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Candida biofilm: A study on characterisation, speciation and a comparative analysis of the effects of antifungal drugs on biofilm producers and non-biofilm producers

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Abstract
Introduction: Microbes build biofilms which is the potential cause of persistent infections which acts as the defense against host immune system and finally results in resistance to antimicrobial treatment. *Candida* is the most common opportunistic fungal pathogen among immunocompromised individuals.
Aims and objectives: To compare the effect of Antifungal drugs on biofilm producers and non-biofilm producers among *Candida* isolates.
Materials and Methods: *Candida* species isolated from the clinical materials received in our laboratory were identified by conventional methods. Biofilm formation was tested by tube method. Antifungal susceptibility was done and results were compared between biofilm producers and non-biofilm producers.
Results: Fifty four *Candida* species were isolated over a period of 3 months, of which *C. albicans* were 63% followed by *C. tropicalis* 24% and *C. krusei* 13%. Out of which 41% of *C. albicans* and 16% of *C. tropicalis* and 9% of *C. krusei* were biofilm producers.
Conclusion: Biofilm producers of *C. albicans* and *C. tropicalis* showed higher resistance to antifungal drugs in this study.

Keywords: Candidiasis, biofilm, antifungal susceptibility testing.

1. Introduction
C. albicans is the most common cause of candidiasis, although there is increased frequency of non-albicans *candida* species isolated from clinical specimens [1]. *Candida* biofilms have recently gained great attention due to their high prevalence and their notorious resistance to antifungal drugs [2, 3]. *Candida* biofilms can contribute to both superficial and systematic candidiasis [4, 5] Invasive medical procedures and long duration of hospital stay are becoming increasingly common, which has led to the increased frequency of candidiasis [6]. All species of *Candida* causes diseases ranging from superficial infections such as oral thrush to invasive disease like endocarditis with marked differences in severity and susceptibility to different antifungal agents.[7] Microbes build biofilms which is the potential cause of persistent infections which acts as the defense against host immune system and finally results in resistance to antimicrobial treatment [8-11]. Extensive use of antimicrobial drugs for prolonged therapeutic courses has led to the change in the relative prevalence pattern of *Candida* species, with increased isolation of various *non-albicans Candida* species [12].
Non-*albicans* species like *C. tropicalis, C. krusei, C. glabrata* and *C. parapsilosis* are exhibiting increased tendency of resistant to antifungal action of azole groups of drugs particularly to fluconazole than *C. albicans*. All this leads to increase in the hospital stay in patients with fungal infections warranting rapid identification and antifungal susceptibility testing at the earliest [13].

2. Materials and Methods
Clinical samples received from out-patient and in-patient departments of K.V.G. Medical College and Hospital and swabs from oral cavity lesions of suspected cases of candidiasis from K.V.G. Dental College and Hospital, were taken up for the study. All the specimens were processed for the isolation of *Candida* spp. using Standard Mycology methods [14].

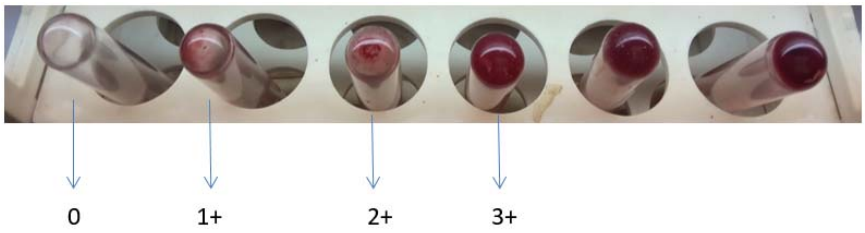
Gram staining was performed from direct specimen and the specimens were inoculated on Sabouraud's dextrose agar and were incubated at 37 ^{0}C for 24 hrs. Germ tube test was done for all the budding yeast cells and the positives identified were either *C. albicans* or *C. dubliniensis*. *C. albicans* were further identified by growth at 45 ^{0}C and chlamydospore formation on corn meal agar. All the isolates were subjected to Sugar fermentation test and Sugar assimilation test for final confirmation of species [15-17]. Biofilm production was detected by tube method described by Brachini *et al.* [18]. A loopful of organisms from Sabouraud's Dextrose agar plate was inoculated into Sabouraud's Dextrose broth supplemented with glucose 8%. The tubes were then incubated at 37 ^{0}C for 24 hours after which the broth was aspirated out gently. The tubes were then washed once with distilled water and then stained with 1% Safranin. The tubes were then kept still for 7 minutes. Safranin then was removed and tubes were examined for biofilm production. Biofilm production was tested twice and read independently by two different observers. The adherent biofilm layer was scored visually as negative (0), weak positive (1+), moderate positive (2+) or strong positive (3+). Antifungal susceptibility testing was performed for all the isolates by disc diffusion method on Mueller Hinton agar supplemented with 2% glucose and 0.5 µg/mL of methylene blue [19]. Commercially available antifungal disc were used and zones of inhibition were measured after 24-48 hours incubation at 37 ^{0}C. Nystatin (100units) amphotericin B (100units), clotrimazole (10mcg), itraconazole (10mcg), fluconazole (10mcg) and voriconazole (1mcg) discs were used. *Candida albicans* MTCC3017, was used as quality control strain [20].

3. Results

A total of 54 *Candida* species were isolated, out of which 34(63%) were *C. albicans* and 20(37%) were non-*albicans Candida* isolated. Among the 54 *Candida* isolates, 19(35%) were from vaginal swabs, 15(28%) were from urine, 10(18%) were from sputum, 8(15%) were from oral lesions and 2(4%) were from blood. Among the isolates, 34(63%) were identified as *C. albicans,* 13(24%) were *C. tropicalis* and 7(13%) were *C. krusei*.

A total of 33(61%) isolates produced biofilm, of which 16(29%) were strong, 7(13%) were moderate, 10(18%) were weak biofilm producers and 21(39%) were non-biofilm producers. (Fig. 1)

3.1 Grading of Biofilm Production



3+- Strong biofilm producer
2+- Moderate biofilm producer
1+- Weak biofilm producer
0- Non biofilm producer

Out of 34 *C. albicans*, 64.7% were biofilm producers, out of 13 isolates of *C. tropicalis* 69.2% were biofilm producers. In case of *C. krusei* out of 7, 28.5% were biofilm producers.

All the isolates of *C. albicans*, *C. tropicalis* and *C. krusei* were 100% sensitive to amphotericin-B. All the isolates of *C. krusei* were 100% sensitive to all the antifungal drugs tested. *C. albicans* were sensitive to nystatin (55.8%), fluconazole (20%), itraconazole (17.6%) and clotrimazole (14.7%). *C. tropicalis* were found to be sensitive to nystatin (100%), fluconazole, itraconazole and clotrimazole (46%) each.

3.2 Percentage of susceptibility of *C. albicans* isolates to various antifungal drugs tested in this study among biofilm producers and non-biofilm producers (Table. 1)

Antifungal drugs	Biofilm producers	Non biofilm producers
Fluconazole	22(9.09%)	5(41.6%)
Itraconazole	1(4.54%)	5(41.6%)
Clotrimazole	1(4.54%)	4(33.3%)
Voriconazole	5(22.72%)	4(33.3%)
Nystatin	13(59.0%)	6(50.0%)

Percentage of susceptibility of *C. tropicalis* isolates to various antifungal drugs tested in this study among biofilm producers and non-biofilm producers. (Table.2)

Antifungal drugs	Biofilm producers	Non biofilm producers
Fluconazole	2(23.0%)	3(75.0%)
Itraconazole	2(23.0%)	3(75.0%)
Clotrimazole	2(23.0%)	3(75.0%)
Nystatin	6(69.2%)	4(100%)

4. Discussion

In the present study, *C. albicans* was the most predominant species isolated from various clinical samples which is similar to the study done by Jin *et al.* [21] followed by *C. tropicalis* and *C. krusei*. These findings are similar to the report published by Dharwad *et al.* [22] and Chaudhary *et al.* [23]. In the current study 66% of the isolates were found to be biofilm producers, of which 41% were *C. albicans* followed by 16% *C. tropicalis* and 9% *C. krusei*. This is in agreement with the studies done by of Dhale *et al.* [24] and Kumar *et al.* [25]. Most of the isolates were susceptible to amphotericin–B as demonstrated by Bhat *et al.* [26] in their study in 2015. Production of biofilm by *C. albicans* and *C. tropicalis* is similar to the study done by Golia *et al.* [27].

5. Conclusion

The biofilm formation is one of the virulence factors which would help the organism to adhere, colonize and cause infection in susceptible host. The biofilm producers have tendency towards more resistance to the antifungals necessitating the need for the early detection of Candidal infection with special reference to identification of various virulence factors and susceptibility testing of antifungal drugs.

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