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BIOFILM PRODUCTION BY VARIOUS CANDIDA SPECIES ISOLATED FROM VARIOUS CLINICAL SPECIMENS IN TERTIARY CARE HOSPITAL.

Medical Microbiology

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ABSTRACT

BACKGROUND: Candida is one of the most commonly encountered opportunistic fungi that cause 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.

MATERIALS AND METHODS: Biofilm formation was performed on a sterile 96-wellmicrotiter plate. A colony of each isolate was inoculated into tubes containing two ml of brain heart infusion broth (BHIB) and incubated at 37°C for 24 hours. Antifungal susceptibility testing was done on SDA petridishes according to CLSI guidelines.

RESULTS AND OUTCOMES: - In our study out of total 310 samples Candida tropicalis shows biofilm positive for 29 samples and biofilm negative for 27 samples of Candida Tropicalis. 8 samples of Candida Parasilopsis shows positive for biofilm production and 23 showed negative. Out of 21 samples of Candida Glabrata 3 samples shows biofilm production and 18 samples doesn't show. In Candida krusei out of 41 samples 27 were found positive for biofilm production and 14 were found negative. In out 161 samples of Candida albicans 70 were found to positive for biofilm and 91 were negative. In our overall scenario 137 samples were found positive for biofilm and 173 were negative.

CONCLUSION: The present study reported a significant shift in incidence of invasive Candidiasis from C.albicans to Non albicans species. Non - albicans Candida has emerged as an important opportunistic pathogen. Speciation of Candida becomes important as the prevalence of NAC is increasing. Antifungal susceptibility testing by the disk diffusion method is cost effective and should be adopted in routine testing as there is an increasing azole resistance, especially in invasive NAC infections. In this study, there was no correlation of antifungal drugs with the biofilm production.

KEYWORDS

Biofilm, Candida, azoles, non-albicans Candida, bloodstream infections.

INTRODUCTION:

A majority of the diseases caused by *Candida* spp. are due to biofilm formation. Biofilms are the group of microorganisms that are embedded in an extracellular matrix (ECM), forming a complex three-dimensional architecture on biotic and abiotic surfaces [32]. Biofilm formation can occur on mucosal surfaces and plastic surfaces of indwelling devices. Biofilms are genetically resistant to antifungal agents including amphotericin B (AMB) and fluconazole (FLU). Biofilm formation varies depending on the *Candida* spp. [33]. Most frequently, pathogenic effects are caused by *Candida albicans* and to a lesser extent by other *Candida* spp. [34].

For many years, C. glabrata was considered a relativelynon-pathogenic saprophyte of the normal flora of healthy humans, and was not readily associated with serious infection. However, C. glabratacan rapidly disseminate throughout the body, and infection with this species is associated with a high mortality rate. Moreover, C. glabratais of added concern because of its inherent resistance to certain antifungal agents[35]. C. parapsilosisis generally regarded as one of the least virulent yeast species, although it is now a frequent cause of candidaemia, commonly

Related to poor hand hygiene of healthcare workers [36].C. tropicalis has emerged as the second or third most common agent of candidaemia, mainly in oncology patients, and is often associated with nosocomial urinary-tract infections [37].

MATERIALS AND METHODS:

The present prospective study will be conduct in Department of Microbiology L. N. Medical College and Research center kolar road bhopal.

SAMPLE SIZE: -

A clinical samples will be submitted to Dept. of Microbiology for routine diagnostic workups were assessed. Candida isolates selection from 310 cases will be fulfilled the diagnostic criteria for Candidiasis will included in the study and will further process by a battery of microbiological investigations aimed at detecting, isolating, identifying and characterizing the Candida sp. so as to determine the spectrum of Candidiasis. A detailed review of Clinical History & clinical examination findings will be taken.

- **Research :- September 2019 to September 2021**

INCLUSION CRITERIA:

1. Clinical manifestations of invasive Candidiasis.
2. Patients associated with Immunocompromised diseases.
3. Presence of associated predisposing conditions and risk factors.
4. Patients under the chemotherapy and steroids.
5. Patients treated with broad spectrum antimicrobial agents for longer duration.

EXCLUSION CRITERIA:

1. Candida found as a commensals on many sites such as skin, mouth, small intestine, large intestine and colon so we excluded these sites from the study except we don't find any lesion at particular site. Candida always has competition to many bacteria on naturally occurring sites so it never overgrow and cause disease.

Note: In this study we are concerned only with Candida infection or invasive Candidiasis and not with Candida colonization. The isolates from cases not fulfilling the inclusion criteria will be considered as Candida colonization and to be excluded from the study.

Processing of the sample

The following protocol will be instituted –

- Direct Microscopy
- KOH wet mount
- Normal Saline preparation
- Gram staining of primary smear.
- Culture on Blood agar, SDA agar & Hi-Chrome agar, LPCB
- Gram staining of culture – smear
- Subculture on Saboraud's Dextrose Agar (SDA) Slants (Containing Cycloheximide and Chloramphenicol).
- Identification on chrome agar

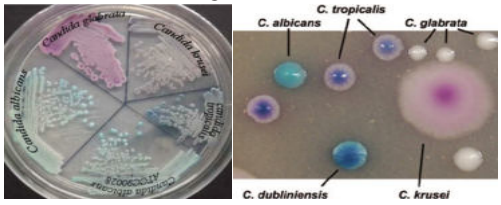


Figure 1 Candida species identification on chrome agar.

Incubation will be at 35°-37° C and 25° C in BOD incubator examined regularly for growth of Candida species. Morphological identification will be done on chrome agar by the development of different types of color according to their species.

Biofilm Production by Micro titer Plate Method (MTP). Biofilm formation was performed on a sterile 96-wellmicrotiter plate. A colony of each isolate was inoculated into tubes containing two ml of brain heart infusion broth(BHIB) and incubated at 37°C for 24 hours. All the broth cultures were diluted at a ratio of 1:20 using fresh BHIB, and200 μl was placed into microtiter plates and incubated at37°C for 24 hours. After the completion of incubation, microtiter plates were emptied, rinsed with distilled water three times, and then inverted to blot. Each well was then filled with 200 μl of 1% crystal violet and incubated for 15 mins. After incubation, the micro plates were again rinsed three times with distilled water. 200 μl of ethanol: acetone mixture (80:20 w/v) was added to each well and was read at 450nm using an ELISA reader and OD were recorded for each well. Sterile BHIB without microorganisms was used as the negative control. Cut-off value was determined by arithmetically averaging the OD of the wells containing sterile BHIB and by adding a standard deviation of +2. Samples with an OD higher than the cut-off value were considered positive, whereas those with the lower optical density than the cut-off were deemed to be negative [13].

The following were the values for the biofilm formation
(OD cutoff value – ODC, average OD of negative control – Avg NC)
Avg NC=0.194 SD=0.013, ODC =Avg NC+3×0.013 (SD).13
0.194+0.039, ODC=0.233 (1)
OD≤ODC=non-adherent
ODC<OD≤2 ODC=weakly adherent
2ODC<OD≤4 ODC=moderately adherent

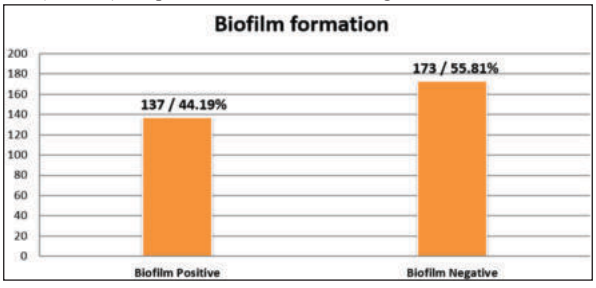
Antifungal susceptibility test: It was done by the Kirby-Bauer disk diffusion method, mainly for azoles like fluconazole (25 µg, HiMedia Laboratories) and voriconazole (1 µg; HiMedia Laboratories). It was performed on Mueller-Hinton agar supplemented with 2% glucose and 0.5 g of methylene blue per milliliter for enhancing the definition of growth margins,14,15 with zone diameter for fluconazole (25 µg) ≥19 mm –susceptible, 15–18 mm – susceptible and dose dependent, and ≤14 mm – resistant and that for voriconazole (1 µg) ≥17 mm – susceptible, 14–16 mm– susceptible and dose dependent, and ≤13 mm – resistant.

Out of total 310 samples Candida albicans 161 (52%) samples and 149 (48%) candida non albicans samples are isolated.

Table 01:- Total Number & Percentage of Biofilm Positive and Biofilm Negative

Biofilm formation		
Biofilm Positive	**Biofilm Negative**	**Total**
137	173	310
44.19%	55.81%	100.00%

Out of 310 samples 137 (44.19%) samples shows biofilm positive and 173 (55.81%) samples were shown biofilm negative.

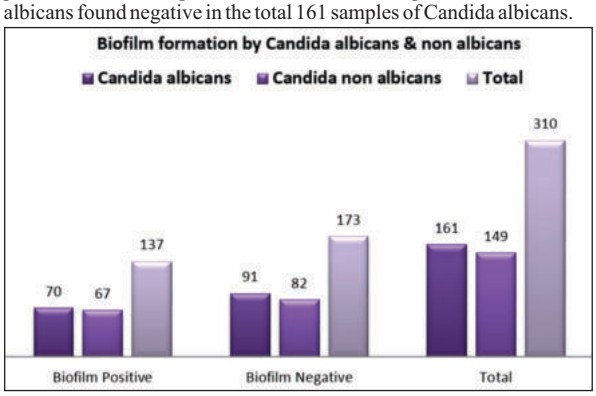


Graph 01:- Total Number & Percentage of Biofilm Positive and Biofilm Negative

Out of 310 samples 137 (44.19%) samples shows biofilm positive and 173 (55.81%) samples were shown biofilm negative.

Candida Spp.	Biofilm formation by Candida albicans & non albicans		
	Biofilm Positive	**Biofilm Negative**	**Total**
Candida albicans	70	91	161
Candida non albicans	67	82	149
Total	137	173	310

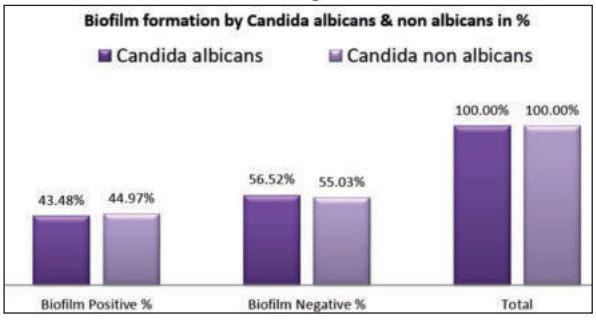
Table 02:- Total Number Biofilm positive and Negative by Candida albicans and Non albicans 70 samples out of Candida albicans found positive for biofilm production out of 161 samples and 91 Candida albicans found negative in the total 161 samples of Candida albicans.



Graph 02 :- Total Number Biofilm positive and Negative by Candida albicans and Non albicans.

Candida Spp.	Biofilm formation by Candida albicans & non albicans %		
	Biofilm Positive %	**Biofilm Negative %**	**Total**
Candida albicans	43.48%	56.52%	100.00%
Candida non albicans	44.97%	55.03%	100.00%
Total	44.19%	55.81%	100.00%

Table 03:- Total Percentages of Biofilm formation by Candida albicans & non albicans % Biofilm positive candida albicans



Graph 03 :- Total Percentages of Biofilm formation by Candida albicans & non albicans %

Candida Spp.	Biofilm formation by various Candida species		
	Biofilm Positive	Biofilm Negative	Total
Candida albicans	70	91	161
Candida kruesi	27	14	41
Candida glabrata	3	18	21
Candia parasilopsis	8	23	31
Candia tropicalis	29	27	56
Total	137	173	310

Table 04:- Total Number Biofilm formation by various Candida species

In our study out of total 310 samples Candida tropicalis shows biofilm positive for 29 samples and biofilm negative for 27 samples of Candida Tropicalis. 8 samples of Candida Parasilopsis shows positive for biofilm production and 23 showed negative. Out of 21 samples of Candida Glabrata 3 samples shows biofilm production and 18 samples doesn't show. In Candida krusei out of 41 samples 27 were found positive for biofilm production and 14 were found negative. In out 161 samples of Candida albicans 70 were found to positive for biofilm and 91 were negative. In our overall scenario 137 samples were found positive for biofilm and 173 were negative.

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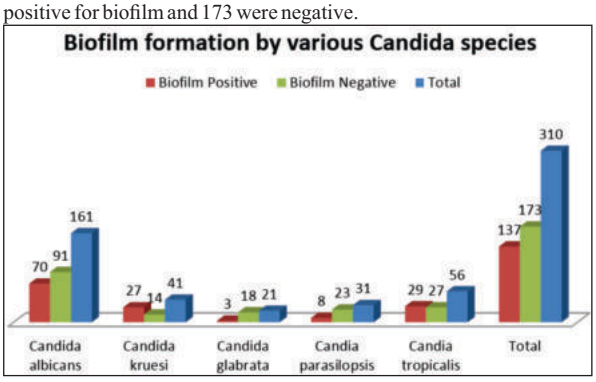

Graph 04:- Total Number of Biofilm formation by various Candida species

Candida Spp.	Biofilm formation by various Candida species %		
	Biofilm Positive %	Biofilm Negative %	Total
Candida albicans	43.48%	56.52%	100.00%
Candida kruesi	65.85%	34.15%	100.00%
Candida glabrata	14.29%	85.71%	100.00%
Candia parasilopsis	25.81%	74.19%	100.00%
Candia tropicalis	51.79%	48.21%	100.00%
Total	44.19%	55.81%	100.00%

Table 05:- Total Percentage of Biofilm formation by various Candida species

DISCUSSION: -

Candida isolates from 310 cases which fulfilled the diagnostic criteria for Candidiasis were selected and included in the study and further processed for species determination. The clinical samples from these cases were processed by a battery of microbiological investigations aimed at detecting, isolating, identifying and characterizing the Candida sp. so as to determine the spectrum of Candidiasis.

The incidence rate of Candidiasis from the clinical specimens received at Dept. of Microbiology for routine diagnostic workup came out to be 35 per thousand samples.

All of our patients had history of administration of Broad spectrum antibiotics for variable periods, 92 % of our patients were having indwelling devices (intravenous cannulae, Central venous catheter, Urinary catheter, Ryle's Tube, Endotracheal Tube), 64% had history of prolonged hospitalization, half of the patients were in extremes of age, 26% wer on steroids, 20% were diabetic, 12% were on mechanical ventilation, 8% were having Acute or chronic renal failure, 8% were on hemodialysis and 6% had undergone major surgery.

CONCLUSION

The present study reported a significant shift in incidence of invasive Candidiasis from C.albicans to Non albicans species. Non - albicans Candida has emerged as an important opportunistic pathogen. Non-albicans species are assuming an increasing role in nosocomial infections particularly in infants. The incidence of Candidaemia caused by non-albicans species is frequent and increasing significantly.

This shift is alarming as the infections caused by non-albicans species are often server, rapidly progressive, refractory to therapy and associated with higher mortality and significant morbidity. The innate and intrinsic resistances of Non-albicans sp. to the commonly used antifungal agents creates problems and complicates the management of the patients. This leads to prolonged hospitalization, increased costs of treatment and delays recovery requiring extra resources for investigations, management and nursing care.

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