e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2026; 18(3); 1384-1388

Original Research Article

Biofilm Production and Antifungal Susceptibility Patterns of Candida Species in Hospitalized Patients in Tertiary Care Hospital: Eastern Odisha

Ansuman Dash[1], Dipti Pattnaik[2], Nirmala Poddar[3], Madhushree Mishra[4]

[1]Professor, Department of Microbiology, Hi-Tech Medical College and Hospital, Rourkela, Odisha, India
[2]Professor, Department of Microbiology, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India
[3]Professor, Department of Microbiology, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India
[4]Assistant Professor, Department of Dentistry, Hi-Tech Medical College and Hospital, Rourkela, Odisha, India

Received: 01-12-2025 / Revised: 15-01-2026 / Accepted: 21-02-2026
Corresponding author: Dr. Ansuman Dash
Conflict of interest: Nil

Abstract

Background: Biofilm formation is a major virulence factor of Candida species and plays a crucial role in persistent infections and reduced antifungal susceptibility. The increasing prevalence of Candida non-albicans species with variable biofilm-forming capacity and antifungal resistance poses significant therapeutic challenges, particularly in hospitalized patients.

Aim: The aim of the study is to contribute clinically relevant data to the evolving understanding of biofilm-associated candidiasis and to support evidence-based therapeutic strategies in hospital settings.

Materials & Methods: A prospective laboratory-based study was conducted in a tertiary care teaching hospital in eastern India from November 2013 to September 2015. A total of 400 clinical samples were processed, yielding 150 culture-positive Candida isolates. Species identification was performed using conventional phenotypic methods, chromogenic media, biochemical tests, and automated identification systems. Biofilm production was assessed using Congo red agar and tube methods. Antifungal susceptibility testing was carried out by the Kirby–Bauer disc diffusion method using polyenes and azole antifungal agents. Data were analyzed descriptively and expressed as frequencies and percentages.

Results: Candida non-albicans species constituted 66% of isolates. Biofilm production was detected in 73 (48.7%) isolates by Congo red agar and in 132 (88%) isolates by the tube method. Strong biofilm formation (3+) was observed in 27.3% of isolates, with Candida non-albicans species demonstrating slightly higher overall biofilm positivity than Candida albicans. Antifungal susceptibility testing showed highest sensitivity to nystatin (89%) and amphotericin B (88%), whereas overall susceptibility to azole antifungals was comparatively low (34%). Voriconazole and clotrimazole were the most effective agents among azoles.

Conclusion: The study highlights a high prevalence of biofilm-forming Candida species, particularly among non-albicans isolates, associated with reduced azole susceptibility. Routine assessment of biofilm production and antifungal susceptibility is essential to guide effective therapy and strengthen antifungal stewardship in hospital settings.

Keywords: Biofilm formation; Candida species; Candida non-albicans; Antifungal susceptibility; Azole resistance; Hospital-acquired candidiasis.

DOI: 10.25258/ijcpr.18.3.220

Introduction

Candidiasis remains one of the most important opportunistic fungal infections worldwide, contributing substantially to morbidity and mortality among hospitalized and critically ill patients. Species of the genus Candida are leading causes of healthcare-associated fungal infections, particularly in intensive care units (ICUs), where invasive procedures, indwelling medical devices, broad-spectrum antibiotic exposure, and host immunosuppression converge to facilitate infection [1,2]. In recent years, the global epidemiology of candidiasis has undergone a marked transition, with Candida non-albicans (NCA) species increasingly replacing Candida albicans as predominant clinical pathogens in many regions [3–5]. Beyond species distribution, biofilm formation has emerged as a

critical virulence determinant in Candida infections. Biofilms are structured microbial communities embedded within an extracellular matrix that adhere to biotic and abiotic surfaces, including vascular catheters, urinary catheters, prosthetic devices, and mucosal tissues [2,6]. Candida biofilms confer significant survival advantages, including enhanced resistance to host immune responses and markedly reduced susceptibility to antifungal agents compared with planktonic cells [1,6]. Consequently, biofilm-associated candidiasis is often persistent, difficult to eradicate, and associated with poor clinical outcomes.

Experimental and clinical studies have demonstrated that both C. albicans and NCA species are capable of biofilm formation; however, species-specific differences in biofilm architecture, metabolic activity, and resistance profiles have been described [4,7]. Notably, Candida tropicalis, which has emerged as a dominant NCA species in many Asian countries, is reported to form dense and metabolically active biofilms, often associated with high levels of antifungal tolerance [4,8]. These characteristics raise significant therapeutic concerns, particularly in regions where C. tropicalis predominates in bloodstream and device-associated infections.

Antifungal resistance further compounds the challenge of managing biofilm-associated candidiasis. Numerous surveillance studies have reported increasing resistance to azole antifungal agents among NCA species, while biofilm growth itself independently reduces antifungal efficacy through multiple mechanisms, including limited drug penetration, sequestration by extracellular matrix components, efflux pump overexpression, and persistence of metabolically quiescent cells [2,6,9]. Although polyenes such as amphotericin B and topical agents like nystatin often retain activity against Candida biofilms, their toxicity profiles and limited systemic applicability restrict widespread use [7,10]. The interplay between biofilm formation and antifungal susceptibility therefore has direct implications for therapeutic decision-making.

Indian and international studies have consistently highlighted regional variation in both species distribution and antifungal resistance patterns, emphasizing the need for locally generated data [8,11,12]. In many low- and middle-income countries, including India, routine laboratory surveillance of biofilm formation and antifungal susceptibility remains limited, and empirical therapy is often guided by historical assumptions rather than current epidemiological evidence [3,11]. This gap is particularly concerning given the rising burden of diabetes, prolonged hospitalization, and device-associated infections, all of which predispose to biofilm-mediated candidiasis [13].

Understanding the relationship between biofilm-forming capacity and antifungal susceptibility across different Candida species is therefore essential for improving clinical management, optimizing antifungal stewardship, and guiding infection control strategies. Recent translational research has also emphasized the potential of antibiofilm-targeted therapies and combination antifungal regimens, underscoring the importance of baseline epidemiological and phenotypic data to inform future interventions [6,14,15].

Against this background, the present study was undertaken to evaluate biofilm production among clinical Candida isolates and to analyze their antifungal susceptibility patterns in a tertiary care setting.

By correlating biofilm-forming ability with species distribution and antifungal response, this study aims to contribute clinically relevant data to the evolving understanding of biofilm-associated candidiasis and to support evidence-based therapeutic strategies in hospital settings.

Materials & Methods

A prospective, laboratory-based observational study was conducted in the Department of Microbiology of a tertiary care teaching hospital in eastern India over a 23-month period (November 2013 to September 2015) at Hi-Tech Medical College & Hospital, Odisha. The study aimed to evaluate biofilm-forming ability and antifungal susceptibility patterns of Candida species isolated from various clinical samples. Ethical approval was obtained from the Institutional Ethics Committee prior to commencement of the study.

A total of 400 clinical specimens were collected from patients attending outpatient departments and from those admitted to hospital wards and intensive care units (ICUs). Specimens included urine, blood, sputum, pus, endotracheal aspirates, catheter tips, other body fluids, vaginal swabs, and throat swabs. Samples representing normal commensal flora were excluded. Of the processed specimens, 150 culture-positive Candida isolates were included for further analysis.

1. Isolation and identification of Candida species: All specimens were processed using standard mycological techniques. Samples were inoculated on Blood agar, MacConkey agar, cystine–lactose–electrolyte-deficient (CLED) agar, and Sabouraud's dextrose agar (SDA) supplemented with antibiotics, and incubated at 37 °C for 24–48 hours.

Blood specimens were additionally processed using an automated blood culture system (BacT/ALERT

3D, bioMérieux), with subcultures performed for up to 10 days. Presumptive Candida isolates were identified by colony morphology, Gram staining, and lactophenol cotton blue mount. Species-level identification was carried out using germ tube test, chlamydospore production on corn meal agar (Dalmau plate technique), chromogenic medium (CHROMagar Candida), sugar assimilation tests, and confirmation by an automated identification system (VITEK, bioMérieux) where applicable.

2. Antifungal susceptibility testing: Antifungal susceptibility testing was performed for all Candida isolates using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar supplemented with 2% glucose and methylene blue, in accordance with standard guidelines. Inoculum suspension was prepared by emulsifying 2–4 colonies in sterile saline to achieve uniform turbidity. Antifungal discs tested included amphotericin B (20 µg), fluconazole (10 µg), itraconazole (30 µg), ketoconazole (10 µg), voriconazole (1 µg), clotrimazole (10 µg), and nystatin (100 µg). Plates were incubated at 35–37 °C for 24–48 hours, and zones of inhibition were measured and interpreted as sensitive or resistant according to standard interpretative criteria.

3. Assessment of biofilm production: Biofilm formation was evaluated using two phenotypic methods: Congo red agar (CRA) method: Isolates were inoculated onto brain heart infusion agar supplemented with glucose and Congo red dye and incubated aerobically at 37 °C for 24–48 hours. Biofilm production was interpreted based on colony colour: black or dark red colonies indicated strong biofilm production, pink colonies indicated weak biofilm production, and white or pale colonies were considered non-biofilm producers.

Tube method: A loopful of growth from SDA was inoculated into Sabouraud's dextrose broth supplemented with 8% glucose and incubated at 37 °C for 24 hours. After incubation, the broth was discarded, and the tubes were washed and stained with 1% safranin. Biofilm formation was assessed visually and graded as negative (0), weak (1+), moderate (2+), or strong (3+).

Statistical analysis: Data were entered into a spreadsheet and analyzed using standard statistical software. Results were expressed as frequencies and percentages. Comparative analysis of biofilm production and antifungal susceptibility between Candida albicans and Candida non-albicans species was performed descriptively.

Results:

Table 1: Antifungal Susceptibility Pattern

Species	Isolates	Amphotericin B	Azoles	Nystatin
C. albicans	51	45 (88%)	18 (35.3%)	46 (90%)
C. tropicalis	61	56 (92%)	17 (27.8%)	57 (93.4%)
C. glabrata	15	13 (86.6%)	9 (60%)	12 (80%)
C. parapsilosis	8	8 (100%)	2 (25%)	5 (62.5%)

Table 2: Azole Sensitivity Pattern

Species	FLC	IT	KT	VRC	CC
C. albicans	42	34	36	45	47
C. tropicalis	39	42	55	50	53
C. glabrata	14	12	15	13	13

Table 3: Biofilm Production (Tube Method)

Species	3+	2+	1+	0	Total
C. albicans	2	23	19	7	51
Non-albicans	39	41	8	11	99

Antifungal susceptibility patterns of the Candida isolates are summarized in Table 1. Overall, Nystatin exhibited the highest susceptibility rate (89%), followed closely by Amphotericin B (88%). Sensitivity to azole antifungal agents was comparatively lower (34%). Candida tropicalis demonstrated the highest sensitivity to Amphotericin B (92%) and Nystatin (93.4%), while C. albicans showed 90% sensitivity to Nystatin. Notably, C. haemulonii isolates exhibited complete resistance to Amphotericin B and azoles but remained sensitive to Nystatin.

A detailed analysis of azole susceptibility patterns is presented in Table 2. Among the azole group, Voriconazole showed the highest overall activity (86%), followed by Clotrimazole (85.3%). Fluconazole and Ketoconazole demonstrated moderate effectiveness (74%), whereas Itraconazole showed the lowest susceptibility (70%). Species-wise analysis revealed that C. albicans was most sensitive to Clotrimazole, C. tropicalis to Ketoconazole, and C. glabrata exhibited relatively better susceptibility across multiple azoles compared to other NAC species.

Biofilm-forming ability of Candida isolates assessed by the tube method is depicted in Table 3. Strong biofilm production (3+) was observed in 27.3% of isolates, while moderate (2+) and weak (1+) biofilm formation were seen in 42.6% and 18% of isolates, respectively. Candida non-albicans species demonstrated a higher overall rate of biofilm production (89%) compared to C. albicans (86.2%). These findings suggest a greater virulence potential of NAC species, which may contribute to persistent infections and reduced antifungal susceptibility.

Discussion

This study evaluated biofilm production and antifungal susceptibility among 150 Candida isolates from clinical specimens, revealing that robust biofilm formation (3+) occurred in 27.3% of isolates and that Candida non-albicans (NCA) exhibited slightly higher overall biofilm production than C. albicans (89% vs 86.2%), while polyenes (amphotericin B, nystatin) retained the highest in-vitro activity and azole susceptibility was comparatively lower (Tables 1–3). These findings align with a growing body of evidence that links biofilm formation with reduced antifungal susceptibility and clinical persistence of Candida infections [1–3]. A recent meta-analysis reported high rates of biofilm formation among bloodstream Candida isolates and strong associations between biofilm phenotype and resistance to fluconazole, voriconazole and echinocandins, supporting our observation of biofilm-associated therapeutic challenges [1].

Species-specific tendencies observed here—particularly the predominance of biofilm-forming NAC such as C. tropicalis—are corroborated by experimental and clinical studies that show C. tropicalis frequently produces dense biofilms with high extracellular matrix content, leading to elevated antifungal tolerance [4,5]. In vitro life-cycle analyse or demonstrates that C. tropicalis biofilms mature rapidly and achieve high biomass, a feature that is associated with resistance phenotypes in clinical isolates [4]. Mechanistically, biofilm resistance is multifactorial, encompassing limited drug penetration, extracellular matrix sequestration, quorum-sensing mediated phenotypic shifts, upregulation of efflux pumps, and persister cell formation, as extensively reviewed in recent literature [2,6].

The antifungal susceptibility profile in our dataset showed high activity of nystatin (89%) and amphotericin B (88%), with much lower aggregate susceptibility to azoles (34%) (Table 1). Comparable surveillance studies from diverse settings report similar patterns, with polyenes often retaining activity when azole resistance increases among NCA species [7–9].

For example, multi-centre and single-centre Indian studies documented substantial fluconazole resistance among C. tropicalis and C. glabrata, while amphotericin B largely preserved activity in vitro [8,9]. These parallels emphasize that empirical azole therapy may be unreliable against biofilm-forming NAC in high-prevalence regions.

The clinical implications of biofilm-associated resistance are substantial. Devices such as urinary and central venous catheters are frequent niduses for biofilm establishment, and biofilm-embedded cells can seed bloodstream infections that are difficult to eradicate without device removal [1,10]. Our finding that urinary specimens were a major source of isolates in the parent dataset underscores the clinical relevance of catheter-associated biofilms in the studied population (see dataset).

Several translational studies spotlight potential strategies to overcome biofilm tolerance. Adjunctive approaches—combining antifungals with biofilm-disrupting agents, antifungal peptides, or novel small molecules—have shown promise in vitro and in animal models [11–13]. Recent reviews highlight that targeting and efflux mechanisms can sensitize biofilms to conventional agents [2,11]. Emerging antibiofilm agents, including antimicrobial peptides, lipopeptides, and nanoparticle formulations, have demonstrated eradication or disruption of mature C. tropicalis biofilms in preclinical studies, suggesting plausible future adjuncts to therapy [14,15]. Nevertheless, regional heterogeneity in species distribution and resistance complicates generalization. Some reports show higher echinocandin resistance or emerging polyene non-susceptibility in particular locales, highlighting the need for continuous local surveillance [7,9,16]. Our data thus contribute a timely regional snapshot but must be interpreted alongside contemporaneous surveillance to guide empirical therapy.

Several limitations warrant mention. First, biofilm assessment employed phenotypic methods (Congo Red Agar and tube method) rather than quantitative microtiter plate biomass/viability assays or confocal imaging; phenotypic scoring is semi-quantitative and may lack granularity. Second, antifungal susceptibility was assessed by disc diffusion rather than broth microdilution (CLSI/EUCAST MIC determination), which is the reference standard for correlation with clinical breakpoints.

Third, molecular characterization of resistance mechanisms (e.g., ERG11 mutations, efflux pump gene expression) and sequencing-based species confirmation were not performed for all isolates, limiting mechanistic inference. Fourth, the data derive from a single tertiary centre and a historical timeframe, which may not reflect evolving post-

COVID ICU epidemiology or emergent species such as Candida auris.

Future recommendations: To address these gaps, future studies should employ standardized quantitative biofilm assays (e.g., XTT/Alamar Blue, CFU enumeration, confocal microscopy) and MIC-based antifungal testing (broth microdilution) to better correlate biofilm biomass with antifungal susceptibility. Integrating molecular analyses including genotyping, resistance gene sequencing, and transcriptomics will illuminate mechanisms underpinning biofilm tolerance and resistance.

Multicentre prospective surveillance across geographic regions, including ICU networks and post-COVID cohorts, is needed to map species dynamics and resistance trends. Finally, clinical trials testing adjunctive antibiofilm strategies (matrix-degrading enzymes, AMPs, lipopeptides, or combination regimens) are warranted to translate in vitro promise into improved patient outcomes.

Conclusion

This study reinforces that biofilm formation is common among clinical Candida isolates particularly NAC species—and is associated with diminished azole susceptibility and reliance on polyenes in vitro. These findings underscore the urgent need for integrated diagnostic, stewardship, and therapeutic strategies targeting biofilm-associated candidiasis in hospital settings.

References:

1. Atiencia-Carrera MB, Cabezas-Mera FS, Tejera E, Machado A. Prevalence of biofilms in Candida spp. bloodstream infections: A meta-analysis. PLoS One. 2022 Feb 3;17(2):e0263522.
2. Kaur J, Nobile CJ. Antifungal drug-resistance mechanisms in Candida biofilms. Current opinion in microbiology. 2023 Feb 1;71:102 237.
3. Fan F, Liu Y, Liu Y, Lv R, Sun W, Ding W, Cai Y, Li W, Liu X, Qu W. Candida albicans biofilms: antifungal resistance, immune evasion, and emerging therapeutic strategies. International journal of antimicrobial agents. 2022 Nov 1;60(5-6):106673.
4. Atiencia-Carrera MB, Cabezas-Mera FS, Vizuete K, Debut A, Tejera E, Machado A. Evaluation of the biofilm life cycle between Candida albicans and Candida tropicalis. Frontiers in cellular and infection microbiology. 2022 Aug 18;12:953168.
5. Chaabane F, Graf A, Jequier L, Coste AT. Review on antifungal resistance mechanisms in the emerging pathogen Candida auris. Frontiers in microbiology. 2019 Nov 29;10: 2788.
6. Borman AM, Muller J, Walsh-Quantick J, Szekely A, Patterson Z, Palmer MD, Fraser M, Johnson EM. Fluconazole resistance in isolates of uncommon pathogenic yeast species from the United Kingdom. Antimicrobial Agents and Chemotherapy. 2019 Aug;63(8):10-128.
7. Shrief R, Zaki ME, El-Sehsah EM, Ghaleb S, Mofreh M. Study of antifungal susceptibility, virulence genes and biofilm formation in Candida albicans. The Open Microbiology Journal. 2019 Sep 30;13(1).
8. Sumana MN. Retrospective analysis on distribution and antifungal susceptibility profile of Candida in clinical samples: a study from Southern India. Frontiers in Public Health. 2023 May 12;11:1160841.
9. Chaudhary P, Singla A, Mamoria VP, Mittal S, Bacchani D. Prevalence of Candida non-albicans and its Antifungal Susceptibility at a Tertiary Care Hospital, Jaipur. Int J Contemp Med Res. 2020;7(7):G7-10.
10. Arsić Arsenijević V, Gerginić V, Jurišić A, Otaševic S, Ranđelović M, Petričević L. Prevalence of Candida and Other Yeasts in Vulvovaginal Infections during Pregnancy: A 10-Year Serbian Survey. Mycopathologia. 2025 Oct;190(5):86.
11. Cedeño-Pinargote AC, Jara-Medina NR, Pineda-Cabrera CC, Cueva DF, Erazo-Garcia MP, Tejera E, Machado A. Impact of Biofilm Formation by Vaginal Candida albicans and Candida glabrata Isolates and Their Antifungal Resistance: A Comprehensive Study in Ecuadorian Women. Journal of Fungi. 2025 Aug 25;11(9):620.
12. Hitkova HY, Georgieva DS, Hristova PM, Marinova BT, Borisov BK, Popov VG. Antifungal susceptibility of Candida non-albicans species in a tertiary care hospital, Bulgaria.
13. Xiong H, Zhao R, Han S, Liu Z, Zhang X, Jia Z, Cui J, Zhang Y, Wang X. Research progress on the drug resistance mechanisms of Candida tropicalis and future solutions. Frontiers in Microbiology. 2025 May 12;16:1594226.
14. Madduri M, Rudramurthy SM, Roy U. Bacillus-derived Lipopeptides Inhibit and Eradicate Candida tropicalis Biofilms: An in Vitro exploration. Biofilm. 2025 Jun 14:100294.
15. Atiencia-Carrera MB, Cabezas-Mera FS, Tejera E, Machado A. Prevalence of biofilms in Candida spp. bloodstream infections: A meta-analysis. PLoS One. 2022 Feb 3;17(2):e0263522.