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RESEARCH ARTICLE

DETECTION AND QUANTIFICATION OF BIOFILM FORMED BY CANDIDA SPECIES ISOLATED FROM CLINICAL SAMPLES AT TERTIARY CARE HOSPITAL.

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Abstract

Candida speciesis a part of commensalin healthy humans but they can cause opportunistic infections, especially in immunocompromised individuals, patient admitted in ICUs(Intensive care units) and HIV patients. biofilm act as protective shield of microorganism. Candida species form most common fungal biofilm, which is extremely difficult to treat.

Purpose: The biofilm forming candida is difficult to eradicate with usual antifungal drug and often cause chronic infections. Understanding of biofilm process is very important for effective control strategies of biofilm associated infections and improvement in patient management.

Method: The observational cross sectional prospective study was conducted on forty patient's samples including urine, BAL/sputum, blood culture, body fluids, pus, swab, indwelling devicesand tissue showing candida species growth. Biofilm was detected using pre-sterilized 96 well polystyrene microtitre plate method.

Results: Among 40 isolates, 22 isolates were detected positive, whereas 18 isolates were negative for biofilm formation. C. tropicalis had formed maximum strong biofilms among all species isolated. These biofilm acts resisting antifungal treatment and withstanding the competitive pressure from other organisms, these are difficult to treat.Changing trend with shift toward non albicans.

Conclusion: Candida tropicalis as the predominant pathogen causing candidia infectionsand it had highest capacity to form biofilm.

Introduction:-

Candida species is a yeast like fungus and it is a part of commensal, generally present in healthy humans on skin, nails and internal organs of the body. They are known to cause opportunistic infections, especially in immunocompromised individuals, patient admitted in ICUs(Intensive care units) and HIV patients. The increasing use of total parenteral nutrition, intravenous catheters, invasive procedures, unnecessary use of broad-spectrum antibiotics, cytotoxic chemotherapies and transplantation are factors that contribute increase risk of candida

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infections. Infections due to fungi belonging to candida genus are increasingly reported in recent years. Candida albicans is most prevalent among all candida species, which causes both superficial and systemic infections.

As biofilm is a polysaccharide matrix act as protective shield of microorganism, it acts as virulence factor. Microorganisms irreversibly attach and grow on a surface and produce extracellular polymers that enables attachment and matrix formation, resulting in an alteration in phenotype of the organisms with respect to alteration in growth rate and transcription gene, by this process formation of biofilm takes place[10].

As candida species form most common fungal biofilm, which is extremely difficult to treat. C. albicans and C. tropicalis have high capacity to cause infection in highest amount and therefore are highest biofilm producer. The biofilm forming candida is difficult to eradicate with usual antifungal drug and often cause chronic infections.

In vitro detection of biofilm by various methods like loose needle connectors methods, tissue culture plate method (TCP) / microtitre plate method (MTP), tube method, congored agar method (CRA), bioluminescent assay, piezoelectric sensors.

Biofilms arehighly importantfor public health because of its role in certain infectious diseases and importance in various device-related infections. A greater understanding of biofilm processes should lead to novel, effective control strategies for biofilm control and a resulting improvement in patient management.[8,9]

Material And Method:-

The observational crosssectional prospective study was conducted at microbiology department for six months of time period after ethical approval by human research ethical committee. Total 40 samples of patients having indwelling devices were included in study a after taking consent of the indoor patient of Tertiary Care Hospital.

Sample size:
Sample size is 40

Study period:
Six months (from July-2020 to December 2020)

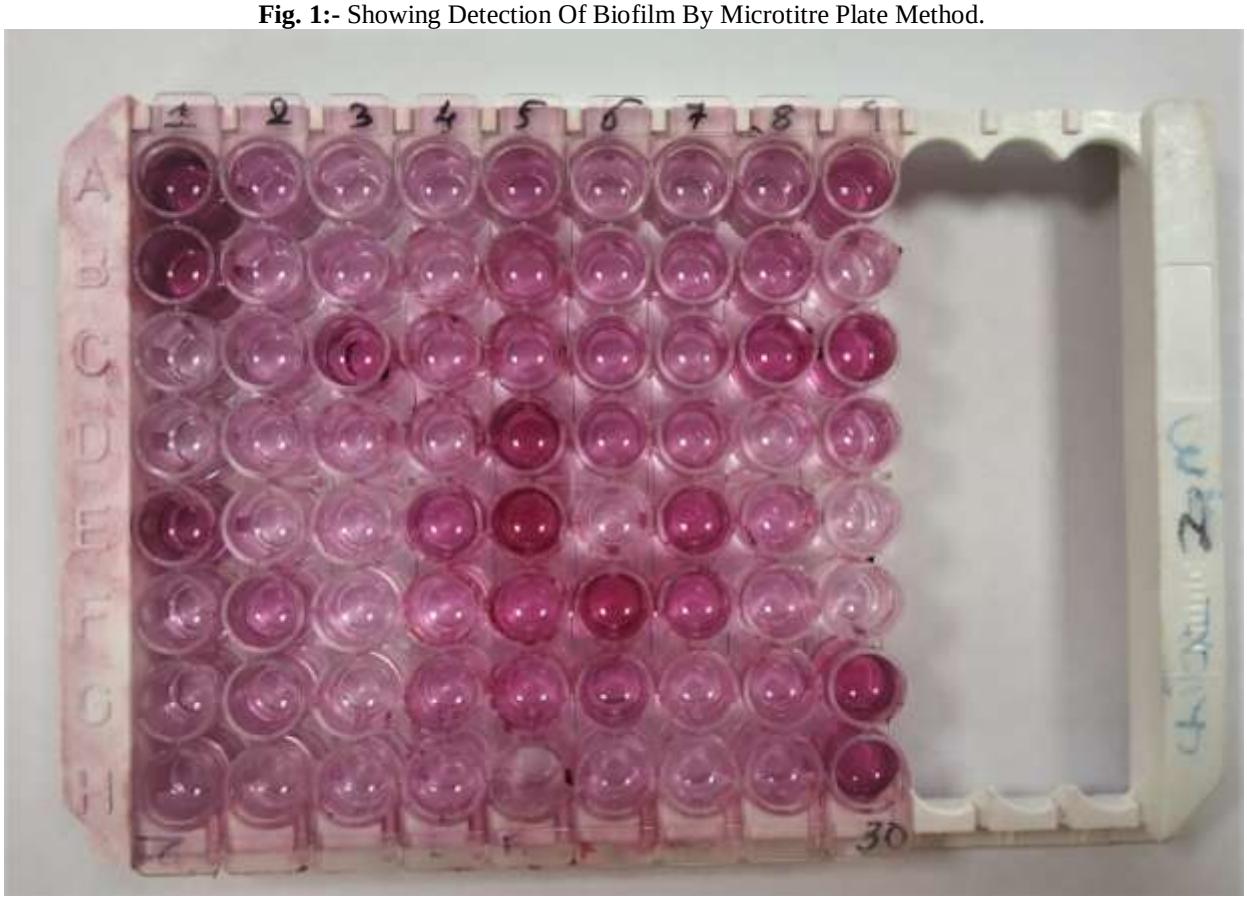
Inclusion criteria:
a) Samples like urine, BAL/sputum, blood culture, body fluids, pus, swab, tissue, oral thrushfrom indoor patients with indwelling device showing growth of yeast like fungi (candida).

Exclusion criteria:
a) Samples which did not show growth of candida.
b) Growth of commensals candida on culture plates.
c) Repeated sample from same patients.
d) Patients having HIV infection.
e) Outdoor patient's samples.

All samples from indoor patients received in microbiology department were proceed according to standard protocol of microbiology department. Gram staining of colony smear confirmed as yeast like organism and these candida species were further isolated and identified.

Biofilm Detectionby Microtitre Plate Method:
Candida isolates were stored by freezing method for further proceed for detection of biofilm formation. Biofilm production was detected by using pre sterilized 96 well polystyrene microtitre plates. For each isolate and controls, a suspension from an overnight culture on SDA plate was prepared in sterile distilled water and it was adjusted to 1 Mcfarland. Each well of microtitre plates were filled with 180 microlitre of SDA broth with 8% glucose and 20 microlitre of prepared suspension was added. Then covered microtitre plates were incubated at 37°C for 24 hours. Empty the wells and manually washed thrice with distilled water. After that, plates were stained with 1% safranin for 5 minutes and read transmittance (%) at 630 nm by ELISA reader [1,10].

Fig. 1:- Showing Detection Of Biofilm By Microtitre Plate Method.



Test was done thrice on candida isolates and the mean OD were calculated and cut off was calculated as three standard deviations above the mean OD of controls. Biofilm of each isolate was quantified as follows [2]:

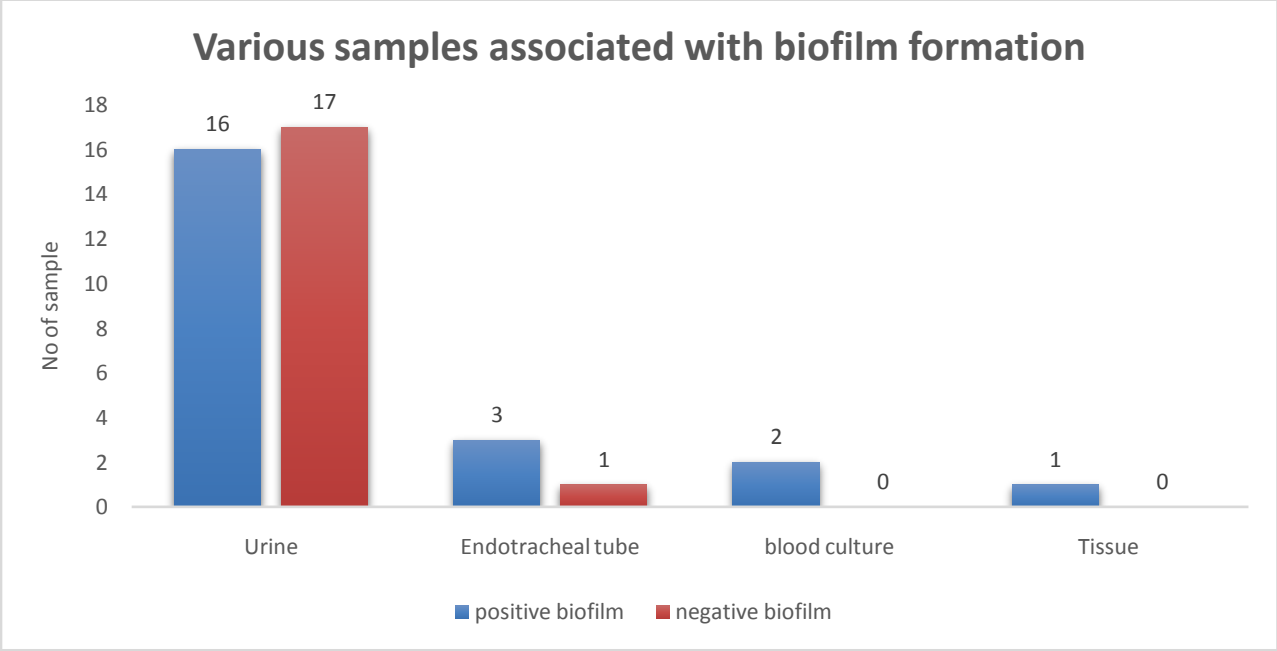
1. Negative (clear well);
2. ODc < OD < 2x ODc = Weak
3. 2x ODc < OD = 4x ODc = Moderate
4. OD > 4x ODc = Strong

Controls:
Positive: C. parapsilosis ATCC 22019
Negative: sterile SDA broth

Results And Observations:-

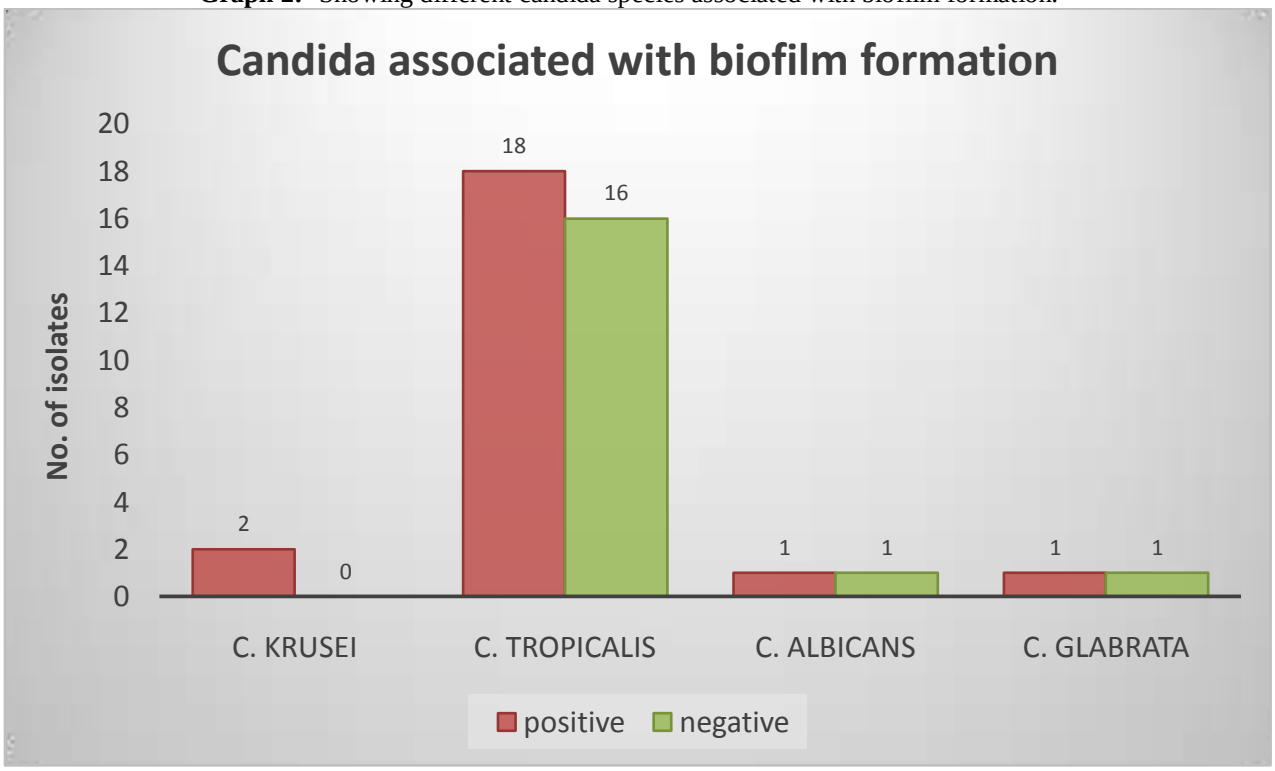
Data of the present study was collected in microsoft excel version 2301 and analysed using epiinfo version 7.0. Total 40 Candida isolates from various clinical samples of the patients having indwelling devices. Among 40 isolates, 22 isolates were detected positive, whereas 18 isolates were negative for biofilm formation.

Graph 1:- Showing biofilm formation in Various samples.

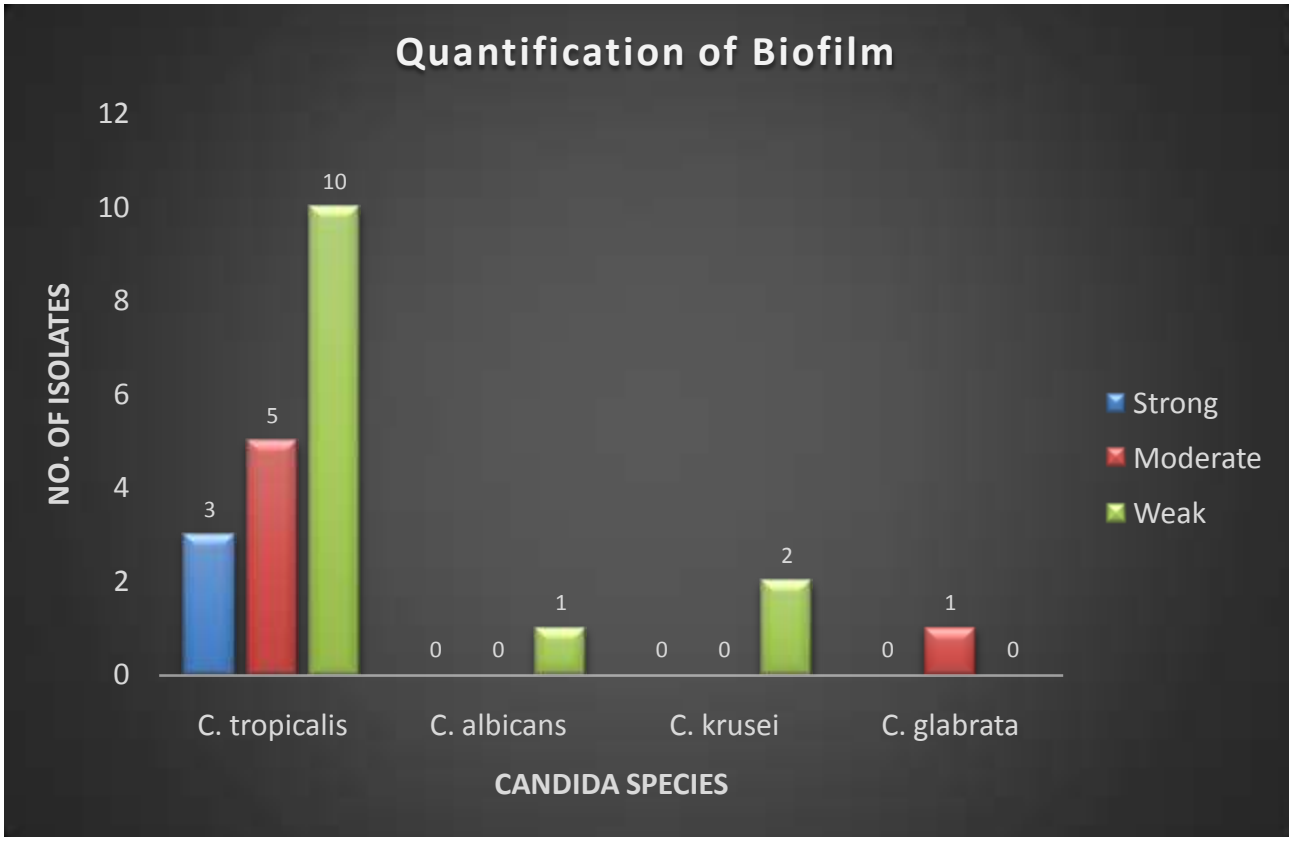


40 samples including thirty three urine from patient having urinary catheters, four were endotracheal tube, two were blood culture from patient having intravenous catheter and one was tissue from patient having prosthetic implant. Out of 33 isolated candida from urine, 16 candida species (48.5%) were associated with biofilm formation and 17 (51%) were not formed biofilm. Out of 33 isolated candida from Endotracheal tube, three candida species (75%) formed biofilm and one had not formed and one candida species isolated from prosthetic joint (100%) had formed biofilm. Two (100%) biofilms formed by candida isolated from blood.

Graph 2:- Showing different candida species associated with biofilm formation.



Graph 3:- Showing Quantitative assessment of biofilm formation by different candida species.



Among 40 samples subjected for biofilm formation, 22 isolates(55%) were positive for biofilm formation. In this study, highest number of biofilms were formed by C. tropicalis (18, 53%), sixteen C. tropicalis had not formed biofilm. Two C. krusei (100%) formed biofilm, one C. albicans (50%) formed biofilm and one C. albicans had not formed out of two C. albicans isolates and one C. glabrata (50%) had formed biofilm and one had not formed biofilm among two isolates of C. glabrata.

Among twenty two positive biofilms only three biofilms were strong, which were formed by C. tropicalis. Five C. tropicalis had formed moderate biofilm and ten C. tropicalis had formed weak biofilm. Whereas C. glabrata formed moderate strength biofilm and C. albicans and C. krusei formed weak biofilm.

Discussion:-

Table 1:- Comparison of candida species biofilm formation in different studies.

	Present study		**Munmun B. Marak et al (2018)[3]**		**B. Janakiram et al (2016)[4]**	
Isolated candida	**Biofilm positive**	**Biofilm negative**	**Biofilm positive**	**Biofilm negative**	**Biofilm positive**	**Biofilm negative**
C. KRUSEI	67%	33%	61%	39%	100%	0%
C. TROPICALIS	53%	47%	60%	40%	75%	25%
C. ALBICANS	50%	50%	51%	49%	83%	17%
C. GLABRATA	50%	50%	0%	100%	-	-

In present study, out of three isolates of C. krusei, two (67%) isolates were able to form biofilm while one (33%) isolate was negative for biofilm generation. Whereas, study of Munmun B. Marak et al (2018)[3] detected 61% positive biofilm and 39% negative biofilm by C. krusei. B. Janakiram et al (2016)[4] detected 100% biofilm formation by C. krusei. In our study, out of thirty four isolates of C. tropicalis, eighteen (53%) isolates were able to form biofilm while sixteen (47%) isolates were negative for biofilm generation. Whereas, study of Munmun B. Marak et

al (2018)[3] detected 60% positive biofilm and 40% negative biofilm by C. tropicalis. B. Janakiram et al (2016)[4] detected 75% positive biofilm formation and 25% negative biofilm by C. tropicalis. In our study, out of two isolates of C. albicans, one (50%) isolate was able to form biofilm while one (50%) isolate was negative for biofilm generation. Whereas, study of Munmun B. Marak et al (2018)[3] detected 51% positive biofilm and 49% negative biofilm by C. albicans. B. Janakiram et al (2016)[4] detected 83% positive biofilm formation and 17% negative biofilm by C. albicans. In our study, out of two isolates of C. glabrata, one (50%) isolate was able to form biofilm while one (50%) isolate was negative for biofilm generation. Whereas, study of Munmun B. Marak et al (2018)[3] detected no any isolate positive for biofilm by C. glabrata. Dharmendra kumar et al (2015)[5]conducted study on 115 samples, majority samples were from ICU, NICU and immunocompromised patients and HIV patients. Most common species was C. albicans and second common was C. tropicalis. C. tropicalis showed high capacity to form biofilm according to their absorbance in the study. Capacity of biofilm formation of C. albicans was less than C. tropicalis. C. glabrata was found to be Lowest capacity of biofilm formation. i.e. less biofilm producer.

Table 2:- Comparison of quantitative assessment of biofilm formation by candida species in different studies.

	Present study			**Shilpa khatri et al (2016)[6]**		
Isolated candida	**Strong**	**Moderate**	**Weak**	**Strong**	**Moderate**	**Weak**
C. KRUSEI	0%	0%	100%	50%	50%	0%
C. TROPICALIS	17%	28%	56%	40%	31%	29%
C. ALBICANS	0%	0%	100%	32%	18%	50%
C. GLABRATA	0%	100%	0%	0%	33%	67%

In this study, highest number of biofilms are formed by C. tropicalis (18), followed by C. krusei (2), C. albicans (1), C. glabrata (1). Among twenty two positive biofilms only three biofilms are strong, which were formed by C. tropicalis. Five C. troicalis had formed moderate biofilm and ten C. tropicalis had formed weak biofilm. Whereas C. glabrata formed moderate strength biofilm and C. albicans and C. krusei formed weak biofilm.

In present study, C. krusei formed weak strength biofilm, while study of Shilpa khatri et al (2016)[6] detected 50% strong and 50% moderate strength biofilm. In our study, C. tropicalis formed 17% strong, 28% moderate and 56% weak strength biofilm, while study of Shilpa khatri et al (2016)[6] detected 40% strong, 31% moderate and 29% weak strength biofilm. In our study, C. albicans formed weak strength biofilm, while study of Shilpa khatri et al (2016)[6] detected 32% strong, 18% moderate and 50% weak strength biofilm. In our study, C. glabrata formed moderate strength biofilm, while study of Shilpa khatri et al (2016)[6] detected 33% moderate and 67% weak strength biofilm. Manal Gomaa Ismail et al. (2020)[7] observed that C. tropicalis were the highest biofilm producers (100%), among this 78% were strong producer and 22% were moderate producer versus 8% of C. albicans was strong biofilm producer, 8% was moderate biofilm producer and the 69% were weak biofilm producer. However, 90% of C. glabrata were weak biofilm producer and 10% was negative biofilm producer.

Conclusion:-

In recent time, the use of indwelling devices such as urinary catheter is the most significant risk factor in hospitals. Additionally, modern medical procedures including implantation of various prosthetic devices in the body, contribute to the risk factors for developing candidia infection.

Indwelling devices associated candida infection is the one of the most common cause of devices associated fungal infection by forming biofilm on biotic and abiotic surfaces. Biofilm may resist the antifungal treatment.

As major strong biofilm producer was C. tropicalis among isolated Candida species, may help to maintain the role of fungi as pathogen, by evading host defense mechanisms, resisting antifungal treatment and withstanding the competitive pressure from other organisms, these are difficult to treat.

Changing trend with shift toward non albicans Candida species as the predominant pathogen causing candidial infections, detection of ability of different candida species to form biofilm will help in treatment. So, species identification and biofilm detection must be performed for early and effective treatment of the patient.

Limitations

We were unable to perform antifungal MIC testing on biofilm formed candida due to unavailability of necessary media and antifungal drugs and it is also cumbersome process and require more budget.

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