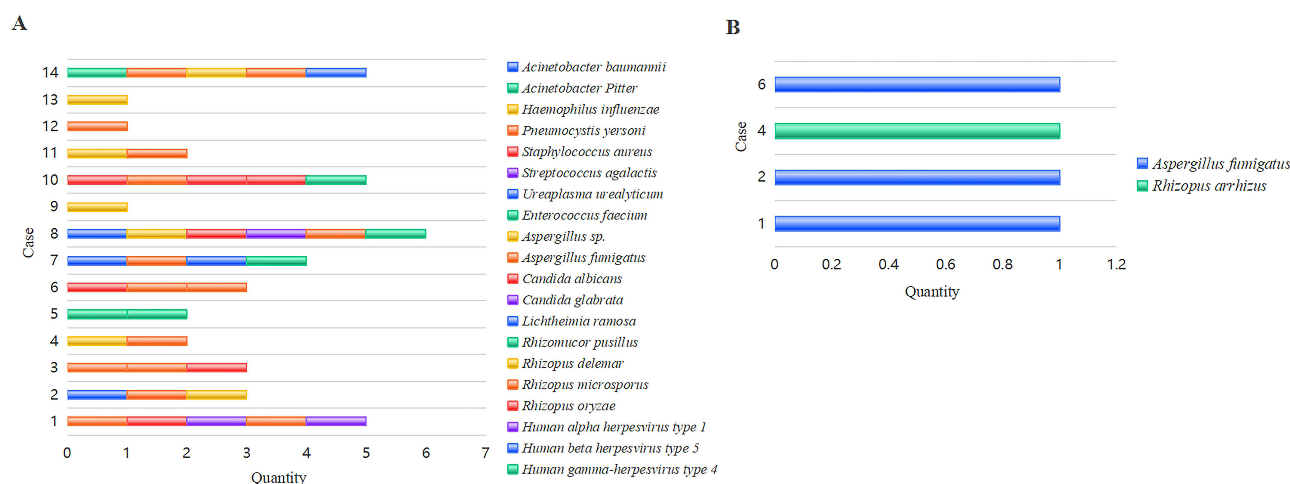


**Figure 2** The types of pathogens detected by mNGS and fungal culture in all enrolled patients. (**A**) Each number (1–14) represents a patient and shows the pathogens detected by mNGS. (**B**) Numbers 1, 2, 4, and 6 represent patients and show the pathogens detected by fungal culture.

## Impact of mNGS on Treatment and Outcomes

Among the 14 enrolled patients, all had received treatment at local hospitals prior to admission, with 9 (64.29%, 9/14) prophylactically administered voriconazole for antifungal therapy upon admission. Based on the mNGS results, clinicians promptly adjusted the specific antifungal therapies for all patients. Case 3 and 8 underwent nebulized administration of amphotericin B, with each dose set at 35 mg and administered twice daily. For case 9 and 11, intravenous infusion of amphotericin B was utilized, with a dosage of 150 mg per administration, delivered once daily. Oral isavuconazole was prescribed for case 2 and 7, with a dosing schedule of 2 capsules per administration, twice daily. Cases 5, 12, and 14 received oral posaconazole, with each administration consisting of 3 tablets, given twice daily. A combination therapy approach was adopted for case 4, involving intravenous amphotericin B alongside oral posaconazole. Similarly, Case 13 was treated with a combination of intravenous amphotericin B and oral isavuconazole. Notably, cases 1, 6, and 10 presented with mixed infections involving both *Aspergillus* sp. and *Mucorales*. These cases were managed with a combination therapy of voriconazole and amphotericin B. The voriconazole dosage was meticulously set at 200 mg per administration, with the medication being administered twice daily (Table 2). In addition to the antifungal treatments, all enrolled patients were concurrently administered broad-spectrum antibiotics to address potential bacterial co-infections and to provide comprehensive antimicrobial coverage.

Among the 14 patients, cases 4 and 13 were diagnosed with disseminated mucormycosis. Case 4 involved the lungs and brain, while case 13 involved the lungs and pleura. In case 4, chest CT upon admission indicated severe pneumonia, and the patient was initially treated with meropenem for infection. mNGS results identified *R. oryzae* and *R. microsporus* and cranial CT revealed a brain abscess, likely due to *Rhizopus* invasion. The clinician immediately initiated intravenous amphotericin B and posaconazole for antifungal treatment. However, due to the severity of the condition and high treatment costs, the family decided to discontinue treatment, and the patient died 26 days after discharge. In case 13, piperacillin-tazobactam was initially administered for infection. mNGS results detected *R. delsoni*, prompting the clinician to add isavuconazole for antifungal therapy. Subsequently, the patient developed chest pain, suspected to be caused by *Mucorales* invading the pleura. The treatment was adjusted to include isavuconazole and amphotericin B cholesteryl sulfate complex infusion. Follow-up chest CT after one week showed improvement in lung infection and abscesses, and the patient was discharged after 10 days of treatment.

After active symptomatic treatment, 9 patients improved, 2 cases (2 and 5) were transferred to higher-level hospitals due to lack of improvement, and 3 cases (4, 6, and 8) discontinued treatment for financial reasons. A 90-day telephone follow-up revealed that all 9 patients who improved survived. Among the 5 patients who were discharged due to severe illness, only case 2, who was transferred to a higher-level hospital and survived. The remaining 4 cases (4, 5, 6, and 8) died due to the severity of their conditions. The mortality rate in this study was 28.5%. All 4 deceased patients had at least two underlying conditions, including diabetes and myocardial infarction. Case 5 also had acute myeloid leukemia and did not undergo full-course treatment.

# Discussion

Mucormycosis, caused by *Mucorales*, is characterized by rapid filamentous growth, widespread distribution, spore production, unique structural features, adaptability to diverse environments, and interactions with host immune defenses. These factors collectively drive infection establishment and progression, establishing mucormycosis (most commonly caused by *R. arrhizus, R. microsporus*, and *Mucor circinelloides*) as the second most common angioinvasive fungal disease after invasive aspergillosis (predominantly *A. fumigatus*). PM, the second most prevalent form, occurs through the inhalation of airborne sporangiospores. These spores secrete lytic enzymes and toxins (such as mucorubin) and can spread through lung parenchyma to the chest wall, central nervous system, or gastrointestinal tract, inducing tissue thrombosis and necrosis. This can lead to complications such as encephalitis, brain abscesses, and gastrointestinal ulcers,[21,22] due to its severe infection, rapid progression, and high mortality rate,[1,11] early etiological diagnosis is critical for timely treatment.

PM is commonly observed in immunocompromised individuals with conditions such as diabetes, hematologic malignancies, solid organ transplantation, neutropenia, long-term corticosteroid use, and other underlying diseases.[23] Numerous studies have identified diabetes as the most frequent predisposing factor, present in 50–70% of patients

diagnosed with mucormycosis.[8,24] Hyperglycemia impairs phagocyte function, intracellular killing, and chemotaxis, while hypoxia in lung diseases creates a favorable environment for fungal spore germination and proliferation, increasing the risk of mucormycosis. Notably, mucormycosis can also occur in individuals without any predisposing conditions.[10,25] In this study, 92.85% of patients had underlying diseases, with 85.71% having diabetes, consistent with previous studies,[8,9,24] this highlights diabetes as a significant risk factor for PM, while other comorbidities (eg, coronary heart disease, hypertension) may further increase susceptibility. Previous studies have also reported a higher prevalence of mucormycosis in males,[8,9] which aligns with our findings (57.14% male vs 42.86% female). *Mucorales* can invade not only the lungs but also the brain, nose, orbits, gastrointestinal tract, kidneys, skin, and other tissues. In this study, 12 cases involved only the lungs, while 2 cases involved the lungs and pleura or central nervous system, diagnosed as disseminated mucormycosis.

The clinical symptoms of PM, including high fever (>38°C), cough, dyspnea, and chest pain, are nonspecific. In this study, 85.71% of patients presented with fever, 50.00% with chest pain, and all patients exhibited cough, sputum production, and dyspnea. In the early stages, imaging may only show perivascular ground-glass opacities, progressing to pulmonary nodules, consolidation, cavities, and the reverse halo sign in later stages,[24] The primary imaging findings in our patients were cavities (50.00%) and consolidation (21.43%). Based solely on clinical manifestations and imaging, it is challenging for clinicians to distinguish PM from other pulmonary diseases.

In this study, the positive rates of G test and GM test were 35.71% and 42.86%, respectively. While the GM-test was positive in 3 culture-positive cases (1, 2 and 6), it was also positive in culture-negative cases (5, 7 and 8), possibly due to low fungal concentrations or prior antifungal prophylaxis. Although the G test and GM test used for fungal screening cannot be directly applied to the diagnosis of mucormycosis, they hold certain value in differentiating invasive aspergillosis or in excluding or co-diagnosing *Aspergillus* in mixed infections.[26,27] However, they cannot be used as a basis for identifying *Mucorales*. Negative results cannot rule out mucormycosis. When using these tests, clinicians need to combine them with other diagnostic methods to comprehensively assess the patient's condition and formulate the most appropriate treatment plan.

Although definitive diagnosis of PM can rely on BALF fungal culture and lung histopathology, invasive procedures may not be initially suitable for patients without risk factors, and culture results take considerable time. In this study, all cases underwent BALF fungal culture, with only 4 yielding positive results, including just 1 case of *Rhizopus*, resulting in a diagnostic positivity rate of only 7.14%. Although fungal hyphae were detected in all 4 cases with pulmonary tissue histopathological examination, this method is a traumatic diagnostic procedure and is not applicable to all patients.

mNGS directly sequences all microbial nucleic acids in a sample without prior knowledge of the pathogen, increasing the likelihood of detecting *Mucorales* by identifying specific gene sequences. It serves as an important complement to histopathology and microbiological diagnostics. Recently, mNGS has been increasingly used to assist in the clinical diagnosis of invasive fungal infections, including mucormycosis.[28–30] Wang C. et al[27] studied 310 patients with suspected invasive pulmonary fungal infections and found that mNGS had a sensitivity and specificity of 86.76% and 86.98%, respectively, outperforming traditional microbiological methods in accuracy and speed (AUC=0.967), which was consistent with our research findings. Wei E. et al[28] analyzed 3 pediatric cases of rare invasive mold brain abscesses, detecting *Rhizomucor miehei* and *Rhizomucor pusillus* in cerebrospinal fluid via mNGS, enabling timely and precise treatment and discharge. In this study, all enrolled patients underwent BALF mNGS, which detected 25 fungal strains, including 15 cases of *Mucorales*. The most common pathogen was *R. microsporus*, followed by *R. pusillus* and *R. delemar*, consistent with previous studies.[31,32] However, regional variations in pathogenic organisms may exist due to environmental factors and temperature-dependent growth characteristics. Additionally, metagenomic sequencing can detect other microorganisms coexisting with *Mucorales*. Studies have shown that mucormycosis can co-occur with other invasive mold infections, most commonly aspergillosis, as reported in hematologic malignancies,[31] COVID-19-associated mucormycosis (CAM),[32] and other immunocompromised patients.[33] In this study, 50.00% of patients had co-detected *Aspergillus* sp. Besides fungi, mNGS also identified bacteria and viruses, including *U. urealyticum*, which are difficult to detect using traditional methods. Among the 11 cases with mixed infections, two or more pathogens were detected. These results demonstrate that mNGS outperforms traditional methods in terms of reporting speed, comprehensiveness, and positive rate, providing more timely, comprehensive, and accurate diagnoses of PM, particularly in mixed infections, consistent with previous studies.[34]

In this study, all 14 patients had their treatment regimens adjusted based on mNGS results. Targeted antifungal therapies were administered individually or in combination as needed. Amphotericin B is the most active drug against *Mucorales* in vitro and has shown efficacy in treating mucormycosis when used alone.[35,36] In this study, 8 of the 14 patients received amphotericin B alone or in combination with other antifungals. It is worth noting that voriconazole is effective against *Candida* infections but not against *Mucorales*. In cases of mixed infections, combination therapy or the use of drugs with activity against both types of fungi is necessary. For PM, patients who received combined medical and surgical treatment had significantly better prognosis.[37,38] Surgical debridement of necrotic tissue may allow better penetration of antifungal drugs, thereby improving the outcome.[1] However, considering the surgical risks, none of the patients in this study underwent surgical treatment. 4 patients died due to severe illness, delayed treatment, and underlying comorbidities, resulting in a mortality rate of 28.57%, this may be attributed to differences in the study population. Further, the use of improper antifungals such as voriconazole for management seen in a sizeable proportion of our participants, highlights the lacunae in knowledge among physicians. mNGS has certain advantages in the diagnosis and prognostic evaluation of invasive mucormycosis, enabling clinicians to adjust treatment strategies in a timely manner and may play a significant role in improving prognosis.

This study has certain limitations. First, as a single-center retrospective analysis with a small sample size, the conclusions may be subject to bias. There is a need for us to conduct larger-scale, prospective, multicenter studies to validate the results. Secondly, *Mucorales* are ubiquitous in the environment, which makes it difficult to distinguish between colonization and infection. This requires clinicians to accurately assess the clinical manifestations. Thirdly, mNGS lacks a standardized method for calculating sequence thresholds when identifying true infections, which means that different studies lack a certain degree of comparability. In addition, the high cost and limited availability of mNGS will also affect its position in routine clinical use. In conclusion, despite the challenges that mNGS still faces in clinical application, it can serve as a rapid complementary method in cases of mixed infections or infections caused by special pathogens such as *Mucorales*.

## Conclusion

As an emerging pathogen detection technology, mNGS shows a high positive rate in the detection of *Mucorales* pathogens. It is particularly suitable for infectious diseases with negative results from traditional detection methods or complex clinical conditions. mNGS may assist clinicians in timely adjusting treatment plans, thereby playing a significant role in improving prognosis.

## Ethics Statement

In accordance with the “Administrative Measures for Ethical Review of Biomedical Research Involving Human Beings”, this study complies with the Declaration of Helsinki and has been approved by the Institutional Medical Ethics Committee of Affiliated Hospital of Jining Medical University (Ethics Approval Number: 2025-01-C024). All patients signed the informed consent for data publication personally during their hospital stay.

## Author Contributions

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.

## Funding

This work was supported by the Research Fund for Shandong Provincial Natural Science Foundation (No. ZR2023MH325), Jining Medical University Affiliated Hospital Doctoral Research Fund (No. 2022-BS-01), Research Fund for Academician Lin He New Medicine (No. JYHL2022MS05), Jining Medical University Affiliated Hospital “Zhi-Xing” Project (No. ZX-ZD-2023-01), and the Jining City Key Research and Development Plan Project (No. 2023YXNS178).

## Disclosure

The authors declare no conflicts of interest. This paper has been uploaded to ResearchSquare as a preprint: https://www.researchsquare.com/article/rs-6308222/v1.

## References


1. Cornely OA, Alastruey-Izquierdo A, Arenz D, et al. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of medical mycology in cooperation with the mycoses study group education and research consortium. *Lancet Infect Dis*. 2019;19(12):e405–21. doi:10.1016/S1473-3099(19)30312-3
2. Hassan MIA, Voigt K. Pathogenicity patterns of mucormycosis: epidemiology, interaction with immune cells and virulence factors. *Med Mycol*. 2019;57(Suppl 2):S245–56. doi:10.1093/mmy/myz011
3. Singla K, Samra T, Bhatia N. Primary cutaneous mucormycosis in a trauma patient with morel-lavallée lesion. *Indian J Crit Care Med Peer-Rev off Publ Indian Soc Crit Care Med*. 2018;22(5):375–377.
4. Rodriguez CJ, Tribble DR, Malone DL, et al. Treatment of suspected invasive fungal infection in war wounds. *Mil Med*. 2018;183(suppl_2):142–-146. doi:10.1093/milmed/usy079
5. Muthu V, Agarwal R, Rudramurthy SM, et al. Multicenter case–control study of covid-19–associated mucormycosis outbreak, India. *Emerg Infect Dis*. 2023;29(1):8–19. doi:10.3201/eid2901.220926
6. Prakash H, Chakrabarti A. Global epidemiology of mucormycosis. *J Fungi*. 2019;5(1):26. doi:10.3390/jof5010026
7. Sahu M, Shah M, Mallela VR, et al. COVID-19 associated multisystemic mucormycosis from India: a multicentric retrospective study on clinical profile, predisposing factors, cumulative mortality and factors affecting outcome. *Infection*. 2023;51(2):407–416. doi:10.1007/s15010-022-01891-y
8. Manade K. A clinical study of rhino-orbital-cerebral mucormycosis during the COVID-19 pandemic in western Maharashtra. *J Fam Med Prim Care*. 2024;13(9):3730–3734. doi:10.4103/jfmpc.jfmpc_2_24
9. Kumari K, Rathod D, Meshram T, et al. Perioperative anesthesia challenges and outcomes of patients with rhino-orbito-cerebral mucormycosis during the second wave of COVID-19 pandemic: an observational study. *J Anaesthesiol Clin Pharmacol*. 2023;39(4):615–621. doi:10.4103/joacp.joacp_169_22
10. Jeong W, Keighley C, Wolfe R, et al. The epidemiology and clinical manifestations of mucormycosis: a systematic review and meta-analysis of case reports. *Clin Microbiol Infect*. 2019;25(1):26–34. doi:10.1016/j.cmi.2018.07.011
11. Danion F, Duval C, Séverac F, et al. Factors associated with coinfections in invasive aspergillosis: a retrospective cohort study. *Clin Microbiol Infect*. 2021;27(11):1644–1651. doi:10.1016/j.cmi.2021.02.021
12. Stone N, Gupta N, Schwartz I. Mucormycosis: time to address this deadly fungal infection. *Lancet Microbe*. 2021;2(8):e343–4. doi:10.1016/S2666-5247(21)00148-8
13. Millon L, Caillot D, Berceanu A, et al. Evaluation of serum mucorales PCR for the diagnosis of mucormycoses: the modimucor prospective trial. *Clin Infect Dis*. 2022;75(5):777–785. doi:10.1093/cid/ciab1066
14. Liu Y, Wu W, Xiao Y, Zou H, Hao S, Jiang Y. Application of metagenomic next-generation sequencing and targeted metagenomic next-generation sequencing in diagnosing pulmonary infections in immunocompetent and immunocompromised patients. *Front Cell Infect Microbiol*. 2024;14:1439472. doi:10.3389/fcimb.2024.1439472
15. Gu W, Miller S, Chiu CY. Clinical metagenomic next-generation sequencing for pathogen detection. *Annu Rev Pathol*. 2019;14:319–338. doi:10.1146/annurev-pathmechdis-012418-012751
16. Liu H, Zhang Y, Chen G, et al. Diagnostic significance of metagenomic next-generation sequencing for community-acquired pneumonia in Southern China. *Front Med*. 2022;9:807174. doi:10.3389/fmed.2022.807174
17. Zhang Z, Wang M. Three case reports of pulmonary mucormycosis with a review of the literature. *Front Med*. 2025;12:1580912. doi:10.3389/fmed.2025.1580912
18. Danion F, Coste A, Le Hyaric C, et al. what is new in pulmonary mucormycosis? *J Fungi*. 2023;9(3):307. doi:10.3390/jof9030307
19. Li H, Durbin R. Fast and accurate short read alignment with burrows-wheeler transform. *Bioinformatics*. 2009;25(14):1754–1760. doi:10.1093/bioinformatics/btp324
20. Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. *Genome Biol*. 2019;20(1):257. doi:10.1186/s13059-019-1891-0
21. Steinbrink JM, Miceli MH. Clinical review of mucormycosis. *Infect Dis Clin North Am*. 2021;35(2):435–452. doi:10.1016/j.idc.2021.03.009
22. Soliman SSM, Baldin C, Gu Y, et al. Mucoricin is a ricin-like toxin that is critical for the pathogenesis of mucormycosis. *Nat Microbiol*. 2021;6(3):313–326. doi:10.1038/s41564-020-00837-0
23. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and update of the consensus definitions of invasive fungal disease from the european organization for research and treatment of cancer and the mycoses study group education and research consortium. *Clin Infect Dis off Publ Infect Dis Soc Am*. 2019;71(6):1367–1376. doi:10.1093/cid/ciz1008
24. Prakash H, Ghosh AK, Rudramurthy SM, et al. A prospective multicenter study on mucormycosis in India: epidemiology, diagnosis, and treatment. *Med Mycol*. 2019;57(4):395–402. doi:10.1093/mmy/myy060
25. Zhang Y, Wei E, Niu J, et al. Clinical features of pediatric mucormycosis: role of metagenomic next generation sequencing in diagnosis. *Front Cell Infect Microbiol*. 2024;14:1368165. doi:10.3389/fcimb.2024.1368165
26. Lass-Flörl C. Zygomycosis: conventional laboratory diagnosis. *Clin Microbiol Infect*. 2009;15:60–65. doi:10.1111/j.1469-0691.2009.02999.x
27. Smith LD, Ahmad M, Ashraf DC, et al. Cutaneous mucormycosis of the eyelid treated with subcutaneous liposomal amphotericin B injections. *Ophthal Plast Reconstr Surg*. 2024;40(2):e42–5. doi:10.1097/IOP.0000000000002545
28. Boulware DR, Atukunda M, Kagimu E, et al. Oral lipid nanocrystal amphotericin b for cryptococcal meningitis: a randomized clinical trial. *Clin Infect Dis off Publ Infect Dis Soc Am*. 2023;77(12):1659–1667. doi:10.1093/cid/ciad440
29. Wang C, You Z, Fu J, et al. Application of metagenomic next-generation sequencing in the diagnosis of pulmonary invasive fungal disease. *Front Cell Infect Microbiol*. 2022;12:949505. doi:10.3389/fcimb.2022.949505
30. Wei E, Niu J, Zhang M, et al. Metagenomic next-generation sequencing could play a pivotal role in validating the diagnosis of invasive mold disease of the central nervous system. *Front Cell Infect Microbiol*. 2024;14:1393242. doi:10.3389/fcimb.2024.1393242

31. Pham D, Howard-Jones AR, Sparks R, et al. Epidemiology, modern diagnostics, and the management of mucorales infections. *J Fungi*. 2023;9(6):659. doi:10.3390/jof9060659
32. Özbek L, Topçu U, Manay M, et al. COVID-19–associated mucormycosis: a systematic review and meta-analysis of 958 cases. *Clin Microbiol Infect*. 2023;29(6):722–731. doi:10.1016/j.cmi.2023.03.008
33. Miller MA, Molina KC, Gutman JA, et al. Mucormycosis in hematopoietic cell transplant recipients and in patients with hematological malignancies in the era of new antifungal agents. *Open Forum Infect Dis*. 2020;8(2):ofaa646. doi:10.1093/ofid/ofaa646
34. Sasani E, Pakdel F, Khodavaisy S, et al. Mixed aspergillosis and mucormycosis infections in patients with COVID-19. *Case Series and Literature Review. Mycopathologia*. 2024;189(1):10. doi:10.1007/s11046-023-00808-z
35. Pomorska A, Malecka A, Jaworski R, et al. Isavuconazole in a successful combination treatment of disseminated mucormycosis in a child with acute lymphoblastic leukaemia and generalized haemochromatosis: a case report and review of the literature. *Mycopathologia*. 2019;184(1):81–88. doi:10.1007/s11046-018-0287-0
36. Ashkenazi-Hoffnung L, Bilavsky E, Levy I, et al. Isavuconazole as successful salvage therapy for mucormycosis in pediatric patients. *Pediatr Infect Dis J*. 2020;39(8):718–724. doi:10.1097/INF.0000000000002671
37. Skiada A, Lass-Floerl C, Klimko N, Ibrahim A, Roilides E, Petrikkos G. Challenges in the diagnosis and treatment of mucormycosis. *Med Mycol*. 2018;56(suppl_1):93–101. doi:10.1093/mmy/myx101
38. Patel A, Kaur H, Xess I, et al. A multicentre observational study on the epidemiology, risk factors, management and outcomes of mucormycosis in India. *Clin Microbiol Infect*. 2020;26(7):944.e9–944.e15. doi:10.1016/j.cmi.2019.11.021

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