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Research Article

Drug Resistance Profiles of *Aspergillus Species* in Cases of Invasive Pulmonary Aspergillosis

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A B S T R A C T

Background: Invasive pulmonary aspergillosis (IPA) is a life-threatening fungal infection, particularly affecting immunocompromised individuals. The increasing emergence of antifungal resistance among *Aspergillus* species poses significant challenges to effective therapy, especially in regions with limited surveillance data.

Objectives: To investigate the distribution of *Aspergillus* species isolated from IPA patients in Baghdad and evaluate their susceptibility patterns to commonly used antifungal agents.

Methods: This cross-sectional study included 250 patients diagnosed with IPA at Medical City Hospital, Baghdad, between September 2024 and May 2025. Clinical diagnosis was confirmed through imaging, galactomannan, and (1,3)-β-D-glucan assays. Isolates were identified and tested for susceptibility to nine antifungal drugs, including triazoles, echinocandins, amphotericin B, and 5-fluorocytosine, using the Microorganism Identification and Antimicrobial Susceptibility Testing (ID&AST) System MA120 following Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Aspergillus flavus was the most prevalent species (58%), followed by *A. fumigatus* (30%), *A. niger* (8%), and *A. terreus* (4%). All isolates exhibited 100% resistance to fluconazole and 5-fluorocytosine. Moderate to high resistance was observed against triazoles (16.0–21.2%), echinocandins (56–58%), and amphotericin B (38%). *A. flavus* demonstrated the broadest resistance spectrum, whereas *A. fumigatus* retained better susceptibility to triazoles but showed emerging resistance.

Conclusion: High levels of antifungal resistance in *Aspergillus* species, especially *A. flavus*, were revealed by this study, highlighting the rise of multidrug resistance. To treat IPA, it is critically important to implement new treatment approaches and routine susceptibility testing.

Keywords: Aspergillosis, Disease, Antifungals, Resistance, Azoles

Introduction

One of the most prevalent types of fungi in the world is *Aspergillus*. In regard to abiotic growth circumstances, they lack a high degree of selection; *Aspergillus* species are frequently isolated from different geographic places.[1] Inhalation of *Aspergillus* spores can cause an allergic reaction in certain individuals; other people may develop lung infections that range from moderate to severe. When the infection spreads to blood vessels and beyond, it results in invasive aspergillosis, the most dangerous type of the disease.[2]

Invasive pulmonary aspergillosis (IPA), a serious fungal infection, is brought on by the filamentous *Aspergillus* species. *Aspergillus* infection is reliant on immunologic response and host variables. All individuals are exposed to the fungus spores, but only those with compromised immune systems can develop invasive infections. People with weakened immunity, such as those receiving chemotherapy, organ transplant recipients, or those with advanced HIV/ AIDS, are frequently impacted.[3,4] Risk factors are constantly changing and have been seen after a COVID-19 infection. These factors include post-influenza infection and novel biological agents that target the immune system.[5] COVID-19-associated pulmonary aspergillosis (CAPA) has emerged as a recognised consequence, particularly in patients in the intensive care unit (ICU) who are on corticosteroids or mechanical ventilation. Although advantageous for COVID-19, these immune-modulating treatments also make people more vulnerable to opportunistic fungal infections like IPA.[6–8]

IPA is becoming more common over time. A 2023 published study ,conducted in 2023, has shown that the overall one-year mortality rate for IPA patients was 32%,[9] with overall mortality rates in invasive forms of the disease ranging from 35.6% to 70.0%[10,11]. *Aspergillus fumigatus* (80%) is the primary cause of IPA, followed by *A. flavus* (15–20%).[12] Recently, *A. fumigatus* was added to the World Health Organization fungal priority pathogen list as a part of the critical priority group of the list (*Cryptococcus neoformans*, *Candida auris*, *Aspergillus fumigatus*, and *Candida albicans*). Regarding their burden on public health, *Aspergillus fumigatus* and *Candida albicans* were ranked highly; both of them placed in the top four.[13] For the majority of clinical forms of aspergillosis, triazoles are the primary choice. Triazoles and amphotericin B, two kinds of antifungal medications now used to treat aspergillosis, target ergosterol. Echinocandins, a third class of chemicals, prevent the formation of beta-1,3-glucan, a crucial component of fungal cell walls.[14,15] As members of the triazole class, voriconazole, isavuconazole, and posaconazole are the first-line treatments for invasive infections, and voriconazole or itraconazole are the first-line treatments for chronic illnesses.[16–18]

Aspergillus acquired resistance to azoles has been documented since 1990. Data on resistance, particularly for *A. fumigatus*, has grown dramatically in recent years, with up to 20% of *Aspergillus* isolates exhibiting entirely newly developed resistance to widely used antifungal medications.[19,20] Nevertheless, the global distribution of antifungal-resistant strains and resistance patterns is still unclear. The prolonged use of these medications, especially during long-term treatment, may result in antifungal pressure, which is what selects non-susceptible clones. A combination therapy using medications with distinct modes of action, such as voriconazole, amphotericin B and echinocandin, is recommended for the treatment of resistant aspergillosis. The increased use of second-line medicines, such as echinocandins, in monotherapy is a result of *Aspergillus* species' resistance to first-line drugs.[21–23]

The current study offers region-specific insights into the prevalence of *Aspergillus* species and their antifungal resistance patterns, particularly in the Middle Eastern context (Baghdad, Iraq), revealing the antifungal susceptibility profiles of IPA patients.

Materials and Methods

Patients and Sampling

The cross-sectional study included 250 patients diagnosed with IPA who were treated at Medical City Hospital, Baghdad, between September 2024 and May 2025. Patients were diagnosed with IPA using clinical symptoms, Computed Tomography (CT) scans and laboratory tests, including galactomannan and (1,3)-β-D-glucan assays. The Ethical Committee at the College of medicine, Al-Iraqia University (Reference: CMEC/0356/0020). A written informed consent was obtained from all participants. The research was performed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Antifungal Susceptibility Test

Every isolate was sent for identification and testing for resistance to nine antifungal medications represented by triazoles (fluconazole, itraconazole, voriconazole, posaconazole, and isavuconazole), echinocandins (caspofungin and micafungin), amphotericin B, and 5-fluorocytosine. The Microorganism Identification and Antimicrobial Susceptibility Testing (ID&AST) System MA120 (Render, China) was used for identification and resistance testing; the susceptibility test was carried out in compliance with Clinical and Laboratory Standards Institute (CLSI) guidelines.[24]

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Statistical Analysis

The Statistical Packages of Sciences (SPSS) programme (2019) was used to detect the effect of different factors on study parameters. Chi-square test was used to significantly compare the percentages (significant at 0.05 and 0.01) in this study.

Results

Demographics

Among the 250 IPA patients, 74% (185) of isolates were male, while 26% (65) were female. The p value was less than 0.01, which indicated that there was a significant difference between the number of males and females and not just a random difference (Table 1).

Age Distribution

The largest age group was more than 60 years of age (75 individuals, 30%); the second largest age group was 20–30 years (57 individuals, 22.80%). The groups of less than 20 years (42 individuals, 16.80%) and 40–50 years (38 individuals, 15.20%) also contributed significantly; the smallest group was 30–40 years (10 individuals, 4%). This indicated a bimodal distribution, where younger (20–30 years) and older (> 60 years) individuals were more represented. Since $p \leq 0.01$, the result was highly significant. This suggests that the age distribution is not random and differs significantly from an expected uniform distribution (Table 2).

The most prevalent species in the sample of 250 isolates was *Aspergillus flavus*, accounting for 58% of the total sample. *Aspergillus fumigatus* followed with 30% of the isolates. *Aspergillus niger* and *Aspergillus terreus* were less common, making up 8% and 4%, respectively (Table 3).

Table 1.Distribution of Study Sample According to Sex

Sex	Number	Percentage
Male	185	74.00
Female	65	26.00
Total	250	100.00
Chi-square: χ^2 (p value)	-	57.60**(0.0001)

**p ≤ 0.01

Table 2.Distribution of Study Sample According to Age Groups

Age Groups (Years)	Number	Percentage
< 20	42	16.80
20–30	57	22.80
30–40	10	4.00
40–50	38	15.20
50–60	28	11.20
> 60	75	30.00
Total	250	100.00
Chi-square: χ^2 (p value)	-	61.063**(0.0001)

**p ≤ 0.01

Table 3.Distribution of Study Sample According to Species

Species	Number	Percentage
Aspergillus flavus	145	58.00
Aspergillus fumigatus	75	30.00
Aspergillus niger	20	8.00
Aspergillus terreus	10	4.00
Total	250	100.00
Chi-square: χ^2(p value)	-	184.40**(0.0001)

**p ≤ 0.01

Identification of Antifungal Susceptibility Profiles

This study tested four *Aspergillus* species against five triazole antifungals, including fluconazole (FLC), itraconazole (ITR), voriconazole (VRC), isavuconazole (ISA), and posaconazole (POS), two types of echinocandins, namely caspofungin (CAS) and micafungin (MCF), amphotericin B (AMB), and 5-fluorocytosine (5-FC). The statistical analysis showed the studied *Aspergillus* species had very high resistance toward FLC and 5-FC with all (100%) isolates being resistant; for the other types of triazole (ITR, VRC, ISA, and POS), *Aspergillus* species showed significant resistance, with 20%, 16%, 20%, and 21.2% of isolates being resistant, respectively, while towards AMB, moderate resistance was indicated (38%), and for echinocandins (MCF and CAS), there was a high resistance of 56.4% and 58.0%, respectively (Table 4).

Aspergillus spp. Susceptibility Profiling for FC and 5-FC

All studied *Aspergillus* species showed resistance to FLC; minimum inhibitory concentrations (MICs) for all isolates were ≥ 64 µg/mL, and there was no breakpoint for this drug by CLSI, as *Aspergillus* spp. are considered intrinsically resistant to FLC. For 5-FC, all (100%) isolates were resistant; MICs for all isolates were ≥ 2 µg/mL, and there was no breakpoint for this drug by CLSI, as *Aspergillus* spp. are considered intrinsically resistant to 5-FC (Table 5).

Aspergillus flavus Susceptibility Profiling

In the current study, *A. flavus* susceptibility profiling of triazoles was as follows: For ITR, 37 (25.5%) were non-wild type (NWT) and 108 (74.5%) were wild type (WT) (Epidemiological Cutoff Value (ECV) 1 µg/mL). For VRC, only 27 (18.6%) were NWT and 118 (81.4%) were WT (ECV 2 µg/mL). For ISA, 37 (25.5%) were NWT and 108 (74.5%) were WT (ECV 1 µg/mL). For POS, 40 (27.6%) isolates were NWT, and 105 (72.4%) were WT (ECV 0.5 µg/mL) (Table 5). *A. flavus* susceptibility profiling of echinocandins showed that for CAS, 88 (60.7%) were NWT, and 57 (39.3%) were WT (ECV 0.5 µg/mL), and for MCF, 93 (64.1%) were NWT, while 52 (35.9%) were WT (ECV ≤ 0.5 µg/mL). CLSI has not established a specific ECV for MCF against *A. flavus*; thus, this study used a suggested ECV from a previous work.[25]

A. flavus susceptibility profiling of AMB revealed that 66 (54.5%) were NWT, and 79 (45.5%) were WT (ECV 4 µg/mL) (Table 6).

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Table 4.Results of Antifungal Susceptibility of Samples

	FLC	ITR	VRC	ISA	POS	CAS	MCF	AMB	5-FC	p Value
S (WT) n (%)	0 (0.00)	200 (80.00)	210 (84.00)	200 (80.00)	197 (78.80)	105 (42.00)	109 (43.60)	155 (62.00)	0 (0.00)	0.0001 **
R (NWT) n (%)	250 (100.00)	50 (20.00)	40 (16.00)	50 (20.00)	53 (21.20)	145 (58.00)	141 (56.40)	95 (38.00)	250 (100.00)	0.0001 **
p value	0.0001 **	0.0001 **	0.0001 **	0.0001 **	0.0001 **	0.0031 **	0.0044 **	0.0001 **	0.0001 **	-

FLC: Fluconazole, ITR: Itraconazole, VRC: Voriconazole, ISA: Isavuconazole, POS: Posaconazole, WT: wild type, NWT: non-wild type
**p ≤ 0.01

Table 5.Susceptibility Profile of *Aspergillus* Isolates for Triazole Antifungals

	FLC	ITR	VRC	ISA	POS
Aspergillus flavus n (%)	145 (R) (100.00)	37 (NWT) (25.50)	27 (NWT) (18.60)	37 (R) (25.50)	40 (NWT) (27.60)
	0 (S) (0.00)	108 (WT) (74.50)	118 (WT) (81.40)	108 (S) (74.50)	105 (WT) (72.40)
Aspergillus fumigatus n (%)	75 (R) (100.00)	13 (NWT) (17.30)	13 (NWT) (17.30)	13 (NWT) (17.30)	13 (NWT) (17.30)
	0 (S) (0.00)	62 (WT) (82.70)	62 (WT) (82.70)	62 (WT) (82.70)	62 (WT) (82.70)
Aspergillus niger n (%)	20 (R) (100.00)	20 (WT) (100.00)	20 (WT) (100.00)	20 (WT) (100.00)	20 (WT) (100.00)
Aspergillus terreus n (%)	10 (R) (100.00)	10 (WT) (100.00)	10 (WT) (100%)	10 (WT) (100.00)	10 (WT) (100.00)
All isolates n (%)	R 250 (100.00)	NWT 50 (20.00)	NWT 40 (16.00)	NWT 50 (20.00)	NWT 53 (21.20)
	S 0 (0.00)	WT 200 (80.00)	WT 210 (84.00)	WT 200 (80.00)	WT 197 (78.80)

FLC: Fluconazole, ITR: Itraconazole, VRC: Voriconazole, ISA: Isavuconazole, POS: Posaconazole, CAS: Caspofungin, MFC: Micafungin, AMB: Amphotericin B, 5-FC: Fluorocytosine WT: wild type, NWT: non-wild type

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Table 6.Susceptibility Profile of *Aspergillus* Isolates for Echinocandins, Amphotericin B, and 5-fluorocytosine Antifungals

	CAS	MCF	AMB	5-FC
Aspergillus flavus n (%)	88 (NWT) (60.70)	93 (NWT) (64.10)	66 (NWT) (54.50)	145 (NWT) (100.00)
	57 (WT) (39.30)	52 (WT) (35.90)	79 (WT) (45.50)	
Aspergillus fumigatus n (%)	42 (NWT) (56.00)	42 (NWT) (56.00)	24 (NWT) (32.00)	75 (R) (100.00)
	33 (WT) (44.00)	33 (WT) (44.00)	51 (WT) (86.00)	
Aspergillus niger n (%)	5 (NWT) (25.00)	1 (NWT) (5.00)	5 (NWT) (25.00)	20 (NWT) (100.00)
	15 (WT) (75.00)	19 (WT) (95.00)	15 (WT) (75.00)	
Aspergillus terreus n (%)	10 (NWT) (100.00)	5 (NWT) (50.00)	10 (WT) (100.00)	10 (NWT) (100.00)
		5 (WT) (50.00)		
All isolates n (%)	NWT 145 (58.00)	NWT 141 (56.40)	NWT 95 (38.00)	NWT 250 (100.00)
	WT 105 (42.00)	WT 109 (43.60)	WT 155 (62.00)	WT 0 (0.00)

CAS: Caspofungin, MFC: Micafungin, AMB: Amphotericin B, 5-FC: Fluorocytosine , WT: wild type, NWT: non-wild type

Aspergillus fumigatus Susceptibility Profiling

In the current study, the susceptibility profiling of *A. fumigatus* to triazoles was as follows: for all tested triazoles except FLC (ITR, VRC, ISA, and POS), 13 (17.3%) isolates showed the NWT phenotype, while 62 (82.7%) showed the WT phenotype (ECV 1 µg/mL for ITR, VRC, and ISA and 0.5 µg/mL for POS). *A. fumigatus* susceptibility profiling of echinocandins showed that for CAS, 42 (56%) were NWT and 33 (44%) were WT (ECV 0.5 µg/mL); for MCF, 42 (56%) were NWT and 33 (44%) were WT (ECV 0.25 µg/mL). CLSI has not established a specific ECV for MCF against *A. fumigatus*; thus, the present study used a reasonable ECV suggested in a previous study.[26,27] For AMB, 24 (32%) were NWT, and 51 (86%) showed a WT phenotype (ECV 2 µg/mL).

Other *Aspergillus spp.* Susceptibility Profiling

The other isolated species included *Aspergillus niger* and *Aspergillus terreus*. For all tested triazoles except for FLC, all isolates (100%) showed the WT phenotype. The ECV values against *A. niger* were 4 µg/mL (ITR), 2 µg/mL (VRC), 4 µg/mL (ISA), and 2 µg/mL (POS). In contrast, *A. terreus* had ECV values of 2 µg/mL (ITR), 2 µg/mL (VRC), 1 µg/mL (ISA), and 1 µg/mL (POS). *A. niger* susceptibility profiling of echinocandins revealed that for CAS, only 5 (25%) isolates were NWT and 15 (75%) were WT (ECV 0.25 µg/mL), while for MCF, 1 (5%) was NWT and 19 (95%) were WT (ECV 0.25 µg/mL). The ECV value used was obtained from a previous study.[25] For AMB, only 5 (25%) isolates were NWT and 15 (75%) were WT (ECV 2 µg/mL). *A. terreus* susceptibility profiling of echinocandins revealed that for CAS, all isolates (100%) showed NWT phenotype (ECV 0.12 µg/mL), while for MCF, 50% of isolates were NWT and the other 50% were WT (ECV 2 µg/mL), depending on ECV determined from a previous study.[25] For AMB, all isolates showed the WT phenotype (ECV 4 µg/mL).

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Table 7.Antifungal Susceptibility Profile of *Aspergillus* Species Isolates

		No. of Isolates at Each Determined MIC Value (µg/mL)																	
	Drug	≤ 0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	MIC Range (µg/mL)	GM MIC (µg/L)
A. flavus	FLC	-	-	-	-	-	-	-	-	-	-	-	-	-	38	79	28	64.000–256.000	128.00
	ITR	-	-	-	-	19	89	-	*	9	14	-	-	14	-	-	-	0.120–32.000	1.50
	VRC	-	-	13	7	61	23	14	-	*	-	-	13	14	-	-	-	0.030–32.000	0.54
	ISA	-	11	5	51	23	18	-	*	-	-	-	23	14	-	-	-	0.015–32.000	0.32
	POS	-	-	21	33	46	5	*	9	-	-	3	14	14	-	-	-	0.030–32.000	0.82
	CAS	33	-	-	14	10	-	*	-	14	9	-	65	-	-	-	-	≤ 0.008–16.000	0.44
	MCF	28	-	-	14	46	5	*	-	-	-	5	47	-	-	-	-	≤ 0.008–16.000	0.35
	AMB	-	-	-	-	-	-	5	51	23	*	14	23	29	-	-	-	0.500–32.000	4.00
	5-FC	-	-	-	-	-	-	-	-	33	47	25	-	5	19	16	-	2.000–128.000	16.00
A. fumigatus	FLC	-	-	-	-	-	-	-	-	-	-	-	-	-	33	23	19	64.000–256.000	128.00
	ITR	-	9	10	-	5	38	-	*	-	-	-	-	13	-	-	-	0.015–32.000	0.21
	VRC	-	19	7	18	10	2	6	*	-	-	-	6	7	-	-	-	0.015–32.000	0.34
	ISA	-	25	-	27	5	5	-	*	-	5	-	-	8	-	-	-	0.015–32.000	0.57
	POS	-	23	-	33	6	-	*	-	2	-	1	6	4	-	-	-	0.015–32.000	0.98
	CAS	12	11	2	3	5	-	*	4	8	-	9	21	-	-	-	-	≤ 0.008–16.000	0.34
	MFC	22	-	7	-	4	*	-	-	-	3	11	28	-	-	-	-	≤ 0.008–16.000	0.49
	AMB	-	-	-	-	11	6	9	25	*3	3	9	9	-	-	-	-	0.120–16.000	1.40
	5-FC	-	-	-	-	-	-	-	-	8	3	12	18	19	15	-	-	2.000–64.000	11.31

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A.niger	FLC	-	-	-	-	-	-	-	-	-	-	-	-	-	14	3	3	64.000–256.000	128.00
	ITR	-	-	6	4	3	7	-	-	-	*	-	-	-	-	-	-	0.030–0.250	0.08
	VRC	-	-	-	3	12	5	-	-	*	-	-	-	-	-	-	-	0.060–0.250	0.12
	ISA	-	3	7	1	6	3	-	-	-	*	-	-	-	-	-	-	0.015–0.250	0.06
	POS	-	3	1	5	11	-	-	-	*	-	-	-	-	-	-	-	0.015–0.120	0.04
	CAS	3	5	7	-	-	*	3	1	1	-	-	-	-	-	-	-	≤ 0.008–0.030	0.12
	MFC	4	6	6	3	-	* 1	-	-	-	-	-	-	-	-	-	-	≤ 0.008–0.060	0.03
	AMB	-	-	-	-	1	1	2	11	-	*	-	3	2	-	-	-	0.120–1.000	1.40
	5-FC	-	-	-	-	-	-	-	-	-	-	7	13	-	-	-	-	8.000–16.000	11.30
A. terreus	FLC	-	-	-	-	-	-	-	-	-	-	-	-	-	3	4	3	64.000–256.000	128.00
	ITR	-	-	-	2	5	3	-	-	*	-	-	-	-	-	-	-	0.060–0.250	0.12
	VRC	-	-	1	1	1	7	-	-	*	-	-	-	-	-	-	-	0.030–0.250	0.08
	ISA	-	2	1	2	5	-	-	*	-	-	-	-	-	-	-	-	0.015–0.120	0.04
	POS	-	-	3	7	-	-	-	*	-	-	-	-	-	-	-	-	0.030–0.060	0.04
	CAS	-	-	4	1	*	-	-	-	-	-	-	5	-	-	-	-	0.030–16.000	0.30
	MFC	-	-	-	1	4	-	-	-	*	-	2	3	-	-	-	-	0.060–16.000	0.97
	AMB	-	-	-	-	-	6	2	2	-	*	-	-	-	-	-	-	0.250–1.000	0.50
	5-FC	-	-	-	-	-	-	-	-	-	-	-	-	-	2	8	-	46.000–128.000	90.50

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Discussion

A. flavus was the most common species of *Aspergillus* in the current study. This contrasts with many Western studies, which showed that *A. fumigatus* predominates, while *A. flavus* is the second most isolated species in Europe and the United States.[28,29] However, the present study aligns with regional trends in Asia and the Middle East, where *A. flavus* is more prevalent due to environmental and climatic conditions.[30,31]

The *in vitro* susceptibility profile of nine antifungals against four isolated *Aspergillus* species was evaluated in this work (Table 7) . The testing revealed that all the isolated species were completely resistant to FLC and 5-FC, with MICs of 64 µg/mL or higher for FLC and 2 µg/mL or higher for 5-FC. Since fluconazole has low efficacy against moulds like *Aspergillus* due to either fungal cell wall variations or the inability of fluconazole to attach to their target enzyme, *Aspergillus* is known to be inherently resistant to fluconazole.[32,33] Numerous studies have demonstrated that intrinsic resistance to 5-FC is universal. This resistance may be brought about by the downregulation of the *FCYB* gene, which codes for the enzyme that facilitates 5-FC absorption.[34] A study showed that the complexity of 5-FC resistance in *Aspergillus* species is highlighted by the fact that changes in tRNA can help *A. fumigatus* become resistant to 5-FC.[35]

Other triazole (ITR, VRC, ISA, and POS) resistance profiles were 20.0%, 16.0%, 20.0%, and 21.2%, respectively. *A. flavus* showed higher resistance to triazole than *A. fumigatus*; this may be attributed to environmental azole exposure or long-term prophylaxis in immunocompromised patients.[36]

For AMB, moderate resistance was observed (38%) overall, with *A. flavus* showing more than 45% resistance. This result aligns with an Iranian study, where a decreased resistance to the drug was observed.[37] Resistance to AMB is concerning due to its role as a second-line therapy, especially in azole-resistant cases. High resistance rates for echinocandins (CAS and MFC) were reported (56–58% overall). *A. terreus* showed 100% resistance to CAS. This challenges the use of echinocandins in monotherapy and supports combination therapy in resistance cases. The echinocandin resistance pattern in the current study is consistent with three regional studies from Iran and Vietnam.[38–40] *A. flavus* showed the broadest resistance spectrum, including notable resistance to echinocandins and AMB in addition to triazole. *A. fumigatus* retained better susceptibility to azole but still demonstrated emerging resistance. *A. niger* and *A. terreus* had relatively lower resistance to azoles, but showed variable responses to echinocandins. These findings align with those of Arastehfar et al.'s study.[41]

In this study, *Aspergillus fumigatus* showed a significant resistance to several common triazole antifungals (ITR, VRC, ISA, and POS), with 17.3% of samples showing the NWT phenotype for all of them. Even though the majority of isolates (82.7%) were still susceptible, the consistent NWT rate across all triazoles pointed to the establishment of multidrug resistance (MDR) in the strains of *A. fumigatus*. Triazole's cross-resistance is especially worrisome because both drugs , itraconazole and voriconazole, have a shared target, lanosterol 14-demethylase. MDR significantly reduces available treatments. When voriconazole, the first-line medication, proves ineffective, people turn to amphotericin B or echinocandins; considerable resistance to these was also exhibited in the current study.[42,43]

In the post-COVID-19 era, where IPA has become a major secondary illness (CAPA), the results of our study are very pertinent. The observed resistance patterns may be attributed to heightened vulnerability to *Aspergillus* infection caused by the immunosuppressive effects of corticosteroid medication, extended intensive care unit stays, and the use of mechanical ventilation during COVID-19. Furthermore, our isolates' high *A. flavus* prevalence and resistance to several antifungals could be the result of environmental exposure and increased selective pressure during the pandemic.[44,45]

Conclusion

The high resistance rates underscore the need for routine susceptibility testing to guide targeted therapy. Voriconazole remains a first-line treatment, but its effectiveness is threatened by rising resistance. Combination antifungal therapy should be considered for resistant or mixed-species infections. The species distribution should guide empirical therapy in local settings. Routine antifungal susceptibility testing should be implemented in clinical laboratories to guide effective species-specific treatment of IPA; empirical use of FLC and 5-FC should be avoided in suspected IPA cases. Triazole resistance also warrants cautious use and may necessitate alternative combination therapies. Expanded multicentre studies across different regions of Iraq and the Middle East are needed to validate these findings, assess resistance trends over time and inform national treatment protocols.

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