https://doi.org/10.18502/jad.v20i2.22401

Original Article

Drug Resistance Patterns of Fungi Isolated from Hospital-Associated Moth Flies (Diptera: Psychodidae) in Babol, Iran

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(Received 11 May 2026; accepted 16 June 2026)

Abstract

Background: The moth fly *Clogmia albipunctata* (Diptera: Psychodidae) has received little attention as a potential mechanical vector of drug-resistant pathogens in hospital settings. This study aimed to evaluate the antifungal susceptibility profile of fungal isolates recovered from moth flies collected in teaching hospitals of Babol, Iran.

Methods: Antifungal susceptibility profile evaluation was performed on 50 fungal isolates (including 36 filamentous fungi and 14 yeasts) collected from moth flies in teaching hospitals of Babol city, using the broth microdilution method in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The concentrations of antifungal drugs ranged from 0.032 to 64 µg/mL. Geometric mean (G-mean), $MIC_{50,}$ and MIC_{90} values were calculated for statistical analysis.

Results: Luliconazole and posaconazole exhibited the lowest MIC values against most *Aspergillus* isolates. Similarly, posaconazole and voriconazole displayed potent antifungal activity against *Candida albicans* isolates, while up to 90% of *C. albicans* exhibited high-level fluconazole resistance (MIC ≥ 8 µg/mL).

Conclusion: Hospital-associated moth flies were found to carry pathogenic fungi with unusual antifungal resistance patterns. These findings highlight that such insects may serve as potential mechanical carriers of drug-resistant fungi. Nonetheless, monitoring synanthropic insects could be important as a complementary measure in hospital infection control programs.

Keywords: Moth fly; Pathogenic fungi; Antifungal susceptibility; Hospital-acquired infection; *Candida albicans*

Introduction

Some arthropods, particularly insects, act as mechanical vectors of pathogens and pose a potential threat to healthcare settings worldwide (1). By carrying infectious agents on their exoskeletons or in their digestive tracts, these insects can contribute to the spread of healthcare-associated infections (HAIs) (2). Synanthropic insects such as cockroaches, house flies, and non-biting moth flies *Clogmia albipunctata* (Diptera: Psychodidae), known as drain flies, are of particular concern among hospital pests. House flies have been found to harbour over 100 human pathogens, and moth flies have recently received increased attention due to their persistent presence in moist, organically enriched hospital environments such as restrooms, patient washrooms and kitchens (3–6).

Concurrently, the incidence of hospital-acquired fungal infections has shown an upward trend, which is a matter of concern. According to data reported to the Centers for Disease Control and Prevention (CDC), the prevalence has risen from 2.0 to 3.8 cases per 1,000 discharged

https://doi.org/10.18502/jad.v20i2.22401

patients (7, 8). Multiple studies demonstrate that synanthropic insects can mechanically transmit clinically significant fungi, including *Aspergillus* spp., *Candida* spp., Mucorales and *Rhodotorula* spp., thereby increasing the risk of opportunistic infections in immunocompromised or hospitalized patients (7). Furthermore, despite advances in antifungal pharmacotherapy, treatment options remain limited by narrow spectra, toxicity, complex pharmacokinetics and the emerging threat of antifungal resistance (9). Fungal resistance is influenced by pathogen species, drug class and host factors, often complicating clinical outcome predictions (10). These challenges underscore the necessity of continuous surveillance of antifungal susceptibility patterns in clinical settings and the control of potential environmental reservoirs of resistant strains.

Considering previous studies on the potential role of moth flies as carriers of pathogenic microorganisms in healthcare facilities (11–13) and given the lack of regional data on the antifungal susceptibility profile of fungal isolates from these insects, the present study was designed to evaluate the antifungal susceptibility of fungal strains recovered from *C. albipunctata* collected from teaching hospitals in Babol.

Materials and Methods

Sample collection and fungal culture

The fungal isolates used in this study included 50 fungal isolates, including filamentous fungi and yeasts from moth flies (*C. albipunctata*) that were collected in a previous study from teaching hospitals in Babol City (Shahid Beheshti Hospital, Shahid Yahya Nejad Hospital and Ayatollah Rouhani Hospital) (11). All isolates had been previously identified using morphological features and were stored at -20 °C. For reactivation, the samples were cultured on Sabouraud Dextrose Agar (SDA) medium containing chloramphenicol and were incubated at 25 °C and 37 °C for 48–72 hours. The fungal colonies were identified by macroscopic and microscopic criteria (Fig. 1).

Preparation of culture media and fungal suspension

Liquid RPMI-1640 medium containing L-glutamine but lacking sodium bicarbonate was supplemented with 0.165 M MOPS buffer solution adjusted to pH 7.0 and sterilized by filtration through a 0.22 µm membrane filter. The prepared medium could be stored at 4 °C for up to one month.

To prepare the yeast suspension, 48-hour *Candida albicans* colonies were harvested from SDA plates. After allowing any large particles to settle, the initial suspension was adjusted spectrophotometrically at 530 nm to achieve a percent transmittance of 75–77%, which corresponds to a final concentration of 1-5 × 10^6 cells/mL. This suspension was then diluted 1:1000 with RPMI-1640 medium to obtain the working inoculum.

For filamentous fungi (primarily *Aspergillus* spp.), suspensions were prepared from 7-day-old colonies grown on potato dextrose agar (PDA). After sedimentation of large debris, the supernatant was vortexed to ensure homogeneity and the turbidity adjusted spectrophotometrically to ≤ 82% transmittance at 530 nm. The suspension was then diluted 1:50 with RPMI-1640 medium to achieve the required final inoculum concentration for susceptibility testing.

Antifungal susceptibility testing

Antifungal susceptibility testing for yeasts and filamentous fungi was performed using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines M38-A2 and M27-A2, respectively (14, 15). All assays were conducted in sterile 96-well microdilution plates.

The antifungal agents tested included fluconazole, itraconazole, voriconazole, posaconazole, luliconazole, amphotericin B and anidulafungin, all purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions were pre-

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Published Online: June 30, 2026

https://doi.org/10.18502/jad.v20i2.22401

pared in dimethyl sulfoxide (DMSO) and stored at -80 °C until use. On the day of testing, stock solutions were diluted with RPMI-1640 medium to prepare two-fold serial dilutions ranging from 0.032 to 64 µg/mL.

In each well, 100 µL of fungal suspension at twice the final desired concentration was added to 100 µL of antifungal dilution, yielding a final volume of 200 µL per well with the target concentrations of both drug and inoculum. The twelfth well of each row served as a drug-free growth control.

Incubation and endpoint determination

Microdilution plates containing yeast isolates were incubated at 35 °C for 24 hours, whereas plates containing filamentous fungi were incubated for 48–72 hours under identical conditions. In cases of insufficient growth in positive control wells, incubation was extended up to a maximum of 7 days.

The Minimum Inhibitory Concentration (MIC) results were determined after 24–72 hours of incubation at 35 °C as the lowest drug concentration that caused complete inhibition of growth (100% for amphotericin B) or a ≥ 50% reduction in growth for azoles and echinocandins, compared to the control well.

All endpoints were read visually with the aid of a convex mirror under standardized lighting conditions. The experiments were performed in duplicate on two independent occasions, and the MIC results were read independently by two trained investigators. The *C. parapsilosis* ATCC 22019 type strain was used for quality control of antifungal susceptibility.

Quantitative susceptibility data were analyzed by calculating MIC_{50}, MIC_{90} and geometric mean (G-Mean) values. All statistical analyses were performed using SPSS software version 16 (IBM Corp., Armonk, NY, USA) and Microsoft Excel 2010.

Results

In this study, the antifungal susceptibility profiles of 50 fungal isolates recovered from moth flies in a hospital setting were evaluated. The isolates comprised 36 filamentous fungi and 14 yeasts. The filamentous fungi included *Aspergillus niger* (n=17), *A. flavus* (n=7), *A. fumigatus* (n=4) and *Aspergillus* spp. (n=8). All yeast isolates were identified as *C. albicans* (n=14).

Antifungal susceptibility testing was performed using the broth microdilution method according to CLSI guidelines M38-A2 (for filamentous fungi) and M27-A2 (for yeasts). Minimum Inhibitory Concentration (MIC) values were determined for seven antifungal agents: posaconazole, luliconazole, voriconazole, itraconazole, fluconazole, amphotericin B and anidulafungin.

Susceptibility patterns of filamentous fungi

Among filamentous fungi, luliconazole and posaconazole exhibited the lowest MIC values and demonstrated the highest antifungal activity. For *A. flavus* isolates, luliconazole was identified as the most effective compound, with MIC_{50} and MIC_{90} values of 0.032 µg/mL. Amphotericin B displayed a broad MIC range (0.5–32 µg/mL), indicating considerable variability in isolate responses. The results of this study showed that luliconazole had excellent *in vitro* activity against *A. niger* with a lower MIC value than the triazoles tested. Posaconazole and voriconazole also exhibited favorable activity, whereas itraconazole showed reduced susceptibility in several isolates, with MIC values reaching 16 µg/mL. Amphotericin B demonstrated comparatively better activity against *A. niger* than against *A. flavus* (Table 1) (Fig. 2).

Due to the small sample size in some groups (*A. fumigatus* n=4), MIC_{50}/MIC_{90} evaluations and comparative statistical analyses could not be performed for these species. Luliconazole exhibited the highest antifungal activity, with a consistent MIC of 0.032 µg/mL across all isolates, followed by posaconazole (0.125–0.25 µg/mL). Voriconazole and itraconazole also

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Published Online: June 30, 2026

https://doi.org/10.18502/jad.v20i2.22401

showed good activity with MIC ranges of 0.125–0.5 and 0.063–0.5 µg/mL, respectively.

Susceptibility patterns of *Candida albicans* isolates

Among *C. albicans* isolates, posaconazole and voriconazole demonstrated the highest antifungal efficacy, with all isolates falling within the susceptible range. Itraconazole also exhibited good activity; however, the presence of one isolate with an MIC of 4 µg/mL suggests reduced susceptibility or acquired resistance. Amphotericin B showed potent and consistent activity with a narrow MIC range. Notably, anidulafungin displayed elevated MIC values in several isolates, indicating variable or reduced responsiveness to this echinocandin-class agent (Table 2) (Fig. 2).

According to the Clinical Laboratory Standards Institute (CLSI) guidelines, fluconazole resistance (R) in *C. albicans* is defined by a minimum inhibitory concentration MIC ≥ 8 µg/ml. In this study, 90% of *C. albicans* isolates exhibited MIC ≥ 8 µg/mL to fluconazole.

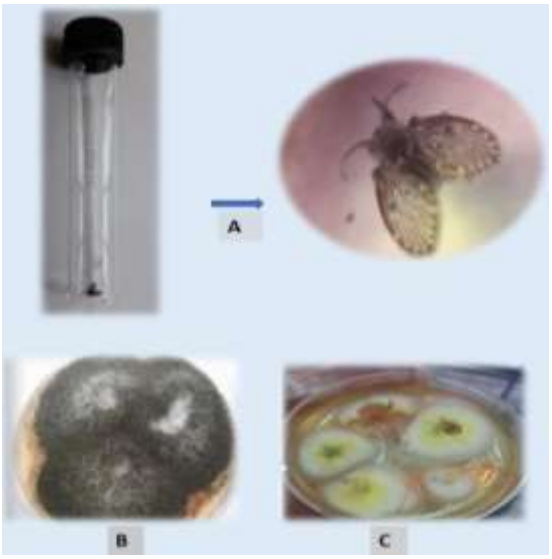

Fig. 1. Adult moth fly (*Clogmia albipunctata*) collected from hospital environment, A). Macroscopic colony morphology of *Aspergillus niger*, B) and *Aspergillus flavus*, C) on Sabouraud dextrose agar

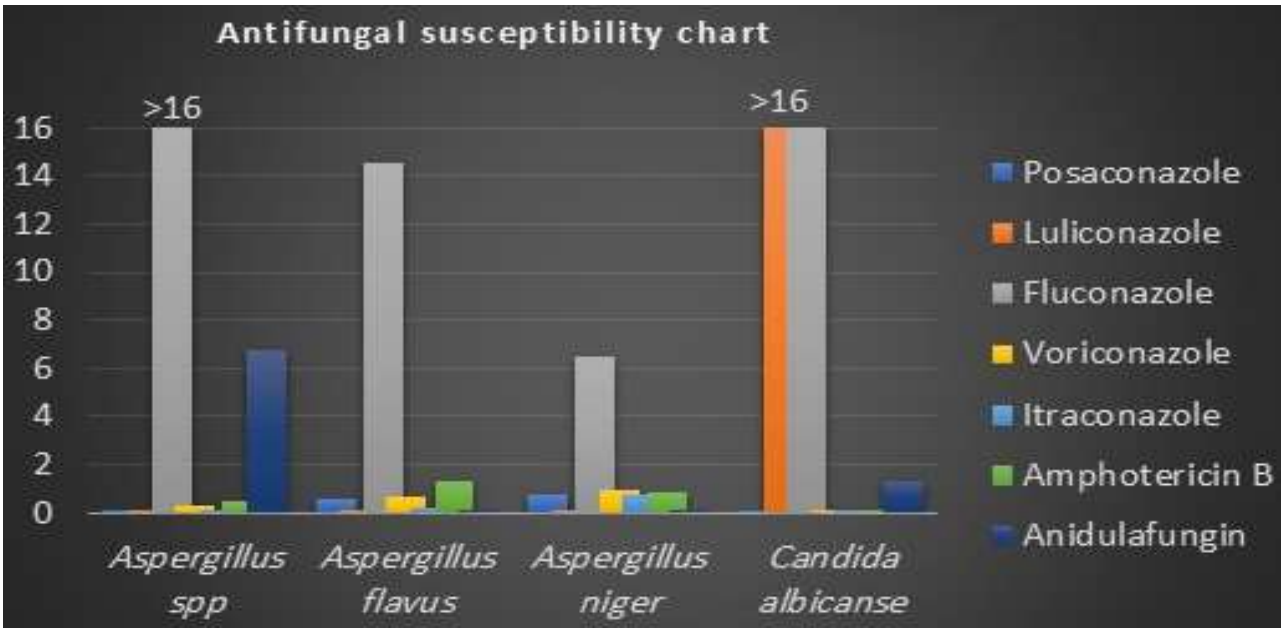

Fig. 2. Antifungal susceptibility chart of *Aspergillus* species (n=36) and *Candida albicans* (n=14) isolated from hospital-associated moth flies (*Clogmia albipunctata*). Bars represent geometric mean MIC values (µg/mL) for each antifungal agent tested against

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Published Online: June 30, 2026

https://doi.org/10.18502/jad.v20i2.22401

Table 1. Descriptive statistics of antifungal susceptibility data for *Aspergillus* isolates recovered from moth flies (*Clogmia albipunctata*)

Isolate	Antifungal agents	MIC (µg/mL)					
		MIC_{50}	MIC_{90}	MIN	MAX	MODE	G-Mean
Aspergillus flavus	Posaconazole	0.5	1	0.25	1	1	0.552
	Luliconazole	0.032	0.032	0.032	0.032	0.032	0.032
	Fluconazole	8	64	4	64	64	14.491
	Voriconazole	1	1	0.25	1	1	0.672
	Itraconazole	0.125	0.5	0.063	0.5	0.5	0.186
	Amphotericin B	0.5	1	0.5	32	0.5	1.345
	Anidulafungin	0.016	0.125	0.016	16	0.016	0.097
Aspergillus niger	Posaconazole	1	1	0.125	2	1	0.721
	Luliconazole	0.032	0.032	0.032	0.032	0.032	0.032
	Fluconazole	4	64	1	64	0.032	6.524
	Voriconazole	1	1	0.5	2	1	0.921
	Itraconazole	0.5	16	0.125	16	1	0.782
	Amphotericin B	0.5	8	0.5	8	0.5	0.815
	Anidulafungin	0.016	0.059	0.016	16	0.016	0.027
***Aspergillus* spp.**	Posaconazole	0.125	0.125	0.032	0.125	0.125	0.074
	Luliconazole	0.125	0.125	0.032	0.125	0.125	0.074
	Fluconazole	64	64	16	64	64	53.817
	Voriconazole	0.5	0.5	0.063	0.5	0.5	0.272
	Itraconazole	0.125	0.237	0.125	0.5	0.125	0.148
	Amphotericin B	1	1	0.125	1	1	0.500
	Anidulafungin	0.016	16	0.016	16	0.016	0.090

Table 2. Descriptive statistical analysis of antifungal susceptibility profiles of *Candida albicans* recovered from moth flies (*Clogmia albipunctata*)

Isolate	Antifungal agents	MIC (µg/mL)					
		MIC50	MIC90	MIN	MAX	MODE	G-Mean
Candida albicans	Posaconazole	0.125	0.125	0.032	0.125	0.125	0.076
	Luliconazole	1	2	1	2	1	1.280
	Fluconazole	64	64	2	64	64	49.965
	Voriconazole	0.063	0.063	0.063	0.125	0.063	0.069
	Itraconazole	0.063	0.125	0.063	4	0.063	0.093
	Amphotericin B	0.125	0.125	0.125	0.125	0.125	0.125
	Anidulafungin	1	1	1	16	1	1.280

Discussion

This study investigated the antifungal susceptibility profiles of pathogenic fungi isolated from moth flies in a hospital setting. The findings demonstrate that these insects are not merely carriers of infectious agents but can also serve as mechanical vectors for fungal isolates that exhibit unusual patterns of drug resistance. The emergence and spread of drug-resistant fungi, particularly among immunocompromised patients, has recently become a serious public health threat. The rising prevalence of invasive fungal infections, alongside resistance to one or more classes of antifungal agents, poses significant therapeutic challenges and increases mortality rates in high-risk groups. Therefore, identifying the sources and mechanical carriage of these pathogens, particularly in sensitive and crowded hospital en-

https://doi.org/10.18502/jad.v20i2.22401

vironments, is of paramount importance (16, 17).

Clogmia albipunctata is recognized as a common insect in urban and sanitary environments, thriving in plumbing systems, moist showers and biofilm-rich areas. Although it does not directly contact human feces, it can mechanically transfer various pathogens, including bacteria, fungi and protozoa, from contaminated surfaces to indoor environments and human contact points, positioning it as a potential mechanical vector of healthcare-associated infections (13).

In the present study, the drug susceptibility pattern of fungal isolates obtained from moth flies, including clinically important species such as *A. flavus*, *A. niger* and *C. albicans*, was investigated.

While most studies have focused on bacterial carriage by houseflies and cockroaches, the potential of *C. albipunctata* to transmit pathogenic fungi remains underexplored (1, 11). A recent study from Brazil by Vieira-Alcântara et al. (18) isolated the emerging pathogenic yeast *Trichosporon asahii* from *C. albipunctata* collected in a tertiary hospital. Our study complements this finding by demonstrating that Iranian moth flies harbor not only *C. Albicans* but also filamentous fungi such as *Aspergillus* species, all of which exhibit concerning antifungal resistance patterns. Furthermore, Rupprecht et al. (19) in Germany documented that 41.1% of moth flies carried MDR bacteria and confirmed the presence of *C. albicans* biofilms in hospital moth pipes, suggesting that these insects acquire microorganisms directly from wastewater biofilms. In the Iranian context, Mirhasani et al. (20) recently isolated *Fusarium* species from hospital water systems in Tehran and reported non-wild-type susceptibility to itraconazole and caspofungin, further confirming that hospital wastewater environments serve as reservoirs for antifungal-resistant fungi. Collectively, these recent studies underscore the urgent need to include moth fly surveillance as a component of comprehensive hospital infection control programs.

In the current study, susceptibility testing revealed that luliconazole and posaconazole exhibited the lowest MIC values against most species, indicating strong antifungal activity against filamentous fungi. Notably, luliconazole demonstrated the highest efficacy against *A. flavus*, with a geometric mean (G-Mean) and MIC of 0.032 µg/mL.

The current study is aligned with the results of Zabihzadeh et al. (12), who investigated the drug susceptibility of fungi isolated from hospital ants and highlighted the potential of luliconazole as a next-generation triazole for treating aspergillosis, particularly in regions with rising resistance to conventional antifungals. Regarding other azoles, voriconazole and itraconazole also showed potent activity, consistent with the findings of Akbari et al. (21), who reported universal susceptibility of *A. flavus* isolates to these agents. However, their report of 14.7% resistance to amphotericin B (MIC > 2 µg/mL) is concerning. In the current study, the broad MIC range of amphotericin B (0.5–32 µg/mL) further underscores heterogeneity in isolate responses and suggests the potential presence of resistant strains.

Regarding yeast isolates, all *C. albicans* strains were susceptible to posaconazole, voriconazole and itraconazole, whereas fluconazole exhibited low efficacy. Notably, 90% of *C. albicans* isolates exhibited MIC ≥ 8 µg/mL to fluconazole, an alarmingly high rate. These results may be related to the origin of the yeast isolates. In this study, the yeasts that entered the current study were isolated from the outside surfaces of moth flies. It seems that the heavy use of disinfectants in hospitals, particularly in bathrooms, where these flies proliferate, might be one reason why this resistance has developed.

In contrast to our findings, Mloka et al. (22) reported only a 16.3% fluconazole resistance rate among *Candida* isolates. Conversely, Hussein et al. (23) in the United States found high fluconazole efficacy, contrasting with our results. This discrepancy may reflect the growing prevalence of fluconazole resistance in

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Published Online: June 30, 2026

https://doi.org/10.18502/jad.v20i2.22401

Iranian hospital settings, potentially driven by widespread or inappropriate use of the drug in recent years.

Furthermore, comparison with the study by Rezaei et al. (24) in Arak, which reported limited resistance to itraconazole and ketoconazole among *Aspergillus* species, indicates that antifungal resistance patterns in Iran are evolving and warrant continuous surveillance. In contrast, Álvarez-Pérez et al. (25) in Spain reported no resistance among yeast isolates recovered from animals and insects, which may reflect differences in antifungal selection pressure or infection control policies across regions. In this study, high MIC values for the echinocandin were observed in several *Aspergillus* isolates. Resistance to echinocandins in *Aspergillus* species, although rare, has been reported in a previous study. This could be due to natural mutations in the FKS target gene or intraspecific variation. However, since there is no known clinical breakpoint for anidulafungin against *Aspergillus*, these results were reported as high MIC values and not resistance (26).

Conclusion

The findings of this study confirm that moth flies not only harbor clinically relevant pathogenic fungi but may act as potential reservoirs or mechanical carriers of drug-resistant strains within hospital environments. This poses an increased risk of difficult-to-treat infections, particularly among immunocompromised patients. Luliconazole and posaconazole emerge as promising therapeutic options against resistant isolates and warrant greater clinical attention. Conversely, the declining efficacy of fluconazole and the heterogeneous response to amphotericin B highlight the importance of conducting routine antifungal susceptibility testing before initiating empirical therapy. Moreover, this study serves as a critical warning regarding the underappreciated role of overlooked vectors, such as moth flies, in the transmission cycle of drug-resistant pathogens in sensitive healthcare settings. Therefore, effective vector control, regular maintenance of plumbing systems, and ongoing surveillance of antifungal resistance patterns in insect-borne pathogens should be integrated into comprehensive hospital infection control programs.

Acknowledgements

This study was supported and conducted as part of a research project (Tracking Code: 724135787, Babol University of Medical Sciences. The authors sincerely thank the Vice Chancellor for Research at Babol University of Medical Sciences for their financial support and collaboration.

Ethical considerations

The study protocol was approved by the Ethics Committee in Biomedical Research at Babol University of Medical Sciences (Ethics Code: IR.MUBABOL.HRI.REC.1403.373).

Conflict of interest statement

The authors declare that there is no conflict of interest.

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