RESEARCH ARTICLE

Isolation of Candida Species in Clinical Specimens and its Virulence Factor: The Biofilm

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ABSTRACT

Background: Candida species are now recognized as major causative agents of hospital-acquired infection. One of the major factors contributing to the virulence of Candida is its ability to form surface-attached microbial communities known as "biofilms". The importance of Candida biofilms is because of its increased resistance to antifungal therapy and the ability of cells within biofilms to withstand host immune defenses.

Objective: This study was undertaken with the objectives of isolating the Candida species and identifying its virulence factor – the biofilm and to determine the role of biofilm in pathogenicity.

Materials and Methods: A cross sectional study was conducted amongst the clinical specimens collected from the critical care wards of a tertiary care Hospital at Navi Mumbai from Jan 2009- Feb 2010. Care was taken to collect the samples before any anti fungal treatment. Candida spp were isolated and identified by standard techniques.

Results: Out of total 200 different clinical specimens collected and processed, the most commonly isolated species was C. albicans(61.36 %) along with non albicans like C. parapsilosis (9.1%) C. pseudotropicalis (13.64 %) and C. glabrata (15.9%).

Conclusion: The data suggests that the capacity of Candida species to produce biofilm appears to be a reflection of the pathogenic potential of the isolates. Isolates of Candida parapsilosis, Candida pseudotropicalisand Candida glabrata all gave significantly less biofilm growth then C. albicans.

Key Words: Biofilm; Candida Species; Clinical Specimens

INTRODUCTION

Pathogenic fungi in the genus *Candida* can cause both superficial and serious systemic disease. Many *Candida* infections involve the formation of biofilms on implanted devices such as indwelling catheters or prosthetic heart valves. Biofilms of *Candida albicans* formed in vitro on catheter material consist of matrix-enclosed micro colonies of yeasts and hyphae, arranged in a bilayer structure. The biofilms are resistant to commonly used antifungal agents like amphotericin B and fluconazole. In natural environments, many microorganisms exist predominantly as biofilms. Biofilms are structured microbial communities that are attached to a surface and encased in a matrix of exopolymeric material and are important in developing clinical infection.[1] One of the major factors contributing to the virulence of *Candida* is its ability in acclimatize to a variety of different habitats for growth and formation of surface-attached microbial communities known as "biofilms". Biofilms are defined as microbial communities encased in a matrix of extracellular polymeric substance (EPS), which display phenotypic features that differ from their planktonic or free-floating counterparts

Individual microorganisms in biofilms are embedded within a matrix of often slimy extracellular polymer. The first example of a biofilm to be recognized in medical systems was a dental plaque on tooth surfaces, but recent estimates suggest that a substantial proportion of human infections involve biofilms.[2] Many of these are implant related infections in which adherent microbial populations are found on the surfaces of devices such as catheters, prosthetic heart valves and joint replacements.[3]

A relatively small number of *Candida* species are pathogenic for humans. These organisms are capable of causing a variety of superficial and deep-seated mycoses that are distributed worldwide. All are opportunistic pathogens. The principal pathogen is *Candida albicans*. *Candida* species are now recognized as major agents of hospital-acquired infection. Their emergence as important nosocomial pathogens is related to specific risk factors associated with modern medical procedures, notably the use of immunosuppressive and cytotoxic drugs, powerful antibiotics that suppress the normal bacterial flora, and implanted devices of various kinds. Non-device-related infections like *Candida* endocarditis and *Candida* vaginitis are associated with biofilm production.[1]

METHODS

A cross sectional study was planned at a tertiary care hospital at Navi Mumbai from January 2009 to February 2010. Specimens to be collected were sputum, urine, blood, bronchial secretions, endotracheal secretions, vaginal secretions and from endotracheal tubes and indwelling catheter tips. Inclusion criteria were that the specimens must be collected from critical care wards and the samples must be collected before any anti fungal treatment. The distribution of clinical specimens collected were as follows - Sputum (40), urine (40), blood (40), bronchial secretions (10), Endotracheal secretions (10),vaginal secretions (10), and devices like Endotracheal tubes (25) and Indwelling Catheter tips (25).

A standard KOH wet mount was performed followed by a Gram's staining on all specimens. Inoculation was carried out on Sabouraud's dextrose agar (SDA) and incubated at 37°C for 7 days or more if required.

Out of 200 specimens, those specimens which came positive for Candida were further processed for biofilm production. Biofilm production was determined by using a method proposed by Brachiniet al., wherein a loop full of organisms from the SDA was inoculated into a tube containing 10ml Sabouraud's liquid medium strains supplemented with glucose (to the final concentration of 8%).[4] The tubes were incubated at37°C for 24 h after which the broth was aspirated out and the walls of the tubes were stained with saffranin. Biofilm production was scored as negative (0), weak positive (1+), moderate positive (2+) and strong positive (3+). Data were entered in excel worksheets and analyzed using suitable statistical methods.

RESULTS

Candida species have the ability to cause a variety of superficial and deep seated mycoses. The present study shows the distribution of candida species in different clinical specimens and the predominance of candidia albicans in them.

Out of the total 200 clinical specimens, candida species were isolated from 88 specimens. Amongst these 88 positive specimens for Candida, majority were C. albicans (61.36%). The rest identified species in decreasing order were C. glabrata (15.9%), C. pseudotropicalis (13.64%) and C. paraspilosis (9.1%).

Table-1: Distribution of Candida species among clinical specimens

Name of the Candida Species	Number of Isolates	95% Confidence Interval
Albicans	54 (61.36 %)	0.5092 – 0.7085
Paraspilosis	08 (9.1 %)	0.0468 – 0.1692
Pseudotropicalis	12 (13.64%)	0.0798 – 0.2234
Glabrata	14 (15.9 %)	0.0972 – 0.2495
Total	88 (100%)	0.9582 – 1.0000

Amongst the C. albicans, 68.51% showed moderate to strong positivity (2+ to 3+),

whereas only 7.42% were negative for biofilm production.

Table-2: Distribution of Candida Species According to Biofilm Production

Candida Species	Biofilm Production			
	0	1+	2+	3+
albicans	04 (7.42%)	13 (24.07%)	32 (59.26%)	05 (9.25%)
paraspilosis	01 (12.5%)	05 (62.5%)	01 (12.5%)	01 (12.5%)
pseudotropicalis	02 (16.67%)	07 (58.33%)	02 (16.67%)	01 (8.33%)
glabrata	01 (7.15%)	09 (64.28%)	04 (28.57%)	00 (0)
Total	8	34	39	7

Table-3: Distribution of Specimens According to Candida Species Isolates and Biofilm Production

Source of the Specimen (Number of Samples)	Proportion of Specimens Positive for		
	Candida isolates	Candida albicans	Candida non albicans
Sputum (N=40)	30 (75%)	17 (56.7%)	13 (43.3%)
Urine (N=40)	18 (45%)	12 (66.7%)	6 (33.3%)
Blood (N=40)	18 (45%)	9 (50%)	9 (50%)
Bronchial Secretions (N=10)	6 (60%)	5 (83.3%)	1 (16.7%)
Endotracheal Secretions (N=10)	3 (30%)	2 (66.7%)	1 (33.3%)
Vaginal Secretions (N=10)	2 (20%)	1 (50%)	1 (50%)
Endotracheal Tubes (N=25)	4 (16%)	3 (75%)	1 (25%)
Indwelling Catheter Tips (N=25)	7 (28%)	5 (71.4%)	2 (28.6%)

Isolates of C.albicans showed moderate to high biofilm production compared to the non albicans group. Majority of C.paraspilosis (62.5%), C. glabrata (64.28%) and C.pseudotropicalis (58.33%) showed weak positivity.

Amongst all the specimens collected for isolating Candida species, majority of the Candida were isolated from sputum (75%), bronchial secretions (60%), urine (45%) and blood (45%).

As shown in table 3, it can be said that out the total Candida isolates proportion of Candida albicans was more in this study as compared to Candida non albicans.

DISCUSSION

Biofilms are a protected niche for microorganisms, where they are safe from treatment and can create a source of persistent infection.

Candida albicans remains the fungal species most commonly associated with biofilm formation, and the increase in Candida infections in the last decades has almost paralleled the increase and widespread use of a broad range of medical implant devices, mainly in populations with impaired host defenses.[1,5,6] The formation of Candida biofilms carries important clinical repercussions because of their increased resistance to antifungal therapy and the ability of cells within biofilms to withstand host immune defenses. Also, biofilm formation on medical devices can negatively impact the host by causing the failure of the device and by serving as a reservoir or source for future continuing infections.[5] The net effect is that Candida biofilms adversely impact the health of these patients with increasing frequency and severity and with soaring economic squeal.[7] Quantitatively albicans spp. produces larger and more complex biofilms than any other spp.[8] Based on the results of this study, biofilm-forming ability was found greater for albicans species, isolated from clinical specimens than the non albicans group of Candida.

CONCLUSION

The data suggests that the capacity of Candida species to produce biofilm appears to be a reflection of the pathogenic potential of the isolates. Isolates of Candida parapsilosis, Candida pseudotropicalisand Candida glabrata all gave significantly less biofilm growth then C. albicans.

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