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Original Research Article

Detection of Biofilm Production and its Quantification in *Candida* Isolates in a Tertiary Care Hospital

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Abstract

Introduction: *Candida* biofilms adversely impact the health of the patients with increasing frequency and severity of disease and with soaring economic sequel. ***Objective:*** Qualitative detection of biofilm production and its quantification was performed in *Candida* isolates from patients infected with health care associated infection (HCAI). ***Method*:** A total of 55 Candida isolates were included in the study. Biofilm production was estimated by Tube method (TM) and Tissue culture plate method (TCP). Further quantification of the biofilm produced was performed by XTT (2, 3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) reduction Assay and Dry weight measurement method. ***Result:*** All Candida isolates were found to be biofilm producers by all three (TM, TCP and XTT) methods**.** Quantity of biofilm produced by *C. albicans* ranged between 2.3 to 9.1 mg/disk. Among *non-albicans Candida Candida tropicalis*) it was between 2.2 to 7.3 mg/disk whereas *non-albicans Candida* (except *C. tropicalis*) weight of the biofilm was 2.0 to 7.1 mg/disk. ***Conclusion:*** Dry weight (DW) is the actual quantity of biofilm produced. *Candida albicans* produced higher quantity of biofilm than *non-albicans Candida* in the study. It is also concluded that quantitative detection of biofilm is definitely help clinician in deciding modality of treatment.

Keywords: Biofilm, XTT, Dry weight, *Candida albicans*, *non-albicans Candida*.

INTRODUCTION

Candida species is one of the most frequently encountered opportunistic fungi that cause severe infection in humans because of its virulence factors. Biofilms have great significance in public health, because biofilm-associated *Candida* exhibit dramatically decreased susceptibility to antifungal agents. *Candida* biofilms adversely impact the health of the patients with increasing frequency and severity of disease and with soaring economic sequel. Fungal infections develop frequently in immune-compromised patients, particularly in patients with prolonged, severe neutropenic episodes and with indwelling medical devices [1]. In one of the earliest studies documenting the ability of *Candida* to form biofilms, Marrie and Costerton reported formation of *Candida parapsilosis* biofilms on vascular catheters [2]. Initial studies also reported that *Candida* biofilms formed on different surfaces including Hickman catheters [3], soft contact lenses, ureteral stents [4], and corneas [5]. Subsequent studies have demonstrated that *Candida* biofilms can form on a wide variety of indwelling medical devices including dentures, central venous catheters (CVCs), and urinary catheters. These biofilms exhibit decreased susceptibility to most antimicrobial agents, which contributes to the persistence of infection [6].

Various methods are available for the qualitative and quantitative detection of *Candida* biofilms. Some of the commonly used qualitative methods of biofilm detection methods are visual and spectrophotometric methods [7-9] (Congo Red Agar, Tube method and Micotitre plate method, whereas quantitative method includes biochemical assay, i.e., the 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT) reduction assay and dry weight (DW) measurements [10].

OBJECTIVE OF THE STUDY

The study was conducted to quantify biofilm production of *Candida* spp. isolated from different clinical samples of admitted patients of GIPMER, New Delhi.

METHODS

Various clinical samples received from patients as per merit of the case (admitted in ICUs and wards) were processed as per standard microbiological methods. These *Candida* isolates were identified by conventional methods, CHROM agar and VITEK II.

Candida Biofilm Detection

After identification of the isolates they were further processed for determination of biofilm production. For qualitative detection of *Candida* biofilm production methods used were visual (Tube Method) and spectrophotometric methods (Tissue Culture Plate Method) [7-9].

Quantification of Biofilm production by the *Candida spp.* was performed by XTT reduction assay and Dry weight (DW) measurement [10].

- **Dry weight:** DW measurements represent total biofilm mass, including fungal cells and extracellular matrix.
- **Wet weight (WW)**: WW measurements represent the entire, hydrated mass of biofilm.

RESULTS

This prospective study was conducted in the department of microbiology; G. B. Pant Institute of Post Graduate Medical Education and Research (GIPMER), New Delhi over a period of one year. Total 55 Candida isolates from patients of hospital acquired infections (HAI) were included in the study.

Maximum number of *Candida* isolates were from urine (catheterized) 47 (85%) followed by respiratory tract samples 02 (3%), body fluids 02 (4%), implants 02 (4%), blood 01 (2%) and tissue 01 (2%).

Table-1: Distribution of *Candida* species in clinical samples

Type of sample	No. of samples	*C. albicans*	*non-albicans Candida*
Urine (Catheterized)	47	12	35
Fluids	02	01	01
Implants(valves)	02	00	02
Blood	01	00	01
Respiratory samples	02	00	02
Tissues	01	01	00
Total	55	14	41

Maximum numbers of *Candida* isolates were from urine sample (47), out of which 12 were *C. albicans* and 35 were *non-albicans Candida.*

Detection of *Candida* biofilm

Qualitative detection of biofilm production was performed by Tube method (TM) and tissue culture plate (TCP) method.

Detection of *Candida* biofilm by TM

Results of biofilm production detected by TM depicted in Table 2.

Table-2: Biofilm production by tube method

		Candida sp.				
	Grading of biofilm	***C. albicans***	***non-albicans Candida***	**Total**	**Pearson Chi-Square**	**p-value**
Biofilm production by tube method	M	2	6	8	1.38	**0.501**
	S	10	33	43		
	W	2	2	4		
Total		14	41	55		

Note: *P* value less than 0.05 is significant.

By Tube Method (TM) 55 (100%) *Candida* isolates were detected as biofilm producers. Out of which, 43 (78.2%) were strong, 8(14.5%) were moderate and 04(7.3%) were weak biofilm producers.

Of the 14 *Candida albicans*, 10 (71.4%) were strong biofilm producers and 2 (14.3%) isolates each are moderate and weak biofilm producers whereas of the 41 isolates of *non-Candida albicans* 33 (80.5%), 6 (14.6%) and 2 (4.9%) were strong, moderate and weak biofilm producers respectively.

Biofilm production detected by TCP method

Findings of detection of biofilm production in *Candida* isolates by TCP method are given in the Table 3.

Table-3: Biofilm production by TCP method

		Candida sp.		Total	Pearson Chi-Square	p-value
	Grading of biofilm	***C. albicans***	***non-albicans Candida***			
Biofilm production by TCP method	M	1	5	6	0.352	**0.839**
	S	12	34	46		
	W	1	2	3		
Total		14	41	55		

Note: *P* value less than 0.05 is significant.

All 55 (100%) *Candida* isolates were detected as biofilm producers by Tissue Culture Plate (TCP) method. Out of which, 46 (83.63%) were strong, 6 (10.9%) were moderate and 3 (5.5%) were weak biofilm producers.

12 (85.7%) *C. albians* and 34 (82.9%) of *non-albicans Candida* (NAC) were strong biofilm producers. These 34 (NAC) strong biofilm producers included *C. tropicalis* 26, *C. famata* 2, *C. haemulonii* 2, *C. glabrata* 1, *C. lusitaniae* 1, *C. aspergilus* 1 and *C. rugosa* 1.

***Candida* Biofilm assay (Quantitative)**

Quantification of biofilm production of Candida sp. was carried out by XTT reduction assay.

***Candida* Biofilm detection by XTT reduction assay**

All Candida strains were biofilm producers by this method showing XTT activity above cutoff value, which was 0.025.

XTT activity observed in *Candida* strains are depicted in Table 4 and Figure 1.

Table-4: XTT activity of *Candida spp*

Candida spp.	**No of strains**	**XTT activity range**
Candida albicans	14	0.09-0.24
Candida tropicalis (*non-albicans*)	29	0.06-0.2
Non albicans Candida (except *C. tropicalis*)	12	0.06-0.192

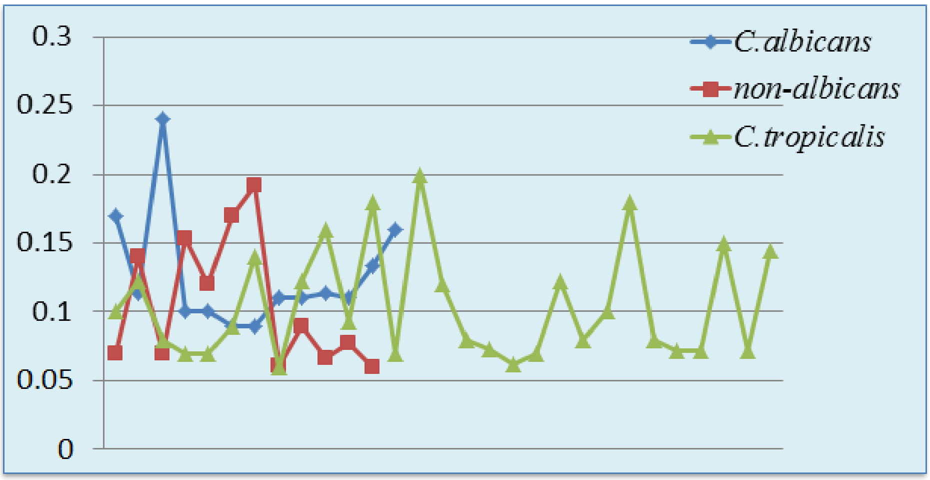

Fig-1: XTT activity of *Candida spp*

Maximum XTT activity shown by *Candida albicans* was 0.24 and minimum was 0.09, while in case of *non albicans Candida* it was observed that *C. tropicalis* exibits highest XTT activity (0.2) and lowest as 0.06. Among *non-albicans Candida* group (other than *C. tropicalis*) the maximum value of XTT activity was of *C. famata* (0.192) and minimum was of *C. aspergilus* (0.06).

Weight measurement (Quantification) of *Candida* biofilm

Quantification of biofilm produced by *Candida* isolates was performed by dry weight measurement assay and the findings are depicted in table 5 and Figure 2.

Table-5: DW and WW of biofilm formed by *Candida spp*

Candida spp.	No of strains	DW (mg/disk)	WW (mg/disk)
Candida albicans	14	2.3-9.1	2.8-10
Candida tropicalis (*non-albicans*)	29	2.2-7.3	2.4-7.5
Non albicans Candida (except *C. tropicalis*)	12	2.0-7.1	2.5-7.1

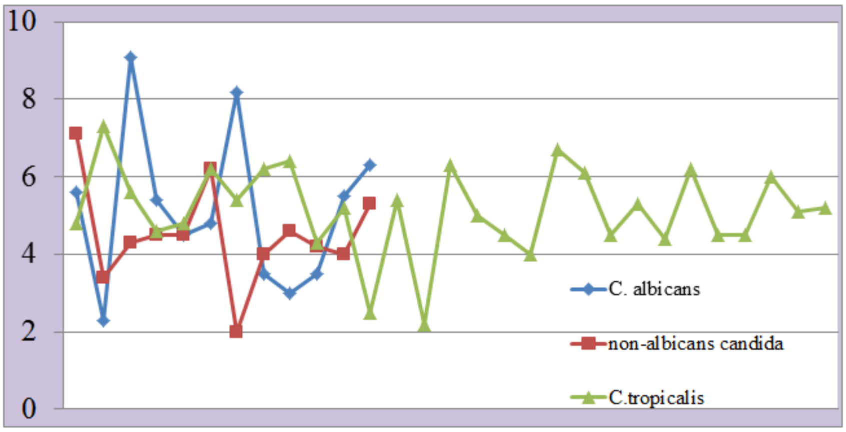

Fig-2: Quantity of biofilm produced by *Candida spp.* (Dry weight in mg/disk)

Actual quantity of biofilm production is the dry weight (DW) of the biofilm. Quantity of biofilm produced by *C. albicans* ranged between 2.3 to 9.1 mg/disk. Among *non-albicans Candida Candida tropicalis*) it was between 2.2 to 7.3 mg/disk whereas *non-albicans Candida* (except *C. tropicalis*) weight of the biofilm was 2.0 to 7.1 mg/disk.

Table-6: Comparative result of TCP method and dry weight method

Biofilm producing *Candida spp.*	No. of isolates	Range of dry weight of biofilm in mg/disk
Weak	3	2.3-3.4
Moderate and strong	52	>3.5 mg/disk

Quantity of dry weight (which is the actual amount of biofilm produced by an organism) was below 3.5 mg/disk for all weak biofilm producers whereas in quantity of dry weight was above 3.5 mg/disk for moderate and strong biofilm producers. In other words we can say that results of both correlated very well with each other in the present study.

DISCUSSION

Candida is one of the most frequently encountered opportunistic fungi that cause severe infection in humans because of its virulence factors. The ability of *Candida* to form biofilms and adhere to host tissues and biomaterial surfaces is an important factor in its pathogenesis. The main characteristics of biofilm are that they are resistant to broad spectrum anti-fungal drugs.

Among 55 *Candida* isolates from patients having Hospital acquired infections, 14 (25.45%) were *Candida albicans* whereas 41(74.55%) were *non-albicans Candida*. Amongst *non-albicans Candida, C. tropicalis* 29 (52.72%) was the most common strain isolated followed by *C. haemulonii* 3 (5.45%), C. *famata* 3 (5.45%), *C. glabrata* 02 (3.64%), *C. parapsilosis* 01 (1.82%), *C. rugosa* 01 (1.82%), *C. lusitaniae* and *C. aspergillus* 01 (1.82%).

The main underlying condition in patients with biofilm producing *Candida* species was catheterization and prolonged antibiotic therapy. All the 55 isolates tested for biofilm formation were found to be positive for biofilm production by both TM and TCP methods.

Shin *et al*. [11] observed that 39 % of Candida strains were biofilm producers, of which only 8% *C. albicans and* 61% *non-albicans Candida were* biofilm producers. Girish Kumar and Menon [12] observed in a study that of the 58 *Candida* isolates 82.8% were biofilm producers, which is almost similar to the finding of the present study. Dag *et al*. [13] study showed that 39.3% *Candida albicans* were biofilm producers, which is slight higher in comparison to *non-albicans Candida* 37.7% of which were biofilm producers.

In a study by Mohandas and Bhallal [14] it was detected that of the 111 *Candida* isolates 73% were biofilm producers, which is lower than present study. Muni *et al*. [15] reported biofilm production in 64% of the 50 *Candida* isolates by Tube method. According to a study by Nascimento *et al*. [16] 60.6% (36.4% high and 63.6% weak) were biofilm producers amongst 327 *Candida* isolates. Among them 43.1% of *C. albicans* and 75.8% of *non-albicans Candida* isolates were found to be positive for biofilm production. They also found that 94.6% of *C. tropicalis* were biofilm producers. These reported findings are less than present study finding.

Khatri *et al*. [17] in their study isolated 80 *Candida* isolates of which 61.25% isolates were positive biofilm producers by TCP method and TM detected 57.50% isolates as biofilm producers, which is less than the result of present study.

It was observed in most of the studies that *C. tropicalis* is the commonest isolates, which is similar to the present study. In this study, all of the *Candida* strains were biofilm producers, which may be due to the fact that all of them are isolated from hospital acquired infections.

XTT Reduction Assay

In the present study 100% of *Candida* isolates were found to be biofilm producers by XTT reduction assay. In a study by Dhale *et al.* 16.94% isolates were detected as biofilm positive strains by XTT reduction assay [18], which is in contrast with the present study, but their findings with other methods were also low. Whereas in the present study all isolates were biofilm producers as detected by two different methods. Nweze EI *et al*. observed that all the *Candida* isolates were biofilm producers by XTT reduction assay [19]. This is in accordance with the present study.

Quantification of Biofilm

In the present study all of the *Candida* isolates showed XTT activity which indicates that all of them are biofilm producers which was well supported by Fluorescent microscopic examination of Silicon elastomer disk. Highest XTT activity was seen in *Candida albicans* strain whereas least activity was recorded in *non- albicans Candida* strain (*Candida tropicalis*) in the present study. Biofilm production measured by dry weight (DW) measurement method were 2 mg/disk to 9.1 mg/disk.

Candida albicans produced maximum amount (DW) of biofilm (9.1mg/disk) and *non-albicans Candida* produced minimum amount of biofilm quantitatively (*Candida Famata*, 2.0 mg/disk) in the present study.

Kuhn et al observed similar results in their study that C. albicans produces quantitatively more biofilm than other *Candida* species, as measured by the XTT and DW methods. 3 to 4 mg/disk of biofilm were detected by DW measurement method after 48 hours of incubation. In particular, biofilm DW measurements for *C. parapsilosis* were consistently smaller than those for *C. albicans*. Kuhn *et al*. also found that the level of XTT activity of biofilm formed by non-invasive isolates was higher than the invasive ones, whereas in case of invasive (DRC) isolates they detected higher DW, which represents the actual biofilm formation [10]. It was also observed by them that biofilm formation ability of *C. albicans* is higher than *S. cervisiae*, as their dry weight were 3.7 ± 0.001 mg/disk and 1.6 ± 0.004 mg/disk respectively. In the present study only one isolate of *Candida parapsilosis* was isolated, whose biofilm DW measurement was 3.4 mg/disk less than most of the *Candida spp* [20, 21].

In the present study dry weight of biofilm detected in weak producers was below 3.5 mg/disk and in case of strong biofilm producers was above 3.5 mg/disk. Hence it is concluded that findings of both methods correlated very well.

CONCLUSION

Biofilm production measured by dry weight (DW) measurement method ranged between 2 mg/disk to 9.1 mg/disk. *Candida albicans* produced maximum amount of biofilm (9.1mg) while *C. famata* produced 2.0 mg/disk.

Quantification of Candida biofilm should be performed and reported as it can help clinicians in deciding dose and duration of antifungal drugs for better and fruitful outcome.

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