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Research article

PREVALENCE OF BIOFILM PRODUCING MDR *CANDIDA ALBICANS AND NON CANDIDA ALBICANS* ISOLATE FROM MEDICAL DEVICES

P. Rajeswari[1], P.Vijayalakshmi[1], D. Jegadeeshkumar[2]

[1]Department of Microbiology, Vivekanandha College of Arts and Sciences for Women, Tiruchengode, Tamilnadu, India.

[2]Chromopark Research centre, Namakkal, Tamilnadu, India.

ABSTRACT : Totally 56% of occurrence was observed from 6 types of sources. Among them highest prevalence was observed from urinary catheter (68%) next in line is intravenous tubes (66.66%), venflon needles (65%), and blood bags (53.33%) respectively. Four types of *Candida* species were identified by using selective media and biochemical tests. The *Candida albicans* was predominant isolates in all sources especially in urinary catheter. In this study, 60.2% of non *Candida albicans* were observed. All isolates were subjected to antifungal stability test, 6 antifungal agents were used. Among the 6 antifungal agents Itraconazole had highly resistance activity and Fluconazole had highly sensitive activity against the isolates. The antifungal resistance of isolates were highly observed in non *Candida albicans* such as *Candida tropicalis* (83.3%) and followed by *Candida glabrata* (74.5%). All isolates were have the ability to produce biofilm, among them 37.4% of isolates were strong biofilm producer and 100% of protease producing isolates were observed in the last part of the study.

Key words: Nosocomial infections, Medical devices, *C.albicans*, non *C.albicans*, Biofilm, MDR.

INTRODUCTION

An infection acquired during the hospitalized period is called nosocomial infections. These infections can be bacterial, viral, and fungal or even parasitic. During the past two decades fungi have become increasingly important causes of nosocomial infections and have emerged as a frequent cause of mortality and morbidity in hospital patients. Since nosocomial fungal infections (NFI) are often severe, rapidly progressive and difficult to diagnose or treat, there is a critical need for more efforts to be directed toward prevention, early diagnosis and aggressive treatment of these infections (Kordbacheh *et al*., 2005).

Fungal diseases became recognized as being of clinical importance in the second half of the last century, mainly due to advances in medical technologies. With the remarkable modern advances in medicine, there has been an increase in the number of immunocompromised individuals who need extensive care in hospitals. This has resulted in a rise in the incidence of fungal infections, especially those due to *Candida* species (Rizvi and Malik 2011). Now a day's number of the medical devices was used during the treatment. At least half of all cases of nosocomial infections are associated with medical devices. The medical consequences of device-related infections can be disastrous; they include potentially life-threatening systemic infections and device malfunction that may require device removal, often complicated by tissue destruction. Management of device-related infections can be difficult and is costly. An increasing proportion of device-related infections, particularly those involving the bloodstream and urinary tract are being caused by *Candida* spp (Erna, 2004). An implanted device such as a urinary catheter is associated with nosocomial infections and biofilms can be detected on such devices. Urinary catheters have been responsible for 80 % of the hospital acquired urinary tract infections. Surveillance data (1986 to 1996) from the United States National nosocomial infections surveillance system has shown that *Candida albicans* is the fourth most common cause urinary tract infections. (NNIS report 1996). The other medical devices totally implanted in the body that are liable to *Candida* infection are Intravenous tubes, Blood bags, dentures and prosthetic heart valves. Organisms may be introduced form the hands o f nursing staff (or) the patient's skin micro flora (Douglas, 2003).

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One of the important factors contributing to the virulence of *Candida* is the formation of surface-attached microbial communities known as ''biofilm'' (Seneviratne *et al.*, 2008). Eradication of biofilms is difficult and biofilm producing *Candida* species are significantly less susceptible to antimicrobial agents (Mathur *et al*., 2006; Douglas, 2003). This study was undertaken to find out the *Candida* in the medical device using patients of an ICU and to determine the species distribution and the antifungal susceptibility profile of isolates.

MATERIALS AND METHODS

This study was carried out from ICU patients of hospitals in and around Namakkal district during the study period (July 2011 to March 2012). The clinical isolates were collected from different medical devices like urinary catheters and intravenous tubes and associated devices.

Isolation and identification

Total of 100 samples were collected in hospitals in and around Namakkal district. Collected devices were cut into pieces and incubate with tube containing peptone broth, after the incubation period a loopful of cultures were streaked on specific (Sabouraud and Candida blue green agar) and selective (Hicrome Candida differential agar medium for confirmation of *Candida* species) medium. Further confirmation was done by germ tube, cornmeal agar, and other biochemical tests (sugar fermentation and sugar assimilation).

Germ tube test (Haley, 1971)

Special species level confirmation was done by germ tube test. Suspend the yeast in the human serum and incubate at 35ºC for 2 to 3 hrs. Use the high power objective to confirm the presence or absence of germ tube.

Chlamydospore formation (Joshik *et al.*, 1993)

Corn meal agar with 1% tween 80, after incubation the colonies were examined, it shows pseudohyphae with blastoconidia and terminal vesicles.

Fermentation reaction (Jawetz *et al.*, 1978)

The 5 ml of carbohydrate (pH- 7.4) containing 1 % peptone, 1 % sugar (glucose, maltose, sucrose and lactose),0.3 % beef extract and 0.5 % NaCl, 0.2 % Bromothymol blue in distilled water medium was dispensed in sterilized Durham tube and 0.2 ml of saline suspension of the test organism was added and incubated at 37ºC for 10 days.

Sugar assimilation (Germain and Beauchesne 1991.)

The main stay of yeast identification to the species level is the carbohydrate assimilation test, which measures the ability of yeast to utilize a specific carbohydrate as the sole source of carbon in the presence of oxygen. Sugars used for assimilation tests including dextrose, maltose, sucrose, lactose, galactose, melibiose, cellobiose, inositol (a form of sugar, carbocyclic polyol, cyclohexanehexol), xylose, raffinose, trehalose and dulcitol (or galactitol, a sugar alcohol, the reduction product of galactose.

Antibiotic sensitivity test (Bauer and Kirby, 1966).

Sensitivity /resistant pattern of isolates were done by Kirby-Bauer method. All the test cultures were inoculated into brain heart infusion broth and incubated at room temperature for 5 hours. Each strains of isolate were spreaded over the Muller Hinton Agar (MHA) plates. The panel of antifungal agents were selected and placed aseptically over the MHA plates. The plates were incubated at room temperature for 18-24hours. Then the plates were examined for the presence of zone of inhibitions and results were interpreted according to the standard chart (Hi-media, India).

Determination of virulence factor

Biofilm tube method (Christensen *et al.*, 1982)

A loopful of organism from a surface of sabouraud dextrose agar plate was inoculated into a polystyrene tube containing sabouraud liquid medium supplemented with glucose 8%. The tubes were incubated at 35ºC for 24hrs. After which the broth was aspirated and walls of the tubes were stained with saffranin.

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Proteinase assay

Proteinase activity of *Candida* species was checked as per method described by Gokce *et al.*, 2007. Ten µl of sample (suspension) was introduced on a sterile paper disk placed on the surface of bovine-serum albumin agar medium (pH 5.0). The inoculated plates were incubated at 37^0 C for two days and diameters of zones of inhibition around the disks were measured for determination of Proteinase activity.

RESULT

All isolates of *Candida* species were obtained by selective media, Sugar fermentation and biochemical tests (Plate1). The results were tabulated in table. 1. In this study totally six types of samples were subjected for isolation of *Candida* species. Among them urinary catheters (68%) are highly infected with *Candida* species, next in line is intravenous tubes (66.66%), venflon needles (65%), and blood bags (53.33%) respectively (Table.2). Totally 56% of isolates were observed from all types of sources. Among them *Candida albicans* (39.5%) showed highly prevalent in all sources and second most *Candida glabrata* (27.0%) followed by *Candida tropicalis* and *Candida parapsilosis* (16.6%). In case of source wise the highest prevalence were observed in urinary catheters (68%) and followed by Intravenous tubes (66.6%), Venflon needle (65%) and blood bags (53.33%).

Table- 1: Morphological characteristics of Candida species

S.No	Name of the test	Media	*Candida albicans*	*Candida glabrata*	*Candida parapsilosis*	*Candida tropicalis*
1	Colony morphology	SDA	Colonies are white to cream colored, smooth, glabrous and yeast-like in appearance.	Small glossy, convex, and smooth white to cream	white to cream colored, smooth, glabrous, although sometimes lacy in appearance	White to cream colored, smooth, glabrous and yeast-like in appearance.
		Candida BCG	Cream to light green homogeneous free flowing powder	Cream to light green homogeneous free flowing powder	Cream to light green homogeneous free flowing powder	Cream to light green homogeneous free flowing powder
		Hichrome	Light green	the colonies will be pink to mauve in color, often with a darker center.	Creamish to pink-	Metalic Bluish green
2	Germ tube	Serum	+	-	-	-
3	Cornmeal agar	Cornmeal agar with tween 80	Pseudohyphae with blastoconidia and terminal vesicles (chlamydoconidia).	cells will appear under the microscope in very closely packed groupings, without any separations	crooked or slightly curved appearance of relatively short pseudohyphae and occasional giant pseudohyphae that is produced	Abundant long, wavy, branched pseudohyphae with numerous ovoid blastoconidia budding off. Terminal vesicles (chlamydoconidia) are not produced.
4	Sugar fermentation	Glucose	+	+	+	+
		Maltose	+	-	-	+
		Sucrose	-	-	-	+
		Lactose	-	-	-	-
		Trehalose	+	+	-	+
5	Sugar assimilation	Glucose	+	+	+	+
		Maltose	+	+	+	+
		Sucrose	+	-	+	+
		Lactose	-	-	-	-
		Trehalose	+	+	+	+

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In my present study 4 different age groups (25-30, 31-35, 36-40 and 40 above) were selected, among these 4 age groups 25-30 (33%) age group peoples were highly infected with *Candida* species. Next in line is above 40 (31.25%), 31-35 (18.75%), and 36-40 (16.66%) peoples were infected with *Candida* species. In 25-30 age groups, *Candida albicans* (41.66%) was most prevalent species, followed by *Candida glabrata* (25%), *Candida parapsilosis* and *Candida tropicalis* (16.66%).

In another hand Denture swabbing and contact lens also collected and subjected to isolation of *Candida* isolates. Out of 15 samples 6 (40%) isolates were obtained from denture swabs. Among them, 50% of *Candida albicans* and 16.6% of non *Candida albicans* were obtained. This prevalence was mostly occurrence in above 75 age groups peoples (100%) and followed by above 45 age group peoples (50%). In case of contact lens 20 % of isolates only observed. In our current study this prevalence were lowest compared other medical devices. This prevalence was equally distributed in each age group of 20-40 and above 40 (Table 3 & 4).

Table-2: Total number of occurrences in medical devices

Name of the devices	N. of isolates	25-30				31-35				36-40				40 above				
		C.a	*C.g*	*C.p*	*C.t*	*C.a*	*C.g*	*C.p*	*C.t*	*C.a*	*C.g*	*C.p*	*C.t*	*C.a*	*C.g*	*C.p*	*C.t*	%
Urinary catheters	17/25	4	1	1	1	1	1	1	0	2	0	0	1	3	1	0	0	68.00
Intravenous tubes	10/15	1	1	1	1	0	0	0	1	0	1	0	0	2	1	0	1	66.66
Venflon needle	13/20	2	0	1	0	1	2	0	0	1	1	0	0	1	2	1	1	65.00
Blood bags	8/15	1	0	1	0	0	1	1	1	0	1	0	1	0	0	1	0	53.33
Total	48/75	8	2	4	2	2	4	2	2	3	3	0	2	6	4	2	2	64

C.a - Candida albicans, C.g - Candida glabrata, C.p - Candida parapsilosis, C.t - Candida tropicalis

Table-3: Total number of occurrences in denture swabbing

Name of the isolate	No of isolates	70+				55+				45+				Percentage
Denture swabbing	6/15	*C.a*	*C.g*	*C.p*	*C.t*	*C.a*	*C.g*	*C.p*	*C.t*	*C.a*	*C.g*	*C.p*	*C.t*	46.66
		1	1	1	1	1	0	0	0	1	0	0	0	

Table-4: Total number of occurrences in contact lenses

Name of the isolates	No of isolates	20-40				40 above				Percentage
Contact lenses	2/10	*C.a*	*C.g*	*C.p*	*C.t*	*C.a*	*C.g*	*C.p*	*C.t*	20.00
		1	0	0	0	0	1	0	0	

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In this study next investigation was antifungal resistance patterns against all isolates of *Candida*. Totally 6 antifungal agents were utilized for this study. Among them Itraconazole had highly resistance activity (98.2%) second most Nystatin (83.9%) followed by Clotrimazole (75%), Amphoteracin B (67.85%) and Ketoconazole (66.0%). The lowest antibiotic resistance was observed in Fluconazole (57.14%) (Fig.1). The Fig.2 showed percentage of antibiotic resistance in *Candida* spp among them highest resistance in *Candida tropicalis* (83.3%) and followed by *Candida glabrata* (74.5%) (Plate.1).

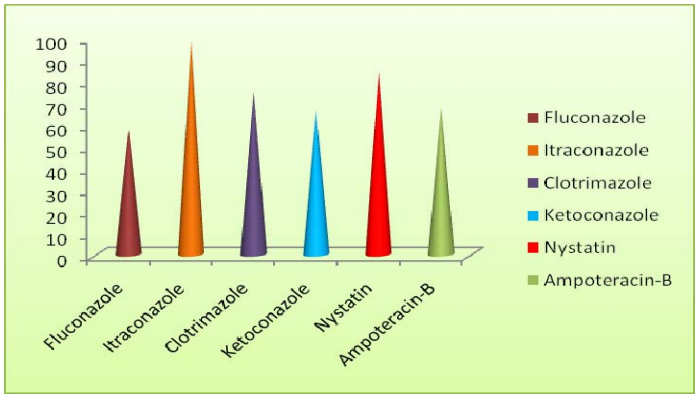

Fig- 1: Percentage of antibiotic resistance

Among the 6 types of sources, Intravenous tubes had highest antifungal resistance (83%) and second most Urinary catheters (81.3%) followed by Venflon needle (80.7%). The increasing resistance patterns were observed in blood bags (49.9%). The lowest resistance was observed in contact lens (41.6%) (Fig.3). According to Table 5 those which were resistant to 2 or more antibiotics considered as MDR strains, in lowest pattern, 2 antibiotic resistances in 4.2% of isolates. Sixteen resistance patterns were recognized among the MDR strains. In this study no one antibiotic sensitive to all isolates. The most frequent phenotypical pattern that was resistant to Itraconazole and Clotrimazole.

The next part of the study was prevalence of biofilm formation. The hundred percentages of biofilm producers were observed but strongly biofilm formation not observed from all isolates. Among the different sources, strongly biofilm producers were highest in denture swab (66.6%) and lowest in blood bag (12.5%). In case of species wise *Candida albicans* had highest strongly biofilm producers (17.8%). The last part of the study was protease enzymes determination. The hundred percentage of result were observed (Plate1).

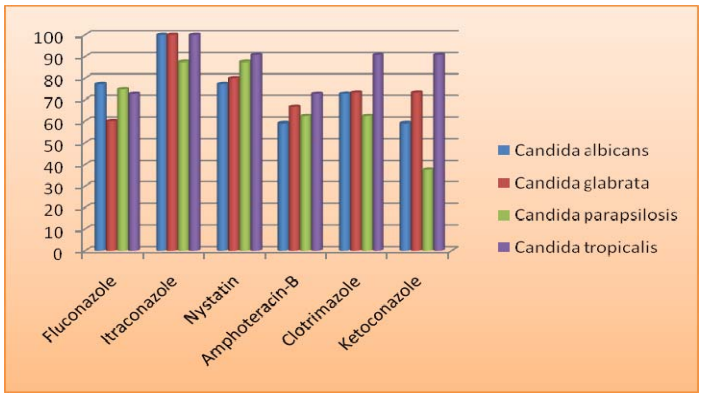

Fig-2 : Antibiotic resistance percentage of *Candida* species

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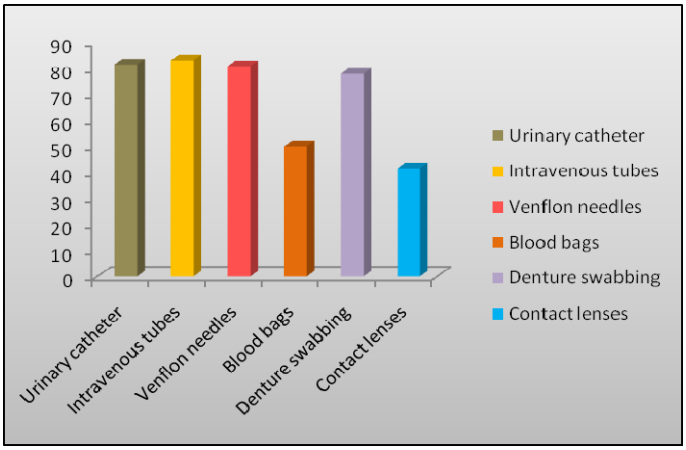

Fig-3 : MDR from different sources of *Candida*

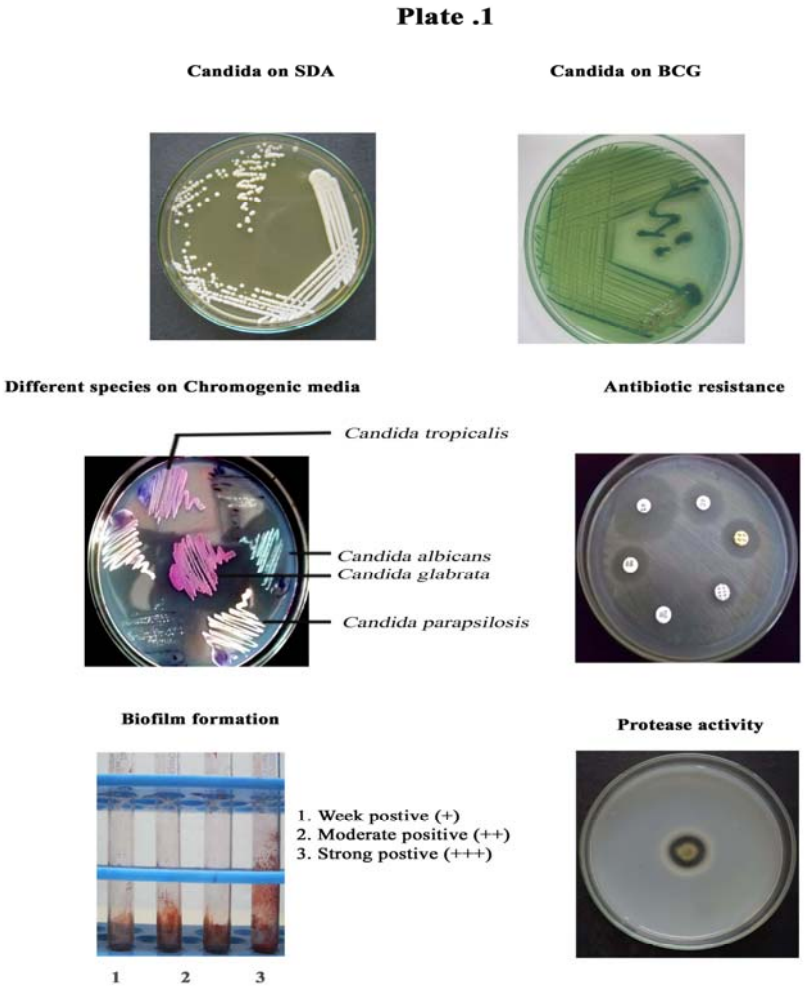

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Table-5: Antibiotic resistance patterns of the isolates

S. No	Antibiotic patterns	No of isolate/ total number of isolates	Percentage
1	It, Kt	1/56	1.785
2	It, Ap	2/56	3.57
3	It, Ct	5/56	8.92
4	It, Nys	3/56	5.35
5	Flu, Ap	1/56	1.78
6	It, Nys, Kt	2/56	3.57
7	It, Nys, Ct	4/56	7.14
8	It, Ct, Kt	1/56	1.78
9	It, Nys, Ap	1/56	1.78
10	It, Nys, Ap, Ct	1/56	1.78
11	It, Nys, Ct, Kt	1/56	1.78
12	It, Nys, Ap, Kt	3/56	5.35
13	Flu, It, Nys, Ap	1/56	1.78
14	Flu, It, Nys, Ap, Ct	1/56	1.78
15	Flu, It, Nys, Ct, Kt	2/56	3.57
16	Flu, It, Nys, Ap, Ct, Kt	27/56	48.24

DISCUSSION

Over the last decades fungal infections are increasing with an alarming rate (Garbino, 2006, Garber, 2001). The increasing rate of fungal infections is a great challenge to healthcare professionals. This increase in incidence of fungal infections is directly related to the increasing number of immuno compromised individuals due to use of intensive chemotherapy and other immunosuppressive drugs (Gokce, 2007). The range of pathogenic yeasts associated with human infections has increased especially *Candida* and *Cryptococcus* (Garber, 2001). Earlier *C. albicans* was regarded the only important fungal agent but now other species are also clinically important. The most common organism implicated in fungal infections is the ubiquitous *Candida*, which is found in the human digestive tract, mouth, and genital region (Eggimann *et al*., 2003). Nosocomial fungal infections are important causes of morbidity and mortality in patients admitted to intensive care units (ICU). Candidemia has been estimated as the fourth most common nosocomial infection with an attributable mortality rate of about 50% *Candida* species may cause severe opportunistic infections, particularly in hospitalized patients (Samia *et al*., 2009). This nosocomial infection mainly occurred by hospital equipments, medical devices and hospital wall, floors, chairs etc., Indwelling medical devices are frequently used in all health setup while critical care units of hospitals use multiple medical devices for treatment and intervention in patient care. Once adhered to fabrics and synthetic materials, both *C. albicans* and *C. parapsilosis* can survive for several days. The adhesion capacity of diverse *Candida* species to different surfaces presumably plays a major role in the pathogenesis of human colonization and invasion (Horn, 2009). In the present study we attempt the different types of medical devices for isolation of *Candida albicans* and non *Candida albicans*. In our results *C. albicans* was the predominant isolates (39.5%) in the all sources and followed by *C. glabrata*, *Candida parapsilosis* and *Candida tropicalis*. *Candida albicans* is the most common and clinically relevant pathogen of the genus. However, there has been a significant trend in the emergence of species other than *C. albicans*, with a particular increase in *Candida glabrata* and *Candida krusei* frequency (Pfaller and Diekema, 2002; Tortorano *et al*., 2004) and to a lesser extent, *Candida parapsilosis* and *Candida tropicalis* (Kao *et al*., 1999). All isolates were obtained by specific (Sabouraud Dextrose agar (Hazen and Howell, 2007 and Larone DH. 2002 and Kreger-Van Rij, N (ed) 1984 and Rippon, 1988) selective media and biochemical test.

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Hichrome Candida differential Agar was useful for detection and identification of *Candida* spp. isolated from different clinical samples (Perry1987 and Rousselle 1994 and Anaparthy Usharani, 2011). Although detailed cost-benefit survey were not carried out, it seems clear that these chromogenic media are economical in terms of labor and time. Moreover, their cost would be more than offset by the decreased need for PCR and other techniques.

In our study urinary catheter had highest fungal prevalence and followed by intravenous tubes. Urinary catheters were used for many indications in hospital like to measure urine output, collect urine during surgery, prevent urinary retention, or control urinary incontinence. Urinary catheters have been held responsible as a cause of 80% of hospital urinary tract infections (UTI) (Wenzel, 1999). The surveillance data from the U.S. National Nosocomial Infections Surveillance system reported *Candida albicans* to be the fourth most common pathogen in UTI. Several researchers have emphasized the role of species other than *C. albicans* as emergent pathogens (Gubbins *et al*., 1993). This point was also verified in our study. We isolated *C. albicans* (58.8%) most often, followed by *C. glabrata* (17%), *Candida parapsilosis* and *Candida tropicalis* had (11%). This high incidence of funguria in this unit should be considered very important. According to some authors, presence of funguria in those patients might be indicative of urinary tract or systemic infection (Nassoura *et al*. 1993). According to our results, the most yeast isolates were obtained from cases and personal samples in hospital indoor in that well known sources of *Candida* contamination were hand touch of the cases followed by hand touch of nurses (Diba *et al*., 2012). Several studies have described the relevance of *Candida* spp. as a nosocomial pathogen (Carlos *et al*., 2008). In these study intravenous needles also contaminated with *Candida* spp, it may be occur by lack of sterile alcohol preparations, and contaminated non-sterile cotton was used for ICU patients. This is in agreement with previous document of Sangita *et al*., (2012). The next part of the study was antibiotic resistance patterns; among the 6 antifungal agents, Itraconazole had highest resistance against *Candida* species. Out of 4 species *C.tropicalis* had highest antibiotic resistance against 6 antibiotics. In case of source wise the highest antibiotic resistance had intravenous tube second most urinary catheter. The emergence of non-*albicans* species may represent selection of less susceptible species by antifungal agents as Fluconazole in particular. Some *Candida* strains as *Candida glabrata* and *Candida krusei* are less susceptible to Fluconazole than *Candida albicans* (Morace *et al*. 1991, Hoppe *et al*. 1994, Nenoff *et al*. 1999). Our result was contrary to that report; *Candida albicans* had highest antibiotic resistance compared to non *albicans*. The main factors responsible for virulence of these yeasts are their ability to adhere to host cells, biofilm production and germ tube formation. Germ tube production was reported only in all *Candida albicans* isolates. Biofilms are a collection of microorganisms surrounded by the slime they secrete. The ability to form biofilms is associated with the pathogenicity and as such should be considered as an important virulence determinant during candidiasis. Biofilms may help maintain the role of fungi as commensals and pathogen, by evading host immune mechanisms, resisting antifungal treatment, and withstanding the competitive pressure from other organisms. Consequently, biofilm related infections are difficult to treat. The biofilm production is also associated with high level of antimicrobial resistance of the associated organisms (Vinitha *et al.*, 2011). In this present study all isolates were produce the biofilm formation but the same time strongly biofilm producing isolates was denture swabs. In case of medical devices venflon needle had highest occurrence. As predicted by earlier work (Silva, 2009), strongly biofilm isolates had highest antibiotic resistance. In the present study *Candida* species were studied for their ability to produce biofilm. Biofilm production was more in *Candida albicans* than other species. There were no significant differences in biofilm production when grouping the strains according to the patients' age, and site of infection. The understanding of microbial biofilm structure and the use of modern technology to bring about modification of the medical devices will lead to decreased microbial infection of medical devices. Proteinase activity of *Candida* species was determined, most of these were found to be Proteinase producers. Proteinase activity was reported in all *Candida* species. The determination of protease and biofilm activity of these will help to understand the pathogenicity and virulence. According to our findings, we believe that *Candida* contamination in medical devices and other materials. It is necessary to more precise and reliable methods such as RAPD-PCR and pulse filed gel electrophoresis to reach the exact sources of clinical isolates within the environment, case and staff materials.

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