IJBPAS, January, 2026, 15(1): 342-350

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF MUCORMYCOSIS CAUSING PATHOGENS AGAINST COMMERCIALLY AVAILABLE ANTIFUNGALS

TANDON T, JAIN P*, KUMAR T AND RANI N

Department of Biotechnology, University Institute of Engineering and Technology, Kurukshetra University Kurukshetra, 136119, Haryana, India

***Corresponding Author: Dr. Pranay Jain: E Mail: drpranayjain@gmail.com**

Received 13th Sept. 2024; Revised 25th Nov. 2024; Accepted 25th Jan. 2025; Available online 1st Jan. 2026

https://doi.org/10.31032/IJBPAS/2026/15.1.9781

ABSTRACT

Susceptibility testing is a tool that clinicians treating patients with fungal infections can use to learn more about the effectiveness of various antifungals against a particular fungus that has been grown. Decisions regarding the course of treatment for a patient may then be made using these results. However, clinical breakpoints have not been determined for a large number of fungal species that have the potential to cause invasive infections. Therefore, it is very important to have knowledge if the antifungals are susceptible or resistant to any mold. This is particularly true for many molds that have the potential to cause severe mycoses. The goal of this study is to give a general understanding of susceptibility testing against mucoralean fungi.

Keywords: Antimicrobial, Susceptibility test, Mucorales, Aspergillosis, Zygomycosis, and Mucormycosis

INTRODUCTION

The population at risk for invasive fungal infections has significantly expanded over the past few decades. This has been linked to the rise in patients in critical care units and those who are immunocompromised, either as a result of illness or the use of immunosuppressive medications. Our understanding of the variety of fungi that can infect humans and cause disease has expanded along with the number of people who are susceptible to fungal infections. Between 1.5 and 5 million distinct fungus species are thought to exist, and about 300 of these are known to be pathogenic to humans **[1–4]**. These figures will only go up in tandem with the growing number of people who are at risk and the increased use of molecular techniques for the identification and diagnosis of novel fungus and fungal illnesses.

Particularly in people with impaired immune systems, invasive fungal infections are significant causes of morbidity and mortality. While *Aspergillus* species, *Candida* species, and *Cryptococcus neoformans* account for the majority of invasive fungal infections, a considerable amount of morbidity and mortality is caused by other less frequent fungal pathogens **[20]**. The infections responsible for mucormycosis, formerly known as zygomycosis, are Zygomycetes, and they are found all over the world and are becoming more common **[21]**. Fungi belonging to the family Mucoraceae and order Mucorales are the cause of mucormycosis **[22, 23]**. 70–80% of all cases of mucormycosis are caused by the most prevalent genera, *Rhizopus*, *Mucor*, and *Lichtheimia* (previously *Absidia*); the remaining <1–5% of cases are known to be caused by *Cunninghamella, Apophysomyces, Saksenaea, Rhizomucor, Cokeromyces, Actinomucor,* and *Syncephalastrum* **[24]**.

New antifungals are not as readily available as they once were due to the discovery of new fungi that can infect people and the emergence of medication resistance. *Candida auris* is one example of a newly discovered fungus in modern times. This new disease was first identified in 2009 **[5]**, and since then, it has swiftly spread to several continents and been linked to multiple outbreaks at various institutions **[6-8]**. This species' isolates are frequently shown to be resistant to several antifungals, and some isolates have also been reported to be resistant to every antifungal now on the market **[7-10]**. Another example is the well-known mold pathogen *Aspergillus fumigatus*, for which resistance to antifungal drugs other than those used clinically is currently a growing issue. The 1990s saw the first reports of Azoleresistant *A fumigatus* in patients who had been exposed to itraconazole on a regular basis **[11, 12]**. However, the usage of azole like substances in the environment was later related to the recovery of azole-resistant strains from patients with invasive aspergillosis more than ten years ago, even though they had not previously been exposed to azoles **[13-15]**. Worldwide reports of azole resistance associated with environmental exposure have been made **[3, 16]**, as have accounts of clinical failures in patients receiving azole treatment for *Aspergillus* infections brought on by resistant isolates **[3, 17-19]**. Due to the emergence of novel pathogenic fungi and the growing risk of antifungal resistance, physicians frequently employ susceptibility testing to direct therapy in patients suffering from invasive mycoses. An overview of antifungal susceptibility testing against molds is the aim of this research.

Among other filamentous fungi, Mucorales is characterized by the fact that it causes infections in reasonably immune-competent people that are disproportionately serious and have extremely high rates of morbidity and mortality **[25]**. Patients with diabetes mellitus, new-borns, and those with burns, trauma, and surgical wounds may exhibit this in particular. The death rate from mucormycosis is significant, ranging from 50–100% depending on the type of the disease, even with vigorous surgical intervention and rigorous antifungal treatment **[25, 26]**.

In contrast, the mortality rates associated with aspergillosis and candidiasis vary from 35–45% and 20–50%, respectively **[27-29]**. Mucormycosis is an infection that can strike anyone at any age, from elderly individuals with various underlying medical disorders to premature new-borns **[30, 31]**. The current data show higher occurrence in a wide variety of distinct immunocompromised patient groups, even if the incidence rates of invasive mucormycosis in these various patient categories have not been thoroughly examined.

MATERIAL AND METHODS

1. Procurement of microbial cultures and antifungal discs

The microbial cultures were procured from MTCC, Institute of Microbial Technology, Chandigarh named *Rhizopus oryzae*, (MTCC NO. 262), *Mucor indicus* (MTCC NO. 918), *Rhizomucor pusilius* (MTCC NO. 973), *Absidia blakseleeana* (MTCC NO. 148). The respective cultures were maintained and preserved in potato dextrose agar slants. Later the culture slants were stored at -20℃ in refrigerator. The minimum inhibitory concentration (MIC) of antifungal discs were 10mcg each with diameter of 6 mm. The following antifungals were used: Amphotericin B, Fluconazole and Clotrimazole.

2. Standardization of Inoculum

On potato dextrose agar, the isolates were sub-cultured from the original broth suspensions. Fresh, mature (3 to 5 day old) cultures grown on potato dextrose agar slants were used to prepare inoculum solutions. The inoculum were prepared by gently scraping the surface of the colonies with a sterile loop. After that, the inoculum was moved to a sterile syringe filled with water that was fastened to a sterile filter with an 11 mm pore diameter. After being filtered, the suspension was gathered in a sterile tube. This process eliminated most of the hyphae, resulting in an inoculum primarily made up of spores.

3. Antimicrobial susceptibility test

The Kirby-Bauer disc diffusion method was used to determine the test microorganisms' antimicrobial susceptibility to the corresponding antimicrobial medications. Forty millilitres after melting and cooling to 50°C, potato dextrose agar was transferred into sterile Petri dishes and given time to solidify entirely. A test pathogen surface was created by uniformly dispersing a 100µl inoculum onto the whole area of the agar plate using a sterile spreader. The antibiotic disc was applied after the plates had had time to dry. Antibiotic discs were allowed to warm to room temperature (29℃ +/- 1) prior to application in order to avoid condensation. After inoculation, the discs were firmly placed on the agar plate surface within 15 minutes. The disk made direct contact with the agar. All agar plates underwent incubation for 24 hours at 30 ℃ +/- 1 in incubator. Agar plates without antibiotic disc were also cultured as a control. Dimethyl sulfoxide (DMSO) was used as negative control. An inhibitory zone encircling the drug disc suggested the presence of antibacterial activity on the plates. Using a HiMedia Zonescale, the inhibitory zones' millimeter diameter at 24 hours was determined. For every test pathogen and drug, the trials were run three times. The diameter of the inhibitory zones was measured for each antibiotic, and the mean and standard deviation were computed. It was determined that an organism was either extremely vulnerable, somewhat susceptible, or resistant to the medications **[32, 33]**.

Table 1: Antibiotic susceptibility test of different Mucoralean fungi against commercially available dugs by Kirby-Bauer disc diffusion method

Sr. no.	Antimicrobial drugs	Minimum inhibitory concentration (µg)	Mean Diameter of inhibition zone (mm)			
			Rhizopus oryzae	***Absidia blakseleeana***	***Mucor indicus***	***Rhizomucor pusilius***
1.	**Amphotericin B**	**10**	**NA**	**NA**	**21.2±0.36**	**10.5±0.08**
2.	**Fluconazole**	**10**	**NA**	**NA**	**28±0.083**	**NA**
3.	**Clotrimazole**	**10**	**NA**	**≤ 10**	**32.10±0.45**	**32.03±0.03**
4.	**Terebinafine**	**10**	**NA**	**20±0.05**	**NA**	**35±0.042**

RESULTS AND DISCUSSION

The Kirby-Baeur disc diffusion method was used to measure the antibacterial activity of three marketed medicines. **Table 1** shows the diameter of the inhibition zones that were created against various mucoralean fungi. The information showed that there were significant differences in the antimicrobial activity of the drugs tested on the agar plates. There was no discernible inhibitory effect from the negative control. According to data *Rhizopus oryzae* and *Absidia blakseleeana,* were found resistant to all the antifungals used.

The data put on views that the zine of inhibition against *Mucor indicus* varied from 21mm to 32mm for all three antifungals i.e., Amphotericin B (21 mm), Fluconazole (28 mm) and Clotrimazole (32 mm) respectively. Thus concludes that clotrimazole and fluconazole is highly susceptible to *Mucor indicus* and moderately susceptible to amphotericin B. *Rhizomucor pusilius* is found to be resistant against fluconazole. The data obtained showed that *Rhizomucor pusilius* is highly susceptible towards clotrimazole. Amphotericin B is least effective to *Rhizomucor sp.* Terebinafine was found most active drug against *Absidia blakseleeana* and *Rhizomucor pusilius.*

For the treatment of mucormycosis, amphotericin B deoxycholate (AmB) is still the sole antifungal medication with a license. However, compared to AmB, lipid formulations of AmB (LFABs) are far less nephrotoxic and can be given at higher doses for longer periods of time without risk. Fluconazole does not consistently work against the pathogens that cause mucormycosis. Mucormycosis responds differently to antifungal medications depending on the host and the place, and it can be especially problematic for people who suffer from hematological disorders. For instance, Shoham *et al*. **[34]** recently reported that 32 patients with hematological malignancies and lung infections had a 32% response rate to LAmB as the primary therapy for mucormycosis. Thus, while determining the prognosis, managing, and designing treatment trials for mucormycosis patients, host-dependent variance in response should be taken into account.

Immuno-compromised patients with invasive fungal infections now have a much better prognosis thanks to the development of novel antifungal medications. However, the invasive nature of mucormycosis results in an overall mortality rate that surpasses 50%, and antifungal therapy alone frequently proves to be ineffective, leading to 100% mortality, especially in patients with disseminated disease. Compared to other invasive fungal illnesses like candidiasis or aspergillosis, mucormycosis has a poorer prognosis. Differences in host-fungus interactions, pathogenetic mechanisms, greater challenges in early diagnosis when the "window" of successful treatment is higher, and wider inadequacies of therapeutic options are all contributing factors to the higher degree of difficulty in curing this devastating infection. Improved treatment strategies, improved diagnostic technologies, and a deeper comprehension of host defense may all help to improve the prognosis of this incurable illness.

Acknowledgments

Authors are grateful to the Director, University Institute of Engineering and Technology, and for providing lab facilities Vice-chancellor of Kurukshetra University Kurukshetra. Thanks, are also due to two anonymous referees for their comments and

suggestions for the improvement of the manuscript.

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