THE BIOFILM PRODUCTION BY VARIOUS CANDIDA SPECIES ISOLATED FROM DIFFERENT CLINICAL SAMPLES

Article Review by Sadaf Tabassum, India
(M.Sc., Ph.D., in Clinical Research Student of Texila American University)
Email: - sadafjasmeen@gmail.com

ABSTRACT

Pathogenic fungi in the genus Candida can cause both superficial and serious systemic disease, and are now recognized as major agents of hospital-acquired infection.

Candida infections involve the formation of biofilms on implanted devices such as indwelling catheters or prosthetic heart valves. Nosocomial infections due to candida are also becoming increasingly important. Early and prompt diagnosis, proper treatment and prevention of candidemia due to biofilms pose a major challenge for microbiologists and clinicians worldwide. Biofilm is an aggregate of microorganism in which cell adhere to each other on a surface. It has been reported that organism in biofilms are more resistant to antimicrobial agents than there planktonic (free) form. Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. These first adhere to the surface initially through weak, reversible adhesion via Vander Waal forces. If the colonies are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pilli. There are five stages of biofilm development such as i) initial attachment ii) irreversible attachment iii) maturation I iv) maturation II v) dispersion.

AIMS AND OBJECTIVES

To study the biofilm production by *Candida albicans* and *Non albicans Candida* species isolated from various clinical samples received in Microbiology Laboratory of a Tertiary Care Hospital.

MATERIALS AND METHODS

A total of 70 Candida species (Candida albicans20 and non-albicans Candida50 species) isolated from various specimens (urine, blood, respiratory tract, genital samples, plastic devices and pus samples) were included in the study. The various candida isolates were identified by using conventional methods and their ability to produce biofilm was detected by the tube method.

RESULTS

Out of the total 70 *Candida* species isolated, 20(39%) were identified as *Candida albicans* and 50 (71%) were identified as *Non albicans Candida* (NAC) species.

Out of 20 *Candida albicans* isolated 6 (30%) isolates produced biofilm and out of 50 *Non albicans Candida* species isolated 22(44%) were biofilm producer. Out of 22 (44%) *Non albicans Candida* species, *Candida tropicalis* 10(45%) was the predominant biofilm producer followed by *Candida glabrata* 7(32%) and *Candida prapsilosis* 5(23%).

CONCLUSION

The present study suggests an increasing prevalence of non-*albicans* Candida species in the various clinical samples isolated and also shows them as strong biofilm producers compared to C.albicans species. These data suggest that, biofilm formation as a potential virulence factor might have a higher significance for non-*albicans* Candida species than for C.albicans and also that the biofilm structure varies with the different species and strains of candida, the nature of the colonized surface and its localization.

KEY WORDS

Candida, biofilm, non-albicans Candida, clinical sample, phospholipase, slime.

INTRODUCTION

Pathogenic fungi in the genus *Candida* can cause both superficial and serious systemic disease, and are now recognized as major agents of hospital-acquired infection.

Candida infections involve the formation of biofilms on implanted devices such as indwelling catheters or prosthetic heart valves.

Biofilm is an aggregate of microorganism in which cell adhere to each other on a surface. It has been reported that organism in biofilms are more resistant to antimicrobial agents than there planktonic (free) form.

Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. These first adhere to the surface initially through weak, reversible adhesion via Vander Waal forces. If the colonies are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pilli. There are five stages of biofilm development such as i) initial attachment ii) irreversible attachment iii) maturation I iv) maturation II v) dispersion.

Although *Candida albicans* remains the most common fungal isolate recovered from the clinical samples a changing trend has been observed. *Non albicans Candida (NAC)* species are emerging

nosocomial pathogens. Biofilm formation has been implicated as a potential virulence factor for at least one *Candida species: Candida parapsilopsis*. *Candida parapsilopsis* can proliferate in high concentration in glucose and form biofilm in prosthetic materials.

It has been reported that the biofilms are resistant to a range of antifungal agents currently in clinical use, including amphotericin B and fluconazole and there appear to be a multiple resistant mechanism. Resistance has been observed both in *Candida albicans* as well as *Non Candida albicans* species.

Bacterial biofilms and their role in disease have been studied intensively in recent years; however biofilm production by *Candida* species has not been studied from this part of the country.

Therefore, a study was planned to detect the biofilm production by *Candida albicans* as well as *Non albicans Candida* species, isolated from different clinical samples received in Clinical Microbiology Laboratory of a tertiary care hospital.

MATERIALS AND METHODS

STUDY PLACE

The present study was carried out in Department of Microbiology, Subharti Medical College, Meerut, for a period of six months.

STUDY SAMPLES

Included 70 clinical isolate of *Candida species.*

METHODS EMPLOYED

A. Identification of *Candida Species*:

1) The isolates of Candida were identified in the laboratory as *Candida albicans* and *Non albicans Candida* species using the following methods:

- Gram staining.
- Culture on Sabourd Dextrose agar (SDA).
- Germ tube test.
- Chlamydospores formation on Cornmeal agar.
- Sugar fermentation.

B. Biofilm production:

The biofilm production in both *Candida albicans* as well as *Non albicans Candida* species was carried out using spectrophotometric analysis of biofilm production.

PRINCIPLE

Polystyrene, a commonly used material for in vitro biofilm studies, is used as a test surface to look the biofilm forming ability of each *Candida species*. The species of Candida would be incubated in proper media on the microtiter plate wells and the produced biofilms on the surface would be quantified by the percent transmittance of light.

PROCEDURE

1. Sterile polystyrene microtiter plate was taken.
2. Each well was inoculated with 20 µl of yeast cell suspension.
3. Two separate wells were inoculated with 20 µl each of the two control strains.
4. To each of the wells 180 µl of Sabouraud dextrose broth with 8% glucose is to be added.
5. The microtiter plate was incubated for 24 hrs at37^0C.
6. The wells were washed twice with 0.15 M of phosphate buffered saline to remove any planktonic cell.
7. Finally, 200 µl of phosphate buffered saline was added to each well.
8. Biofilm formation was directly measured by spectrophtometric reading at 405 nm with microtiter plate reader.

CALCULATION

Transmittance = 1- absorbance of blank

% Transmittance =100 x Transmittance

% Transmittance = % Transmittance of blank - % Transmittance of sample

1. Biofilm is quantified by measuring the percent transmittance (% T) at 405 nm with the microtiter plate reader.
2. The percent transmittance is calculated by subtracting % T value of each test and control strain from the % T value for reagent of blank to obtain a measure of amount of light blacked passing through wells. (% T block)

INTERPRETATION

Biofilm production by each isolates of *Candida species* was scored as:-

% T block < 10	**Negative**
% T block 10-20	1+low biofilm production
% T block 20-30	2+high biofilm production
% T block 35-40	3+high biofilm production
% T block >40	4+high biofilm production

OBSERVATION AND RESULTS

The study was carried in the Department of Microbiology with the aim to study the formation of biofilm production by various *Candida* species. A total of 70 clinical isolates of *Candida* obtained from various samples were processed. The distribution of clinical isolates of *Candida albicans* and *Non albicans Candida (NAC)* species is shown in Table 1.

Table1: Distribution of clinical isolates of *Candida albicans* and *Non albicans Candida (NAC)* species (n=70)

Name	**Number**	**Percentage**
Candida albicans	20	29%
Non albicans Candida (NAC)	50	71%

Out of the total 70 *Candida* species isolated 20 (29%) were identified as *Candida albicans* and 50 (71%) were identified as *Non albicans Candida* species as shown in Table 1.

Out of the total number of *Candida* species studied, biofilm production was seen in 28 / 70 (40%) isolates.

Table 2: Distribution of biofilm producers from total number of isolated species (n=70)

Species	Number isolated	No. of biofilm producers	Percentage
C.albicans	20	06	30 %
NAC	50	22	44 %

Distribution of biofilm producers from total number of isolated species is shown in Table 2.

Out of 20 *Candida albicans* isolated 06 (30 %) were biofilm producers. Among the 50 *Non albicans Candida* species isolated, 22 (44 %) were biofilm producers.

Table3: Distribution of various *Non albicans Candida* species producing biofilm (n=22)

Candida species	Number	Percentage
C. tropicalis	10	45%
C. glabrata	07	32%
C. parapsilosis	05	23%

Among the 22 *Non albicans Candida* species isolated, biofilm production was seen predominantly in *Candida tropicalis* 10(45%), followed by *Candida glabrata* 7 (32%) and *Candida parapsilosis* 5(23%) as shown in Table 3.

Table 4: Score of Biofilm Produced by *Candida albicans* (n=6)

S.No	Name of species	Score
1	*C.albicans*	4+
2	*C.albicans*	4+
3	*C.albicans*	4+
4	*C.albicans*	4+
5	*C.albicans*	4+

6	*C.albicans*	4+

All the 6 clinical isolates of *Candida albicans* produced high score of biofilm (4+) as shown in Table 4.

Table5: Score of Biofilm produced by *Candida tropicalis* (n=10)

	Name of species	**Score**
1	*C.tropicalis*	4+
2	*C.tropicalis*	4+
3	*C.tropicalis*	4+
4	*C.tropicalis*	4+
5	*C.tropicalis*	4+
6	*C.tropicalis*	4+
7	*C.tropicalis*	4+
8	*C.tropicalis*	4+
9	*C.tropicalis*	4+
10	*C.tropicalis*	4+

All the 10 clinical isolates of *Candida tropicalis* produced high score of biofilm (4+) as shown in Table 5.

Table 6: Score of biofilm produced by *Candida glabrata* (n=7)

S.No	**Name of species**	**Score**
1	*C.glabrata*	3+
2	*C.glabrata*	3+
3	*C.glabrata*	3+
4	*C.glabrata*	3+
5	*C.glabrata*	2+
6	*C.glabrata*	2+

7	*C.glabrata*	2+

The clinical isolates of *Candida glabrata* produced biofilm in score of (3+ and 2+) as shown in Table 6

Table7: Score of Biofilm produced by *Candida parapsilosis* (n=5)

S.No	Name of species	Score
1	*C.parapsilosis*	1+
2	*C.parapsilosis*	1+
3	*C.parapsilosis*	1+
4	*C.parapsilosis*	1+
5	*C.parapsilosis*	1+

The 5 clinical isolates of *Candida parapsilosis* produced low score of biofilm (1+) as shown in Table 7

Table 8: Grading of Biofilm production by different *Candida* species isolated from the clinical samples

Species	Score	Grading of Biofilm Productivity
C. albicans	4+	High biofilm producer
C. tropicalos	4+	High biofilm producer
C. glabrata	2+,3+	High biofilm producer
C.parapsilosis	1+	Low biofilm producer

Candida albicans and *Candida tropicalis* showing score of 4+ was Graded as high biofilm producer.

Candida glabrata showing score of 3+and 2+ was graded as moderate biofilm producers.

However, *Candida parapsilosis* showing a score of 1+ was Graded as low biofilm producer.

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 at a tertiary care hospital Meerut, India.

Out of the total 70 *Candida* species isolated, 20(39%) were identified as *Candida albicans* and 50 (71%) were identified as *Non albicans Candida* (NAC) species.

Out of 20 *Candida albicans* isolated 6 (30%) isolates produced biofilm and out of 50 *Non albicans Candida* species isolated 22(44%) were biofilm producer. However, in a study carried out by J.H.Shin et. al. in Gwangu Korea[11] they reported 8% of *Candida albicans* as biofilm producer whereas biofilm was produced by 61% *Non albicans Candida* species. Another study by G.gokce, hayderpasa Istanbul, Turkey reported, 11.8% and 41.93% biofilm production by *Candida albicans* and *Non albicans Candida* species respectively which is almost similar to the present study.

Out of 22 (44%) *Non albicans Candida* species, *Candida tropicalis* 10(45%) was the predominant biofilm producer followed by *Candida glabrata* 7(32%) and *Candida prapsilosis* 5(23%). This positivity is comparatively less than the findings reported by J.H Shin et.al. Gwangu Korea. They reported 80%, 28% and 73% of biofilm produced by *Candida tropicalis*, *Candida glabrata* and *Candida parapsilosis* respectively. Another study carried out by M.tumbarello, Rome, Italy, reported *Candida tropicalis* (71.4%) was the predominant biofilm producer followed by *Candida glabrata* (23%) and *Candida parapsilosis*(21.8%).

The present study was designed as a pilot study so the sample size was small. Further, study in more number of clinical isolates is required to know the exact prevalence and predominant species.

Among the biofilm positive strains the highest relative intensity of biofilm formation ($\%T_{bloc}$>50) by the spectrophotometric method that is Score of 4+ was observed for *Candida albicans* and *Candida tropicalis* followed by *Candida glabrata* ($\%T_{bloc}$ ranging from 20 to 40) showing a score of 3+ and 2+ and least intensity of biofilm formation score 1+ was shown by *Candida parapsilosis* ($\%T_{bloc}$<10). Similar finding have been reported by Shin et al Gwangu, Korea and M.tumbarello et al. Rome, Italy[20] in their respective studies.

Candida albicans and *Candida tropicalis* showing score of 4+ was graded as high biofilm producer and *Candida glabrata* showing score of 3+and 2+ was graded as moderate biofilm producers. However, *Candida parapsilosis* showing a score of 1+ was graded as low biofilm producer.

The microbes residing inside the biofilm shows resistance towards various types of antimicrobial agents causing therapeutic problem. Such microorganisms pose a serious threat even to the pharmaceutical industry. Therefore, it is recommended to prevent the formation of biofilm rather than treatment. Biofilm can be prevented by early aggressive antifungal prophylaxis or therapy

and they can be treated by chronic suppressive therapy. A promising strategy may be the use of enzyme that can dissolve the biofilm matrix (e.g, DNase and Alginate lyase) as well as Quorum sensing inhibitors that increase biofilm susceptibility to antifungals.

The *Candida* species isolated from various clinical samples in our study showed biofilm production in various grades and scores posing a therapeutic problem which definitely is a matter of concern. However, this is a preliminary study carried out in a few number of isolates. To see the exact grading of biofilm production and to pinpoint the predominant species of Candida, we need to carry out the study in more number of isolates.

Further in future the study can be extended by correlating the score and the grade of biofilm production with the resistant pattern and the clinical outcome of infection.

CONCLUSION

- Present study was carried out in Department of Microbiology with aim to study the biofilm production.
- A total of 70 Candida species isolated from different clinical samples were subjected for biofilm formation by spectrophotometric method.
- Out of 70 Candida species 20 isolates were identified as *Candida albicans* and 50 identified as Non *albicans Candida* species.
- Biofilm production was observed in 6/20 (30%) of *Candida albicans* and 22/50 (44%) of NAC species. Showing that NAC species are predominant biofilm producers than *Candida albicans*.
- Among the NAC species, *Candida tropicalis* was the predominant biofilm producer followed by *Candida glabrata,* and *Candida parapsilosis* 45%, 32% and 23% respectively.
- High score and grade (4+) of Biofilm production was found in all isolates of *Candida albicans* and *Candida tropicalis* as T bloc %>40 was obtained with spectrophotometric method.
- *Candida glabrata* was a moderate biofilm producer with grading of 2+ and 3+ as T bloc % >20and<40 was obtained.
- Lowest score and grade (1+) of biofilm was produced by *Candida parapsilosis* as T bloc %<10 was obtained with spectrophotometric method.
- 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.

- To see the exact grading of biofilm production and to pinpoint the predominant species of Candida, we need to carry out the study in more number of isolates. Further in future the study can be extended by correlating the score and the grade of biofilm production with the resistant pattern and the clinical outcome of infection.

REFERENCES

1. Douglas J.L. *Candida* Biofilms and their role in infection, Review Trends in Microbiology, Jan 2003, vol. 11, 30-36.

2. Pfaller M.A**.** Nosocomial Candidiasis: emerging species, reservoirs and modes of transmission. Cl. Infect. Dis., Oxford Jour. 1996 (suppl. 2) 589-94.

3. Chandra J. Kuhn D.et al; Biofilm formation by fungal pathogen *Candida albicans*: development architecture and drug resistance. Jour. Bacteriol. 2001 183(18) 5385-94.

4. Watnick P. Kotler R. Biofilm: city of microbes, Jour. Of Bacteriol. May 2000 182(10) 2675-79.

5. Glasgow/ biofilm 2011.igem.org/Team

6. Berry V. Badyal D. sensitivity of clinical isolates of *Candida* species to antifungal drugs org. art. J.K. science 2006 8(4)214-20.

7. Mary A.J. Falkler W. Fungal biofilm and drug resistance. Emerg. Infect. Dis. 2004 Jan. 10(1); 14-19.

8. Kokare C. Chakraborty S. et al; Biofilm: importance and applications.rev Ind. Jour. of Biotech. 2008 8,159-68.

9. Chander J. Textbook of medical mycology, Mehta publications Ed.3rd, chapter20, Candidiasis, 266-83.

10. Topley and Wilson'S Microbiology and Microbial infection: Medical Mycology Hodder Arnold publication Ed. 10th

11. Shin J. Kee S. et al; Biofilm production by isolates of *Candida* species recovered from non neutropenic patients: comparison of bloodstream isolates from other sources. Jour. Of Cl. Microbiol. apr. 2002 40(4) 1244-48.

12. Paanusorn S. Fernandez V. et al**;** Prevalence of biofilm formation in clinical isolates of *Candida* species causing bloodstream infection. Jour. of mycoses.2013, 56(3) 264-72.

13. Mohandas V.et al**;** Distribution of *Candida* species in different clinical isolates and their virulence: Biofilm formation, proteinase and phospholipase production: a study on hospitalized patients in Southern India. Jour. Glob. Infect.Dis.20113 (1):4-8.

14. Lal P. Agarwal V.et al; Biofilm formation by *Candida* albicans isolated from intrauterine devices. Ind. Jour. of Microbiol. 2008 48:438-444.

15. Hawser S. Douglas J**.** Biofilm formation by *Candida* species on the surface of catheter material in vitro. Jour. of Infection and Immunity 1994 62(3):915-921.

16. Silva S.et al**;** Biofilm formation ability by *Non albicans Candida* species biofilm club 2007 33-41.

17. Gokce G.et al; Acid proteinase, phospholipase and biofilm production of *Candida* species isolated from blood culture J. Mycopathologia 2007 164(6) 265-269.

18. Meleck INC et al; investigation virulence factors of clinical *Candida* isolates in relation to atmospheric condition and genotype. Turk. J. Med.2012 Sci.(sup 2): 1476-1483

19. JPG image niaid.nih.gov; erc.montana.edu.

20. Tumbarello M. Posteraro B. et al**.** Biofilm production by *Candida* species and inadequate antifungal therapy as predictors of mortality for patients with candidemia. Jour. Of Cl. Microbiol. June 2007 45(6) 1843-1850.

21. Seneviratne CJ, Jin L, Samaranayeke LP. Biofilm lifestyle of Candida: A mini review Oral Dis. 2008; 14: 582-590.

22. Aparna MS, Yadav S. Biofilms: Microbes and Disease. The Braz. J. Infect. Dis.2008; 12: 526-530.

23. RM Dominic, S Shenoy, S Baliga. Candida biofilms in medical devices: Evolving trends. Kath Univ Medical J. 2007; Vol. 5, No. 3, Issue 19, 431-436.

24. Baillie GS, Douglas LJ. *Candida* biofilm and their susceptibility to antifungal agents. Methods Enzymol.1999; 310:644–56.

25. Segal, E. and D. Elad. Candida species and Blastoschizomycescapitatus. In: Agillo, L. and R. J. Hay (Eds).1998; Microbiology and Microbial Infection. New York, Arnold, pp: 423-460

26. Chakrabarti A, Singh K, Das S. Changing face of nosocomial candidaemia. Indian J Med Microbiol.1999; 17:160–6.

27. Vinitha, M and Ballal, M. Biofilm as virulence marker in Candida isolated from blood, World J for Medical Sciences 2007;2:46-48.