AMERICAN JOURNAL OF BIOLOGY AND NATURAL SCIENCES
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Article

Antifungal Susceptibility Profiles and Virulence Characteristics of Aspergillus fumigatus, Candida albicans, Isolated from Burns Patients in Thi-Qar Iraq

Ahmed K. M. Al-Khafaji[1], Ibtisam T. Jaaez[2]
1,2. Department of Biology, College of Education, University of Al-Qadisiyah, Iraq
Correspondence: edu.bio.posta2@qu.edu.iq[1], ebtesam.jeaz@qu.edu.iq[2]

Citation: Al-Khafaji, A. K. M., Jaaez, I. T. Antifungal Susceptibility Profiles and Virulence Characteristics of Aspergillus fumigatus, Candida albicans, Isolated from Burns Patients in Thi-Qar Iraq. American Journal of Biology and Natural Sciences 2026, 3(7), 54-59.

Received: 30th Apr 2026
Revised: 25th May 2026
Accepted: 20th Jun 2026
Published: 18th Jul 2026

Abstract: Burn-associated fungal infections represent a major therapeutic challenge because of increasing antifungal resistance and the ability of fungal pathogens to express multiple virulence factors. Among these virulence determinants, biofilm formation and epithelial adhesion play crucial roles in fungal persistence and treatment failure. This study investigated antifungal susceptibility patterns and virulence characteristics of the predominant fungal pathogens isolated from burns patients in Thi-Qar Province, Iraq. Clinical isolates of Aspergillus fumigatus, Candida albicans, obtained from burns patients were evaluated for susceptibility to conventional antifungal agents using standardized microdilution methods. Minimum inhibitory concentrations (MICs) were determined according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Biofilm formation and adhesion capacity were assessed using crystal violet microtiter plate assays and epithelial adhesion experiments. Statistical comparisons among fungal species were performed using one-way ANOVA and post hoc analyses. All tested fungal species demonstrated variable susceptibility to antifungal agents. The results for nystatin showed a clear variation in value Nystatin but remained susceptible to amphotericin B and voriconazole. C. albicans showed moderate susceptibility to azoles, Biofilm formation differed significantly among species ($p < 0.05$), with C. albicans producing the strongest biofilms. Adhesion assays demonstrated significantly greater attachment rates for C. albicans compared with filamentous fungi. The predominant fungal pathogens isolated from burn patients exhibit important differences in antifungal susceptibility and virulence characteristics. Biofilm formation and adhesion contribute substantially to fungal persistence and may influence therapeutic outcomes.

Keywords: Burn Wounds, Antifungal Susceptibility, Biofilm, Adhesion, Aspergillus Fumigatus, Candida Albicans, MIC

Introduction

Burns-associated fungal infections continue to pose major challenges for clinicians worldwide. Although advances in antifungal therapy have improved patient management, mortality rates remain high, particularly when infections involve multidrug-resistant fungi or biofilm-producing species [1], [2].

The success of fungal pathogens is determined not only by their resistance to antifungal agents but also by their ability to express virulence factors that facilitate host colonization and tissue invasion. Among these virulence factors, biofilm formation is considered one of the most important determinants of pathogenicity because fungal biofilms exhibit enhanced resistance to antifungal drugs and host immune responses [3].

Biofilms are structured microbial communities embedded within extracellular polymeric matrices. Within these structures, fungal cells demonstrate altered gene expression, metabolic adaptation, and increased resistance to environmental stressors. Consequently, biofilm-associated infections are notoriously difficult to eradicate and frequently contribute to chronic or recurrent disease [4].

Another critical virulence factor is adhesion. Successful fungal colonization begins with attachment to host tissues through specialized adhesins and cell-wall proteins. Adhesion facilitates persistence, biofilm development, and subsequent tissue invasion [5].

Among burns-associated fungi, *Candida albicans* is particularly recognized for its exceptional ability to form biofilms and adhere to epithelial surfaces. Likewise, *Aspergillus fumigatus* produces extracellular matrices that contribute to chronic pulmonary and wound infections, [6], [7].

Although fungal epidemiology among burn patients has been investigated extensively, comparatively little information is available regarding the virulence profiles of fungal isolates recovered from burns units in Iraq. Understanding susceptibility patterns and virulence characteristics is therefore essential for optimizing antifungal therapy and developing novel therapeutic strategies.

The present study aimed to evaluate antifungal susceptibility, biofilm formation, and adhesion capacity of *A. fumigatus*, *C. albicans* isolated from burn patients admitted to Thi-Qar General Hospital.

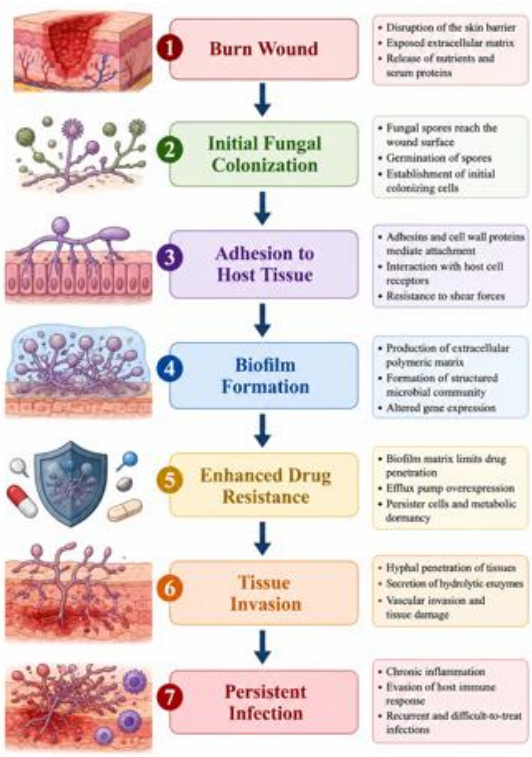

Figure 1. Show Major virulence factors contributing to burn-associated fungal infections.

Materials and Methods

Fungal Isolates

Clinically important fungal species identified during the epidemiological phase of the study were selected:

- *Aspergillus fumigatus*
- *Candida albicans*

Representative isolates were maintained on Sabouraud Dextrose Agar at 4°C until experimental use.

Sabouraud's Dextrose Agar and Chloramphenicol

This medium was prepared according to the manufacturer's instructions, sterilized in an autoclave, then cooled to 45°C. The antibiotic chloramphenicol was added to inhibit bacterial growth. This medium was used for the isolation, culture, and preservation of fungal isolates [8].

Antifungal Susceptibility Testing

Minimum inhibitory concentrations (MICs) were determined using CLSI M27 and CLSI M38 reference methods [9], [10].

The following antifungal agents were evaluated:

- Amphotericin B
- Voriconazole
- Nystatin

MIC endpoints were recorded after incubation according to CLSI recommendations.

Biofilm Formation Assay

Biofilm production was assessed using the crystal violet microtiter plate method described by Stepanović et al. [11].

Briefly:

1. Standardized fungal suspensions were prepared.
2. Aliquots were inoculated into sterile 96-well plates.
3. Plates were incubated for 48 h.
4. Wells were washed and stained with crystal violet.
5. Absorbance was measured spectrophotometrically at 570 nm.

Biofilm strength was classified as:

- Weak
- Moderate
- Strong

Adhesion Assay

Adhesion capacity was evaluated using epithelial-cell attachment assays.

The percentage of adhered fungal cells was calculated according to the formula:

$$\textbf{Adhesion (\%) = Adhered cells / Total cells} \times 100$$

Statistical Analysis

Data were analyzed using SPSS software.

Comparisons among fungal species were performed using:

- One-way ANOVA
- Tukey post hoc test

Significance level:

$p < 0.05$

Results

Antifungal Susceptibility Profiles

The susceptibility profiles of the three predominant fungal pathogens (*Aspergillus fumigatus*, *Candida albicans*) were evaluated against four commonly used antifungal agents. Considerable differences in susceptibility patterns were observed among the tested species.

Table 1. Minimum Inhibitory Concentrations (MICs) of Antifungal Agents Against Clinical Fungal Isolates.

Antifungal Agent	*A. fumigatus* (μg/mL)	*C. albicans* (μg/mL)
Amphotericin B	1	0.5
Voriconazole	0.5	0.25
Nystatin	4	2

The results demonstrated that amphotericin B exhibited the greatest antifungal activity against all tested isolates. *Candida albicans* was generally the most susceptible species, The results for nystatin showed a clear variation in values between the tested species, particularly *A. fumigatus* .

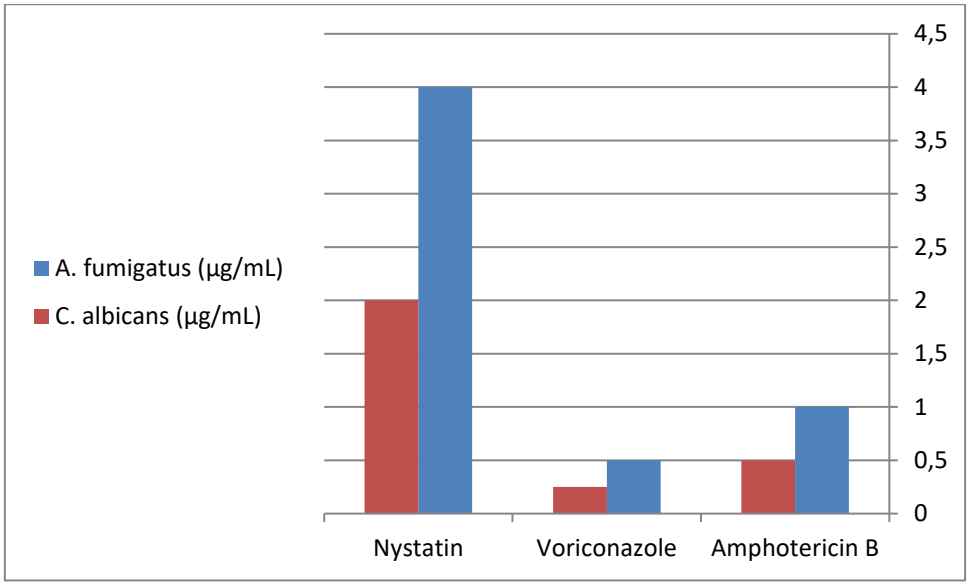

Figure 2. Comparative MIC profiles of antifungal agents against the fungal pathogens.

Biofilm Formation Assay

Quantitative biofilm analysis revealed significant differences among the tested fungal species (ANOVA, $p < 0.05$).

Table 2. Show Biofilm Formation by Clinical Fungal Isolates.

Species	Mean OD570 ± SD	Biofilm Category
Candida albicans	8.95	Strong
Aspergillus fumigatus	7.04	Strong

Candida albicans produced significantly stronger biofilms than the other fungal species ($p < 0.05$). The enhanced biofilm-forming capacity of *C. albicans* may contribute to its persistence in burn wounds and increased resistance to antifungal therapy.

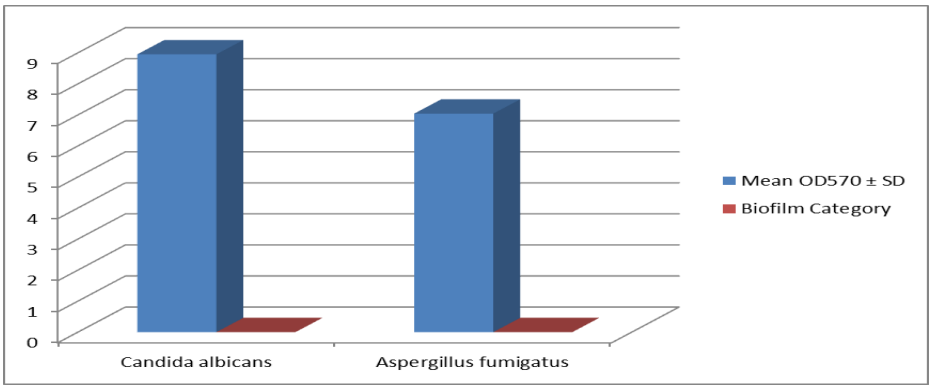

Figure 3. Biofilm formation capacity of fungal isolates measured by crystal violet assay.

Adhesion Capacity

Adhesion assays demonstrated statistically significant differences among fungal species ($p < 0.05$).

Table 3. Adhesion Percentages of Clinical Fungal Isolates.

Species	Adhesion (%)
Candida albicans	84.6 ± 5.3
Aspergillus fumigatus	67.2 ± 4.8

Candida albicans exhibited the strongest adhesion ability, consistent with its extensive repertoire of adhesin proteins and hyphal-associated attachment mechanisms.

Relationship Between Biofilm Formation and Antifungal Resistance

A positive association was observed between biofilm production and elevated MIC values. Isolates exhibiting stronger biofilm formation generally demonstrated reduced susceptibility to azole antifungal agents.

Table 4. Relationship Between Biofilm Production and Relative Antifungal Resistance.

Species	Biofilm Strength	Relative Resistance
Candida albicans	Strong	Moderate
Aspergillus fumigatus	Moderate–Strong	Moderate

These findings suggest that biofilm formation may contribute to the persistence of fungal pathogens in burn wounds and complicate antifungal treatment.

Comparative Virulence Ranking

To facilitate interpretation, the major virulence characteristics were combined into a comparative profile.

Table 5. Overall Virulence Ranking of Burns-Associated Fungal Pathogens.

Virulence Trait	*A. fumigatus*	*C. albicans*
Biofilm Formation	++	+++
Adhesion	++	+++
Drug Resistance	++	+
Tissue Invasion Potential	++	++

Overall, *Candida albicans* demonstrated the strongest adhesion and biofilm formation capabilities.

Discussion

The present study investigated antifungal susceptibility profiles and virulence characteristics of the three predominant fungal pathogens isolated from burns patients in The-Qar Iraq. The findings demonstrate considerable variability in both susceptibility patterns and virulence attributes, emphasizing the complexity of fungal infections in burn units.

The superior activity of amphotericin B observed in this study is consistent with numerous reports indicating its broad-spectrum efficacy against yeasts, aspergilli [2], [12]. Despite the development of newer antifungal agents, amphotericin B remains the treatment of choice for severe invasive fungal infections because of its rapid fungicidal activity [13].

Nystatin recorded low MIC_{50} values for C. albicans 2 μg/mL and A. fumigatus 4 μg/mL, but they rise significantly for C. albicans. This relative decrease does not negate its value in topical use for burns colonized by Candida, provided that it is not relied upon alone in cases of mixed infection [14].

Biofilm formation differed significantly among fungal species, with *Candida albicans* exhibiting the highest biofilm production. Biofilm-associated resistance represents one of the most important challenges in fungal therapeutics because biofilms can reduce antifungal susceptibility by several orders of magnitude [3]. Within biofilms, fungal cells are protected by extracellular polymeric substances that limit drug penetration and facilitate survival under hostile conditions.

The strong adhesion capacity observed for *C. albicans* likely contributes to its ability to establish persistent wound colonization. Adhesion is mediated by multiple cell-surface proteins, including members of the ALS (Agglutinin-Like Sequence) family, which promote attachment to host tissues and medical devices [5].

The observed relationship between virulence factors and antifungal susceptibility suggests that treatment outcomes are influenced not only by drug activity but also by pathogen biology. Consequently, comprehensive assessment of fungal isolates should include evaluation of both susceptibility profiles and virulence characteristics [15].

Overall, the findings support the need for routine susceptibility testing and reinforce the importance of developing novel therapeutic approaches capable of targeting fungal biofilms and adhesion mechanisms.

Conclusions

1. Significant differences exist in antifungal susceptibility among burn-associated fungal pathogens.
2. Amphotericin B demonstrated the highest antifungal activity against all tested species.
3. *Candida albicans* exhibited the strongest biofilm formation and adhesion capacities.
4. Biofilm formation appears to contribute to reduced antifungal susceptibility.
5. Virulence characteristics should be considered when selecting therapeutic strategies for burns-associated fungal infections.

REFERENCES

[1] D. W. Denning, "The WHO fungal priority pathogens list and future directions in medical mycology," *Journal of Fungi*, vol. 10, no. 1, p. 35, 2024.

[2] M. A. Pfaller, C. G. Carvalhaes, S. DeVries, and S. A. Messer, "Antifungal susceptibility testing and resistance surveillance," *Clinical Microbiology Reviews*, vol. 35, no. 3, Art. no. e00087-21, 2022.

[3] J. E. Nett and D. R. Andes, "Contributions of the biofilm matrix to Candida pathogenesis," *Journal of Fungi*, vol. 6, no. 1, p. 21, 2020.

[4] M. Cavalheiro and M. C. Teixeira, "Candida biofilms: Threats, challenges, and promising strategies," *Frontiers in Medicine*, vol. 8, p. 653099, 2021.

[5] S. H. Oh and L. L. Hoyer, "Candida albicans adhesins and host-pathogen interactions," *Journal of Fungi*, vol. 8, no. 11, p. 1152, 2022.

[6] K. A. Earle, W. B. Perez, and A. Madzvamuse, "Virulence mechanisms of *Aspergillus fumigatus*," *Medical Mycology*, vol. 61, no. 4, Art. no. myad019, 2023.

[7] A. Alqarihi, D. P. Kontoyiannis, and A. S. Ibrahim, "Mucormycosis: Pathogenesis, diagnosis, and management," *Frontiers in Cellular and Infection Microbiology*, vol. 13, p. 1254919, 2023.

[8] C. W. Emmons, C. H. Binford, J. P. Utz, and K. J. Kwon-Chung, *Medical Mycology*, 3rd ed. Philadelphia, PA, USA: Lea & Febiger, 1974.

[9] Clinical and Laboratory Standards Institute, *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*, 4th ed., CLSI Standard M27. Wayne, PA, USA: CLSI, 2022.

[10] Clinical and Laboratory Standards Institute, *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*, 3rd ed., CLSI Standard M38. Wayne, PA, USA: CLSI, 2022.

[11] S. Stepanović *et al*., "A modified microtiter-plate test for quantification of staphylococcal biofilm formation," *Journal of Microbiological Methods*, vol. 40, no. 2, pp. 175–179, 2007.

[12] O. A. Cornely *et al*., "Global guideline for the diagnosis and management of mucormycosis," *The Lancet Infectious Diseases*, vol. 19, no. 12, pp. e405–e421, 2019.

[13] K. Akinosoglou *et al*., "Amphotericin B in the era of new antifungals: Where will it stand?," *Journal of Fungi*, vol. 10, no. 4, p. 278, 2024.

[14] H. Raza *et al*., "Synthesis and characterization of hyaluronic acid (HA) modified polymeric composite for effective treatment of wound healing by transdermal drug delivery system (TDDS)," *Scientific Reports*, vol. 13, no. 1, Art. no. 13425, 2023.

[15] F. Rodríguez-Arrondo, B. Almirante, D. Rodríguez, and M. Cuenca-Estrella, "Fungal infections in burn patients: Current epidemiology and management," *Clinical Microbiology and Infection*, vol. 27, no. 5, pp. 673–680, 2021.