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Original Research Article

Clinico-mycological profile and antifungal susceptibility patterns of zygomycosis during the COVID-19 pandemic at a tertiary care center

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Abstract

Background: Zygomycosis has emerged as a significant opportunistic fungal infection, with a marked increase in incidence during the COVID-19 pandemic, particularly among immunocompromised individuals such as those with diabetes mellitus.

Aim: To analyze the clinico-mycological profile of zygomycosis and determine the antifungal susceptibility patterns, including the minimum inhibitory concentrations (MICs) of commonly used antifungal agents.

Materials and Methods: A total of 57 culture-positive clinical isolates suspected of zygomycosis were included. Specimens were collected from paranasal sinuses, bronchoalveolar lavage, bronchial wash, wounds, nasal swabs, and lung and brain tissues. Identification was done via culture, microscopy, and gene sequencing. Antifungal susceptibility testing for itraconazole, posaconazole, voriconazole, and amphotericin B was performed using the CLSI M38-A2 broth microdilution method.

Results: Rhizopus spp. was the predominant isolate (83%), followed by *Apophysomyces variabilis* (7%), *Lichtheimia corymbifera* (7%), and *Cunninghamella bertholletiae* (2%). Amphotericin B showed the highest efficacy among the studied antifungal agents, followed by posaconazole. Itraconazole and voriconazole was largely ineffective.

Conclusion: Zygomycosis continues to be a life-threatening infection, especially in patients with diabetes and a history of COVID-19. Rhizopus spp. remains the most common etiological agent. Amphotericin B and posaconazole are the antifungal agents deemed most likely to be effective, underlining the importance of routine susceptibility testing for optimized treatment outcomes.

Keywords: Zygomycosis, Mucormycosis, *Rhizopus spp*., Antifungal Susceptibility, Amphotericin B, COVID-19, Diabetes mellitus.

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1. Introduction

Zygomycosis (or mucormycosis) has gained significant attention during the COVID-19 pandemic, particularly in India, which witnessed an unprecedented rise in cases. The widespread use of corticosteroids, coupled with the high prevalence of uncontrolled diabetes mellitus—a key risk factor—has contributed to the surge in infections.[1-3] Immunocompromised patients, especially those recovering from COVID-19, are particularly vulnerable to invasive fungal infections like mucormycosis.[2]

India, known as the "Diabetic Capital of the World," faces a unique burden, with diabetic patients disproportionately affected by mucormycosis.[4] While many reports have documented the epidemiological link between COVID-19, diabetes, and zygomycosis,[5] there is a notable scarcity of comprehensive studies evaluating the clinico-mycological profiles and antifungal susceptibility patterns of zygomycetes in this setting.[6]

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Despite numerous case reports during the COVID-19 pandemic, there remains a lack of systematic antifungal susceptibility profiling of zygomycetes in the Indian population. This study aims to address that gap by evaluating the clinico-mycological characteristics and antifungal resistance patterns in patients with suspected zygomycosis.

2. Materials and Methods

2.1. Study design

This prospective observational study was conducted in the Department of Microbiology at a tertiary care center from November 2019 to August 2021.

2.2. Ethical approval

Ethical clearance was obtained from the Institutional Ethics Committee (Ref: CSP-MED/19/NOV/57/188). Informed consent was acquired from all patients or their legal guardians, as applicable.

2.3. Sample collection

Fifty-seven clinical samples from patients suspected of having zygomycosis were collected. Specimens included paranasal sinus tissue, bronchoalveolar lavage (BAL), bronchial wash, infected wound tissue, nasal swabs, lung tissue, and one brain biopsy. Preliminary screening was performed using 10% potassium hydroxide (KOH) mount to detect fungal elements.

2.4. Fungal identification

Samples showing fungal hyphae on KOH mount were cultured on oatmeal agar slants. Initial fungal identification was done using tease mount and slide culture techniques. Final species confirmation was performed via gene sequencing. Histopathological reports were reviewed when available and included in the analysis.

Of the 57 specimens

1. 42 (73.6%) were culture-positive
2. 15 (26.3%) were KOH-positive but culture-negative
3. 43 (75.4%) showed histopathological evidence of fungal infection

Among these, 38 patients (66.7%) had a history of COVID-19 infection, while 19 (33.3%) did not.

2.5. DNA extraction

DNA was extracted from all culture-positive isolates using an in-house protocol. A loopful of fungal culture was suspended in 400 µL of lysis buffer (10 mM Tris pH 8, 1 mM EDTA pH 8, 3% SDS, 100 mM NaCl) and incubated at 100°C for 1 minute. An equal volume of phenol:chloroform was added, and the mixture was centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was collected, washed with chloroform, and DNA was precipitated with cold isopropanol. The pellet was washed with 70% ethanol and resuspended in 30 µL of TE buffer. DNA was stored at –20°C until use.

2.6. PCR amplification and sequencing

PCR was performed using 25 µL of GeNei PCR master mix and 1 µL each of ITS-1 and ITS-4 primers. Amplified products were sequenced via the Sanger method on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA). Sequence alignment and analysis were carried out using BioEdit software and the NCBI BLAST database.

2.7. Antifungal susceptibility testing

Susceptibility testing was conducted using the CLSI M38-A2 (2017) broth microdilution reference method for filamentous fungi. The antifungal agents tested were:

1. Amphotericin B
2. Posaconazole

2.8. Stock preparation

Antifungal stock solutions were prepared at concentrations of ≥1280 µg/mL, depending on drug solubility. For example, 16 mg of amphotericin B was dissolved in 10 mL of 100% DMSO to yield a 1600 µg/mL solution. Aliquots were stored in 2 mL snap-cap tubes at –70°C.

2.9. Reading and interpretation

MICs were read after 46–50 hours of incubation. The Minimum Inhibitory Concentration (MIC) was defined as the lowest drug concentration that completely inhibited visible fungal growth.

3. Results

3.1. Demographics

The study included 57 patients with suspected zygomycosis, aged between 10 and 70 years, with the highest incidence in the 50–60-year age group. Males constituted 74% (42/57) and females 26% (15/57). A history of COVID-19 was noted in 38 patients (66.7%), while 19 (33.3%) had no prior COVID-19 infection. A total of 84% of our patients were diabetic (48/57) and almost 92% of our patients were diabetic patients affected with COVID infection.

3.2. Sample distribution

Out of the 57 clinical specimens, 42 (73.7%) were culture-positive and 15 (26.3%) were KOH-positive but culture-negative. Histopathological examination was performed in 42 cases, all of which confirmed the presence of fungal elements.

3.3. Source of clinical specimens

The majority of samples were from paranasal sinus tissue (41 cases; 72%), followed by bronchoalveolar lavage/bronchial wash (6 cases; 11%), infected wound tissue (5 cases; 9%),

nasal swabs (2 cases; 4%), lung tissue (2 cases; 4%), and brain tissue (1 case; 1%).

3.4. Clinical presentation

Rhino-orbitocerebral zygomycosis was the most common presentation, seen in 44 patients (77%), followed by pulmonary (8 cases; 14%) and cutaneous zygomycosis (5 cases; 9%).

3.5. Fungal species identified

Rhizopus spp. was the predominant fungal isolate, followed by other members of the order Mucorales:

3.6. Antifungal susceptibility patterns

Antifungal susceptibility testing was performed for all 42 culture-positive isolates against amphotericin B, posaconazole.

The MIC interpretive ranges for the antifungal agents tested are summarized in **Table 1**. Amphotericin B is likely to be effective for most isolates, with MICs ranging from 0.5–2 µg/mL. Isolates with MICs ≤1 µg/mL were considered likely to be susceptible, while those with MICs ≥4 µg/mL indicated reduced susceptibility. Posaconazole showed good in vitro activity, with MICs ranging from 0.125–4 µg/mL in most isolates. MICs ≤1 µg/mL were considered likely to be susceptible, whereas values ≥4 µg/mL suggested resistance. Itraconazole and voriconazole were ineffective against all isolates, with MICs >8–16 µg/mL; no susceptibility breakpoints exist for these drugs, and MICs in this range are consistent with their intrinsic resistance against Mucorales. (**Table 2**).

4. Discussion

Zygomycosis has become a prominent opportunistic infection in India, especially during the COVID-19 pandemic. The high prevalence of uncontrolled diabetes mellitus, compounded by the indiscriminate use of corticosteroids for COVID-19 treatment, has created a high-risk population vulnerable to invasive fungal infections.[1,4,5] Our study reinforces this link, as the majority of zygomycosis cases were seen in patients with a history of COVID-19 and diabetes, particularly those with rhino-orbitocerebral involvement.

Rhizopus spp. was the dominant isolate, which aligns with both national and global epidemiological patterns.[6,7] However, the identification of less common species such as *Apophysomyces variabilis* and *Cunninghamella bertholletiae* emphasizes the importance of molecular methods for accurate species-level identification, particularly in atypical or resistant cases.[10]

4.1. Antifungal resistance: Mechanisms and consequences

Amphotericin B, a polyene antifungal, showed the highest efficacy overall, but resistance (MIC ≥8 µg/mL) was noted in a small subset of isolates. Resistance to amphotericin B is generally attributed to alterations in ergosterol biosynthesis or reduced ergosterol content in the fungal cell membrane, which compromises drug binding and efficacy.[11] This can have serious clinical implications, especially in severe infections where delayed or ineffective treatment can be fatal.

Posaconazole, a second-generation triazole, demonstrated good activity against most isolates. However, elevated MICs in a few cases suggest the possibility of efflux pump overexpression or mutations in the CYP51A gene, which reduce drug binding affinity.[10,14] This underlines the importance of therapeutic drug monitoring and individualized dosing strategies, particularly in salvage therapy.[12]

Table 1: Distribution of fungal species isolated and their MIC values for amphotericin B and posaconazole

Isolate	Number (n = 42)	Amphotericin B MIC Range	Posaconazole MIC Range
Rhizopus spp.	36	0.5–16 µg/mL	0.125–8 µg/mL
Apophysomyces variabilis	3	0.5–8 µg/mL	0.125–0.5 µg/mL
Cunninghamella bertholletiae	1	16 µg/mL	Not tested
Lichtheimia corymbifera	2	1 µg/mL and 16 µg/mL	0.12 and 0.5 µg/mL

There were 18 cases diagnosed solely by histopathology that were not sent for culture.

Table 2: Identification of the isolates and their susceptibility pattern

Isolate	Number	MIC value for amphotericin B and posaconazole
Rhizopus *arrhizus*	19	0.5-16 µg /ml 0.125-8 µg /ml
Rhizopus *microsporus*	9	
Rhizopus *delmar*	8	
Apophysomyces *variabilis*	3	0.5 -8 µg /ml 0.125-0.5 µg /ml
Cunninghamella *bertholletiae*	1	16 µg /ml

Itraconazole exhibited inconsistent activity, likely due to limited in vitro efficacy against Mucorales and poor tissue penetration in some anatomical sites. Voriconazole showed universally high MICs, which is expected given the intrinsic resistance of zygomycetes to this azole due to lack of target binding affinity.[2,13] The frequent empirical use of voriconazole, especially in centers with limited diagnostic capacity, risks treatment failure and delayed intervention. The clinical consequence of antifungal resistance is profound: it can lead to delayed response, progression of tissue necrosis, higher rates of surgical intervention, prolonged hospitalization, and increased mortality. In high-burden settings, such outcomes also place a considerable strain on healthcare resources.

5. Conclusion

This study highlights the predominance of *Rhizopus* spp. as the leading etiological agent of mucormycosis in our patient cohort, particularly among individuals with diabetes and recent COVID-19 infection. A smaller proportion of cases were caused by non-*Rhizopus* Mucorales, which exhibited variable antifungal susceptibility patterns, underscoring the need for precise species-level identification. Amphotericin B remained the most likely antifungal to be effective, while posaconazole served as a reliable second-line or adjunct therapy probably to be efficacious, especially in isolates showing resistance or in patients with drug intolerance.

Importantly, routine antifungal susceptibility testing, combined with molecular confirmation, proved essential in tailoring appropriate therapy and improving patient management. The integration of these diagnostic and therapeutic strategies into routine clinical practice is strongly recommended to enhance treatment precision and outcomes in mucormycosis cases.

6. Source of Funding

None.

7. Conflict of Interest

None.

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