

## Mucormycosis: A Tunisian Case Series of Ten Patients and Literature Review

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### Abstract

**Objective:** Mucormycosis is an uncommon and often fatal fungal infection. Few case series studies have been conducted in our country, highlighting the importance of our research. The aim of this study was to analyze the epidemiological, clinical, biological, and therapeutic characteristics of affected patients.

**Design:** Single-center, longitudinal, retrospective and descriptive study of 10 patients admitted in Military Hospital of Tunis and confirmed with mucormycosis over a 5-year period (2019-2023). Mycological diagnosis was made in the laboratory of parasitology-Mycology of the same hospital.

**Setting:** Epidemiological, clinical and mycological data were collected and analysed using Excel software. In cases where mycological diagnosis was difficult, PCR sequencing of ITS 2 region was performed.

**Participants:** Tunisian patients with confirmed mucormycosis were included.

**Primary outcomes:** We investigated in-hospital mortality during the study period.

**Results:** The average annual incidence was 2 patients, with a mean age of 53.6 years and a sex ratio of 1.5. Diabetes was the most common risk factor, affecting 90% (n=9) of patients. Clinical manifestations included rhino cerebral, cutaneous, and pulmonary forms, occurring in 70% (n=7), 20% (n=2), and 10% (n=1) of cases, respectively. Direct microscopy was positive in 90% of cases (n = 9), and fungal culture in 100% (n = 10). Four species were identified. *Rhizopus arrhizus* was the most frequently isolated species, found in 70% of cases (n=7). All our patients were treated with amphotericin B. Surgical excision and hyperbaric oxygen therapy were performed in seven patients each (70%). The mortality rate was 30% (n=3).

**Conclusion:** our study aims to contribute to the analysis of mucormycosis on a national scale, to highlight ideas for research and to alert all healthcare professionals to the problems associated with the management of this potentially fatal infection.

**Keywords:** Mucormycosis, *Rhizopus arrhizus*, diagnosis, Amphotericin B.

### Introduction

Mucormycosis is rare but often fatal invasive fungal infection although. It is caused by filamentous fungi classified in the order Mucorales [1]. This opportunistic infection mainly occurs in particular conditions: severe neutropenia, unbalanced acetoketotic diabetes, immunosuppression following organ transplantation or HIV infection [1,2].

Inhalation of the spores is the most frequent mode of contamination, which explains the areas of predilection for infection, particularly the nasal cavities, sinuses and lungs. There is also the possibility of contamination through the skin or digestive tract [3]. The non-specific clinical manifestations, and the rapid progression of lesions explain contribute to the high mortality rate which ranges from 40 to 50%. Confirmation of the diagnosis is based on mycological and anatomopathological examinations [2].

Mucormycosis currently represents an epidemiological challenge due to the increase in its incidence and the lack of available local data. It also raises diagnostic difficulties, due to the non-specificity of clinical manifestations, which leads to delays in diagnosis and treatment. In Tunisia, there have been

few studies involving case series. This study aimed to analyze the epidemiological, clinical, biological and therapeutic features of ten mucormycosis cases.

### Materials and Methods

#### Study Design and Setting

We conducted a retrospective, descriptive, longitudinal study at a single tertiary care center. The study included ten patients with confirmed mucormycosis diagnosed between 2019 and 2023 at the Laboratory of Parasitology-Mycology, Military Hospital of Tunis, Tunisia.

#### Data Collection

For each patient, we collected age, sex, medical history, clinical manifestations, nonspecific laboratory results, mycological findings, complications, treatment, and clinical outcome. Data were extracted from medical records and analyzed using Microsoft Excel.

#### Mycological Diagnosis

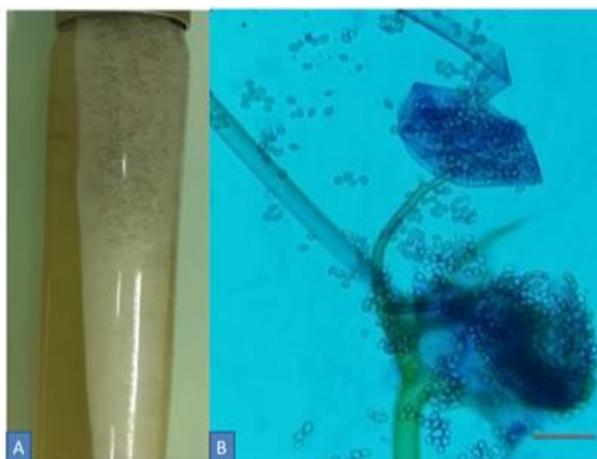
Clinical specimens were obtained aseptically by biopsy or swabbing. Direct examination in 30% potassium hydroxide (KOH) revealed broad, ribbon-like, pauci-septate hyphae with

right-angle branching typical of Mucorales. Cultures were performed on Sabouraud dextrose agar. Species identification was based on macroscopic and microscopic characteristics. When conventional diagnosis was inconclusive, PCR amplification and sequencing of the internal transcribed spacer 2 (ITS2) region were performed and compared with sequences in the NCBI GenBank database [3]. Antifungal susceptibility testing using E-test on RPMI agar was performed for two isolates (*Rhizopus arrhizus* from patient 2 and *Rhizomucor miehei* from patient 3).

## Results

The average annual incidence was two cases per year over the five-year period, with a peak of five cases in 2021. The mean age was 53.5 years ( $\pm 14.7$ ), ranging from 29 to 74 years. The sex ratio was 1.5, with 60% males (n=6) and 40% females (n=4). Mucormycosis was distributed as follows: rhinocerebral (n = 7, 70%), cutaneous (n = 2, 20%), and pulmonary (n = 1, 10%). Diabetes mellitus was the most common underlying condition (n = 9, 90%), followed by intensive care unit admission (n = 3, 30%) and renal failure (n = 3, 30%). Other risk factors included postoperative wounds or trauma (n = 2 each, 20%), neutropenia (n = 1, 10%), and acute myeloid leukemia (n = 1, 10%). Half of the patients (n = 5, 50%) had hypertension, and two had a recent history of COVID-19 infection (20%).

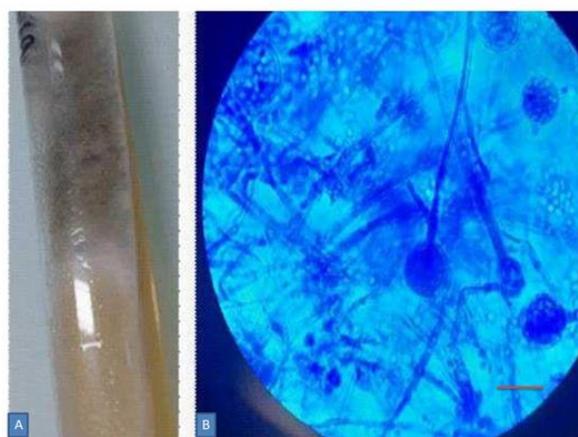
Direct microscopy was positive in 90% of cases (n = 9), and fungal culture in 100% (n = 10). *Rhizopus arrhizus* was the most frequently isolated species (n = 7, 70%) (figure 1). *Lichtheimia* spp. (figure 2), *Rhizomucor miehei* (figure 3), and *Saksenaea vasiformis* (figure 4) were each identified in a single case (10% each).



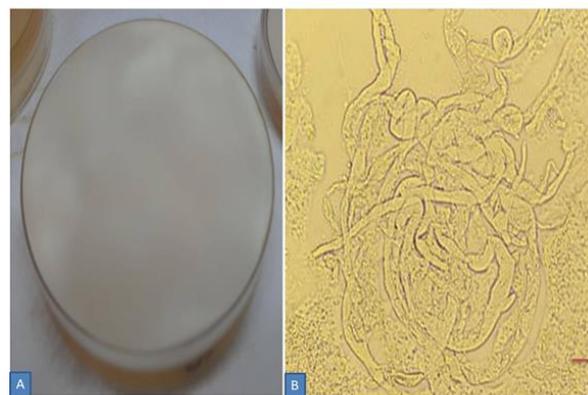
**Figure 1:** Macroscopic (A) and microscopic (B) aspects of *Rhizopus arrhizus* spp; Scale bar 50  $\mu$ m (case 2).



**Figure 2:** Macroscopic (A) and microscopic (B,C) aspects of *Lichtheimia* spp; Scale bar 100 $\mu$ m (case 1).



**Figure 3:** Macroscopic aspect of the culture (A) and microscopic aspects (B) of *Rhizomucor miehei*; scale bar: 100 $\mu$ m (case 3).



**Figure 4:** Macroscopic aspect of the culture (A) and microscopic aspects (B) of *Saksenaea vasiformis*; scale bar: 100 $\mu$ m (case 6).

*Rhizopus arrhizus* showed MICs of 0.25  $\mu$ g/mL for amphotericin B and 8  $\mu$ g/mL for voriconazole, with resistance to fluconazole (>256  $\mu$ g/mL) and caspofungin (>32  $\mu$ g/mL). *Rhizomucor miehei* exhibited MICs of 0.25  $\mu$ g/mL for amphotericin B and 2  $\mu$ g/mL for voriconazole, and was resistant to fluconazole (>256  $\mu$ g/mL) and caspofungin (>32  $\mu$ g/mL).

All patients received amphotericin B. Surgical debridement was performed in eight cases (80%) and hyperbaric oxygen therapy in seven (70%). The mortality rate was 30% (n=3). **Table 1** summarizes the epidemiological, clinical, biological, and outcome data of the cases included in our study.

**Table 1:** Summary table of the collected clinical cases.

Patient	Year	Age (years)/ Sex	Medical history	Clinical signs	Location of infection	Sample	Direct examination	Culture and Species identification	Treatment	Evolution
1	2020	74/F	Diabetes type 2 Hypertension Femoral neck fracture Osteoporosis	Irregular erythematous-scaly and painful rash near the surgical site	Cutaneous	Swab at the site of the operation	Presence of mycelial filaments of mucorales	<i>Lichtheimia spp</i>	- Amphotericin B	Death
2	2020	53/M	Diabetes type 2 Hypertension Chronic kidney failure Dyslipidemia Gastritis	Acute invasive sinusitis of the right maxillary sinus	Rhinocerebral	Biopsy	Presence of mycelial filaments of mucorales	<i>Rhizopus arrhizus</i>	- Liposomal Amphotericin B - Surgical excision - Hyperbaric oxygen therapy	Favorable
3	2020	29/F	Acute myeloid leukemia	Persistent fever Dyspnea Hemoptysis	Pulmonary	Biopsy	Negative	<i>Rhizomucor miehei</i> (PCR)	- Amphotericin B	Death
4	2020	39/M	Diabetes type 2 Gout disease	Fever Purulent rhinorrhea Right nasal obstruction Jugular swelling extending to the eye Lower eyelid edema	Rhinocerebral	Biopsy	Presence of mycelial filaments of mucorales	<i>Rhizopus arrhizus</i>	- Amphotericin B - Surgical excision - hyperbaric oxygen therapy	Favorable
5	2021	68/M	Diabetes type 2 Hypertension asthma	Purulent rhinorrhea Necrotic lesions of the palate	Rhinocerebral	Biopsy	Presence of mycelial filaments of mucorales	<i>Rhizopus arrhizus</i>	- Amphotericin B Local debridement - hyperbaric oxygen therapy	Favorable
6	2021	48/M	Diabetes type 2 Trauma	Extension of necrosis leading to amputation	Cutaneous	Biopsy	Presence of mycelial filaments of mucorales	<i>Saksenaeva vasiformis</i> (PCR)	- Surgical excision - Liposomal Amphotericin B	Death
7	2021	68/M	Diabetes type 2 Hypertension Kidney failure	Necrotic lesion of the palate with pus	Rhinocerebral	Biopsy	Presence of mycelial filaments of mucorales	<i>Rhizopus arrhizus</i>	- Amphotericin B Local debridement - hyperbaric oxygen therapy	Favorable
8	2021	47/F	Diabetes type 2 COVID-19 infection	Alteration of the general condition Swelling around the left orbit fever	Rhinocerebral	Biopsy	Presence of mycelial filaments of mucorales	<i>Rhizopus arrhizus</i>	- Amphotericin B - Local debridement - hyperbaric oxygen therapy	Favorable
9	2021	45/M	Diabetes type 2	Left facial pain Left headache facial paralysis Purulent secretions Edema left hemiface	Rhinocerebral	Biopsy	Presence of mycelial filaments of mucorales	<i>Rhizopus arrhizus</i>	- Amphotericin B - Local debridement - hyperbaric oxygen therapy	Favorable
10	2022	63/F	Diabetes type 2 Hypertension Kidney failure Obstructive sleep apnea syndrome	Headache Ptosis of the right eye Binocular diplopia Right blindness Lesions at the level of the middle turbinate	Rhinocerebral	Biopsy	Presence of mycelial filaments of mucorales	<i>Rhizopus arrhizus</i>	- Amphotericin B - Local debridement - hyperbaric oxygen therapy	Favorable

**Discussion**

Our study is monocentric, allowing us to provide uniformity in sample collection, analysis and processing procedures, which enhances the reliability of the results obtained. The number of cases in our series (10 cases over a period of 5 years) is both an advantage, given the rarity of this infection, and a disadvantage, as the number of cases reduced the power of the statistical analyses performed. Nevertheless, certain limitations of our work must be mentioned. The data must be interpreted with caution due to the limitations inherent in the retrospective nature of the data and the risk of bias.

In Spain, the incidence of mucormycosis increased from 1.2 to 3.3 cases/100,000 admissions between 2005 and 2017 [4]. In France, the incidence of zygomycosis was raised from 0.7 to 1.2 cases per million [5]. The Tunisian studies showed an increasing number of cases per year [6-10]. Several factors explain this increase, including the expansion of the population at risk, such as patients with diabetes, haematological malignancies, solid organ transplants and other immunocompromising conditions. In addition, the use of prophylactic voriconazole may modulate the virulence of mucorales [11].

The mean age was 53.5 years, and a male predominance with a sex ratio of 1.5 was found in our study. Our results are comparable with those published by the majority of Tunisian series, which also show a male predominance (Table II). This male predominance may be partly explained by the higher prevalence of diabetes and its complications among men, as reported in previous studies [12,13].

Nine of our patients had diabetes mellitus. Diabetic ketoacidosis is a well-recognized risk factor for invasive fungal sinusitis. Under conditions of metabolic acidosis, the reduced affinity of transferrin for iron and the activity of ketone reductase which converts ferric to ferrous iron lead to increased levels of free iron, thereby promoting fungal growth [14].

Kidney failure was the second most common risk factor (30%) after diabetes in our series. This result is consistent with Tunisian studies. Global epidemiological data have highlighted kidney failure as an emerging risk factor [1]. Table 2 presents the clinical and epidemiological characteristics of patients reported in the Tunisian literature.

**Table 2:** Presentation of patients in Tunisian literature according to epidemiological data.

Authors	Year	Study period (years)	Number of cases	Cases/year	Average age (years)	sex-ratio M/F	Risk factors	
							Diabetes (%)	Other risk factors (%)
Battikh et al [10]	2002	6	3	0,5	52,33	2	100%	Kidney failure (30%)
Ferchichi et al [11]	2006	4	4	-	51	1	100%	-
kacem et al [12]	2014	14	12	0,85	-	0,7	60%	-
Bellazreg F et al [13]	2015	13	5	0,38	60,8	4	60%	Leukemia (20%)
Frikha et al [14]	2016	18	23	1,27	57,3	1,5	82,6%	Kidney failure (22%) Immunodeficiency (4%)
<b>Our study</b>	2023	5	10	2	53,6	1,5	90%	Kidney failure (30%) Postoperative injury/wound (20%) Acute myeloid leukemia (10%)

In our study, a patient with acute myeloid leukaemia developed neutropenia following chemotherapy, leading to the development of pulmonary mucormycosis. Haematological malignancy is the most common underlying condition for mucormycosis in Europe and the USA, with an incidence ranging from 38% to 62%. Individuals with acute myeloid leukaemia, myelodysplastic syndrome, haematopoietic stem cell transplantation and acute lymphoblastic leukaemia are at higher risk to develop mucormycosis during the neutropenic phase [1,2].

Two patients with cutaneous mucormycosis had a post-operative wound or trauma as a risk factor. Cutaneous mucormycosis is mainly influenced by traumatic events, such as open wounds, road accidents, surgery, etc. Infection can be classified as localized, deep or partially disseminated. A localized infection is seen in 32-56% of patients, usually confined to skin and subcutaneous tissue, without invading adjacent sites [1,4].

Mucormycosis is usually suspected based on results of direct microscopy of clinical specimens, preferably stained with fluorescent brighteners' calcofluor white or blankophor. Mucorales hyphae have a variable width of 6–16 µm, but may be up to 25 µm, and are non-septate or pauci-septate with 90° branching angle. Culture of specimens is strongly recommended for genus and species identification, and for antifungal susceptibility testing. Homogenisation of tissue should be avoided before culturing. Incubation at 30°C and 37°C separately is strongly recommended [3].

In our research, molecular biology was used for 2 patients. Detection of Mucorales DNA in serum by quantitative PCR (qPCR) is a non-invasive approach that can optimize therapeutic management. Indeed, qPCR can be performed as soon as the first clinical signs appear. Several retrospective studies have already revealed that it can anticipate the diagnosis of mucormycosis by 3 to 68 days in patients suffering from haemopathy, trauma and renal transplantation. This is of vital importance, as early initiation of targeted antifungal treatment is crucial to improving prognosis [15].

A multicentre MODIMUCOR study was carried out prospectively on 232 individuals with suspected invasive fungal infections and revealed a sensitivity of 85.2% and a specificity of 89.8% for quantitative PCR (qPCR). The first quantitative PCR-positive serum for mucorales was detected a median of 4 days before the collection of the first mycology- or histology-positive sample, and a median of one day before the first imaging. Negative mucorales qPCR within seven days of initiation of liposomal amphotericin B was correlated with a reduced mortality rate of 85% [16]. Molecular biology techniques are therefore excellent and highly effective, allowing rapid diagnosis and prognosis. However, PCR is not a substitute for mycological and histological diagnosis, but a complement.

Table 3 provides a comparative overview of literature data based on the identified Mucorales species and site of infection. As worldwide, *Rhizopus arrhizus* is the most widespread species [1,2]. In Tunisia, although the majority of studies that have carried out mycological identification have found the presence of the species. *Rhizopus arrhizus* with percentages exceeding 70% [2,8,17-19]. *Rhizopus arrhizus* was isolated in all cases of rhinocerebral mucormycosis in diabetic patients. We isolated three other species: *Saksenenae vasiformis*, *Lichtheimia* spp. and *Rhizomucor miehei*. This highlights the importance of understanding local epidemiology. In addition, the use of more efficient techniques such as molecular biology has improved diagnosis.

**Table 3:** Comparison of reported cases by Mucorales species and site of Infection.

Authors/Country	Study period	Number of cases	Infection site (%)	Mucorales species (%)
Our study <b>Tunisia</b>	2019-2023	<b>10</b>	<b>Rhinocerebral (70%)</b> Cutaneous (20%) Pulmonary (10%)	<i>Rhizopus arrhizus</i> (70%) <i>Saksenenae vasiformis</i> (10%) <i>Lichtheimia</i> spp. (10%) <i>Rhizomucor miehei</i> (10%)
Kacem et al. [12] <b>Tunisia</b>	2000-2014	<b>12</b>	<b>Rhinocerebral (70%)</b> Pulmonary (30%)	<i>Rhizopus arrhizus</i> (75%)
Anane et al. [17] <b>Tunisia</b>	1992-2007	<b>17</b>	<b>Rhinocerebral</b>	<i>Rhizopus arrhizus</i> (100%)
Ammari et al. [18] <b>Tunisia</b>	1988-2004	<b>4</b>	<b>Rhinocerebral (50%)</b> <b>Sinus (50%)</b>	<i>Rhizopus arrhizus</i> (75%)
Gouzien et al. [2] <b>France</b>	2012-2022	<b>550</b>	<b>Pulmonary (52,4%)</b> Rhinocerebral (14,5%) Cutaneous-articular (17,1%) Disseminated and other sites (16%)	<i>Rhizopus arrhizus</i> (21,2%)
Sakiada et al. [19] <b>Europe</b>	2005-2007	<b>230</b>	<b>Pulmonary (30%)</b> rhinocerebral (27%) cutaneous (26%) disseminated (15%)	<i>Rhizopus</i> spp (34%), <i>Mucor</i> spp (19%) <i>Lichtheimia</i> spp (19%)

The therapeutic approach to mucormycosis is based on treatment of underlying conditions, administration of antifungal agents, and surgery. Medical treatment is based on intravenous administration of amphotericin B. Liposomal formulations of amphotericin B, formulated to reduce renal toxicity, enable high doses to be administered with less side-effects. The duration of treatment varies from six weeks to three months, with high doses of 5 to 15 mg/kg per day [20].

In localized forms, surgery significantly improves outcomes, offering a favorable risk-benefit balance despite possible complications [6]. The combination of an antifungal agent with surgical debridement is essential because of the poor diffusion of antifungal agents in necrotic tissue [17]. Hyperbaric oxygen therapy has been suggested in the literature as a complementary treatment because of its fungistatic properties and its involvement in the neovascularisation of ischemic areas [17].

The mortality rate in our study was 30%. It ranges from 40% to 80%, with rates varying according to underlying conditions and sites of infection. The highest survival rates are observed in patients with a healthy immune status and no comorbidities. The poorest outcomes are observed in patients with haematological malignancies and hematopoietic stem cell transplant recipients, as well as in patients with extensive burns. Although the disease is better understood and more treatment options are available, survival rates in mucormycosis remain low [20].

**Conclusion**

Mucormycosis mainly affects immunocompromised patients, particularly those with uncontrolled diabetes. Despite the limited number of studies in Tunisia, our results align closely with national findings. PCR and sequencing are essential tools for diagnosis and prognosis. However, conventional microscopy has proved to be a valuable method for rapid and reliable diagnosis. Further nationwide studies are needed to improve our understanding of the epidemiology of the disease.

**Ethical Form**

Written informed consent was obtained from the patient or legal guardian(s) for publication of this case series. A copy of the written consent is available for review by the Editor in-Chief of this journal on request.

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**Conflict of Interest**

There are none.

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