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# Characterisation Of Candida Species Isolated From Clinical Samples And Their Antifungal Susceptibility

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**Abstract**

Microorganisms growing in a biofilm are associated with chronic and recurrent human infections and are resistant to antimicrobial agents. Candida species are responsible for the most frequently encountered opportunistic fungal infections due to widespread use of broad spectrum antibiotics, chemotherapy, chronic kidney disease patients and in other immune-compromised patients. This study was undertaken to study different characters of various Candida species isolated from clinical samples including biofilm formation and their antifungal susceptibility .The study was conducted at Department of Microbiology from June 2013 to June 2015. Percentage of biofilm formation was 28% and biofilm formation was higher among non-albicans Candida than *Candida albicans* .Antifungal resistance was higher among biofilm producers than non-biofilm producers. The long-term use of azoles in the prophylaxis of systemic mycoses can result in more resistance of Candida isolates to azole therapy. Hence species identification and susceptibility testing is of great need of today for treating physician.

**Keywords**: Biofilm, Candida species, Antifungal susceptibility

## Introduction

Candida species are significant opportunistic pathogens which cause a wide variety of infections in humans ranging from trivial intertriginous infection to fatal candidemia. The commonest species that are implicated in infections are *C.albicans, C.tropicalis, C.parapsilosis, C.krusei* and *C.glabrata.*

In previous study carried out in our department in 2011 in immunocomprised patients, *C.albicans* was isolated from 96.51% of clinical isolates from patients of candidiasis. Sensitivity of *C.albicans* to fluconazole was 98.79% and resistance was 1.2%.[(1)] Candidiasis is an opportunistic infection occurring in presence of predisposing factors such as extensive and prolonged use of broad-spectrum antimicrobials, corticosteroids, immunosuppressive agents and cytotoxic drugs, diabetes mellitus, immunosuppression, chronic renal failure, hemodialysis, renal transplantation and indwelling urinary catheter.[(2)]Candida spp. are part of the human body's microbiota (i.e. normal flora) but they also have become endemic in most hospital infections which may be caused by endogenous yeasts or may be acquired in the hospital.[(3)]Biofilms are defined as structured microbial communities that are attached to a surface and encased in a matrix of exopolymeric material.[(4)]Biofilms represent the most prevalent type of microbial growth in nature and are crucial to the development of clinical infections. They can serve as a nidus for disease and are often associated with high-level antimicrobial resistance of the associated organisms.[(5)]

## Methodology

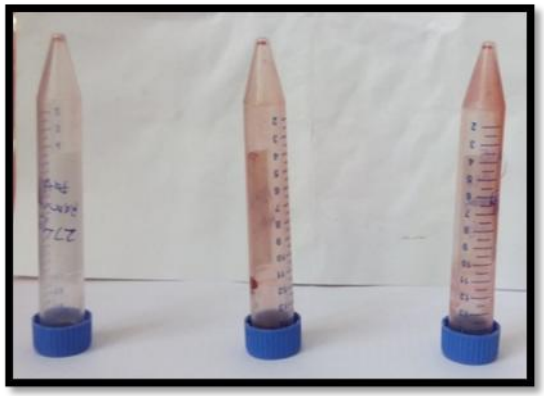
A total of 100 Candida isolates from clinical specimens including urine , sputum, Foleys catheter tip, blood , ET secretions ,stool and other body

fluids were processed in the department of Microbiology during the study period. Candida spps. in clinical specimens were identified on direct microscopic examination as gram positive yeast-like cells with or without pseudohyphae.[6]Clinical specimens were inoculated on Sabouraud's dextrose agar slant and incubated at 37º C for 24-48 hours .Growth of yeast is identified as cream colored, smooth, pasty colonies[6 ] with sweet smell. Germ tube test was done for presumptive identification of *Candida albicans* and *Candida dublinensis* , both when incubated in serum at 37 º C for 2-4 hours produce long tube-like projections extending from yeast cells .[7] Identification of various Candida species was done on CHROME agar. Distinctive patterns of color are used to separate Candida species in mixed cultures[8]

**Biofilm detection by tube method.[9]**

Biofilm production was investigated by tube adherence method proposed by Yigit et al .[9] 10mlof Saborauds Dextrose broth with glucose (8%) was inoculated with test organism .The tubes were incubated at 35ºC for 48 hours. After incubation the broth from the tubes were gently aspirated .The tubes were twice washed with distilled water to remove non-adherent cells. The tubes were stained with 2% safranin for 10 min. Excess of stain was removed by rinsing with distilled water. Tubes were examined for the presence of adherent layer. The isolate was considered positive for biofilm formation when a visible film was seen on the wall and bottom of the tube.(Fig.1).

**Fig. 1 : Biofilm detection by tube method Antifungal susceptibility test**



Fluconazole and voriconazole susceptibility patterns was studied as per CLSI disc diffusion guidelines M44-A2 using fluconazole and voriconazole disc.[10]

Inoculum was prepared by picking 5 distinct colonies on Sabouraud dextrose agar. Colonies were suspended in 5ml of sterile saline and turbidity was adjusted to 0.5McFarland standard. The medium used was Mueller-Hinton agar+2% glucose and 0.5µg/ml methylene blue. Discs were applied and incubated at $37^0$ C. Plates were examined after 24-48 hours. (Fig 2)The sensitivity patterns were determined as per Clinical and Laboratory Standards Institute (CLSI) as shown in table 1.

**Table 1: CLSI guidelines**

| Antifungal agent | disc content (µg) | Resistant (mm) | Susceptible-dose-dependent (mm) | Susceptible (mm) |
|---|---|---|---|---|
| Fluconazole | 25 | ≤14 | 15-18 | ≥ 19 |
| Voriconazole | 1 | ≤ 13 | 14-16 | ≥17 |

**Fig 2: Antifungal susceptibility test**



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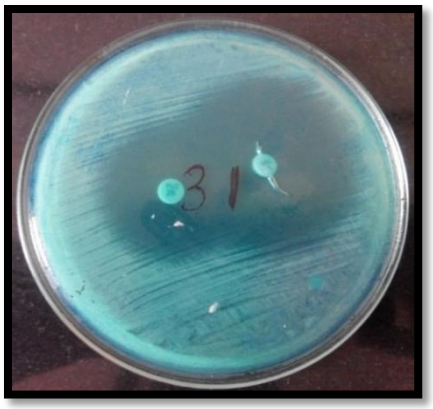

## Results

Out of 100 Candida isolated, 65(65%) were *C. albicans*, 21(21%) were *C. tropicalis*, 10 were *C. parapsilosis* (10%), 3 were *C.krusei* (3%) and 1 (1%) was *C. glabrata*.

Out of 100, 28 isolates were biofilm producers. Out of 28, 15 were *C.albicans* and 13 were non- *albicans Candida.*

Out of 65 *C.albicans*, 15 were biofilm producers (23.07%).

Out of 35 non- *albicans Candida*, 13 were biofilm producers. (37.17%) Out of 21 *C.tropicalis*, 8 were biofilm producers. Out of 10 *C.parapsilosis*, 4 were biofilm producers. Out of 3 *C.krusei*, 1 was biofilm producer. Sensitivity pattern to fluconazole and voriconazole was as shown in Table 2.

**Table 2: Antifungal susceptibility of Candida isolates to fluconazole and voriconazole.**

| Candida spp. | Fluconazole | | | | Voriconazole | | | |
|---|---|---|---|---|---|---|---|---|
| | Sensitive | | Resistant | | Sensitive | | Resistant | |
| | No | % | No | % | No | % | No | % |
| *C.albicans* | 50 | 76.9 | 15 | 23.07 | 60 | 92.3 | 5 | 7.6 |
| *C.tropicalis* | 16 | 76.19 | 5 | 23.8 | 19 | 90.4 | 2 | 9.5 |
| *C.parapsilosis* | 6 | 60 | 4 | 40 | 8 | 80 | 2 | 20 |
| *C.krusei* | 0 | 0 | 3 | 100 | 3 | 100 | 0 | 0 |
| *C.glabrata* | 0 | 0 | 1 | 100 | 1 | 100 | 0 | 0 |

Biofilm producers in *C.albicans* were resistant to fluconazole but 66.66% were sensitive to voriconazole .

In *C.parapsilosis* , biofilm producers were resistant to fluconazole but 50% were sensitive to voriconazole.

In *C.tropicalis*, 37.5% were resistant to fluconazole but sensitive to voriconazole and 25% were resistant to both antifungal drugs.

**Table 3: Antifungal susceptibility pattern by all Candida isolates among various studies to fluconazole**

| Study series | Year | fluconazole susceptibility |
|---|---|---|
| **Adhikary et al[13]** | 2011 | 75% |
| **Amit Kumar et al** | 2013 | 70.83% |



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| | | |
|---|---|---|
| **Mondal S** | 2013 | 70.2% |
| **Jayapriya et al** | 2012 | 62% |
| | | |
| **Nidhi Barot et al** | 2015 | 81% |
| **AnjanaGopi et al** | 2014 | 83.6% |
| **Present study** | 2015 | 72% |

**Table 4: Antifungal susceptibility pattern by all Candida isolates among various studies to voriconazole**

| Study series | Year | Voriconazole susceptibility |
|---|---|---|
| **Adhikary et al (13)** | 2011 | 100% |
| **AnjanaGopi et al** | 2014 | 86.8% |
| **Makhado et al (14)** | 2014 | 93.8% |
| **Barot et al** | 2015 | 97% |
| **Present study** | 2015 | 91% |

In present study 72% of *C.albicans* were sensitive to fluconazole. It is comparable with study conducted by Adhikary et al[14] which reported 75% of fluconazole susceptibility by all Candida isolates. (Table 3)

In present study 91% of all Candida isolates are sensitive to voriconazole which is correlating with study conducted by Makhado et al which reported 93.8% of all Candida isolates to voriconazole[15](Table 4)

**Discussion**

Biofilm formation play an important role in the pathogenicity of Candida species by the adherent to the cell surface to the host. Biofilm formation is the most common mode of fungus growth in nature that lead to a major clinical infection and serious disease in the immunocompromised patients and patients from ICU and NICU. [11] In view of this ,a reliable and easy method for their diagnosis is necessary.

In present study ,Biofilm production in *C.albicans* was (23.07%).It is co-relating with Kaskatepe et al study[12] which shows 21.21% biofilm production in *C.albicans* and Tumbarello et al[13] which study 22.6% of biofilm production in *Candida albicans.*

Biofilm production among NAC in present study was 37.17% which is in comparison Tumbarello et al study which reported 33.33% of biofilm production among Non-*albicans Candida*.[13]

In present study highest biofilm production was seen in *C.parapsilosis* (40%) followed by *C.tropicalis*(38%).

In Kaskatepe et al also, the highest biofilm production was found among *C.parapsilosis* isolates (66.66%) followed by *C.tropicalis* (64.28%).[12]



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## Conclusion

To conclude that biofilm production is 28% in present study .Biofilm production is higher among non-*albicans Candida* (37.17%) than *C.albicans*(23.07%)

Biofilm formation as a virulence factor might have a higher significance for non- *albicans Candida* species than for *C. albicans* and also this ability to form biofilms is intricately linked with the ability of the organisms to adhere, colonize and subsequently cause infection in susceptible individuals and resistance to antifungal drugs.

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