

Original Article

Biofilm As A Virulence Marker In Candida Species In Nosocomial Candiduria Infection And Its Resistance Pattern In A Tertiary Care Hospital Kanpur.

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ABSTRACT

Biofilms are specific and organized communities of cells under the control of signaling molecules, rather than random accumulations of cells resulting from cell division. Biofilms may help maintain the role of fungi as pathogenic by evading host immune mechanisms, resisting antifungal treatment and withstanding competitive pressure from other organisms. In this study *Candida dubliniensis* (100%) was found to be the strong biofilm producer followed by *Candida tropicalis* (23.7%), and *Candida glabrata* (15.38%) respectively. Whereas *Candida krusei* were found non biofilm producer in the present study. It is concluded that *Candida* colonization has a considerable prevalence among the hospitalized patients, especially in the patients with indwelling catheters. And the prevalence is relatively high in Intensive Care Unit (61.53%) Proper infection control practices should be implemented of the critical care unit.

INTRODUCTION

Catheterization is one of the most important aspect of modern medical practice, urinary catheter to monitor urine output is also an important cause of nosocomial urinary tract infection. ^[1,2]

The manipulation of this type of device by health professionals could facilitate the migration of yeast to the bladder, contributing to the appearance of an infection. As *Candida* species are part of the human microbiota, they are often found on biomaterials, implants and various types of catheters. *Candida* on its first interaction with the host cause subsequent colonization of surrounding tissue and disseminate throughout the body. ^[1,3] The significance of indwelling devices in hospitalized patients lies in the fact that these are very frequently associated with formation of biofilm on mucosal surface and plastic surface of indwelling devices, which consist of enclosing micro colonies of yeast, hyphae, pseudo hyphae. ^[4] Biofilms are specific and organized communities of cells under the control of signaling molecules, rather than random accumulations of cells resulting from cell division. These biological communities can be embedded in an extracellular matrix that is self-produced. ^[5] Biofilms may help maintain the role of fungi as pathogenic by evading host immune mechanisms, resisting antifungal treatment and withstanding competitive pressure from other organisms. Consequently, biofilm related infections are difficult to treat. Biofilm production is also associated with a high level of antimicrobial resistance to antifungal agent including amphotericin B, fluconazole rendering them infective during the treatment of candiduria. The biofilm producing capacity varies among the species of *Candida*. ^[1] Biofilm formation can be affected by growth conditions and coinfection with other pathogens. ^[6] There are several valuable antifungals, such as amphotericine B, itraconazole, fluconazole, ketoconazole, econazole and nystatin, that are effective against *Candida* species. Some of these agents (fluconazole, amphotericine B, ketononazole, econazole, itraconazole) are systemically used to treat urinary tract infections (UTI). Several reports have demonstrated the antifungal fluconazole has been effective for short- tem eradication of candiduria. On the other hand some researchers have found that the susceptibility degree of *Candida* species very towards the used antifungal drugs. For example, *C. krusei* and *C. glabrata* are resistant and less susceptible to fluconazole, respectively. ^[7] Antifungal therapy that is administered without previous determination of the agent incurs the risk of being ineffective, and could also contribute to the number of cases of candiduria due to non-*Candida*

albicans Candida albicans Candida (NCAC) species, and some of them are naturally resistant to azoles. This situation has made necessary to indentify the yeasts involved in order to determine not only the species, but also its susceptibility profile to the available antifungals^[8]. Although several new antifungal drugs have been licensed in recent years, antifungal drug resistance is becoming a major concern during treatment of such patients. The mechanism of drug resistance in microorganisms traditionally takes the path of either identifying a cellular determinant that prevents entry of the drug or removes the drug from the cell of inactivates the drug of prevents the drug from inhibiting the target of various combinations^[9].

These is changing in epidemiology of candiduria with the risk factor of biofilm, therefore, this study is carried out to monitor the distribution of different Candida sp. , its reistance pattern and biofilm production of Candida species from urinary tract infection in order to optimize therapy and outcome.

MATERIAL AND METHODS

INCLUSION CRITERIA

All candida isolates from patients with urinary catheters in Rama hospital more that 48hrs of admission,

- Male
- Female
- All the age group were included in this study.

EXCLUSION CRITERIA

- Patients in hospital less then 48 hours

- Patient demographics
- Underlying diseases,
- Date of admission
- Location of the patient at time of infection
- Therapy

STUDY DESIGN

Prospective study including laboratory investigations and observational analytical design.

STUDY PERIOD

Rama Medical Collage Hospital & Research centre, Kanpur, UP.

STUDY PERIOD

The study was conducted from January 2016 to December 2016.

SAMPLE COLLECTION

Urine specimens were collected as midstream morning sample or from the port of the Catheter. And the sample were repeated after 24hrs and immediately send to the microbiology laboratory for microbiological examinations, culture, identifications and Antifungal sensitivity. If any delay sample should be placed in refrigerator at 8⁰C.

PROCESSING OF SAMPLE

All urine samples will inoculate on CLED (Cysteine Lactose Electrolyte Deficient Agar). And incubated at 37⁰C for overnight, next day organism indentified of the basis of colony morphology, colonies of Candida were appear small, rough and white, for confirmation we were performed gram staining

PROCEDURE OF GRAM STAINING

In the gram staining the Candida seen purple colour oval shaped organism.[6]

PROCEDURE OF GERM TUBE TEST ,SDA (Sabouraud Dextrose Agar) and

CHROM agar was performed.

BIOFILM FORMATION

All the isolated were further test for biofilm formation. Biofilm production was detected by method described by a loopful of organism from sabouraud's dextrose agar (SDA) plate was inoculated in to tube containing 10 ml sabouraud's dextrose broth was supplemented with glucose (final concentration 8%). The tubes were then incubated at 37⁰ C for 24hrs after which the broth was aspirated out and then was of the tubes were stained with 1% safranine. Tube were then kept still for seven minutes. Safranine then was removed and tubes were examined for biofilm production. Slime production was scored by to observers simultaneously twice each to reduce as much as possible intra and inter observer's difference.

It was scored as-

There is no film	Negative
There is film only on the bottom of the tube	Weak positive (1+),
There is weak film on the wall and bottom of the tube	Moderate positive (2+),
There is strong film around the wall	Strong positive (3+),

RESULTS

In the present study, we observed 100 positive isolates with growth in urine sample. Out of them 87% were bacterial isolates and 13% were fungal isolates. In our study the overall prevalence of candiduria is 13% which was comparatively higher to the result of other study done by Meena Mishre et al^[10]

with 10.5% and comparatively lower to the study done by zarei mah moudabadie et al^[11] and mythreyi et al^[12] with 16.5% and 26% of prevalence rate. In our study the prevalence rate is low may be we have taken only true pathogen, which was confirmed by repeat sampling.

The prevalence of candiduria caused by the species other there than *C.albicans* was surprisingly high in our study, our study showed that isolation rare of non albicans candida was 76.93% which is higher than *Candida albicans* 23.7% this finding is in concordance with the study done by Yashavanth R et al with 69.7% of non albicans and 3.30% of aalbicans^[10].

In several studies have shown that candiduria is more frequently is female than male^[6], similarly in our study we found that candiduria were more common among females (69%) than males (31%). Because of candida colonization in vulvo vestibular area females are more prone to UTI.

NOSOCOMIAL ISOLATES

	NUMBER OF ISOLATES	PERCENTAGE
Candida species	13	13%
Other isolates	87	87%

Out of 100 nosocomial UTI 87% were bacterial and 13% were found to be positive for fungal isolates.

BIOFILM PRODUCTION BY DIFFERENT CANDIDA SPECIES

Name of isolates	Total	Biofilm positive	Biofilm grading			Percentage of biofilm positive isolates
			+1	+2	+2	
<i>Candida krusei</i>	01	00	00	00	00	00%
<i>Candida dudlinienses</i>	01	01	00	00	01	100%

Candida glabrata	03	02	01	00	01	66.66%
Candida albicans	03	03	01	00	02	100%
Candida tropicalis	05	03	00	02	01	60%

In this study *Candida dubliniensis* (100%) was found to be the strong biofilm producer followed by *Candida tropicalis* (23.7%), and *Candida glabrata* (15.38%) respectively. Whereas *Candida krusei* were found non biofilm producer in the present study.

INTERPRETIVE CRITERIA FOR SUSCEPTIBILITY OF ANTIFUNGALS

The interpretive criteria for the Fluconazole, Nystati, Pmphtericin B, Ketoconazole, Itraconzole, and Clotrimazole, and Clotrimazole disk were indicated in the table-

INTERPRETATION OF ZONE SIZE-

Zone diameter in mm			
Antifungal drugs	Sensitive (S)	Dose dependent	Resistance

Amphotericin B	<15	10-14	<9
Nystatin	≥25	17-24	<16
Fluconazole	≥19	15-18	≤14
Ketaconazole	≥30	23-29	≤22
Itraconazole	≥16	10-15	≤9
Clotrimazole	≥20	12-19	≤11

SPECIESWISE INTERPRETATIONS OF ANTIFUNGAL DRUGS

S. NO	ANTIFUNGAL		C.albicans n=03	C.tropicalis n=05	C.glabrat a n=03	C.krusei n=01	C.dublienesi n=01
1.	Itraconazole	R	33.33%	80%	66.66%	100%	00%
		S	33.33%	20%	00%	00%	100%
		D D	33.33%	00%	33.33%	00%	00%
2.	Fluconazole	R	66.66%	25%	00%	00%	100%
		S	33.33%	0%	33.33%	100%	00%
		D D	00%	75%	66.66%	00%	00%
3.	Kitoconazole	R	33.33%	40%	00%	00%	00%
		S	33.33%	60%	66%	100%	100%

		D	33.33%	00%	33.33%	00%	00%
		D					
4.	Clotrimazole	R	66.66%	80%	33.33%	100%	00%
		S	33.33%	00%	66.66%	00%	00%
		D	00%	20%	00%	00%	100%
		D					
5.	Nystatin	R	33.33%	00%	33.33%	00%	00%
		S	66.66%	40%	66.66%	100%	00
		D	00%	60%	00%	00%	100%
		D					
6.	Amphotericin B	R	00%	00%	00%	00%	00%
		S	66.66%	100%	100%	100%	00%
		D	33.33%	00%	00%	00%	100%
		D					

Risk factors for nosocomial candiduria, catheterization are the most common risk factor, normal commensals gain ready access to urinary tract in the presence of indwelling catheter. Antibiotic administration was done in all the patients which was most common risk factor diabetes is the most common, diabetes predisposes to candiduria by enhancing fungal growth in the presence of glycosuria in the vulvovaginal area in female and in periurethral area in men, followed by post-surgical stay in the hospital.

DISCUSSION

Urinary tract infection as a result of *Candida* species are becoming increasingly common in hospital settings. The introduction of more efficient diagnostic methods, new techniques in surgery and transplantation, antibiotics and chemotherapeutics more potent and novel materials for prostheses, catheters, catheters and probes significantly increased the life expectancy and quality

of life of critically ill patients, on the other hand, hospital acquired infection emerged as important iatrogenic complication. In general invasive fungal infection are associated with high morbidity and mortality, difficulties in diagnosis, antifungal resistance, length of hospital stay and increased hospital cost. This study was done to study the biofilm as a virulence marker for the Candida species in nosocomial candiduria. The prevalence of Candida spp. was 13% with 100 positive isolates. The species wise distribution among the candida isolates was Candida tropicalis (38.4%), Candida albicans (23.07%), Candida glabrata (23.07%), Candida dubliniensis (7.69%), and Candida krusei (7.69%). The present study revealed the emergence of nosocomial candiduria in which non albicans species are replacing the Candida albicans species. Biofilm productions were seen positive in 69.23% in all the candida isolates. In the present study Amphotericin B were found to be the most sensitive drug and Clotrimazole and Itraconazole were found to be the most resistant drug against Candida infection.

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