Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 1497-1501 | ISSN:2251-6727

Evaluation of Virulence Attributes and Antifungal Susceptibility of *Candida* Species in Patients with Deep Dental Caries

[1] Prachi Srivastava *, [2] Pratibha Sharma, [3] Payal Jaiswal, [4] Laxmi Pillai, [5] Leena Malviya

[1, 2, 3, 4, 5] Department of Microbiology, Institute of Sciences, SAGE University, Indore, Madhya Pradesh, India

(Received: 05 November 2025 Revised: 15 December 2025 Accepted: 23 January 2026)

KEYWORDS

Deep dental caries, *Candida*, Haemolysin, Biofilm, Antifungal resistance.

ABSTRACT:

Candida species are increasingly recognized as important opportunistic pathogens in oral infections, particularly in patients with deep dental caries, where their virulence factors such as haemolysin production and biofilm formation contribute to tissue invasion, persistence, and antifungal resistance[1]. The present study aimed to isolate *Candida* species from patients with deep dental caries and to evaluate their haemolysin activity, biofilm-forming ability, and antifungal susceptibility patterns. Isolation and identification of *Candida* were performed using standard mycological techniques, followed by detection of haemolysin activity and biofilm production using established laboratory assays. A total of 50 clinical samples were collected and processed, and antifungal susceptibility testing was carried out according to CLSI guidelines. Of the samples analyzed, 11 (22%) yielded *Candida* species, including *C. albicans* (54.5%), *C. dubliniensis* (27.3%), and *C. krusei* (18.2%). All isolates exhibited haemolytic activity, while biofilm formation was observed in 90% of isolates, with *C. dubliniensis* showing strong biofilm-producing ability. Antifungal susceptibility testing revealed the highest resistance to clotrimazole (54%), whereas all isolates were sensitive to amphotericin B, highlighting the significant virulence potential and emerging antifungal resistance of *Candida* species associated with deep dental caries and emphasizing the need for early diagnosis and rational antifungal therapy.

1. Introduction

Dental caries is one of the most prevalent oral health problems worldwide and continues to be a major public health concern affecting individuals of all age groups. According to the World Health Organization, untreated dental caries in permanent teeth is the most common health condition globally, affecting nearly 2.5 billion people [1]. In developing countries, including India, the burden of dental caries is steadily increasing due to changes in dietary habits, inadequate oral hygiene practices, and limited access to preventive dental care [2]. Studies from India have reported dental caries prevalence ranging from 50–80% in different population groups, highlighting its significant public health impact [3]. In Madhya Pradesh, and particularly in urban centres such as Indore, dental caries remains a common oral complaint among patients attending dental clinics, often presenting at advanced stages due to delayed diagnosis and treatment [4].

Deep dental caries create a favorable anaerobic and nutrient-rich microenvironment that supports the colonization and proliferation of opportunistic microorganisms, including fungal pathogens such as *Candida* species [5].Although *Candida albicans* is a normal commensal of the oral cavity, alterations in local oral ecology caused by extensive carious lesions, poor oral hygiene, saliva reduction, and compromised host immunity can trigger its transition from a harmless commensal to a pathogenic organism[6]. In recent years, non-albicans *Candida* species such as *C. dubliniensis* and *C. krusei* have been increasingly isolated from oral infections and are of particular concern due to their reduced susceptibility or intrinsic resistance to commonly used antifungal agents [7].

Virulence factors play a critical role in the pathogenicity of *Candida* species. Haemolysin production enables fungal cells to acquire iron from host tissues, facilitating survival and tissue invasion, while biofilm formation enhances adhesion to oral surfaces, persistence within the host, and resistance to antifungal drugs [8] Biofilm-associated *Candida* infections are particularly difficult to

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 1497-1501 | ISSN:2251-6727

eradicate and are often implicated in chronic and recurrent oral infections, including those associated with deep dental caries. Reports from India and central Indian regions have also indicated an increasing involvement of *Candida* species in oral lesions, emphasizing the need for continuous surveillance of their virulence potential and antifungal resistance patterns [9,10].

The present study was therefore undertaken to evaluate the virulence attributes, including haemolysin activity and biofilm-forming ability, along with the antifungal susceptibility pattern of *Candida* species isolated from patients with deep dental caries in Indore, Madhya Pradesh. This investigation aims to provide insight into the pathogenic potential of oral *Candida* species and to address emerging therapeutic challenges associated with antifungal resistance.

2. Materials and Methods

Study Design and Sample Collection

A hospital-based cross-sectional study. A total of 50 patients clinically diagnosed with deep dental caries were included in the study. Patients of both sexes and different age groups were enrolled after obtaining written informed consent. Individuals who had received antifungal therapy within the previous four weeks were excluded from the study. Dental caries samples were collected aseptically using sterile swabs and transported immediately to the microbiology laboratory for further processing [11].

Isolation and Identification of *Candida* Species

Collected samples were inoculated onto Sabouraud Dextrose Agar (SDA) plates and incubated at 37°C for 48 hours for the primary isolation of yeast colonies. Presumptive *Candida* isolates were identified based on colony morphology and microscopic examination. Further species-level identification was carried out using standard mycological methods, including the germ tube test, CHROM agar *Candida*, sugar fermentation tests, and microscopic morphology using Gram staining [12,13].

Detection of Haemolysin Activity

Haemolysin production was assessed using blood agar medium supplemented with 3% glucose. The *Candida* isolates were spot inoculated onto the agar plates and incubated at 37°C for 48 hours. Plates were examined for the presence of translucent or clear zones around the colonies, indicating haemolytic activity. The haemolytic index was recorded based on the diameter of the haemolysis zone relative to the colony diameter, as described previously [8].

Biofilm Production Assay

Biofilm-forming ability of the *Candida* isolates was evaluated using the tube method. Briefly, isolates were inoculated into Sabouraud dextrose broth supplemented with glucose and incubated at 37°C for 48 hours. After incubation, the broth was decanted, tubes were washed with phosphate-buffered saline, and biofilms were stained using crystal violet. Based on the intensity of the stained biofilm layer, isolates were categorized as strong, moderate, or weak biofilm producer [14,15].

Antifungal Susceptibility Testing

Antifungal susceptibility testing was performed using the disc diffusion method on Mueller–Hinton agar supplemented with glucose and methylene blue, following the guidelines of the Clinical and Laboratory Standards Institute. The antifungal agents tested included nystatin, itraconazole, clotrimazole, ketoconazole, fluconazole, and amphotericin B. After incubation at 37°C for 24–48 hours, zones of inhibition were measured and interpreted as sensitive, intermediate, or resistant according to CLSI interpretative criteria [16, 17].

Data Analysis

The results were analyzed to determine the distribution of *Candida* species, prevalence of virulence factors (haemolysin production and biofilm formation), and antifungal susceptibility patterns among the isolates. Data were expressed in terms of frequencies and percentages for descriptive interpretation.

3. Results and Discussion

In the present study, *Candida* species were isolated from 11 out of 50 patients with deep dental caries, indicating an overall prevalence of 22%. This finding supports the growing evidence that deep carious lesions provide a favorable anaerobic and nutrient-rich environment for opportunistic fungal colonization [11]. Similar isolation rates of *Candida* species from deep dental caries have been reported in earlier studies from India and other developing countries, emphasizing their role in oral infections [18]

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 1497-1501 | ISSN:2251-6727

Among the isolates, *Candida albicans* was the predominant species, accounting for 54.5% of cases, followed by *C. dubliniensis* (27.3%) and *C. krusei* (18.2%). The predominance of *C. albicans* is consistent with previous reports identifying it as the most common oral *Candida* species due to its strong adhesive properties and adaptability to the oral environment[6]. However, the notable presence of non-*albicans Candida* species is clinically significant, as these species are increasingly associated with enhanced virulence traits and intrinsic or acquired resistance to commonly used antifungal agents [17]

All *Candida* isolates in the present study demonstrated haemolysin production, indicating a high virulence potential. Haemolysin activity facilitates iron acquisition from host tissues, which is essential for fungal growth and tissue invasion [17]. Universal haemolytic activity observed in this study suggests that *Candida* species associated with deep dental caries possess enhanced pathogenic capabilities, contributing to disease persistence and progression. Similar findings have been reported by other investigators, highlighting haemolysin as an important virulence determinant in oral candidiasis [19,20].

Biofilm formation was observed in 90% of the isolates, further underscoring their virulence potential. Among these, 27% were strong biofilm producers, 36% were moderate producers, and 27% were weak producers. Notably, *C. dubliniensis* exhibited the strongest biofilm-forming ability. Biofilm formation enables *Candida* cells to adhere firmly to oral tissues and dental surfaces, protects them from host immune defenses, and significantly reduces susceptibility to antifungal agents [15]. The high prevalence of biofilm-producing isolates observed in this study aligns with previous reports that link biofilm formation with chronic and recurrent oral infections [21].

Antifungal susceptibility testing revealed variable resistance patterns among the *Candida* isolates. The highest resistance was observed against clotrimazole (54%), followed by fluconazole and ketoconazole (36% each), and itraconazole (27%), while resistance to nystatin was relatively low (9%). Importantly, all isolates were sensitive to amphotericin B. The increased resistance to azole antifungals observed in this study may be attributed to their widespread and often irrational use as topical agents in oral infections, leading to selective pressure and resistance development [7]. The continued susceptibility of all isolates to amphotericin B supports its role as a reliable antifungal agent, although its systemic toxicity limits routine clinical use [22].

Overall, the results of this study demonstrate that *Candida* species isolated from deep dental caries possess multiple virulence factors, including haemolysin production and biofilm formation, along with a concerning level of resistance to commonly used azole antifungals. These findings highlight the importance of early detection of *Candida* involvement in deep dental caries and the need for judicious antifungal therapy to prevent treatment failure and chronic oral infections.

Prevalence of *Candida* Species

Out of 50 samples, 11 (22%) were positive for *Candida* species.

Species	Number (%)
C. albicans	6 (54.5%)
C. dubliniensis	3 (27.3%)
C. krusei	2 (18.2%)

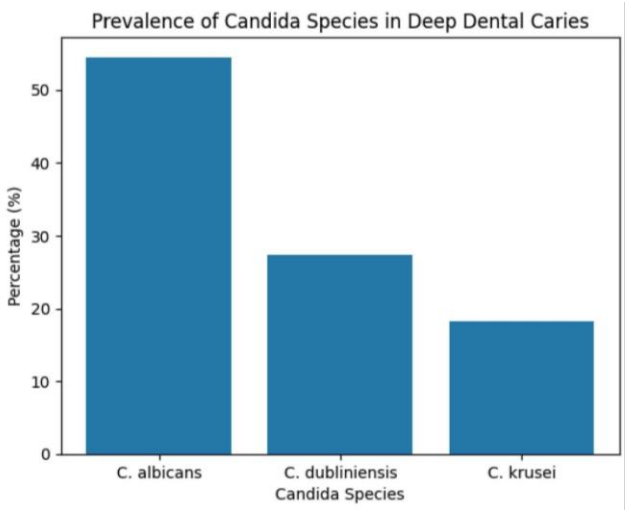

Figure 1. Prevalence of *Candida* Species Isolated from Deep Dental Caries

This bar graph illustrates the distribution of Candida species isolated from deep dental caries patients, showing the predominance of C. albicans *(54.5%), followed by* C. dubliniensis *(27.3%) and* C. krusei *(18.2%).*

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 1497-1501 | ISSN:2251-6727

Haemolysin Production

All 11 isolates (100%) demonstrated haemolytic activity, indicating strong virulence potential.

Biofilm Production

Biofilm production was observed in 10 (90%) isolates.

Biofilm Intensity	Number (%)
Strong	3 (27%)
Moderate	4 (36%)
Weak	3 (27%)

C. dubliniensis showed the strongest biofilm-forming ability.

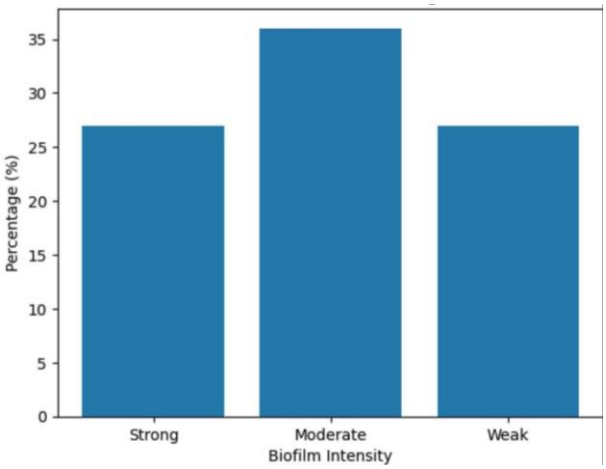

Figure 2. Distribution of Biofilm Production Intensity Among *Candida* Isolates

The graph depicts the biofilm-forming ability of Candida isolates, where moderate biofilm producers (36%) were predominant, followed by strong (27%) and weak (27%) biofilm producers.

Antifungal Resistance Pattern

Antifungal Drug	Resistant (%)
Nystatin	9%
Itraconazole	27%
Clotrimazole	54%
Ketoconazole	36%
Fluconazole	36%
Amphotericin B	0%

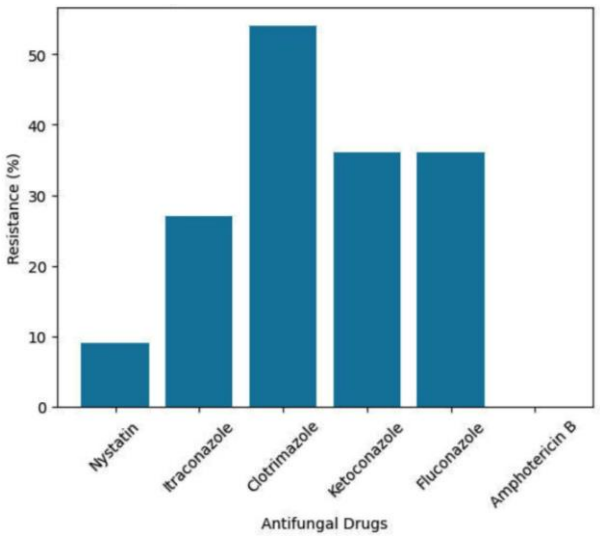

Figure 3. Antifungal Resistance Pattern of *Candida* Isolates

This figure represents the resistance pattern of Candida isolates against commonly used antifungal drugs. Maximum resistance was observed against clotrimazole (54%), while all isolates remained sensitive to Amphotericin B.

Therefore, Figure 1 shows the prevalence of Candida species isolated from deep dental caries, with C. albicans being the most common isolate. Figure 2 demonstrates the biofilm-forming capacity of the isolates, indicating a higher proportion of moderate biofilm producers. Figure 3 highlights the antifungal resistance pattern, revealing significant resistance to azole antifungals, particularly clotrimazole.

4. Conclusion

The present study highlights that *Candida* species isolated from deep dental caries exhibit significant virulence traits, including haemolysin production and biofilm formation, which contribute to their pathogenic potential. Additionally, an increasing trend of resistance to commonly used azole antifungals was observed, while amphotericin B remained fully effective. These findings underscore the importance of routine identification of *Candida* species and antifungal susceptibility testing to guide appropriate therapy, prevent treatment failure, and manage oral fungal infections effectively.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 1497-1501 | ISSN:2251-6727

Acknowledgements

The authors sincerely acknowledge the Department of Microbiology, Institute of Science, SAGE University, Indore for laboratory support.

Conflict of Interest

None declared.

References:

1. World Health Organization. **Global Oral Health Status Report**. Geneva: WHO; 2022
2. Petersen PE, Ogawa H. The global burden of periodontal disease: towards integration with chronic disease prevention and control. *Periodontol 2000*. 2012;60:15–39.
3. Bali RK, Mathur VB, Talwar PP, Chanana HB. **National Oral Health Survey and Fluoride Mapping, India**. New Delhi: Dental Council of India; 2015.
4. Verma SK, Singh P, Jain R. A review paper on rejuvenation of forgotten Kanh River of Indore. *IRJET*. 2020;7(6):2450–2456.
5. Samaranayake LP. **Essential Microbiology for Dentistry**. 2nd ed. London: Churchill Livingstone; 2002.
6. Calderone RA, Fonzi WA. Virulence factors of *Candida albicans*. *Trends Microbiol*. 2001;9(7): 327–335.
7. Pfaller MA, Andes D, Diekema DJ, Horn DL, Reboli AC. Epidemiology and outcomes of invasive candidiasis. *Clin Infect Dis*. 2010;50(4): 547–556.
8. Luo G, Samaranayake LP, Yau JY. *Candida* species exhibit differential in vitro hemolytic activities. *J Clin Microbiol*. 2001;39(8):2971–2974.
9. Tiwari A, Mishra S. Study of some physico-chemical water pollutants of Kanh River at Indore – a review. *Int J Environ Sci*. 2019;10(2):112–121.
10. Patel D, Sharma R, Dubey A. To study the ecology and effect of pollution on Kanh River, Indore. *J Environ Biol*. 2021;42:875–883.
11. Samaranayake LP. **Essential Microbiology for Dentistry**. 2nd ed. London: Churchill Livingstone; 2002
12. Forbes BA, Sahm DF, Weissfeld AS. **Bailey & Scott's Diagnostic Microbiology**. 13th ed. St. Louis: Elsevier; 2016.
13. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. **Koneman's Color Atlas and Textbook of Diagnostic Microbiology**. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2017
14. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, et al. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. *J Clin Microbiol*. 1985;22(6):996–1006.
15. Ramage G, Saville SP, Thomas DP, López-Ribot JL. *Candida* biofilms: an update. *Eukaryot Cell*. 2005;4(4):633–638.
16. Clinical and Laboratory Standards Institute (CLSI). **Reference Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts**. CLSI document M44. Wayne (PA): CLSI; 2017.
17. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev*. 2007;20(1):133–163.
18. Bikandi J, San Millán R, Moragues MD, Cebas G, Clarke M, Coleman DC, et al. Antifungal drug resistance in *Candida* species. *Int J Antimicrob Agents*. 2012;40(2):95–101.
19. Manns JM, Mosser DM, Buckley HR. Production of a hemolytic factor by *Candida albicans*. *Infect Immun*. 1994;62(11):5154–5156.
20. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. *Candida* biofilms: a review. *Med Mycol*. 2012;50(2):109–120.
21. Odds FC. Antifungal agents: their diversity and mechanisms of action. *J Antimicrob Chemother*. 2003;52(1):1–9.