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IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

Determination of the prevalence rates, species distribution and evaluation of virulence remove the determinants of *Candida* Species from clinical samples

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Abstract

Background: Candidiasis predominantly impacts individuals with compromised immune systems, resulting in opportunistic infections that can present in various forms (e.g., skin lesions or systemic involvement). Species of *Candida* are often recognized as significant pathogens in such instances. Biofilm formation serves as a crucial virulence factor, enhancing the organism's adherence to host tissues and facilitating its proliferation. The secretion of proteinases is thought to augment the organism's capacity to colonize and penetrate host tissues, while also evading immune responses. Additionally, phospholipases may greatly assist in the invasion of host tissues during candidiasis lesions by disrupting epithelial cell membranes. The aim of this study was to isolate and identify *Candida* species from clinical samples, as well as to evaluate the distribution of virulence factors among species.

Materials and Methods: A total of sixty *Candida* isolates were gathered from patient samples for this investigation. The evaluation of virulence markers associated with various *Candida* species—including phospholipase, proteinase and biofilm production—was performed using phenotypic assessment techniques. However, challenges arose during the analysis, which complicates the interpretation of results. Although the study was thorough, some limitations were encountered because of the inherent variability within clinical samples.

Results: Among the 60 isolates examined, the species that was most prevalent was *Candida albicans,* which accounted for 67% of the isolates. *Candida parapsilosis* followed closely, comprising 24% of the total. The rates of occurrence for various virulence factors across different *Candida* species were notable: 65% for proteinase production, 55% for biofilm formation and 50% for phospholipase production. Moreover, *Candida* growth was predominantly observed in individuals aged between (50 and 81) years, at a rate of 75%.

Conclusion: The virulence factors linked to *Candida* such as biofilm, proteinase and phospholipase—exhibit variability that is related to species and host immunity. Early detection is crucial (because it aids in evaluating invasiveness and informing clinical decision-making).

Keywords: *Candida* species, Virulence factors, Phospholipase, Proteinase, Biofilm.

Received: 31-01-2025; **Accepted:** 05-03-2025; **Available Online**: 26-03-2025

1. Introduction

Candida, a widespread co-inhabiting yeast, predominates in mucosal regions such as the oral cavity, genital, digestive, vaginal cavities, and well-healthy skin areas, usually without inducing symptoms.[1] Numerous harmful microbes, inclusive of diverse kinds of *Candida*, display an extensive series of pathogenicity elements and distinct methods that facilitate adhesion, penetration, and advancement of illness. Many living things can stick to different things and may lead to quick or spreading diseases. This means that things *Candida* does to be harmful like breaking down proteins and making slimy layers are important for how harmful they can be. Accurate determination of different *Candida* types is crucial for the proper use of antifungal medications.[2,3] The creation of proteinases (enzymes that break down proteins) probably helps the microbe stick to and grow inside body tissues while slipping past the body's defense systems. The instruction specified replacing only a few key words with similar words or phrases (synonyms). The synonyms chosen aim to retain the Many *C.krusei* is widely linked with severe mycotic diseases, whereas other *Candida* varieties have grown more prominent among opportunistic microbes in therapeutic environments.[4,5] Many women have *Candida* species in their vagina, making them more prone to genitourinary

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http://doi.org/10.18231/j.ijmmtd.2025.012

candidiasis. Disruptions in both internal body functions and conditions of the tissue where these organisms are present may disturb the delicate balance.[6] This research seeks to isolate and identify different Candida species from patient samples and assess the distribution of their virulence factors.

2. Materials and Methods

2.1. Type of study

Cross sectional Observational study.

2.2. Study population

60 *Candida* species were studied from patient's samples which were sent to microbiology laboratory for fungal culture and sensitivity.

2.3. Inclusion criteria

Candida species isolated from samples.

2.4. Exclusion criteria

Repeated isolates of *Candida* species from same patients will be excluded.

2.5. Statistical method

All data were collected and recorded in MS Excel format and percentage of prevalence calculation was done in MS Excel.

2.6. Ethical consideration

The study was approved by the Institute Review board, Mahatma Gandhi Medical College & Research Institute, Pillaiyarkuppam, Pondicherry-607402.

2.7. Methodology

For fungal culture, samples were inoculated on to Sabouraud Dextrose Agar (SDA) and incubated at 37°C. Identification of the growth on SDA plates was done by colony morphology, Gram-staining, and standard biochemical reactions. The presumptive identification was carried out by observing the colony colour with reference to the manufacturer's guidelines for Hi CHROM medium. *Candida albicans* - green, *Candida tropicalis* - blue, *Candida krusei* - pink colonies with matt surface, *Candida parapsilosis* - cream to pale pink. *Candida glabrata* - pink to purple[7] (**Figure 1**) Following the separation, the isolates were examined for generating virulence factors, entailing testing phospholipase by cultivating them in egg yolk medium to gauge precipitate zone dimensions and analyzing protease activity and biofilm development.

2.8. Assessment of Virulence Factors

The isolated cells were assayed for apolipoprotein hydrolysis by inspecting the production of a coagulum region following proliferation on cholesterol-containing agar. After a 24-hour culture on SDA, the various *Candida* strains were collected and mixed with sterile *phosphate buffered saline* (PBS) until opaque. A small amount of liquid PBS was put onto an egg yolk mixture in a special dish and left in a warm spot for two days to grow. The phospholipase output (Pz) measurement was determined through the procedure outlined below.[8]

$$Pz = \frac{Colony\ diameter}{Colony\ diameter + Zone\ of\ precipitation}$$

A Pz value of 1 denotes zero phospholipase activity, whereas a decreased Pz value correlates with heightened phospholipase enzyme generation. (**Figure 2**)

2.9. Evaluation of protease activity

We measured how well different proteins break down (protease activities) by following the same steps that Aoki et al. did for testing cow serum protein (BSA) in dishes. The substance was thoroughly purified and brought to a normal room temperature before adding 1 gram of Bovine Serum Albumin (BSA) to the already prepared solution. Variants were seeded into the nutrient mix and cultured for a pair of days at 37°C. Clear zones encompassing the cultured variants signified protease function.[1] (**Figure 3**).

2.10. Determination of biofilm production

Tube method was employed to detect biofilm formation. A colony from the agar plate was transferred into 3 ml of Sabouraud liquid broth enriched with 8% glucose. The tubes were then incubated for 24 hrs at 37°C. After incubation, the broth was aspirated, and phosphate-buffered saline was used to rinse the tubes. Once dried, 1% Safranin was used to stain the test tubes and excess stain was removed by washing with de-ionized water. Finally, then they were inverted and left to dry and checked for stain deposition on the walls of the tube.[9] (**Figure 4**)

3. Result

In the current study the most predominant species was inferred as *Candida albicans* (67%) followed by non albicans *Candida* species (33%). *Candida* colonization was found predominantly between the age group of 50 to 81 years (75%). **Figure 6** depicts the occurrence of various virulence factors in *Candida* species.

3.1. Phospholipase activity, proteinase activity & biofilm formation

Phospholipase activity was evaluated by observing the precipitation zones as illustrated in **Figure 2**, **Figure 3** shows the assessment method of proteinase activity, and biofilm production was considered positive when walls of the tube were visibly stained, as depicted in **Figure 4**. Of the total 60 isolates *Candida albicans* exhibited the highest phospholipase activity (28.3%), a proteinase activity of 45% and a Biofilm formation (41%), followed by *Candida parapsilosis showing* 11.6%, 13.3%, and 15% respectively.

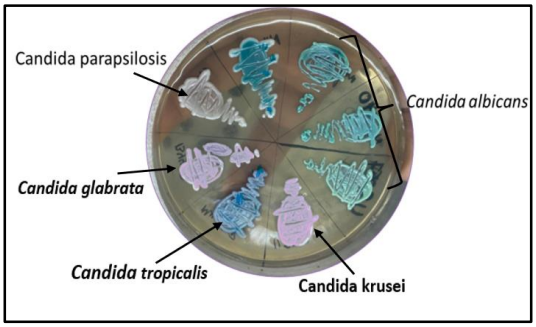

Figure 1: Candida species on CHROM agar

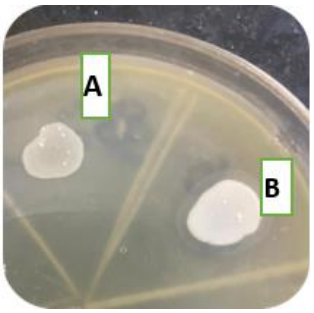

Figure 2: Phospholipase production on egg yolk agar; **A:** Negative for phospholipase; **B:** Positive for phospholipase activity

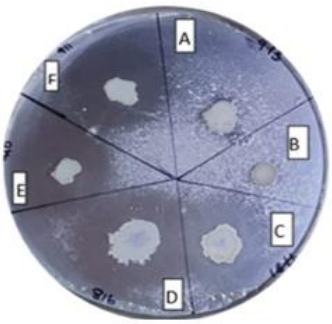

Figure 3: Proteinase production in bovine serum albumin agar (BSA); **A,B:** No proteinase activity; **C** to **F:** Transparent halo→ proteinase activity present.

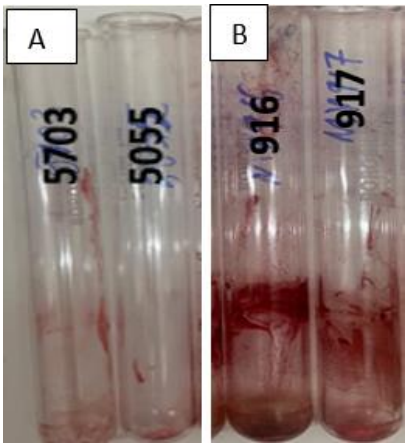

Figure 4: Biofilm formation by tube method; **A:** Negative; **B:** Positive

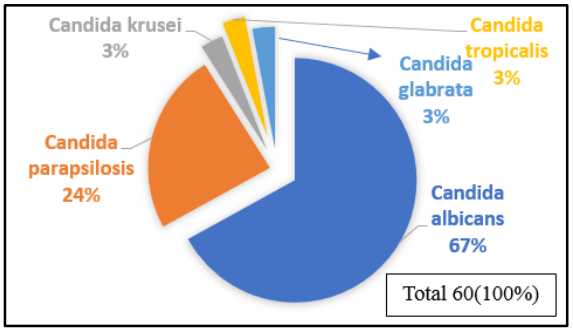

Figure 5: Percentage of Candida species isolated from clinical samples

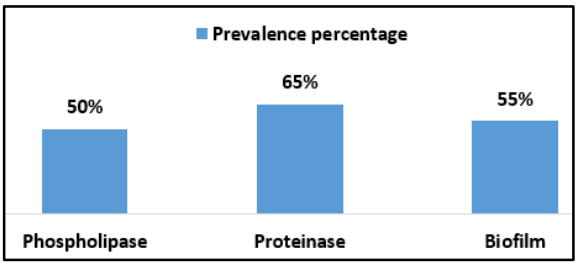

Figure 6: Prevalence of virulence factors

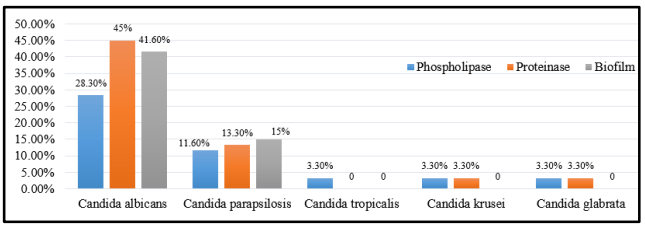

Figure 7: Percentage of virulence factor produced by different species of Candida

4. Discussion

Clinical prevalence of different species of *Candida* is reported; however, C. *albicans* is most implicated species.[5] In our study, the most predominant species isolated was *Candida albicans* (67%), followed by non *albicans Candida* species, which accounts for (33%). Which is comparable to previous others studies.[1,8,10] However, there are few other studies, which have proven that non *albicans Candida* are emerging and showing increased prevalence.[2,11]

The shift of *Candida* from a harmless commensal to a persistent pathogen is a delicate process, driven by an extensive range of virulence factors that are selectively expressed under favourable predisposing conditions[3] biofilm formation, proteinase activity, and phospholipase production play significant roles in the pathogenicity of *Candida* species. Therefore, recognizing these virulence factors early in clinical settings is essential for making effective treatment decisions and managing infections caused by *Candida*. Several factors, including biofilm formation, proteinase activity, and phospholipase production, contribute to its

pathogenicity. Early identification of these virulence factors in *Candida* is crucial for informed clinical decision-making.

Proteinase production is considered to enhance the organism's ability to colonise and penetrate host tissues, and to act on their immune system by Breaking down several key proteins involved in host defence, including immunoglobulins, complement, and cytokines.[5]

Phospholipases may actively aid in infiltration of host tissue in candidiasis lesions by damaging the membranes of epithelial cells.[11] Membrane disruption of the host cells, ability of proceeding to a secondary infection and ability of adherence to host cell surface is executed by the extracellular phospholipase enzyme present in the organism which is a crucial virulence determinant.[6]

Formation of Biofilm is considered as a crucial virulence factor by attaching to body sites and further proliferation. Biofilm formation is a critical factor in determining pathogenicity and should be considered an important virulence determinant in candidiasis.[12] Occurrence of Biofilm was predominantly seen to be frequently occurring in Non albicans Candia than in C.albicans[13,14] and less frequently in vice versa.[15] Biofilm formation, proteinase activity, and phospholipase production play significant roles in the pathogenicity of Candida species. Therefore, recognizing these virulence factors early in clinical settings is essential for making effective treatment decisions and managing infections caused by Candida. Thus, virulence factors help the organisms to evade host defence mechanisms and to establish their pathogenicity.[2] The prompt identification of this can aid in clinical decision-making, as it signals the potential pathogenicity of *Candida* isolates. The biofilms contain extracellular materials, composed of proteins, carbohydrates, and other substances. *Candida* biofilm formation has important clinical ramifications, as biofilms are formed on inert surfaces of indwelling medical devices such as catheters, artificial dentures and prosthetic valves.[8]

5. Conclusion

In conclusion, the presence of virulence factors in Candida species is influenced by various factors such as the infecting species, geographical location, infection type and stage, site of infection, and the host's immune response. Key determinants of pathogenicity, including biofilm formation, proteinase activity, and phospholipase production, play a critical role in Candida's invasiveness. Early detection of these virulence factors is essential for informed clinical decision-making, offering valuable insights into the potential severity and progression of Candida infections.

6. Conflict of Interest

The authors declare that there are no conflicts of interest

7. Source of Funding

No funding was sort for this study.

8. Acknowledgement

The authors would like to express our sincere gratitude to the Head & faculty members of Department of Microbiology, MGMCRI, and Pondicherry for their invaluable guidance and support throughout this research. We would also like to thank Sri Balaji Vidyapeeth deemed-to-be University for the support provided.

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Cite this article: Thejakhrienuo K, Valentina Y, Easow JM. Determination of the prevalence rates, species distribution and evaluation of virulence remove the determinants of *Candida* Species from clinical samples. *IP Int J Med Microbiol Trop Dis.* 2025;11(1):81-85.