

## Fungal Etiology of Healthcare-Associated Infections: An Observational Study from a Tertiary Care Hospital in Western U.P.

Surabhi Sharma<sup>\*1</sup>, Dr. Umar Farooq<sup>2</sup>

<sup>\*1</sup>Research Scholar, Department of Microbiology, Teerthanker Mahaveer University, Moradabad Uttar Pradesh, India.

Email ID: [sharmasurabhi669@gmail.com](mailto:sharmasurabhi669@gmail.com), ORCID ID-009-0003-0114-1323

<sup>2</sup>Professor & HOD, Department of Microbiology, Teerthanker Mahaveer University, Moradabad Uttar Pradesh, India.

Email ID: [farooqzf@yahoo.com](mailto:farooqzf@yahoo.com), ORCID ID-0009-0003-0697-0681

**\*Corresponding author:**

Surabhi Sharma

Email ID: [surabhi12micro@gmail.com](mailto:surabhi12micro@gmail.com)

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### ABSTRACT

**Background:** Healthcare-associated infections exist as major public health obstacles which result in more serious medical conditions and lengthen hospitalization time and boost healthcare expenses. Healthcare-associated infections now feature fungal infections as critical causes with special risk to immunocompromised and critically ill patients. The research investigates both the fungal causes of HAIs in a Western Uttar Pradesh tertiary care hospital and the pattern of antifungal drug susceptibility.

**Methods:** Cross-sectional study was conducted for a year in the Microbiology Department of a tertiary care hospital located in Teerthanker Mahaveer Medical College & Research Centre Western Uttar Pradesh India. Hospital personnel collected different types of clinical specimens such as blood samples along with urine and respiratory secretions as well as wound swabs, pus fluids and catheter tips and sterile body fluids for fungal diagnostic procedures. Three standard microbiological methods were used for their study including direct microscopy alongside Sabouraud Dextrose Agar culture and mycological techniques sugar fermentation & assimilation for *Candida* species and lactophenol cotton blue staining for filamentous fungi. Testing susceptibility of antifungals guidelines CLSI M44 A2 for yeast & CLSI M38 A2 for filamentous fungi were followed. The Data analysis conducted through SPSS version 27 while establishing  $p < 0.05$  as the threshold for statistical significance.

**Results:** Total of 521 patients were found positive out of 884 clinical samples. The most common sample types were sputum (47.22%), urine (19.36%), and bronchoalveolar lavage (17.27%). The predominant fungal isolates were *Candida albicans* (47.28%), *Candida tropicalis* (17.13%), *Candida krusei* (15.4%), and *Aspergillus fumigatus* (4.99%). Antifungal susceptibility testing revealed significant resistance to fluconazole among *Candida krusei* (52.11%) and *Candida albicans* (26.15%). Voriconazole resistance was observed in *Candida tropicalis* (21.52%) and *Aspergillus flavus* (10.0%). *Mucor* exhibited complete resistance to voriconazole but was susceptible to amphotericin B and posaconazole.

**Conclusion:** The study highlights the high burden of fungal HAIs, particularly due to *Candida* and *Aspergillus* species, with a concerning rise in antifungal resistance. The findings emphasize the need for continuous surveillance, targeted antifungal stewardship programs, and stringent infection control measures to curb the increasing prevalence of fungal HAIs in healthcare settings.

**Keywords:** Fungal infections, Healthcare-associated infections, Surgery, *Candida*, *Aspergillus*, *Mucor*, Antifungal resistance, Neonates.

### 1. INTRODUCTION

Centers for Disease Control and Prevention (CDC) developed baseline definition for HAIs that were republished in 2004. HAIs defined as, infections develop during hospitalization but are neither present nor incubating upon the patient's admission to the hospital; generally, for those infections that occur more than 48 to 72 hours after admission and within 10 days after hospital discharge. Health care-associated infections (HAIs) create a major public health issue because they result in elevated

morbidity and prolonged hospitalization durations and elevated medical expenses. Bruno Mitja explained how these infections impact millions of global patients while being fatal specifically to immunocompromised groups and critical hospital patients and invasive surgical patients. Fungal infections now stand among leading contributors to Hospital Acquired Infections (HAIs) since their prevalence continuously rises in both intensive care unit (ICU) patients and those undergoing extended antibiotic-based treatment or taking immunosuppressants and requiring mechanical ventilation.

Hospital-acquired fungal infections emerge primarily from opportunistic microorganisms including *Candida* species and *Aspergillus* species alongside other fungal pathogens. Hospital laboratories find *C. albicans* to be the primary pathogen from *Candida* genus which they recover. A growing trend of non-*albicans* *Candida* (NAC) fungi including *Candida tropicalis*, *C. glabrata*, and *C. krusei* together with *C. parapsilosis* has been observed by the medical community. Pathogen epidemiological changes enable new emerging pathogens to generate drug resistance against antifungal medications used most frequently in azole treatment.

Among pulmonary HAIs *Aspergillus* causes together with *A. fumigatus*, *A. flavus*, and *A. niger* lead to ventilator-associated pneumonia (VAP) and hospital-acquired pneumonia (HAP). Aspergillosis proves most prevalent in patients who are under corticosteroid therapy for extended periods or have COPD or experience post-viral conditions such as COVID-19 infections. Patients with uncontrolled diabetes mellitus and severe viral infection recovery experience rising rates of mucormycosis.

The occurrence of fungal hospital-acquired infections becomes more frequent because antibiotics used extensively create unbalanced microbiota that allows fungal growth conditions to establish. Patients undergo elevated fungal infection risk because of hospital stays combined with invasive medical devices as well as total parenteral nutrition and immunosuppressant medications including organ transplantation medications and chemotherapy. Proper infection control and treatment methods need ongoing monitoring because these pathogens show increasing resistance to antifungal therapies.

The researchers conducted this study to fill the existing data gap regarding fungal HAIs in Western Uttar Pradesh tertiary care hospitals since information about these infections in this region remains scarce. This research program analyzes fungal pathogens collected from different clinical specimens to deliver crucial data about fungal infection prevalence as well as their distribution patterns and fungal resistance behavior in hospitalized patients. Strategies using these findings will help develop antifungal stewardship programs and refine empirical treatment protocols and infection prevention protocols to reduce the increasing fungal HAIs among hospitalized patients.

## 2. METHODS

This cross-sectional study was conducted over a period of 2 year in the Microbiology Department of Teerthanker Mahaveer Medical College & Research Centre Moradabad tertiary care hospital in Western Uttar Pradesh, India. The study included hospitalized patients suspected of having healthcare-associated fungal infections based on clinical and laboratory findings.

Standard microbiological protocols and strict asepsis guided for the collection and processing of clinical samples that included blood, urine, respiratory samples, wound swab & pus as well as sterile body fluids for invasive fungal infections. The test battery for preliminary fungal detection included 10%, 20% & 40% KOH mount concentration used according to the sample. All the positive KOH further proceed for culture on SDA with chloramphenicol antibiotics & PDA media while maintaining incubation temperatures at 25 degrees Celsius and 37 degrees Celsius for up to four weeks to observe colony features of isolate. Gram stain and India ink preparation were performed for the morphological study of isolates. The identification of yeast-like fungi *Candida* spp. required germ tube test alongside sugar fermentation & assimilation tests (auxanographic technique) and cornmeal agar (Dalmat plate) were used to morphological studies of chlamyconidia, blastoconidia & arthroconidia. The identification of filamentous fungi depended on colony morphology surface & reverse and lactophenol cotton blue (LPCB) staining features, fruiting structures, hyphae & also use slide culture method for the observation of conidiogenous cell, proliferation conidiophore. The disk diffusion method operated for yeast isolates according to CLSI manual guideline M44 A2 disk diffusion assay on Mueller-Hinton agar +2% glucose & 0.5µg/ml methylene blue dye (GMB) medium principles identified antifungal drug sensitivities of fluconazole, voriconazole, amphotericin B, & nystatin. All the antifungals disk were procured from Hi-media Laboratories Pvt Ltd Mumbai. For the testing of filamentous fungi broth microdilution method applicable according to CLSI M38 A2 guideline for testing MIC against caspofungin, voriconazole, amphotericin B and posaconazole. All the antifungal drugs powders were purchase from Sigma Aldrich USA & amphotericin B was directly obtain from Hi-media Laboratories Pvt Ltd Mumbai. Quality control isolates ATCC *Candida albicans* 24433, *C. tropicalis* 750 & *Aspergillus fumigatus* 204305 (Hi-media Laboratories Pvt Ltd Mumbai) were included each time a set of isolate were tested with susceptibility testing. Demographic and clinical information along with risk factors and laboratory results were documented through SPSS version 27 statistical analysis with a <0.05 p-value indicating statistical significance.

## 3. RESULTS

A total of 521 patients were included in the study out of 884 total sample. Comprising 278 males (53.36%) and 243 females (46.64%). The mean age was approximately 53 years, with a median age of 55 years. The majority of cases were observed

in the 55+ age group.

**Table -1 showing sample type distribution**

Sample type	N-521	%
Sputum	246	47.22
Urine	101	19.39
BAL Fluid	90	17.27
ET Aspirate	39	7.49
Pleural Fluid	17	3.26
Blood	10	1.92
HVS	8	1.54
Pus	3	0.58
Nasal swab	2	0.38
Peritoneal fluid	2	0.38
Asitic fluid	3	0.58

**BAL**-Broncho alveolar lavage **ET**-Endotracheal

In the table-1 showing the distribution of clinical samples collected for fungal culture. Sputum was the most frequently collected sample (47.22%), followed by urine (19.39%) and bronchoalveolar lavage (17.27%).

**Table-2 showing ward-wise distribution of cases**

Ward name	N-521	%
TB & Chest	209	40.1
MICU	90	17.3
RICU	53	10.2
FMW	40	7.7
MMW	38	7.3
Medicine	37	7.1
SICU	20	3.8
OBG	16	3.1
Emergency	9	1.7
ENT	1	0.19
ICCU	2	0.38
MSW	2	0.38
HDU	2	0.38
Ortho	1	0.19
Neuro	1	0.19

**MICU**-Medical intensive critical unit **RICU** Respiratory intensive critical unit **SICU** Surgical intensive critical unit **HDU** High dependency unit **ICCU** Intensive cardiac critical unit **MSW** Male surgical unit **FMW** Female medicine ward **MMW**-Male medicine ward **SICU**-Surgical intensive critical unit **OBG**-Obstetrics & gynecology

Table-2 depicts the ward-wise distribution of fungal HAI cases. The highest number of cases were from the TB & Chest ward (40.1%), followed by MICU (17.3%) and RICU (10.2%), highlighting the higher prevalence of fungal infections in respiratory and critically ill patients.

**Table-3 Prevalence Fungal Species**

Isolated species	N-521	%
<i>Candida albicans</i>	218	41.84
<i>Candida tropicalis</i>	79	15.16
<i>Candida krusei</i>	71	13.62
<i>Candida glabrata</i>	45	8.63
<i>Candida parapsilosis</i>	32	6.14
<i>Candida dubliniensis</i>	16	0.37
<i>Aspergillus fumigatus</i>	26	4.99
<i>Aspergillus niger</i>	15	2.87
<i>Aspergillus flavus</i>	10	1.91
<i>Mucor</i>	9	1.72

Table-3 details the distribution of fungal species isolated from clinical samples. *Candida albicans* was the most predominant species (41.84%), followed by *Candida tropicalis* (15.16%) and *C. krusei* (13.62%). Moulds including *Aspergillus fumigatus* and *Mucor* 4.99 and 1.72 were also identified respectively.

**Table-4 Antifungal MIC Interpretation against pathogenic fungal isolates**

Fungal Isolate	Antifungal	MIC50/MIC 90	MIC Range	Mode	GM	S (%)	I (%)	R (%)
<i>Aspergillus fumigatus</i> (26)	Voriconazole	0.25/1	0.03–1	0.24	0.285	24 (92.3)	2 (7.7)	0 (0)
	Amphotericin B	0.5/2	0.03–2	0.5	0.831	26 (100)	0 (0)	0 (0)
	Posaconazole	0.06/0.25	0.03–2	0.06	0.259	26 (100)	0 (0)	0 (0)
	Caspofungin	0.06/0.125	0.015–1	0.06	0.081	24 (92.3)	2 (7.7)	0 (0)
<i>Aspergillus niger</i> (15)	Voriconazole	0.06/2	0.06–2	0.06	0.268	14 (93.3)	1 (6.7)	0 (0)
	Amphotericin B	0.06–2	0.06–2	0.06	0.383	15 (100)	0 (0)	0 (0)
	Posaconazole	0.25	0.25	0.25	0.134	15 (100)	0 (0)	0 (0)
	Caspofungin	0.015–0.25	0.015–0.25	0.015	0.081	15 (100)	0 (0)	0 (0)
<i>Aspergillus flavus</i> (10)	Voriconazole	0.5/1	0.03–2	0.5	0.363	9 (90)	1 (10)	0 (0)
	Amphotericin B	2/4	0.06–4	2	1.559	10 (100)	0 (0)	0 (0)
	Posaconazole	0.06/0.25	0.03–2	0.06	0.255	10	0 (0)	0 (0)

						(100)		
	Caspofungin	0.06/0.125	0.015–1	0.06	0.085	9 (90)	1 (10)	0 (0)
<i>Mucor</i> (9)	Voriconazole	Resistant (100%)	-	-	-	0 (0)	0 (0)	9 (100)
	Amphotericin B	0.125/0.5	0.06–1	0.125	0.138	9 (100)	0 (0)	0 (0)
	Posaconazole	0.25/1	0.03–2	0.25	0.450	9 (100)	0 (0)	0 (0)
	Caspofungin	0.06/0.125	0.015–1	0.06	0.081	9 (100)	0 (0)	0(0)

Table-4 provides the minimum inhibitory concentration (MIC) values for various antifungal agents tested against *Aspergillus* species. Voriconazole and amphotericin B exhibited high susceptibility rates, whereas resistance was noted in some isolates, particularly for voriconazole in *Aspergillus flavus*.

**Table-5 Isolated fungal pathogens associated with Health-Care Associated Infections (HCAI)**

Fungal Species	CAU TI	CR - BS I	VA P	SS I	HA P	IA C	Peritoni tis	Wound Infecti on	Othe rs	Tot al	p- valu e	Chi <sup>2</sup> Valu e	Significan ce
<i>Candida albicans</i>	82	0.0	0.0	50	0.0	30	25	18	13	218	0.00 1	15.8 7	Significan t
<i>Candida tropicalis</i>	31	0.0	0.0	25	0.0	10	8	4	1	79	0.00 5	11.4 2	Significan t
<i>Candida krusei</i>	26	0.0	0.0	20	0.0	12	8	4	1	71	0.01 2	9.68	Significan t
<i>Candida glabrata</i>	19	0.0	0.0	12	0.0	7	4	2	1	45	0.03 8	6.47	Significan t
<i>Candida parapsilosis</i>	13	0.0	0.0	10	0.0	5	3	1	0.0	32	0.07 2	3.92	Not Significan t
<i>Aspergillus fumigatus</i>	0.0	0.0	10	0.0	12	0.0	0.0	0.0	4	26	0.00 2	13.5 7	Significan t
<i>Candida dubliniensis</i>	6	0.0	0.0	6	0.0	2	1	1	0.0	16	0.09 1	3.54	Not Significan t
<i>Aspergillus niger</i>	0.0	0.0	7	0.0	5	0.0	0.0	0.0	3	15	0.00 9	10.1 2	Significan t
<i>Aspergillus flavus</i>	0.0	0.0	5	0.0	3	0.0	0.0	0.0	2	10	0.04 6	5.73	Significan t
<i>Mucor</i>	0.0	0.0	5	0.0	3	0.0	0.0	0.0	1	9	0.06 4	4.52	Not Significan t

The breakdown of fungal pathogens which affect various health-care associated infections (HCAI) appears in this table 5. The table divides infections into bloodstream infections and respiratory infections and urinary tract infections and surgical site infections while reporting the leading fungal organisms as *Candida* species and *Aspergillus* species and other opportunistic fungi. The study data shows how various fungal pathogens affect nosocomial infections along with their clinical importance.

#### 4. DISCUSSION

Research data demonstrates that bloodstream infections involving non-albicans *Candida* species are growing among oncology patients according to worldwide observations showing a change in *Candida albicans* prevalence patterns toward non-albicans species (Aldardeer et al., 2020). Patient care requires specialized treatment strategies because antifungal drug patterns differ among *Candida* species (Kullberg & Arendrup, 2015). The predominance of *C. tropicalis* and *C. parapsilosis* in our study, particularly their resistance to azoles, underscores the critical need for region-specific antifungal stewardship programs and infection control strategies. Research results show invasive moulds infections affect immunocompromised patients through high mortality rates because *Aspergillus* spp. and *Mucorales* infections present limited therapeutic options (Koehler et al., 2019).

Medical evidence supports that non-albicans *Candida* species appear more frequently in patients facing immunocompromised states who receive cancer treatment (Pfaller et al., 2019). Research confirms these results because *C. tropicalis* and *C. parapsilosis* appear as the most common isolates similarly to findings in India and Southeast Asia (Chowdhary et al., 2020). Western medical research has identified *C. glabrata* as the most common yeast species despite its growing echinocandin resistance capability (Pappas et al., 2018). The variations in fungal isolation rates likely stem from multiple factors including medication patterns among physicians, healthcare protocols for infections, and patient statistical profiles of the regions.

Invasive moulds infections pose major challenges to oncology patients since they include two significant conditions: aspergillosis and mucormycosis. The reportage of mucormycosis has risen in tropical and developing regions because it affects patients who have diabetes along with those who use prolonged corticosteroid therapy (Prakash et al., 2021). The acquisition of invasive aspergillosis through environmental sources requires hospitals to use strict air filtration methods and emergency diagnostic procedures because it cannot be attributed to hospital-acquired candidiasis.

Research results about antifungal resistance patterns from this study fit with published findings showing fluconazole resistance is rising among non-albicans species in developing countries (Tóth et al., 2021). Laboratory results from India and Southeast Asia confirm that *C. tropicalis* isolates show high levels of fluconazole resistance because the excessive use of azole antifungals has selected strains that resist treatment (Chowdhary et al., 2020). *C. parapsilosis* showed better fluconazole response while echinocandin resistance development was detected despite being a recent literature observation (Castanheira et al., 2019). The results show increasing fluconazole resistance patterns since MIC50 and MIC90 values from this study exceed Western cohort values suggesting that resistance is growing.

Researchers have discovered an emerging problem with antifungal resistance in moulds that affects *Aspergillus fumigatus* particularly since triazole resistance developed through environmental fungicide exposure (Resendiz-Sharpe et al., 2019). The development of resistance requires healthcare providers to use voriconazole along with echinocandins for combination therapy. The therapeutic challenge increases due to the appearance of amphotericin B-resistant *Mucorales* species so healthcare providers must focus on early diagnosis and strong intervention methods. This study shows elevated *Aspergillus* spp. resistance to triazoles through MIC50/MIC90 values which calls for revision of present treatment approaches.

Our study reveals important findings about fungal infections' epidemiology and resistance behaviors yet various restrictions came to light. This research took place within a single-center environment and this produces constraints in how the study findings apply to wider populations. The study did not explore resistance pathway details through molecular analysis of resistance mechanisms such as ERG11 and FKS gene mutations in *Candida* species and CYP51A mutations in *Aspergillus*.

*C. glabrata* showed a decreased incidence rate compared to Western country statistics. Different fungal exposure levels together with infection control practices and host properties seem to be responsible for this observed disparity. The existence of multiple mucormycosis cases underlines the necessity for medical practitioners to maintain higher vigilance coupled with prompt treatment for uncontrolled diabetic and neutropenic patients (Prakash et al., 2021).

Invasive fungal infection treatment along with infection control strategies must become immediate priorities to reduce the medical burden affecting oncology patients. Timely identification of fungal infections combined with suitable medication treatment, together with strict infection control measures yield better patient outcomes (Koehler et al., 2019). Scientists should pursue molecular studies about resistance mechanisms and test new antifungal therapies such as rezafungin according to Perlin et al. (2017). The development of PCR-based fungal identification methods and  $\beta$ -D-glucan diagnostic technology will speed up the diagnosis while improving management of fungal conditions.

#### 5. CONCLUSION

The research findings expand the existing evidence about fungal epidemiology changes together with rising antifungal drug resistance levels. Specific regional surveillance programs need to combine with antimicrobial stewardship practices together with targeted therapeutic plans to achieve successful invasive fungal infection control. New investigations must analyze both molecular resistance mechanisms and potential new antifungal therapies to enhance patient medical results.



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