Research Article

Distribution of *Candida albicans* and non-albicans Candida *in clinical* samples and their intrinsic biofilm production status

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Abstract

Background: Candida species, mainly *Candida albicans* are traditionally associated with severe and debilitating diseases especially in immunocompromised hosts. Biofilm is emerging virulence factor in fungi and has been correlated with pathogenicity among Candida species. The emergence of *C. albicans* and non-albicans Candida (NAC) species producing biofilms and severe or recurrent infections in hospitalized patients with its attendant treatment failure and poor prognosis has become a great concern globally.

Objective: To determine the species distribution of Candida organisms (*C. albicans* and NAC) from clinical samples and their pathogenic ability to produce biofilms; and to highlight the clinical implications of these extracellular substances to aid preventive measures, chemotherapy, and prognosis.

Materials and Methods: This was a descriptive, cross-sectional study, and was carried out at SRM MCH & SR, Tamil Nadu, India. Between February 2014 and January 2015, a total of 90 Candida fungal isolates recovered from clinical samples including urine, pus, vaginal swab, skin scrapping, sputum, and blood were analyzed. Samples were cultured on Sabouraud Dextrose Agar with gentamycin. Candida organisms were identified by standard methods. Germ tube rapid test was used to differentiate *C. albicans* and *Candida dublinieses* from other Candida species. Further speciation of the isolates was carried out by culture on CHROM agar and Corn meal-Tween 80 agar, including sugar fermentation and assimilation tests. Biofilm production was detected using Congo red method. Results were analyzed statistically.

Result: A total of 90 Candida organisms were recovered from clinical specimens of which 33 (36.7%) were *C. albicans* and 57(63.3%) were NAC species. Majority of the isolates were recovered from urine (42, 46.7%), vaginal swab (20, 22.2%), and pus (11, 12.2%) samples. Among NAC species, the most common isolate was *C. tropicalis* (23, 25.6%) followed by *C. parapsilosis* (15, 16.7%). Of the 90 Candida species analyzed, 26 (28.9%) gave positive results for biofilm production. Overall, biofilm formation was detected more frequently among NAC species (16, 61.5%) than in *C. albicans* (10, 38.5%). Among NAC species, *C. tropicalis* (12, 46.2%) produced biofilm most frequently than other members of the group. Although, most of the Candida isolates strongly producing biofilms were members of NAC species particularly *C. tropicalis* (3, 50%), nonetheless, majority of the weakly biofilm producers were also detected among the strains of *C. tropicalis* (9, 45%).

Conclusion: The outcome of this study shows a notable shift in the pathogenic incidence of Candida species from *C. albicans* to NAC species with significant rate of pathogenic biofilm production. Biofilm production was most common in *C. tropicalis* than other members of NAC species whereas slime formation was not detected in *C. glabrata* species. There is need to create awareness among the populace and stakeholders on healthcare system management about this emerging scenario in Candida species pathogenicity, biofilm production, and clinical repercussions for appropriate measures to checkmate the trend.

KEY WORDS: *Candida albicans*, non-albicans Candida, distribution, biofilms, pathogenicity

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Introduction

Candida species are known to cause serious debilitating diseases especially in immunocompromised hosts, resulting in significant mortality.[1,2] Biofilm has emerged as an intrinsic component virulence factor in fungi and has been associated with pathogenicity among Candida species.[3–5]

Candida infections in the last two decades have been aggravated by increasing and widespread use of various medical implant devices mainly in immunologically debilitated patients.[6,7] The increasing occurrence of opportunistic Candida disease was predicated on the frequent presence of Candida species in the normal ecological niche of human body surfaces. Consequently, this scenario often facilitates close encounter between Candida organisms and most medical implanted devices and host surface resulting in acute, chronic, or recurrent infections.[8,9]

Biofilm production is widely acknowledged as an important component of virulence factors of microbial organisms because (1) it aids the producing organism to withstand or evade host defense mechanism and its destructive effect, (2) it enables the organism to survive and exist as reservoir and recurrent source of infection, as well as development of resistance to antimicrobial agents.[10–15] Consequently, Candida biofilm imposes serious adverse effects on the health and well-being of patients by preventing optimum functioning of implant devices, production of treatment failure resulting in aggravated morbidity, poor prognosis, prolonged hospital stay, and high socioeconomic cost.[1,13,14]

Although *C. albicans* is the species most commonly associated with candidemia and biofilm formation,[15,16] however, various reports[17–19] have indicated the increasing involvement of NAC in fungal opportunistic infections and biofilm production culminating in serious clinical repercussions.

Despite a large volume of reports on *C. albicans* as a pathogen and biofilm producer, however, data on NAC species as emerging pathogens with significant level biofilm production are scanty. Therefore, this study aims to assess the distribution of different species of Candida organisms (i.e., *C. albicans* and NAC species) in clinical samples and their pathogenic ability to produce biofilm; and also highlight the clinical implications of this extracellular substance to aid preventive measures and checkmate the spread of Candida-associated diseases.

Materials and Methods

This was a descriptive, cross-sectional study. A total of 90 Candida species were recovered following culture, from 143 clinical specimens obtained from patients attending Tamil Nadu Tertiary Healthcare Centre, India between February 2014 and January 2015. The clinical samples include urine ($n = 55$), pus ($n = 18$), vaginal swab ($n = 39$), sputum ($n = 16$), skin scrapping ($n = 9$), and blood ($n = 6$). All samples were initially examined using normal wet mount (while potassium hydroxide mount was used for skin scrapings), followed by culture on Sabouraud Dextrose Agar (SDA) with Gentamycin (to prevent bacterial growth) for upward of 48 h at 37 °C. Patients who were on any type of medical implants were included in the study whereas those on any form of antifungal therapy 6 weeks prior to the commencement of the research were excluded from the study.

Identification of Candida species was carried out using standard conventional methods,[17] (as API Candida species identification system could not be accessed during the study period) including Gram staining of developing colonies on SDA. Rapid germ tube test (Reynold's Brande Phenomenon) was used to differentiate *C. albicans and C. dublinenses* from other Candida Species. Further speciation of the isolates was carried out by culture on CHROM Candida differential agar, whereas microscopic morphology of the isolates recovered from Corn meal-Tween 80 agar was noted. The characteristics reactions of the Candida species arising from carbohydrate/sugar fermentation and assimilation tests[20] were observed and recorded accordingly. All isolates were subcultured on SDA before further testing to maintain viability and purity.

Detection of Biofilm Formation

The in vitro screening test for biofilm production in Candida isolates was carried out using modified Congo red agar method as described by Saxena et al.[21] The utilized solid media is composed of Brain Heart Infusion (BHI, 37 g/L) broth supplemented with glucose (80 g/L), agar base No. 1 (10 g/L), and Congo red stain (0.8 g/L).

Briefly, Congo red was prepared as concentrated aqueous solution and sterilized via autoclaving at 121°C for 15 min. The agar medium was allowed to cool to about 55 °C followed by addition of Congo red solution to form a complete media required for the test. Developing colonies of identified Candida species subcultured on fresh SDA was inoculated on plates of Congo red agar and incubated aerobically at 37 °C for 24–48 h. Positive and strong biofilm production by test organism (i.e., Candida species) was indicated by appearance of dark red colonies, whereas biofilm negative Candida species produced white or very light pink colonies. However, weakly biofilm producing organism appeared pink. To validate our results, culture of *C. albicans* (ATCC 90028) and *Staphylococcus epidermidis* (ATCC 12228) were set up as controls for positive and negative biofilm-producing organisms, respectively.

Statistical Analysis

Data were analyzed with Epi info (Version 6.04, CDC, Atlanta, GA). The prevalence of Candida species isolates from clinical samples was expressed in simple proportion or percentages. Comparison of prevalence of *C. albicans* and NAC species, and association between the Candida species and biofilm production were analyzed using χ^2-test. A *P*-value of <0.05 was considered statistically significant. The approval to carry out the study was sought and approved by Ethical Committee of SRM MCH & RC, Tamil Nadu, India.

Result

Analysis of our results shows that of the 90 Candida isolates recovered from clinical samples, 33 (36.7%) were *C. albicans* and 57 (63.3%) were NAC species and the difference was statistically significant ($P < 0.05$; $P = 0.022$). Majority of the isolates were recovered from urine (42, 46.7%), vaginal

swab (20, 22.2%), and pus (11, 12.2%) samples [Tables 1 and 2]. Among NAC species, the most common isolate was *C. tropicalis* (23, 25.6%) followed by *C. parapsilosis* (15, 16.7%) [Table 3]. Of the 90 Candida species analyzed, 26(28.9%) isolates gave positive result for biofilm formation [Table 4]. Overall, biofilm formation was detected more frequently among NAC species (16, 61.5%) than in *C. albicans* (10, 38.5%) and was statistically significant ($P < 0.05$; $P = 0.02$). Among NAC species, *C. tropicalis* (12, 46.2%) formed biofilm most frequently than other members of the group [Table 5] and the difference was found to be statistically significant ($P < 0.05$; $P = 0.04$).

Although, most of the Candida isolates strongly producing biofilms were members of NAC species particularly *C. tropicalis* (3, 50%), nonetheless, majority of the weakly biofilm producers were also recorded among the strains of *C. tropicalis* (9, 45%).

Discussion

Candida species are known to exist as either commensals or opportunistic pathogens with capacity to produce a wide range of superficial and deep systemic diseases especially in immunologically debilitated hosts.[3,22] *Candida albicans* and NAC species producing biofilms have emerged as important agents of Healthcare Associated Infections (HAIs) with increasing severe morbidity and significant mortality.[16,23] Species identification and biofilm formation have become important elements in the determination of Candida pathogenicity and outbreak investigations for necessary clinical and chemotherapeutic interventions.

In this study, 90 Candida isolates from various clinical specimens were analyzed. There was preponderance of NAC

Table 1: Rate of isolation of *Candida albicans* and non-albicans Candida from clinical samples

Clinical Sample	No. of sample examined	No. positive for Candida species	*C. albicans* (*n*, %)	Non-albicans Candida (*n*, %)
Urine	55	42	13 (39.4)	29 (50.9)
Pus	18	11	3 (9)	8 (14)
Vaginal swab	39	20	11 (33.3)	9 (15.8)
Sputum	16	8	2 (6.1)	6 (10.5)
Skin	9	7	2 (6.1)	5 (8.8)
Blood	6	2	2 (6.1)	0 (0)
Total (%)	143	90	33 (36.7)	57 (63.3)

$P < 0.05$; $P = 0.022$.

Table 2: Distribution of Candida species isolates in different clinical specimens

		No. of Candida isolate from clinical sample					
Candida spp.	**No (%) of isolate**	**Urine**	**Pus**	**Vagina swab**	**Skin**	**Blood**	**Sputum**
C. albicans	33(36.7)	13	3	11	2	2	2
C. tropicalis	23(25.6)	13	3	2	3	0	2
C. parapsilosis	15(16.7)	7	3	3	1	0	1
C. krusei	11(12.2)	5	2	2	0	0	2
C. glabrata	8(8.8)	4	0	2	1	0	1
Total (%)	90(100)	42(46.7)	11(12.2)	20(22.2)	7(7.8)	2(2.2)	8(8.9)

Table 3: Distribution of Candida species isolated from different clinical samples

Clinical sample	No. of isolate	No (%) of Candida species isolate				
		C. albicans	Non-albicans Candida			
			C. tropicalis	*C. parapsilosis*	*C. crusei*	*C. glabrata*
Urine	42	13(39.4)	13(56.5)	7(46.7)	5(45.5)	4(50)
Pus	11	3(9)	3(13)	3(20)	2(18.2)	0(0)
Vaginal swab	20	11(33.3)	2(8.7)	3(20)	2(18.2)	2(25)
Skin	7	2(6.1)	3(13)	1(6.7)	0(0)	1(12.5)
Blood	2	2(6.1)	0(0)	0(0)	0(0)	0(0)
Sputum	8	2(6.1)	2(8.7)	1(6.7)	2(18.2)	1(12.5)
			23(40.4)	**15(26.3)**	**11(19.3)**	**8(14)**
Total (%)	90	33(36.7)	57(63.3)			

Table 4: Biofilm production among *Candida albicans* and non-albicans Candida species

Candida spp	No. (%) of isolate	No. (%) of biofilm producing isolate	No (%) of Biofilm negative isolate	No. (%) of biofilm positive:	
				Strong producer	Weak producer
C. albicans	33(36.7)	10(38.5)	23(69.7)	2(20)	8(80)
Non-albicans Candida	57(63.3)	16(61.5)	41(71.9)	4(25)	12(75)
Total (%)	90	26(28.9)	64(71.1)	6(23.1)	20(76.9)

$P < 0.05$; $P = 0.02$.

Table 5: Biofilm formation among Candida species isolates

Candida spp.	No. (%) of isolate	No. (%) of biofilm negative	No (%) of biofilm positive	No. (%) of biofilm positive:		
				Strong producer	Weak producer	
C. albicans	33(36.7)	23(69.7)	10(38.5)	2(33.3)	8(40)	
C. tropicalis	23(25.6)	11(47.8)	12(46.2)	3(50)	9(45)	
C. parapsilosis	15(16.7)	12(80)	3(11.5)	1(16.7)	2(10)	
C. krusei	11(12.2)	11(100)	0(0)	0(0)	0(0)	
C. glabrata	8(8.9)	7(87.5)	1(3.8)	0(0)	1(5)	
Total (%)	90	64(71.1)	26(28.9)	6(23.1)	20(76.9)	26(28.9)

$P < 0.05$; $P = 0.04$.

species (57, 63.3%) in our clinical isolates as compared with *C. albicans* isolates (33, 36.7%) and the difference was statistically significant ($P < 0.05$). This result corroborates the outcome of the studies published by Sida et al.[17] and Mijica et al.[18] which underscored the shifting trend of Candida species infection toward NAC organisms. It is therefore evident that NAC species are increasingly assuming important and widely recognized opportunistic pathogens particularly in HCAIs. The implication of the current trend is that hospitalized patients with certain risk factors (including immunosuppression as in HIV/AIDS, cancer, diabetes mellitus, organ transplant, or medical implantation etc.) are at greater risk of being exposed to multiple infections caused by NAC species other than *C. albicans* resulting in increased morbidity and fatal consequence.[17]

Biofilm formation is increasingly recognized as an important virulence factor of Candida species.[24,25] Production of these extracellular slimes or biofilms by microorganisms generally is associated with serious clinical implications and may influence the outcome of chemotherapy, complete recovery of patient, and prognosis. Investigations[12,26] with biofilm-producing fungal organisms have revealed that Candida species have (among other clinical repercussions) a substantially reduced susceptibility to antifungal agents due to reduced drug penetration into biofilms and may lead to treatment failure.

Analysis of our results showed that biofilm production was more common among NAC species (16, 61.5%) than *C. albicans* (10, 38.5%) and was statistically significant ($P < 0.05$). This result is in agreement with the reports published by Girish and Menon[27] as well as Muni et al.,[28] who recorded similar results in their studies. This is not surprising because the catheter disc (from which most of the NAC species were isolated via the urine originating from it) (data not shown) is widely known to increase the synthesis of biofilm consisting of mono- or multilayer of cells embedded within a matrix of extracellular polymeric material.[29] Release of microorganisms from the biofilm may initiate acute disseminated infections as well as chronic or recurrent diseases.[12,30–32] Such scenario has been documented by other investigators in catheter-associated candidemias caused by NACs.[2,17,29]

In this light, biofilm production among NAC species may add pathogenic credential to this group and aid its potential to further cause severe and difficult to resolve systemic infections, evade host defenses, resist chemotherapy, and thus seriously jeopardizing the health recovery of infected patients.[31,33]

Among NAC species, *C. tropicalis* showed a much greater propensity for biofilm formation (12, 52.2%) than other members of the group. This outcome, however, contrasts the reports by Sida et al.[17] who noted significantly higher rate of biofilm production in *C. parapsilosis* as compared to *C. tropicalis*. However, it was in agreement with the data published by other workers[11,29,31] elsewhere in the same region.

In this study, 29 (28.9%) of our Candida species isolates were found to produce biofilms. We however, observed that the rate of biofilm production by our isolates was significantly lower than those Candida species isolated elsewhere as reported by Muni and colleagues (64%),[28] including Mohandas and Ballal (73%).[11] On the other hand, biofilm formation was not detected in *C. krusei* species, whereas one isolate of *C. glabrata* species produced non-elaborate or limited extracellular polymeric substance during our study. However, Hawser and Douglas[26] reported that the rate of biofilm production among Candida species isolates may be influenced by various factors including sources of isolates, culture, and incubation methods (gentle shaking versus static incubation); media constituents

or sugar utilization by the organism (e.g., the use of glucose, galactose, etc.). These factors may have influenced differences in results of various researchers during their investigations on Candida biofilm formation including the results of this study. Moreso, various reports[5,10,12,15] have reiterated that biofilm production is a new phenomenon in fungi; its mechanism of pathogenicity, evasion of host's defenses, and resistance to antimycotic agents are still poorly understood.

Conclusion

The results of this study have shown a notable shift in the pathogenic incidence of Candida species from *C. albicans* to NAC species with significant rate of pathogenic biofilm production. Biofilm production was most common in *C. tropicalis* than other members of NAC species whereas slime formation was not detected in *C. krusei* species. Hence there is need for improved knowledge of Candida species from various clinical sources in terms of biofilm production and associated pathogenicity or clinical repercussions that will aid the adoption of appropriate management and control strategies to limit the associated morbidity and mortality.

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