Original Research Article

Biofilm Production by Various Candida Species Isolated From Various Clinical Specimens

Archana[1], Dinesh Kumar[1], Mukesh Kumar[1],Ratnesh Kumar[2]

[1]Tutor, Department of Microbiology, Patna Medical College and Hospital, Patna, India
[2]Assistant Professor, Tutor, Department of Microbiology, Patna Medical College and Hospital, Patna, India

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Abstract

Background: Candida is one of the most frequently encountered opportunistic fungi that cause severe infection in humans. Incidence of candidiasis is increasing worldwide. The ability of Candida albicans to form biofilms and adhere to host tissues and biomaterial surfaces is an important factor in its pathogenesis. One of the main characteristics of biofilms is their resistant to broad- spectrum anti-microbial drugs. Biofilm formation can act as a reservoir of agents, allow co-infection with other pathogens. **Aim:** The aim of the study was to know the biofilm formation by various Candida species isolated from various clinical specimens. **Materials and Method:** The study was carried out over a period of 1 year from January 2019 to December 2019 at the Department of Microbiology of our institute. A total 100 candida strains were isolated from various clinical specimens. Speciation of candida was done by standard yeast identification protocol and Hichrom Candida agar. Biofilm formation was detected by tube method and microtitre plate method. **Results:** Out of total 100 candida strains studied, biofilm production was seen in 58/100 (58%) isolates by microtitre plate method. While comparing tube method of biofilm production with microtitre plate method, microtitre plate method detected 5 more biofilm producers than tube method. **Conclusion:** Candida has certain species which have the capability to produce high grade of biofilm and others which produce low grade of biofilms. We can conclude that microtitre plate assay is seen to be the most sensitive and specific method to detect the biofilm production as compared to tube method.

Keywords: *Candida* species,Biofilm production

Introduction

Nosocomial infections due to *Candida* species have increased significantly during the past three decades. Use of a wide range of biomaterial instruments (urinary and in- dwelling vascular catheters, denture appliances, orthopaedic prostheses and heart valves) in clinical practice accelerates infection by *Candida* species[1-4]. In addition, Nosocomial *Candida* infections are more prevalent among immune compromised individuals and those with a history of diabetes, malignancy, neutropenia, cancer chemotherapy, organ transplantation, hemodialysis, use of broad-spectrum antimicrobial agents and prolonged hospitalization[5-9].Candiduria, vulvovaginal candidiasis and oral candidiasis are the most important forms of the disease. *Candida albicans*is still considered as the major etiologic agent in candidiasis and several factors are associated with its pathogenesis. The ability of *C. albicans*to form biofilms and adhere to host tissues and biomaterial surfaces is an important pathogenesis factor[10,11].Biofilm formation can act as a reservoir of agents, allow co-infection with other pathogens, promote persistence of infection and increase mortality[12,13]. One of the main characteristics of biofilms is their resistance to broad-spectrum anti-microbial drugs[11,14,15]. Several studies have shown that sessile yeasts (biofilm) are more resistant to amphotericin B, fluconazole, azoles, and echinocandins when compared to planktonic cells[5,11,14]The aim of the study was to know the biofilm formation by various Candida species isolated from various clinical specimens.

**Correspondence*
Dr. Mukesh Kumar
Tutor, Department Of Microbiology, Patna Medical College And Hospital, Patna, India
E-mail: drmukesh03@rediffmail.com

Materials & Methods

This study was conducted in Department of Microbiology, at Patna Medical College and Hospital, Patna. Ethical clearance was obtained from the institutional ethical and research committee.

The study was carried out over a period of 1 year (January 2019 to December 2019) . Various Clinical specimens were taken from Patient attending our institute. All chronic purulent exudates were studied for bacterial and fungal growth. Specimens which do not show any pus cells were excluded from further studies. Total 1152 Clinical specimens were taken for this study out of which 100 *Candida* strains were isolated.

Samples collection & processing:Various clinical samples like Urine, Stool, Vaginal swabs, Sputum, Throat swab, Endotracheal tube, Skin scrapings, Nail clippings, Eye swabs, Ear swabs, Wounds & Pus samples were collected in a sterile, properly labelled container with aseptic precautions. The various clinical samples were collected and processed as per the standard microbiological procedures.

Organisms Identification: All samples were screened for budding yeast like cells with the help of Gram stain, 10% KOH, and Specimens were culture on Sabourad's Dextrose Agar with chloramphenicol and Hichrome candida agar, & incubated at 37ºC for 48 hours. Growth was then processed for gram staining. Gram positive budding yeast cells with or without pseudohyphae were considered as *Candida* species. Speciation of *Candida* was done by germ tube test, carbohydrate fermentation & assimilation test, colony colour on Hichrom Candida agar and microscopic morphology on corn meal agar & 45°C thermo-tolerance tests[16].

Biofilm Formation:

Biofilm formation was detected by two methods as described below.

Microtitre plate method: Biofilm formation was also determined using preseterilized polystyrene 96-well microplates (Himedia, India) (as described by Yigit N *et al.*[17]. For each isolate, a suspension from an overnight culture on SDA was prepared in sterile distilled water and adjusted to 1 McFarland. Each well of the microplate was

filled with 180 µl of Sabouraud dextrose broth (SDB) (Himedia, India) supplement with 8% glucose and then 20 µl of the standard suspension of tested isolates was inoculated. Microplates were covered with lids and incubated at 35°C for 24 hours. The medium in wells was removed and washed three times with sterile phosphate buffer solution (PBS). Microplates were stained with 1% Safranin for 5 minutes and then percentage transmittance (%T) was read at 630 nm by an Elisa reader. All tests were done in duplicates and means were calculated. Finally, adherent biofilm layers were scored as either negative; weak (+) (percentage transmittance (%T ≤ 20)); moderate, (++) (%T = 20-35); strong (+++) (%T = 36-50) and very strong (++++) (%T ≥ 50).

Tube method: Biofilms was assessed by the tube method (as described by Christensen G.D. *et al[18]*and Gokce G *et al*[19] Colonies of Candida species from Sabouraud's dextrose agar were inoculated in saline and incubated overnight at 37ºC. 0.5 ml of this saline suspension was added into screw capped conical polystyrene tubes containing 5mL of Sabouraud's dextrose broth supplemented with glucose (final concentration of 8%). The tubes were incubated at 35ºC for 48 h without agitation. After incubation the broth from the tubes was aspirated gently using Pasteur pipette. The tubes were washed twice with distilled water and stained with 1% safranine. The stain was decanted after 10 min. The tubes were rinsed with distilled water to remove excess stain. Presence of visible adherent film on the wall and at the bottom of the tube indicated biofilm formation. Ring formation at the liquid interface was not considered as an indication of biofilm production. The test was conducted in triplicate and results were expressed as negative –, weak +, moderate ++ and strong +++ as described by Gokce *et al*., 2007[19]. *Staphylococcus epidermidis* ATCC 35984 and *C. albicans*ATCC 10231 were used as positive and negative controls respectively.Candida ATCC control strains were obtained from MicrobiologicsInc, USA.

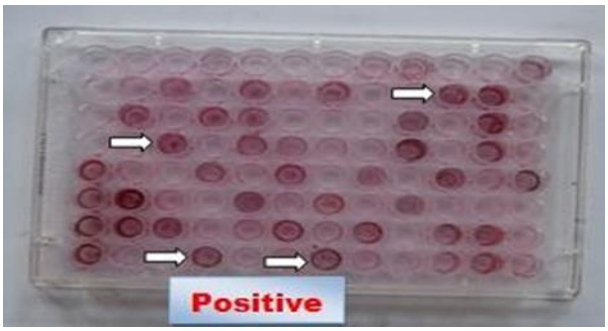

Fig 1: Biofilm formation by Candida species (Microtitre plate method)

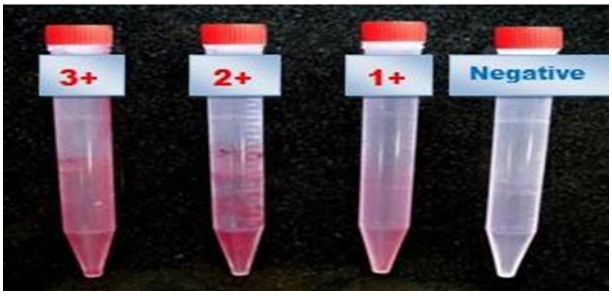

Fig 2: Biofilm formation by Candida species (Tube method)

Results

A total 100 specimens showing culture positive for *Candida* were taken for the study. Out of these 100 *Candida* strains isolated, 44 (44%) were identified as *Candida albicans* and 56 (56%) were identified as *Non-albicans Candida* species. These 100 isolates were studied for biofilm production. Out of total 100 *Candida* species studied, biofilm production was seen in 58/100 (58%) isolates by microtitre plate method as shown in (Table 1).

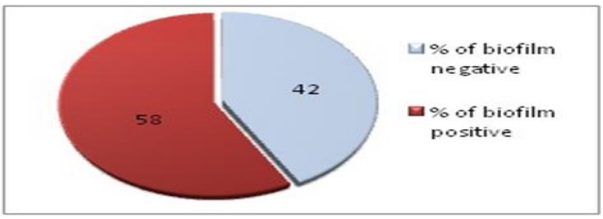

Fig 3: Biofilm formation by Candida species in Clinical specimens

Table 1: Biofilm formation by various Candida spp.

S.N	Candida spp.	No. of biofilm positive					
		1+	2+	3+	4+	Total	Percentages
1	*C. albicans*(n=44)	4	6	6	7	23	52.3%
2	*C. tropicalis*(n=30)	2	2	5	9	18	60.0%
3	*C. glabrata*(n=15)	1	2	2	5	10	66.7 %
4	*C. krusei*(n=7)	0	1	2	3	6	85.7%
5	*C. gulliermondii*(n=4)	1	0	0	0	1	25.0%
Total (N=100)		8(13.8%)	11(19%)	15(25.9%)	24(41.4%)	58	58.0%
Chi-square =8.493; df=3; P-value=0.0368; (significant at P < 0.05)							

Table 2: Biofilm production among Candida species

No. of isolates tested for biofilm	No. & % of biofilm Producers	No. & % ofC. albicans producing biofilm	No. & % ofNAC producing biofilm
100	58 (58%)	23 (52.3%)	35 (62.5%)

Out of 44 *Candida albicans* isolated 23 (52.3%) were biofilm producers. Among the 56 *Non-albicans Candida* species isolated, 35 (62.5%) were biofilm producers. Among the 29 *Non albicans Candida* species isolated, biofilm production was seen predominantly in *C. krusei* 85.7% followed by *C. glabrata*64.7% and *C. tropicalis* 60% as shown in Table 1. This shows biofilm formation is higher in *Non-albicans candida* than *Candida albicans*.

Table 3: Biofilm formation in various clinical samples

S.N	Samples & (No. of Candida spp.)	No. of biofilm positive				
		1+	2+	3+	4+	Total (%)
1	Urine (n=30)	2	5	6	8	21 (70%)
2	Pus (n=3)	0	1	0	0	1 (33.3%)
3	Sputum (n=25)	2	3	3	7	15 (60%)
4	Stool (n=10)	1	0	2	3	6 (60%)
5	Vaginal swab (n=11)	2	1	2	3	8 (72.7%)
6	Throat swab (n=6)	0	1	1	1	3 (50%)
7	Skin scraping (n=5)	0	1	0	0	1 (20%)
8	Nail clipping (n=3)	0	0	0	0	0 (0%)
9	Endotracheal tube (n=2)	1	0	0	0	1 (50%)
10	Ear swabs (n=2)	0	0	1	0	1 (50%)
11	Eye swabs (n=3)	1	0	0	0	1 (50%)
Total (n=100)		9	12	15	22	58 (58%)
Chi-square =8.493; df=3; P-value=0.0368; (significant at P < 0.05)						

Maximum Biofilm formation was seen in High vaginal swabs (72.7%) followed by urine sputum, stool, throat swabs, ET, ear swabs, pus, eye swabs, skin scrapings & nail clippings sample. While comparing tube method of biofilm production with microtitre plate method, microtitre plate method detected 5 more biofilm producers than tube method.

Discussion

Biofilms are notoriously difficult to eliminate and are a source of many recalcitrant infections. Although, bacterial biofilms and their role in disease have been investigated in detail over a number of years, much less is known about fungal biofilms. Present study was carried out in Department of Microbiology with aim to study the biofilm production by various *Candida species* isolated from different clinical samples.

It is increasingly obvious that infections caused by *Candida* species are an escalating clinical problem, and with a limited arsenal of antifungal and a growing menace of biofilms, a lot has to be done for proper disease management. In the present study, biofilm production was found to occur most frequently in *Non-albicans candida* than *C. albicans*. This finding is in contrast to an earlier report that suggested that pathogenic *Non-albicans candida* were more likely to produce biofilms than *C. albicans*.In our study, biofilm production was seen in 58% of *Candida* isolates which is close to the findings of S. Golia et al[20], Mythreyl SR. et al[21], MelekInci et al[22], Nisha VJ et al[23] Sahar Ali M et al[24], Saurabh M et al[25]&ArineBruder et al[26]However lower percentage and higher percentage of biofilm production by *Candida* is shown by some researchers. In our study we found 52.3% of *C. albicans*were biofilm producers which correlate well with the findings of MelekInci et al[22], Nisha VJ et al[23], Sahar Ali M et al[24], Saurabh M et al[25]&Vinitha Mohandas et al[27] Similarly, 62.5% of *Non-albicans candida* were biofilm producers which is close to the findings of S. Golia et al[20], Mythreyl SR. et al[21]& Sahar Ali M et al[24].In our study we found that NAC was more biofilm produces as compared to C. albicans.

In our study, among the five *Candida* species which were most commonly isolated from clinical specimens (*C. albicans*44%, *C. tropicalis* 30%, *C glabrata*15%, *C krusei*7% and *C. guillermondii* 4%), biofilm production was most frequently observed for the isolates of *C. krusei*85.7% followed by *C. glabrata*(64.7%), *C. tropicalis* (60%), *C. albicans*(52.3%) and *C. guillermondii*(25%). In our study, *C. krusei* isolates were high biofilm production 85.7%, S. Golia et al[20] and Vinitha Mohandas et al[27]. also reported maximum biofilm production by *C. krusei*. In our study, *C. glabrata* isolates showed 64.7% biofilm production which is close to the finding of Alessandra R et al[28].In our study, *C. tropicalis*isolates showed 60% biofilm production which is close to the finding of Vivek A et al[29]. In our study, *C. guillermondii* showed (25%) biofilm production which is close to the finding of Rahul P Dahale et al[30]. In our study, biofilm production was most frequently seen in vaginal swabs (72.7%) followed by urine (70%), sputum (60%), stool

(60%), throat swabs (50%), endotracheal tube (50%), ear swabs (50%), pus (33.3%), eye swabs (33.3%) and skin scrapings (20%).

Different authors had reported different percentage of *Candida* biofilm in various clinical samples. Our study shows 70% of *Candida* isolated from urine were biofilm producers which is close to the findings of Sachin C.D. et al[31], Vivek A et al[29], Nisha VJ et al & Sahar Ali M et al[24]60% of *Candida* isolated from sputum were biofilm producers which is close to the findings of Nisha VJ et al[23]& Sahar Ali M et al[24]72.7% of *Candida* isolated from vaginal swabs were biofilm producers which is close to the findings of Vivek A et al[29], Nisha VJ et al[23]& S. Golia et al[20].

Conclusion

For the detection of biofilm production, we can conclude that, microtitre plate assay is seen to be the most sensitive, most reproducible, accurate, efficient and specific method to detect the biofilm production as compared to tube method/visual detection assay. To conclude, genus *Candida* has certain species which have the capability to produce high grade of biofilm and others which produce low grade of biofilms. It has been seen that organism in biofilm are more notorious to treat than their free or planktonic form, so, it is essential to prevent biofilm formation for easy treatment and improving mortality and morbidity rate.

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