A Menace of *Candida* biofilms: Prospective study among the intensive care unit patients in tertiary health care centre in North east India

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Abstract

Objective of this study is to know the incidence of biofilm formation by *Candida spp* among the intensive care unit patients and to identify the relationship between various *Candida* species with their antifungal susceptibility at North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences (NEIGRIHMS), Shillong.

Materials and Methods: A prospective study conducted at NEIGRIHMS, among patients admitted to the ICU during the period between January 1st and December 31st 2015. Different samples like endotracheal secretion, urine, blood and pus were collected under sterile conditions and standard fungal tests were performed for identification and appropriate statistical methods were employed to look for association between biofilm and *Candida* species.

Results: Out of the 396 samples included in the study from ICU, 117 samples showed evidence of *Candida* infections yielding an infection rate of 29.5%. Among the 189 isolates (63 *Candida albicans* and 126 Non albicans *Candida)* subjected for biofilm production, 76 (40.2%) were positive. Out of total *Candida* isolates 6.2% were multidrug resistant.

Conclusion: Non albicans *Candida* infection has drastically increased in healthcare centres. Biofilms have significant role in the perpetuation of these infections and antifungal resistance primarily with respect to their ability to adhere to various medical devices. Regular monitoring on the sensitivity and biofilm formation will be helpful in optimising therapy and outcome.

Keywords: *Candida* spp, Biofilms, Antifungal Resistance.

Introduction

Candida species are frequently encountered in the normal microbiota of humans, however, in cases of immunocompromised host these fungal pathogens facilitates their invasion over medical devices and host surfaces. Non albicans *Candida* species are now emerging as major agents of hospital acquired infections due to dramatic rise in organ transplant patients, cancer patients and HIV patients. Although *C. albicans* is the predominant etiologic agent of candidiasis, other *Candida* species that tend to be less susceptible to the commonly used antifungal drugs such as *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. lusitaniae*, etc have emerged as substantial opportunistic pathogens. Non albicans *Candida shares* many virulence factors with *C. albicans* such as biofilm production and phenotypic switching.[1] Medical devices like stents, shunts, implants, endotracheal tubes and catheters have shown to support colonization and biofilm formation by *Candida*.

Biofilms are specific and organized communities of cells under the control of signalling molecules, rather than random accumulations of cells resulting from cell division. Biofilm produced on medical devices can negatively impact the host immune defences by causing the failure of the device, enhanced resistance against most antifungal agents and by serving as a reservoir or source for future continuing infections.[2] Azole antifungal agents considered as important treatment for candidiasis in immunocompromised patients. In the expanding population of immunocompromised persons and injudicious use of antifungals, there is a drastic increase in antifungal drug resistance leading to morbidity and increase mortality of patients.[3, 4]

There is paucity of studies to the best of our knowledge, relating to burden of the disease and the resistance pattern prevalent in this part of the world. Therefore, the rationale of this study was to determine the incidence of biofilm formation by various *Candida* species isolated from clinical specimens among intensive care unit (ICU) patients and determination of their antifungal susceptibility pattern.

Materials and Methods

This was a hospital based prospective study conducted in the Department of Microbiology, NEIGRIHMS, Shillong. Ethical clearance was duly obtained from Institute Ethics Committee, NEIGRIHMS for conducting the study. A total of 396 clinical samples like blood, urine, pus and endotracheal secretions from ICU were included in the study. All the suspected *Candida* isolates were confirmed using the tests mentioned and only those isolates with confirmed identity were included for the further antifungal susceptibility study and analysis.

The Candida isolates were inoculated on SCCA, SDA and incubated at 25℃ and 37℃ for 7 days respectively. Colony morphology was observed with difference in colony, size and margin for different species on the SDA, Hicrome agar (Fig. 1) and CMA.[5]

Isolated Candida spp. were identified by colony morphology, gram staining, germ tube test, sugar assimilation and appearance of chlamydospore on cornmeal agar (Fig. 2).

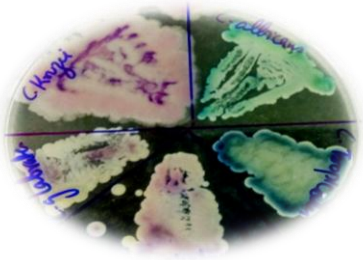

Fig. 1: Gross morphology in CHROMagar

Fig. 2: Microscopic morphology in CMA under 40X

Biofilm Formation: Assessment of biofilm formation was determined qualitatively by inoculating 5ml of brain heart infusion broth (BHIB) with colonies of *Candida spp* from 24h culture plates incubated at 37℃. The tubes were washed with phosphate buffered saline (PBS, pH 7.3) followed by drying. Tubes were then stained with crystal violet (0.1%) and excess stain was removed by washed with distilled water. Tubes were dried in inverted position and observed for biofilm formation. Biofilm production was reported positive on presence of visible film (Fig. 3).

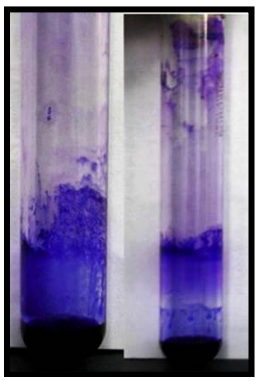

Fig. 3: Biofilm Production using 0.1% Crystal violet

Antifungal Susceptibility Testing: The antifungal susceptibility of these isolates were determined using VITEK 2 system. AST-YS07 cards were used and manufacturer's instructions were followed for the study of antifungal susceptibility profile. The activity of fluconazole, voriconazole, itraconazole, caspofungin, amphotericin B and flucytosine against our isolates were studied.[6] To evaluate the differences in antifungal susceptibility among *C. albicans* and non albicans Candida spp appropriate statistical tests were used like Fisher's test and p value less than 0.05 was taken as significant.

Results

During this study period, total of 189 isolates of *Candida* species were obtained from 117 (32%) samples out of 396 different clinical specimens of patients admitted in ICU. Of these, 103 (54.5%) were from Endotracheal secretions, 38 (20%) from urine of patients with in dwelling urinary catheter, 23 (12.2%) from vaginal discharge, 16 (8.5%) from blood and 9 (4.8%) from pus.

The study indicated slight preponderance of females (53%) in *Candida* infection than males (47%). The most common age group of patients which was affected by *Candida* infections in ICU were 40-60 years (36.8%) followed by elderly age group more than 60 years (26.5%).

The distribution among 189 *Candida* species isolated were *C. tropicalis* [73 (38.6%)], *C. albicans* [63 (33.3%)], *C. glabrata* [20 (10.6%)], *C. parapsilosis* [13 (6.9%)], *C. lusitaniae* [8 (4.2%)], *C. krusei* [5 (2.6%)], *C. kefyr* [4 (2.1%)] and *C. dubliniensis* [3 (1.6%)].

Among the 189 isolates subjected for biofilm production, 76 (40.2%) were positive. Biofilm was strongly produced in 52 strains most commonly by *C. tropicalis, C. albicans* and *C. Parapsilosis*, however, 24 strains identified as weak biofilm producers like *C. glabrata, C. lusitaniae* and *C. Krusei* (Fig. 4).

As the cases were from the ICU, the presence of various risk factors like renal failure, diabetes mellitus, use of broad- spectrum antimicrobial agents etc were observed for biofilm production enhancement. The most common factors associated with *Candida* infections were the presence of devices, implants and various types of catheters in 72 (61.5%) followed by presence of co-morbid conditions like diabetes mellitus and renal failure 45 (38.5%). The association was observed to be statistically significant with $p < 0.05$. *Candida* infections were also found to be associated with the increased duration of ICU stay in these patients, with most of the patients staying for more than 2 weeks.

5-Flucytocine was found to be the most resistant drug (80 isolates; 42% of total isolates) followed by fluconazole (45 isolates; 23.8%) on performing antifungal susceptibility testing. Among the 76 biofilm producing isolates, 50 (65.8%) were 5-flucytosine resistant and 24 (31.6%) were fluconazole resistant (Table 1). The most susceptible antifungal agent was Amphotericin B (177 isolates; 94%) in the present study. However, 6% of total isolates were observed to

be multi drug resistant and all these 12 isolates were biofilm producers.

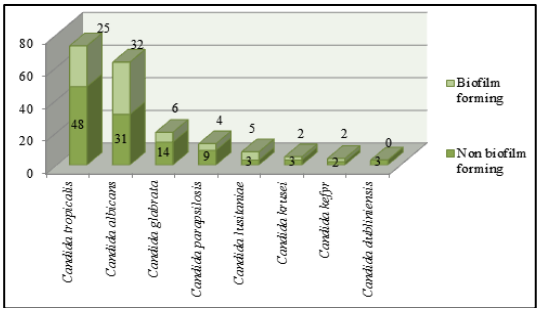

Fig. 4: Biofilm forming Candida species

Table 1: Antifungal resistance pattern among biofilm producers and non-producers (AmB – Amphotericin B, Vori – Voriconazole, Itra – Itraconazole, Flu – Fluconazole, 5-FU – flucytosine)

Isolates n= 189	Resistant Isolates				
	AmB	**Vori**	**Itra**	**Flu**	**5 FU**
Biofilm forming (n=77)	**12**	**14**	**17**	**24**	**50**
Non-biofilm forming (n=112)	**0**	**5**	**15**	**21**	**30**
P value <0.05	**0.0005**	**0.002**	0.16	0.06	**0.0001**

Discussion

NAC spp. once dismissed or ignored as non-pathogenic, commensal has emerged as potential pathogens in last two decades. Among NAC spp. *C. tropicalis* alone, or in association with other species, is implicated more frequently in human infections.

This study highlights the prevalence of *Candida* infection among the hospitalized patients in ICU, biofilm production and its correlation to antifungal susceptibility testing. In the present study, the predominance of non-*albicans Candida* species over *C. albicans* was a notable feature as more than 60% of infections were caused by non-*albicans Candida*, which is in accordance with the published report from various parts of the world. The most common isolate in our study was *C. tropicalis* (38.6%) followed by *C. albicans* (33%). The finding in our study was similar with the results reported by Nidhi et al., 2003,[7] Jagdish et al., 2013.[8]

Candida spp. have many virulence attributes which assist in invasion of host tissues. These include adherence to host tissues, release of extracellular enzymes, production of hyphae to aid in evasion of host immune defences and biofilm production. In comparison to Candida albicans, NAC isolates were shown to produce biofilms in higher proportion. Many studies have reported production of biofilms in range of 20%-60% of NAC isolated.[9-12] In our study biofilm production was seen in 34.9% of the NAC isolates and 50.7% of *C. albicans* isolates.

CLSI has standardized and recommended broth microdilution method for Antifungal susceptibility testing of *Candida* spp. In the present study we performed the antifungal susceptibility profile of the isolates using Vitek 2 compact system. Amphotericin B had been the mainstay of therapy against systemic fungal infections and had shown good susceptibility. Resistance (MIC >2 µg/ml) is rare and only few strains have been reported with high level resistance to this agent. In our study 12 isolates (6%) showed MIC >2µg/ml.

The present study showed increased resistance of biofilm forming *Candida spp* to 5 flucytosine and fluconazole (42% and 23.8%, respectively) although voriconazole, itraconazole and Amphotericin-B showed good efficacy on antifungal susceptibility testing by VITEK 2 compact system. This finding was consistent with study conducted by Bhatt et al.[13]

In developed countries, remarkably sensitivity to polyenes, flucytosine, azoles and echinocandins reported in *Candida spp*. However, in contrast to our study, Adhikari et al.,[14] 2011 had reported increased resistance to voriconazole (56%) and fluconazole (36%). As reported by several authors, we also observed immense degree of resistance to fluconazole in biofilm producers (65%) when compared to non-producers (26.7%). Possible reasons for this resistance may involve decreased penetration of drug through matrix of biofilm, release of extracellular hydrolytic enzymes and increase expression of genes for phenotypic switching.

Conclusion

C. tropicalis appears to be the emerging non-*C. albicans* species at our setup and Non-*albicans Candida* predominate over *C. albicans* in nosocomial *Candida* infections. The overall morbidity and mortality is high especially in critically ill patients and the study highlights the clinical significance of nosocomial *Candida* infections. Biofilms play a

significant role in the perpetuation of these infections primarily with respect to their ability to adhere to various medical devices. Constant monitoring of Candida infections and better interaction between the clinicians and the microbiologists can help in developing strategies and new products with antifungal abilities to control this emerging threat.

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Conflicts of Interest
There are no conflicts of interest.

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