DOI: 10.7860/NJLM/2025/69715.2891

Case Series

Microbiology Section

A Case Series on *Rhizopus homothallicus*: An Emerging Zygomycete causing Mucormycosis

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ABSTRACT

Mucormycosis is an acute opportunistic infection caused by various fungi belonging to the family Mucorales. The most common species causing this infection in humans are *Rhizopus (R)* species (spp.,) with *R. oryzae* and *R. microsporus* being the most prevalent. The authors here report a case series of infections caused by a very rare emerging zygomycete, *Rhizopus homothallicus*, in a tertiary care Institution in Southern India. The first case involved a 26-year-old male who developed cutaneous mucormycosis following trauma and surgery, with no underlying co-morbidities. The second case was a 40-year-old male, a diabetic, who had recovered from Coronavirus Disease 2019 (COVID-19) and subsequently developed COVID-19-associated rhino-orbital mucormycosis. The third case was a 36-year-old male with a known history of insulin-dependent diabetes mellitus who developed pulmonary mucormycosis. In all three cases, the authors isolated a very rare zygomycete that was identified as *Rhizopus homothallicus*. The first patient was successfully discharged, while the second and third patients succumbed to the infection on days 12 and 8, respectively. There has been an increased incidence of mucormycosis in all forms post-COVID-19, and fungi that were previously considered rare, such as *R. homothallicus*, are now being encountered more frequently. Microbiologists and clinicians must maintain vigilance for these cases to ensure appropriate and prompt diagnosis and treatment, as current evidence suggests that the mortality rate associated with *R. homothallicus* is lower compared to other Mucorales when diagnosed and treated early. The present case series was aimed to highlight the importance of identifying fungi to the species level in all situations and to emphasise that, with a larger population living with immunosuppression and the emergence of new pandemics like COVID-19 compared to previous years, there is a heightened risk of infections from lesser-known pathogenic fungi such as *R. homothallicus*.

Keywords: Cutaneous mucormycosis, Emerging fungi, Pulmonary mucormycosis, Rhino-orbital mucormycosis

INTRODUCTION

Fungi are found ubiquitously in the environment and are increasingly implicated in various invasive and non invasive diseases. Mucormycosis is an acute opportunistic infection caused by various fungi belonging to the family Mucorales. The increase in the number of diabetics and individuals living with other disorders that cause immunosuppression has led to a rise in the incidence of mucormycosis, posing a significant health threat that is still underestimated. Mucormycosis manifests in various forms, including rhinocerebral mucormycosis, cutaneous mucormycosis and pulmonary mucormycosis. In particular, cutaneous and soft tissue infections can occur following trauma, burns, and invasive procedures in previously healthy individuals.

Rhizopus oryzae is the most common species among the Rhizopus fungi causing human infections, followed by *Rhizopus microsporus, Rhizopus azygosporus* and others [1]. The treatment of mucormycosis involves the surgical debridement of infected tissue, appropriate and timely antifungal therapy, and the management of the underlying disease. Amphotericin B (AmB) is the first-line drug of choice; subsequently, posaconazole and isavuconazole are used as salvage therapy in the treatment of mucormycosis [1].

The authors here report a case series of infections caused by an emerging rare zygomycete, *Rhizopus homothallicus*, in a tertiary care Institution in Southern India, and informed consent was obtained from all the patients.

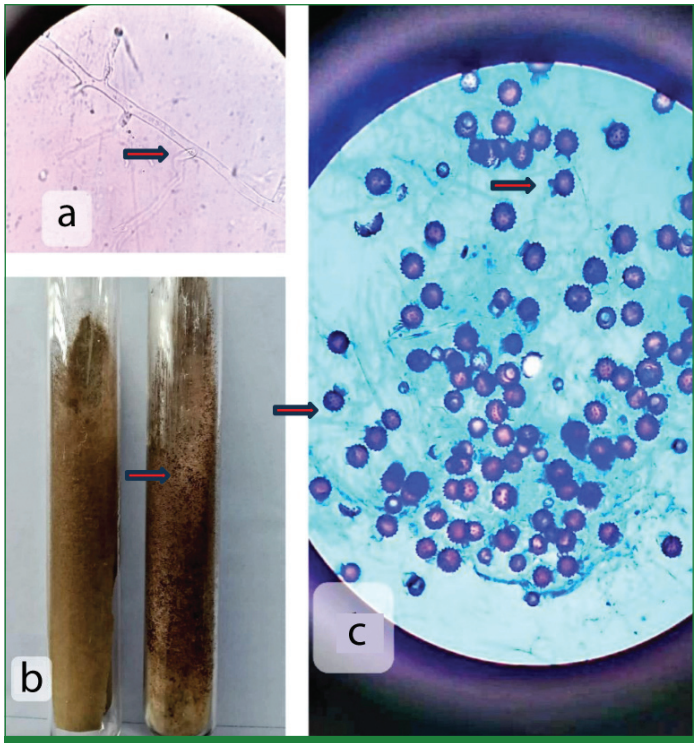
CASE SERIES

Case 1

A 26-year-old male patient was admitted to the Casualty Unit after suffering a penetrating injury to the abdomen caused by scissors. He underwent exploratory laparotomy, during which no bowel injury was found. Haemostasis was achieved, and the wound was closed. Two days postoperatively, he developed a wound infection and was started on broad-spectrum antibiotics. Two swabs were sent for bacterial culture. Gram stain revealed plenty of pus cells with scattered Gram negative bacilli. The patient was already receiving injection Piperacillin-tazobactam 4.5 g intravenously every six hours. The patient's aerobic culture did not grow any bacteria, and by day 4, a black discolouration was noted at the wound site. Suspecting a possible fungal infection, a repeat sample along with a tissue specimen was collected under aseptic precautions, and a 10% Potassium Hydroxide (KOH) mount was prepared. It showed broad, aseptate hyphae with obtuse angle branching [Table/Fig-1a].

Sabouraud's Dextrose Agar (SDA) (Himedia, Mumbai, India) was prepared in duplicate and incubated at 37°C. On day 2, fast-growing, cotton-wooly, fluffy colonies filling the entire tube of SDA were observed. The colonies appeared floccose white, which later turned grayish with black speckles [Table/Fig-1b]. There was no pigment on the reverse side of the colonies. Phenotypic identification of the isolate was performed based on colony morphology and microscopic findings using lactophenol Cotton Blue Mount (LPCB) [1].

Microscopic examination with lactophenol Cotton Blue mount revealed broad, aseptate hyphae with sparse sporangiophores bearing globose sporangia and sporangiospores. Abundant golden-brown zygospores with unequal suspensor cells were also observed [Table/Fig-1c]. The isolate demonstrated growth at a temperature of 45°C, in addition to 37°C, confirming the diagnosis of *Rhizopus homothallicus*. A diagnosis of *Rhizopus homothallicus* was made based on the work of Hesseltine CW and Ellis JJ [2], and the isolate was sent to the National Culture Collection of Pathogenic Fungi (NCCPF) at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India under the Indian Council



[Table/Fig-1]: a) Obtuse angle branching (arrow) (KOH, 400x); b) Floccose grey colony with black speckles (arrow) on the obverse and no pigmentation on the reverse in SDA; c) LPCB mount showing zygospores with suspensor cells (arrows).

of Medical Research (ICMR) for confirmation, where it was indeed confirmed as *Rhizopus homothallicus* (Isolate accession ID: IL-2122).

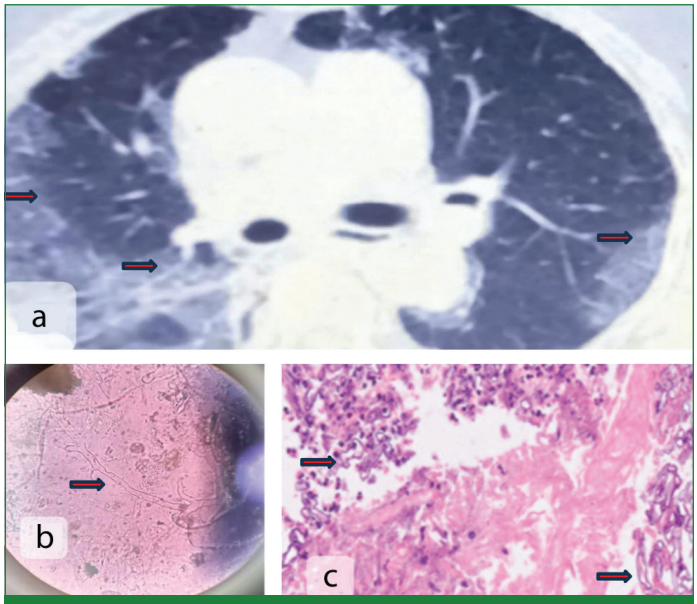
Since there was no intra-abdominal infection, thorough wound care was performed, including wide excision of the affected skin for the patient. The patient was started on liposomal amphotericin B (0.5 mg/kg/day), and liposomal amphotericin B cream was applied locally for four weeks. The patient recovered without any undue complications, and no relapse was noted during a six-month follow-up. This isolate has been confirmed as *R. homothallicus* and deposited with the NCCPF, ICMR (Accession no: IL-2122).

Case 2

A 40-year-old male patient was admitted to the Emergency Ward with a history of malaise and progressive breathlessness for two days during the second wave of the COVID-19 pandemic. He had been diabetic for two years and was on oral hypoglycaemics. Upon examination, his pulse rate was 90 beats per minute, and his blood pressure was 160/90 mmHg. He was afebrile on admission, with a respiratory rate of 32 breaths per minute and an oxygen saturation of 84% despite oxygen supplementation at 10 liters per minute. Auscultation revealed bilateral crepitations at both lung bases. Examination of other systems was normal. His blood glucose level was 454 mg/dL upon admission, and urine ketones were negative. A Reverse-transcriptase Polymerase Chain Reaction (RT-PCR) test from a nasal swab was positive for the SARS-CoV-2 virus. A Computed Tomography (CT) scan of the chest showed multiple patchy ground-glass opacities in both lungs, particularly involving the lower lobes and the left upper lobe, strongly suggestive of COVID-19 infection [Table/Fig-2a].

He was started on antivirals, anticoagulants and a low dose of steroids (dexamethasone 4 mg twice daily), along with general supportive care. His diabetes mellitus was managed with insulin. On day three, left lid oedema with left eye prominence was noted, prompting consultations with an otolaryngologist and ophthalmologist. A preliminary diagnosis of invasive fungal sinusitis and left orbital cellulitis was made. A CT scan of the paranasal sinuses showed significant mucosal thickening in the left frontal, maxillary and ethmoidal sinuses, as well as, hyperintensity in the left retrobulbar space. A diagnostic nasal biopsy with paranasal sinus endoscopy from the left middle turbinate was performed. The tissue was subjected to a 10% KOH mount preparation, which revealed broad, aseptate, filamentous ribbon-shaped fungal hyphae suggestive of mucormycosis [Table/Fig-2b]. A diagnosis of COVID-19 Associated Mucormycosis (CAM) or Rhino-orbital Mucormycosis (ROM) was made, and he continued on antivirals with the addition of amphotericin B (0.5 mg/kg/day). Steroids were withheld from day five of admission. Extensive debridement of the sinuses was performed, and intraoperative tissue samples were sent for fungal culture. The culture grew a fast-growing zygomycete by day two of the culture on SDA (Himedia, Mumbai, India). The colony was initially floccose white and turned grayish with black speckles. There was no pigment on the reverse side of the colony.

Microscopic examination using lactophenol cotton blue mount of the colony showed broad, aseptate hyphae with sparse sporangiophores bearing globose sporangia with sporangiospores. Abundant golden-brown zygospores with unequal suspensor cells were observed. The isolate also grew at a temperature of 45°C in addition to 37°C, confirming the diagnosis of *Rhizopus homothallicus*. Histopathology of tissue samples from the nasal turbinate and sinuses demonstrated tissue necrosis, angioinvasion, and non septate fungal hyphae, consistent with angioinvasive mucormycosis [Table/Fig-2c].



[Table/Fig-2]: a) Computed Tomography (CT) of the chest showing multiple patchy ground-glass opacities (arrows) in both lungs involving both lower lobes; b) Ribbon shaped broad hyphae (KOH, 400x); c) Haematoxylin and eosin section showing non septate hyphal forms (arrow).

Despite extensive efforts, the patient had to be intubated for respiratory failure and succumbed on day 12 of admission, with the cause of death being respiratory failure following SARS-CoV-2 infection.

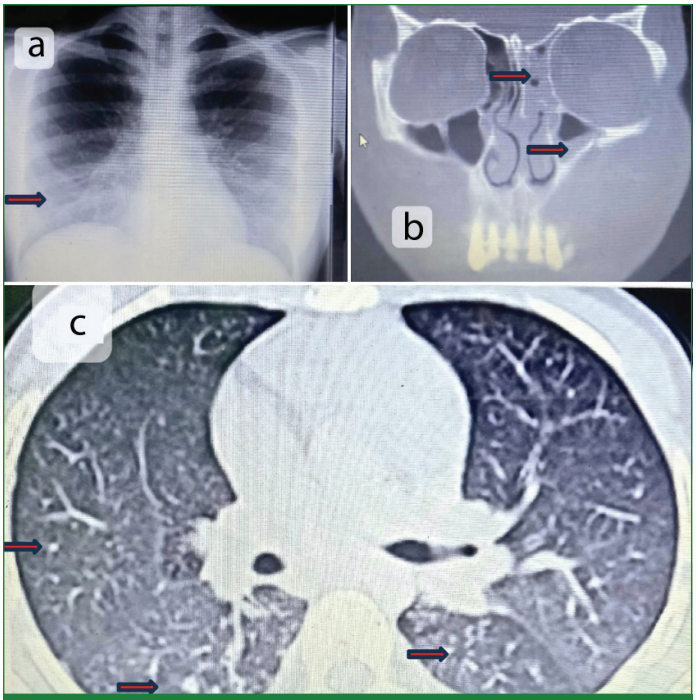
Case 3

A 36-year-old male patient was admitted with a cough that included expectoration for one week, along with one episode of haemoptysis. He experienced two episodes of low-grade fever. The patient was a known type I diabetic on insulin and had a history of habitual alcohol use. During his admission, the patient was investigated, and blood sugar levels ranged from 112-256 mg/dL. A chest X-ray revealed right middle lobe pneumonia [Table/Fig-3a]. Sputum was sent for GeneXpert testing, which returned negative. However, sputum culture grew *Klebsiella pneumoniae*, and he was placed on a regimen of ceftazidime 2 gm Intravenous (IV) every 8 hours, while his insulin levels were titrated according to his blood sugar levels.

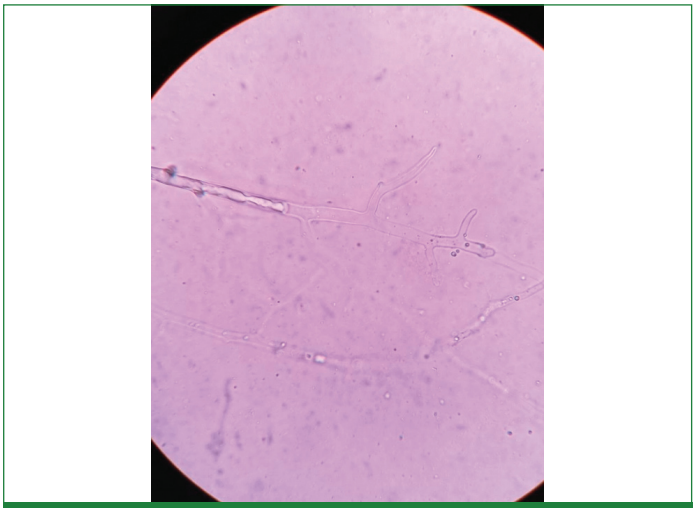
Despite four days of antibiotic treatment, the patient did not show any clinical or radiological improvement. He was subsequently

taken for bronchoscopy, which revealed nodules in both lungs. The bronchoalveolar lavage fluid was sent for microscopy which showed broad aseptate hyphae with obtuse angle branching on subjection to 10% sodium hypodroxide (10% KOH) [Table/Fig-4] and culture, leading to a diagnosis of probable pulmonary mucormycosis. On day 5 of admission, the patient developed right facial cellulitis. A computed tomography scan of the sinuses revealed right maxillary and ethmoid sinusitis [Table/Fig-3b]. There was no bony erosion of the orbit, and a presumptive diagnosis of associated rhinocerebral mucormycosis was made. Functional endoscopic sinus surgery was planned.

A Contrast-enhanced Computed Tomography (CECT) scan of the thorax revealed right middle lobe pneumonia and multiple nodules in both lungs [Table/Fig-3c]. Culture of the bronchoalveolar lavage fluid on SDA (SDA, HiMedia, Mumbai, India) grew a fast-growing zygomycete by day 2. The colony was initially floccose white, turning grayish with black speckles. No pigmentation was observed on the reverse of the colony.



[Table/Fig-3]: a) X-ray chest showing right middle lobe pneumonia (arrow); b) Computed tomography of the sinuses showing right maxillary and ethmoid sinusitis (arrows); c) Computed tomography of the thorax showing multiple nodules (arrows) in both lungs.



[Table/Fig-4]: Broad aseptate hyphae with obtuse angle branching on 10% KOH (KOH, 400x).

Microscopic examination using a lactophenol cotton blue mount of the colony showed broad, aseptate hyphae with sparse sporangiophores bearing globose sporangia and sporangiospores. Abundant golden-brown zygospores with unequal suspensor cells were also observed. The isolate grew at a temperature of 45°C in addition to 37°C, confirming the diagnosis of *Rhizopus homothallicus*.

The isolate was identified as *Rhizopus homothallicus*, resulting in a final diagnosis of pulmonary mucormycosis. The patient was started on liposomal amphotericin B (0.5 mg/kg/day) on day 6 of admission. The facial cellulitis showed drastic improvement, and the patient began to demonstrate clinical improvement. However, on day 8, he suddenly experienced a severe bout of haemoptysis and collapsed. Immediate resuscitation efforts were undertaken, but the patient could not be revived.

DISCUSSION

Zygomycosis, previously a rare disease, is caused by filamentous fungi, primarily affecting the nose and paranasal sinuses, though it can involve other systems, as well. The estimated prevalence of mucormycosis is 70 times higher in India than in the rest of the world [1]. Zygomycetes are opportunistic pathogens commonly found in immunocompromised individuals. The fungal agents causing zygomycosis grow rapidly and aggressively, often leading to a fulminant and life-threatening disease. Early intervention is crucial to save lives and prevent permanent complications. Zygomycosis typically presents as an acute fungal infection in most cases, although chronic presentations have also been described, which are indolent and slowly progressive, spanning over several weeks.

The most common risk factors for zygomycosis include diabetic ketoacidosis, severe burns, solid organ transplantation, prolonged corticosteroid therapy, Human Immunodeficiency Virus (HIV) infection, neutropenia, malnutrition, hematologic malignancies and others [2,3]. Among the etiological agents of zygomycosis, *Rhizopus spp.* are the most commonly implicated in human infections. *R. oryzae* is the predominant species, accounting for 90% of reported cases of invasive zygomycosis [1,4-6], followed by *R. microsporus* [7] and others.

The present fungal species of interest, *Rhizopus homothallicus*, is mentioned in the literature less frequently than the more common and significant species, *R. oryzae*. In 1961, Hesseltine CW and Ellis JJ described *R. homothallicus*, which was isolated from a soil sample in Guatemala [2]. Schipper MAA, and Schipper MAA and Stalpers JA classified *Rhizopus* species into three groups: the *R. stolonifer* group, the *R. oryzae* group, and the *R. microsporus* group, based on phenotypic characteristics and maximum growth temperatures [8,9]. *R. homothallicus* closely resembles *R. microsporus* in general morphology, particularly in its asexual sporangiophores, sporangia, sporangiospores, and maximum growth temperatures [9]. The main difference is the abundant zygospores produced by *R. homothallicus*. *R. homothallicus* can also be confused with another homothallic species, *R. sexualis*, which also produces abundant zygospores. The two can be differentiated by the fact that *R. homothallicus* can grow at temperatures as high as 46-48°C, while *R. sexualis* does not grow at 37°C [9].

Human infections caused by the homothallic fungus *R. homothallicus* were previously comparatively lower than those caused by other mucorales. However, the above case series suggests that cases attributed to *R. homothallicus* are increasingly being documented. The prevalence of *R. homothallicus* as a cause of mucormycosis is reported to be 2.5% (Prakash H et al.,) [10], 7.6% (Patel A et al.,) [11], and 6.8% (Rudramurthy SM et al.,) [3] in various studies.

Very few studies have described the agent *Rhizopus homothallicus*, as detailed below [Table/Fig-5] [2-4,12-17]. It is evident that the present case species of concern, *Rhizopus homothallicus*, has been documented in a total of 56 cases [2-4,12-17]. Of which 30 cases were classified as COVID-19 Associated Mucormycosis (CAM), 21 cases as ROM, while pulmonary mucormycosis and cutaneous mucormycosis accounted for 4 and 1 cases, respectively. Among

the cases identified in the post-COVID-19 era, only 30 cases were COVID-19 positive; the others were either COVID-19 negative or had unknown status. The authors could not ascertain whether these individuals developed mucormycosis after recovering from COVID-19 infection. It is noteworthy that only six cases were reported before the COVID-19 pandemic [4,16,17]. Therefore, the authors inferred that this rare fungus has been increasingly isolated in the post-COVID-19 era, although the exact association remains to be proven. In the present case series, one case was associated with COVID-19 and was diagnosed as CAM.

Author name	Year and place	Site of isolation	Patient survival status	Diabetes
Rudramurthy SM et al., [3]	2023/ Chandigarh	ROM (18 cases) CAM (23 cases)	30 day mortality 4/41 cases expired	39/41
Gupta MK et al., [12]	2023/North India	CAM (6 cases)	All survived	Yes (6)
Kaur H et al., [13]	2021/ Chandigarh	CAM (1 case)	Survived	Not known
Bhanu K et al., [14]	2021/ Himachal pradesh	ROM (1 case)	Survived	Yes (1)
Taneja J et al., [15]	2023/North India	Pulmonary mucormycosis (1 case)	Death	Yes (1)
Compain F et al., [16]	2017/France	Pulmonary mucormycosis/ Cavitary pneumonia (1 case)	Death	Yes (1)
Kokkayil P et al., [4]	2017/North India	ROM (2 cases) Cutaneous mucormycosis (1 case)	2 cases-death 1-survived	Yes (3)
Chakrabarti A et al., [17]	2010/North India	Cavitary pulmonary zygomycosis (2 cases)	1 Survived 1 death	Yes (2)
Hesseltine CW and Ellis JJ [2]	1961/ Guatemala/ Central America	Soil	Not applicable	Not applicable

[Table/Fig-5]: Review of literature of documented cases of *R.homothallicus* [2-4,12-17].
ROM: Rhino-orbital mucormycois; CAM: COVID-19 associated mucormycosis

According to the literature summarised in [Table/Fig-4], out of the 56 cases, 53 were diabetic, indicating a strong association between this infection and diabetes. In the present case series, two out of the three cases were known diabetics.

The ROM is the most common presentation among the various categories of zygomycosis. Pulmonary zygomycosis is considered the second most frequent type after rhino-orbital mucormycosis and has rarely been reported without any predisposing factors [15]. In the present case series, the authors report one case of ROM, one case of pulmonary mucormycosis, and one case of cutaneous zygomycosis. Pulmonary zygomycosis may present with a wide variety of lesions, including isolated solitary nodules, lobar involvement, and cavitary or disseminated lesions [18,19]. Pulmonary consolidation or effusion is less frequently observed [16,17]. The patients in the present report exhibited consolidation lesions along with nodules.

The mortality rate for mucormycosis may vary with the causative agents. Rudramurthy SM et al., observed a significantly better survival rate with *R. homothallicus* infection, which they attributed to early presentation due to higher visual disturbances [3]. In present series, the mortality rate was 2 out of 3 cases. Pulmonary mucormycosis is associated with higher mortality rates, as discussed by Muthu V et al., [20] in the present case of pulmonary mucormycosis, the patient did not survive.

Rudramurthy SM et al., suggest that *R. homothallicus* produces more heavy sexual spores than asexual spores and undergoes less dispersion in the air, thereby resulting in fewer infections compared to other *Rhizopus* species [3]. Hence, the rising trends in this fungal infection may indicate the presence of some environmental niche where the agent can produce asexual spores abundantly and disperse in the air. Some studies have already confirmed the presence of *R. homothallicus* in hospital air and in air samples obtained from the residences of patients with mucormycosis [21,22]. Future studies may attempt to recreate in-vitro conditions to induce the fungi to produce asexual spores more abundantly.

The fungus *Rhizopus homothallicus*, which was very rare before the COVID-19 era, is now increasingly being isolated and documented, as suggested by the present case series. Preliminary evidence suggests that infection with this emerging fungus has a better prognosis than its more common counterpart, *R. oryzae* [3]. Therefore, attempts to achieve a speedy diagnosis and prompt treatment will influence the morbidity and mortality associated with the disease. A high degree of suspicion is essential in high-risk individuals to accomplish a multifaceted approach [23].

CONCLUSION(S)

Since the major victims of invasive mucormycosis are immunocompromised individuals, they should be considered for early diagnosis through constant suspicion and prompt intervention. Knowledge of mucormycosis and the agents causing this fatal infection aids in early diagnosis and increases survival rates. A high degree of suspicion is essential in high-risk individuals to accomplish a multi-faceted approach to diagnosing and managing this rare infection.

In the present case series, the authors present a relatively rare pathogen, *Rhizopus homothallicus*. There has been an increased incidence of mucormycosis in all forms post-COVID-19, and fungi that were once considered rare, such as *R. homothallicus*, are now more prevalent. Microbiologists and clinicians need to be vigilant for these cases and ensure appropriate and timely diagnosis and treatment, as current evidence suggests that the mortality rate associated with *R. homothallicus* is lower compared to other mucorales, if identified and treated early.

The present case series aimed to highlight the importance of identifying fungi at the species level in all situations and to emphasize that, with a larger number of individuals living with immunosuppression and the emergence of new pandemics such as SARS-CoV-2, there is a heightened risk of infection by lesser-known pathogenic fungi like *R. homothallicus*.

Acknowledgement

The authors gratefully acknowledge the support by NCCPF for confirming the identification of the present case series isolate and for depositing the isolate.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Jan 22, 2024
- Manual Googling: Sep 04, 2024
- iThenticate Software: Sep 10, 2024 (15%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Jan 19, 2024**
Date of Peer Review: **Feb 14, 2024**
Date of Acceptance: **Sep 11, 2024**
Date of Publishing: **Jan 01, 2025**